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Effect of Mutagens and Antibiotics on Some Traits of The Ladybird Beetle Epilachna vigintioctopunctata (Coleoptera: Coccinellidae)

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University of Rajshahi

Ph.D
THESIS

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SUBMITTED TO THE UNIVERSITY OF RAJSHAHI IN THE FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN GENETIC ENGINEERING AND BIOTECHNOLOGY

SUBMITED BY

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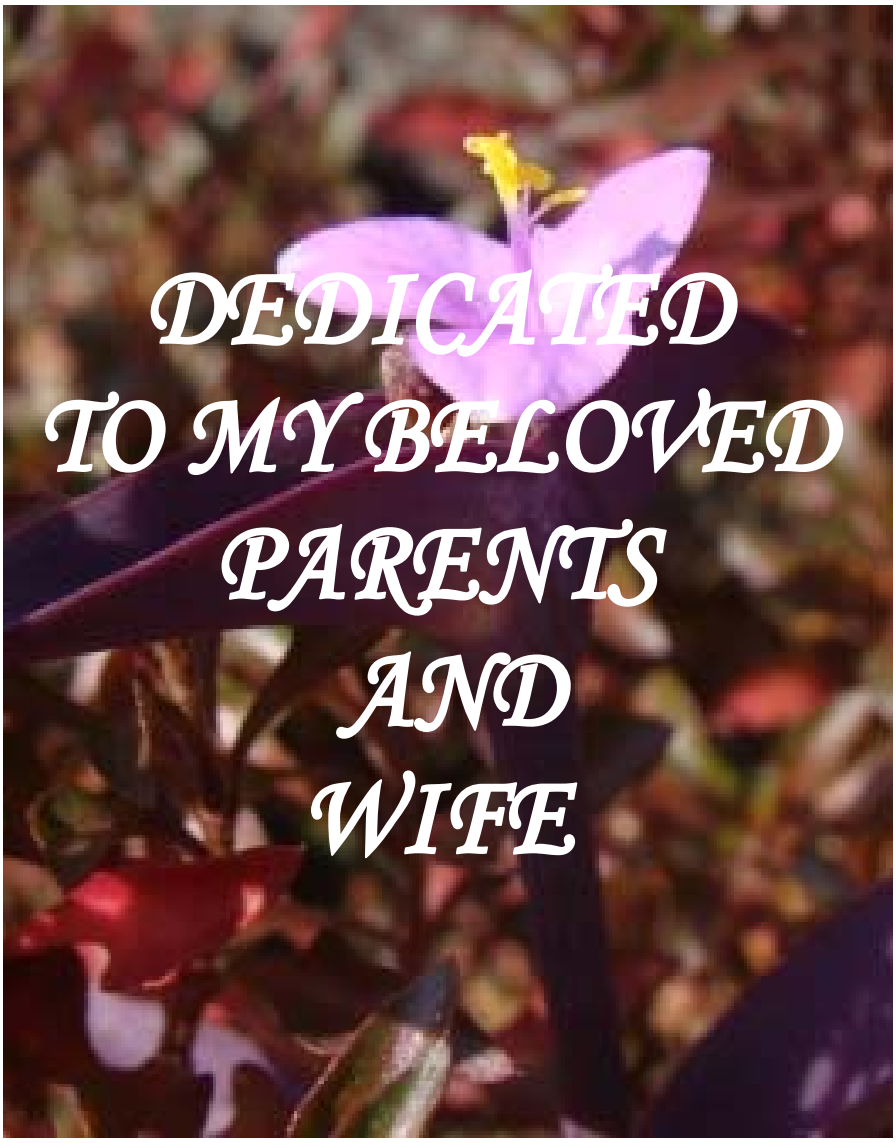
DEDICATED
TO MY BELOVED
PARENTS
AND
WIFE

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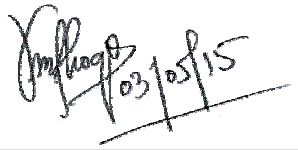
DECLARATION

I do hereby declare that the whole work submitted as a thesis entitled ***EFFECT OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF THE LADYBIRD BEETLE Epilachna vigintioctopunctata (Coleoptera: Coccinellidae)*** *in to the Department of Genetic Engineering and Biotechnology, University of Rajshahi, for the degree of Doctor of Philosophy is the result of my own investigation and was carried out under the guidance of principal supervisor Professor Dr. Md. Abdus Salam, Department of Zoology, University of Rajshahi, Rajshahi and co-supervisor Professor Dr. Shahriar Zaman, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi. The thesis has not been submitted in the substance for any other degree.*



(Kazi Md. Faisal Hoque)

Candidate

Date: 03.05.2015

CERTIFICATE

This is to certify that the thesis entitled ***EFFECT OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF THE LADYBIRD BEETLE Epilachna vigintioctopunctata (Coleoptera: Coccinellidae)*** *is an original work done by Kazi Md. Faisal Hoque for the degree of Doctor of Philosophy in Genetic Engineering and Biotechnology. The references cited in it have duly been acknowledged. The style and contents of the thesis have been approved and recommended for submission.*

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The Author

ABSTRACT

Effects of methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) and actinomycin-D (ACM-D) on the quantitative traits such as, dominant lethal mutation (DLM), number of eggs, number of larvae, number of pupae, sex-ratio, and incubation period, larval and pupal period and longevity of adult males and female were studied in *Epilachna vigintioctopuntata*. Four different doses of MMS, EMS *viz*. 1.0ml, 2.0ml, 3.0ml and 4.0ml were used, five types of crosses were designed and the experiments were carried out for three generations. In crosses of *Epilachna vigintioctopuntata* it was found that all the doses of MMS and EMS induced dominant lethal mutations. However, this effect was not significant in all the crosses. There was a linear relationship between the doses of mutagen and dominant lethal frequency. The mutagenic effect of actinomycin-D on the dominant lethal mutations revealed that it induced dominant lethal mutations (significant or not) but it produced weak effect in the induction. The effects of actinomycin-D on the frequency of MMS, EMS-induced dominant lethal mutations showed that actinomycin-D did not play any role to modify the effects of alkylating agent on the induction of dominant lethal mutations. Effects of MMS, EMS and actinomycin-D on the number of eggs, larvae and pupae were studied. It was observed that the number of eggs, larvae, and pupae were decreased in the treated crosses in comparison to the controls of the parental, F_1 and F_2 generations. It was also noted that the combined action of MMS or EMS and actinomycin-D significantly decreased the number of eggs, larvae and pupae in the parental generation. Mutagenic effects of MMS, EMS and actinomycin-D on the number of male and female were also studied. In the F_1 and F_2 generation numbers of male and female were decreased significantly in some treated crosses than in the controls. The incubation period was decreased in the treated crosses compared to the controls but the value was increased in only one treated cross in the parental generation. In the F_1 and F_2 generations, the incubation period was found to increase in some crosses than the controls.

For the larval period, pupal period, longevity of male and female it was observed that in the parental, F_1 and F_2 generations, the larval period was increased for the treated

crosses in comparison to the control cross. But larval period did not change in the cross ACM-D♂ x control♀. For the effects of MMS and EMS on the pupal period was found to gradually increase with doses. The longevity of adult males and females were also gradually increased by the increased mutagenic doses and antibiotic in three generations. It was recorded that the longevities of males were longer than the females, but the longevity of adult males and females did not change in the cross ACM-D♂ x control♀.

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INTRODUCTION

1.1 Ladybird beetles

The family Coccinellidae was first established by Laterile in 1807 and in 1843 Redtwenbatcher established the genus *Epilachna*. Coccinellid beetles, commonly known as "Ladybird beetles" for long times have attracted attention of entomologists, because of their common occurrence, generally beautiful colouration and great economic importance. It is very important to us for being both beneficial and harmful to our agricultural crops, depending upon their food habits. The family Coccinellidae is classified into two groups, phytophagous and entomophagous. The ladybird beetles locally known as 'Katale-poka' are well known to the people of Bangladesh. Most of the beetles of this class are distinguished by its characteristic bright contrasting colour pattern. Some are red with black spots, some are yellow with black or maroon spots and some have stripes instead of spots and still some have no spot at all. In many myths and legends, ladybird beetles are considered as the symbol of good fortune. The term 'lady' is in reference to biblical mother Mary (Roache, 1960). The red colour is said to represent her clock which in early painting and sculptures was usually depicted as being red.

Ladybirds are probably the most benignly considered insects in the world. Although many people dislike beetles ladybirds are excluded from this distaste, initially as a result of their bright colours. Due to its colourful features it has gained its access in the motifs on children books as well as attractive prints in clothes and other products. Children are introduced with this lovely creature at their early ages, both from seeing them in the wild and from their attractive illustrations on various items. They even hear of them in rhymes. In many cultures this magnificent creature has religious connections, often being associated with scared figures (Richards, 1983). Indeed, out own name for this family of beetles reflects this, as they are called after the Lady, the Virgin Mary.

Ladybirds have already been used to study of the female choice, sexually transmitted diseases in invertebrates, habitat specificity and the evolutionary consequences of

cannibalism. They are also used as the model system for the study of male-killing endosymbionts. By increasing our knowledge of these colourful characters, their beneficial characters can be observed in nature as well as in culture media.

There are over 5000 species in the ladybird family varying in wide range in respect of size and colour. Their size may range from less than 2.0mm to over 15.0mm (Deshmukh *et al.,* 2012). In terms of colour they cover the whole spectrum of the rainbow. Many species spots at least two strongly contrasting colour in a spotted or striped pattern, although some of the smaller species are black or brown and lack bright markings. In the United Kingdom the most common species is the red one with chestnut pale yellow stripes while some species with black spots. In our country the most common one is *Epilachna vigintioctopunctata*. The ladybirds exhibit aposematism (warning colouration), where bright or contrasting colour patterns warn away potential predators. They are also able to secrete unpleasant or toxic alkaloids, pyrazines and glucosides to make them unpalatable or even poisonous to the predators. When attacked they secrete filtered haemolymph containing these defensive chemicals through pores in their legs, forming small droplets of yellow fluid on the edge of their pronotum elytra. This fluid smells and tastes foul and deters most of the vertebrate predators from swallowing the intended prey. As they are extremely resistant to injury, most of the rejected ladybird survives from these initial attacks. The predator comes with a nasty experience associated with the bright contrasting colours reducing the likelihood of attacks on similarly coloured insects (Dieke, 1947). Therefore, not surprisingly, a great many beetles and other insects mimic these chemically protected species.

There are still many areas of ladybird ecology and behavior which are not fully understood, however, they are one of the better research groups of invertebrates in these respects. This is important for two reasons: firstly, ladybirds are major predators of some plant pests. The more understanding of these insects, the more to utilize them in integrated pest management schemes. Secondly, our knowledge of this beetles makes them useful as model organism in a range of research fields and increases our ability to interpret research findings cogently. For example, the 2-spot ladybird has recently been

used to look at the knock-on effects on pest predators of insect-resistant lectin gene from snowdrop into potato (Birch *et al.,* 1999).

1.2 Description of *Epilachna*

According to Dieke (1947), about one sixth of all the described species of the Coccinellidae belong to the sub-family Epilachninae and almost all are single genus *Epilachna.* The members of this sub-family are recognized by certain morphological characteristics and are distinguished from the rest of the Coccinellidae not only by their structural differences but also by marked difference in their food preferences. The species of *Epilachna* almost exclusively feed on leaves of plant species belonging to Solanaceae and Cucurbetaceae, with a few species attacking plants of other families, particularly the Fabaceae and the Composite. Many of our important cultivated plants, such as potato, tomato, brinjal, squash and bean are members of these plant families and are subjected to devastating attack by the species of *Epilachna*.

The adults present apparently a great uniformity in external structure, almost all of the known species had been placed in one genus, *Epilachna*, but later there has been a tendency to divide this sub-family into many natural groups based on external structures of the genitalia (Kapur, 1950). The elytron of *Epilachna* is variable to their body size, spot patterns of large and small black colour. Katakura *et al.,* (1988) mentioned the individual beetles of *Epilachna,* possess 6-14 spots on each elytron. This *Epilachna* is also widely distributed in all over Bangladesh.

1.3 Distribution

The phytophagous ladybird beetles of the sub-family Epilachninae are most diversified in tropical and subtropical regions of the world (Katakura *et al.,* 1988) and only a scarce number are distributed in cooler region (Richards, 1983). Phytophagous ladybird beetle *E. vigintioctopunctata* is widely distributed from tropical to temperate regions throughout Asia and Oceania and its host plants are Solanaceae (CAB, 1992). About 4500 species under 500 genera in 21 tribes of ladybirds have been described worldwide where most of the species are predacious and are used for biological control of aphids, scale insects, white

flies and phytophagous mites etc. which are serious pests of agricultural crops. To make best use of these beetles we have to know about their bio-ecology. For the proper identification of any species of this genus, one has to acquire taxonomic knowledge about the relevant insect group. Morphology is of prime importance for taxonomical studies (Rajagopal and Trivedi 1989; Mondal and Ghatak, 2007; Ghatak and Mondal 2008; Swaminathan *et al*., 2010).

E. vigintioctopunctata is widely distributed in South-East Asia, Srilanka, India, China and Australia (Kapur, 1950). Several workers have investigated 52 species under 33 genera of Coccinellidae as they have been in the northern region of Bangladesh (Ahmed and Quayum 1985). In India the beetles are present in the hills tracts of Jammu and Kashmir, Punjab, Himachal Pradesh, Uttar Pradesh, West Bengal and also in the plains.

1.4 Damage of crop

Both the grubs and adults of *Epilachna* feed on the leaves of host plants to make the leaves a net like structure and later the leaves dry away which ultimately weaken the plants (Islam, 2001). Khatun (2006) stated that both the adults and grubs of *E. dodescastigma* (Wied) and *Henosepilachna vigintioctopunctata* (F) feed on green chlorophyll and skeletonize the leaves which present a lace like appearance and later dry away. Walker (1957) stated that the adults and larvae of *Epilachna* feed on the foliage of potato, wheat, maize, oats, barley, sunflower and cotton. Islam (2001) reported that only *E. vigintioctopunctata* consumed 178.63 cm^2 of leaf area equivalent to about 3 to 5 number of leaves in total lifetime of their own host plants.

1.5 Life cycle

Epilachna vigintioctopunctata passes the winter as a hibernating adult among heaps of dry plants or in cracks and crevices in the soil. It resumes activity during March-April and lays yellow cigar-shaped eggs, mostly on the undersurface of leaves, in small batches of 5-40 (Atwal, 1976), 34 (Chue, 1930) or 45 (Krishnamurti, 1932) eggs in each batch. A single female can lay up to 400 eggs in her lifetime. In laboratory feeding studies on Australian populations of *E. vigintioctopunctata,* Richards and Filewood (1988) found a

maximum fecundity of 2882 eggs laid, with an average of 1692 eggs, when pairs of beetles were reared on *Solanum nigrum*. Mating takes place soon after emergence. The instinct is a little more precocious in the males, which look for females as early as the age of 4-5 days, while the females accept the males a little later, preferably about the seventh day of life, although they sometimes mate on the sixth and even on the fifth day.

The hatchability is highest (94.5%) at about 35°C. The eggs hatch in 5, 3.5 and 2.9 days at 25°C, 30°C, 35°C, respectively (Atwal, 1976). There are four larval instars. The speed of larval growth and development depends on the abundance of food supply and temperature. Larval development is completed in 11-15 days (Chue, 1930). Krishnamurti (1932) found that developmental period was 14-16 days in potato leaves, and took 29-31 days in cucurbitaceous plants. However, two different species may have been involved in this study. Richards and Filewood (1988) found that larval development at 25°C averaged 23 days when *S. nigrum* was the host, but only 17.4 days when *S. tuberosum* was the host. In laboratory feeding studies on an Indian population, Singh and Mukherjee (1988) found larval development periods of 12-15 days at about 27.5°C, and survival rates of 36-80%, both depending on the host.

Pupation occurs on the leaves, stems and at the base of the plants. Prior to pupation the fourth instars larvae may migrate away from their larval habitats to drier ground. However, the periphery of breeding places may be sufficiently dry for larvae to pupate there. The pupal stages were reported as 13.4, 6.7 and 5.1 days at 25°C, 30°C, 35°C, respectively. The adult beetle escapes from its puparial case by pushing off its posterior end, crawling out and flying away. The longevity of males and females varies from 50-180 days. Beetles are most active at high temperature and at low humidity and least active at low temperature and humidity. The preferred temperature for adults lies between 30°C to 35°C. The pest passes through several broods from March to October and its population is at a maximum at the end of April or in early May. During the hot and dry months, the number declines greatly but the population again builds up in August (Atwal, 1976).

1.6 Genetic variability

The existence of genetic variability of component of fitness in natural populations and that of genetic correlation between these components are two important factors in the evolution of the life history for a given population. Without genetic variability no evolution will occur. Moreover, the existence of genetic correlation between life history traits that are beyond the control of selection may, however, evolve because they are linked to other traits that are selected for or against. Though genetic influences on life history traits have been repeatedly emphasized in *Drosophila* (Lints, 1988), a very few experiments have been conducted to verify the existence of genetic variability in natural populations.

Important quantitative genetic properties of a population may be changed in multiple ways by changing the environment it inhabits. Environmental changes can affect the average expression of traits, the amount of genetic variance and environmental variance for traits, the heritability of those traits and the level of inbreeding depression (Hoffmann and Parsons, 1991). Moreover, the effect of environmental change has sometimes been assumed to be greatest in stressful environments, for example, those to which the organisms are not well adapted (Hoffmann & Merila, 1999).

The genetic variation in growth performance in a population of the herbivorous ladybird beetle *Epilachna vigintioctopunctata* Motschulsky (Coccinellidae, Epilachinae) on both the normal host and a novel host were studied by Ueno *et al.,* (2001). They found higher heritability for growth performance on the novel host than the normal host. The difference in heritability was caused by smaller among-family variance components on the normal host compared to those on the novel host. The results are compatible with the view that natural selection, which improved the ability of the population to use a particular host plant, has reduced genetic variation within that population in the ability to use that host plant.

1.7 Uses of mutagens and antibiotic against pest insect

In recent years a new area of research on the public health issues have emerged and gained enormous interest, which is known as genetic toxicity. Genetic toxicity refers to the ability of substances or physical agents to damage the DNA and/or chromosomes of cells. Such

damage can lead to mutations that increase the likelihood of certain diseases, such as cancer and birth defects. In contrast to other toxicology, the focus of this new field is not only the evaluation of the biological activity of certain chemical compounds that may be harmful to any individual but also to his future progeny. Concerns over the general problems of environmental mutagenesis began with the discovery of supermutagenes in the mid-sixties (Rapoport *et al*., 1966). Supermutagenes are chemicals which can produce gene mutation at high levels resulting in either low or negligible levels of toxicity to the cell. As a matter of fact there are numerous man made chemicals in the environment with such potent mutagenic activity in the range of experimental organisms which now raises a widespread concern over their effect on human populations. Therefore, the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, and the U.S. Consumer Product Safety Commission have testing requirements and guidelines in place for assessing the genetic toxicity of regulated products.

And due to civilization, urbanization and industrialization large number of wastes is generated which is dumped into the environment annually. Approximately 6×10^6 chemical compounds have been synthesized, with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water (Tiwari G and Singh SP 2014) most of them have mutagenic effect on the flora and fauna of any given place. The contaminants causing ecological problems leading to imbalance in nature is of global concern. The environmentalists and the genetic toxicologists of the world are trying to overcome it by several means. However, they are raising their voices at international platforms regarding the depletion of natural resources; little attention is given to their words and continues to use them without caring the adverse consequences (Shukla *et al,* 2010). The exploratory experiments which were started in the late sixties to test chemical in our environment have shown that mutagenic chemical can be found in all major categories: food additives, drugs, pesticides, cosmetics, air and water pollutants, as well as, household and industrial chemicals.

With the large variety of genotoxic agents in our environment, it is particularly urgent to know the action of above chemical compounds, their dose effects, combined effects etc. This will allow a better assessment of benefit versus risk, moreover, can also be learned from this study of this kind is that the mechanisms of how a particular chemical structure produces mutations (Sables, 1982). The responsibility of the genetic toxicologist is rather unusual in that, first he must consider the effects of exposure to a genotoxic agent on individuals in the present population. Then scientist must develop effective measures to determine the likelihood of successful transmission of any damage that may be produced in the germ cells and to evaluate its effect on future generations. This is necessary not only to evaluate the effects of exposure on the individual but also to his offspring which is technically difficult. Because man's most precious heritage is his genome, it is an awesome responsibility. The vitality of the human population and indeed its very existence, as it is known now, is dependent in a large part on the successful transmission of an undamaged genome from one generation to another.

It is our prime obligation to identify the environmental mutagens in the current context of growing number of chemical mutagens emerging everyday which are adding increasing risk of mutagenesis and carcinogenesis for our life (Zimmering, 1981a). To resolve this issue, a series of short-term *in vitro* and *in vivo* tests are employed for mutagenicity assessment which ultimately may help in effective and affordable protection against an active chemical.

With this approach, thousand of untested environmental chemicals could be assayed to identify those agents in our environment with potential mutagenic and carcinogenic effects on man. Researchers are not ready, however, to extrapolate directly from data obtained in short-term tests for mutagenicity directly to man. This is simply because researchers do not have a sufficiently large database to be confident that a genetically active compound in the short-term tests will produce significant levels of genetic damage in man. Because of the lack of such data the short term tests are best viewed as qualitative indicators of genetic activity. To get the quantitative data that are required for estimating risk to man, tests on other organism will have to be performed. This is not only to obtain further information on the total spectrum of genetic alterations produced, but also to

determine the rate of increase over the spontaneous background for each class of genetic alterations (De Serres, 1976).

Drosophila has been considered for long time as the model animal to perform these short-term tests besides the prokaryotes because of its genome simplicity and shorter life span. A total spectrum for identification and determination of genetic damage in germinal as well as in somatic cell types are well developed (Nguyen *et al.,* 1979) in *Drosophila.* The total genome sequence of *Drosophila* is known. Numerous loci (genes) which may express themselves as phenotypic or lethal mutations have been documented and mapped (King, 1975). In *Drosophila* most of the genotoxic end-points relevant to human hazard can be detected either simultaneously or in separate assays *viz*. point mutations, small deletions, duplications, chromosome damage and aneuploidy. Furthermore, genetic events can be assayed either in the soma or germ line (Abrahamson and Lewis, 1971; Vogel and Sobels, 1976).

In assessing priorities for mutagenecity testing of environmental agents MMS, EMS and ACM-D, by most conventional criteria, would be considered one of the most important chemicals to evaluate. A central consideration in the establishment of a firm data base from which inferences may be drawn concerning the mutagenic activity of these chemicals and their chemical allies, and an ultimate decision as to it's potential as a genetic hazard in man, is availability of adequate procedures for assessing DNA altering agents, repair modification. It is obvious that these mutagens and antibiotic does not represent a simple case study in determining genetic hazards, however, they will serve to illustrate the more practical problem of identifying multi-factorial effects of several substances in association, with isolated studies with single substances that can readily control more in experimental procedures (Legator and Zimmering, 1979).

In the study of chemical mutagenesis in biological systems, it is important to establish accurate dissymmetric techniques relating the administered doses of chemical agents to the number of potentially mutagenic events occurring in individual cells (Sega *et al.,* 1974). The relationship between dosage and mutational response has been used extensively in radiation genetics for distinguishing between different mechanisms of

mutation induction and for establishing a basis for estimating risk at low dosage for results at moderate to higher dosage. Unfortunately, as a quantitative science chemical mutagenesis has not progressed rapidly as did radiation genetics. Lee (1975) suggested that dose should be defined as the amount of the chemical that reacts with a selected target molecule such as DNA in the germ line. Some parameters such as dominant lethal mutations, sex-linked and autosomal recessive lethal mutations were generally used to test the effect of chemical mutagens.

Dominant lethal mutations were first recognized early in this century and their basic properties were explored in 1930's by Strandskov (review, Russel, 1954). The studies were reopened in the 1950's to explore various properties of ionizing radiations (Radman, 1979). The method proposed by Bateman (1966) has been widely used in many academic and industrial laboratories for testing chemicals for mutagenesis. The dominant lethal test measures genetic damage to the germ cells. It is an *in vivo* test to determine risk to germ cells from a suspect mutagen. The test is usually conducted after genetic activity has been observed *in vitro* with other mutagenecity tests. A positive result in dominant lethal test provides evidence for damage transmitted via gametes.

The test for sex-linked recessive lethal has been used to detect induced mutations, however, the early experiments were mainly carried on *Drosophila melanogaster*. The advantage of testing both screening and hazard evaluation is transmissible mutations in the germ cells of a eukaryote. Statistical criteria for both negative and positive mutagenecity at the highest concentration tested under a particular exposure condition were developed by a work group, and a recommended protocol for future testing was agreed upon (Lee *et al.,* 1983). In the years after world war II, the mutagenic effects of radiation were studied extensively (Sankaranarayanan and Sobles, 1976). In the early 1940's, Charlotte Auerbach and her co-workers (1946) discovered that certain chemicals were mutagenic using the sex-linked and autosomal recessive lethal assay in *Drosophila*. Since that time an increasing number of chemicals have been tested for mutagenic activity in various organisms using this assay (Lee, 1976a) and has been proved to be an excellent screening test for the detection of chemical mutagens (Vogel and Sobels, 1976;

Donner *et al.,* 1983). The value of the recessive lethal test was further increased by demonstration of xenobiotic metabolizing enzymes in adult flies (Baars *et al.,* 1977; Baars, 1980) and in larva (Hallstrom *et al.,* 1981; Zijistra *et al.,* 1979). The metabolic capacity reaches to the germ cells, *i.e*. the targets of the sex-linked recessive lethal test (Vogel, 1975).

The mutational response of different germ cell stages is usually studied by generation pattern analysis. Adult male insects are treated with a mutagenic agent and then are mated daily to a succession of females. The testis of adult male insect contains all germ cell stages from spermatogonia to spermatozoa. By mating treated males to a succession of tester females one obtains a succession of broods representing germ cells that were successively younger at the time of treatment. This "brood pattern analysis" provides information about the treatment of different germ cell stages (Ashburner and Novitski, 1976). With the large variety of potential hazards in our environment, it is particularly urgent to understand the section of above chemical compounds, their dose effects on different generations, their combined effects etc. which will allow a better assessment of benefit versus risk.

1.8 Objective

Bangladesh is an agro-based economy. The control of pests of agricultural crops, fruit-trees etc. are vital to boost up its economy. Global warming has cautioned us and the adverse consequences of using pesticides are always alarming and also induce pest outbreak because of pest resistance and mass propagation. The entomological backlashes have compelled the scientists to be concerned with compatible pest management programme. The beneficial Coccinellidae may be an alternative and complementary means to insecticidal spray. Moreover, they are also used in fields of biology, population ecology, genetics, cytology and bio-geography.

This species is fairly common throughout the country and causes considerable damage to a number of solanaceous, cucurbitaceous and leguminous crops (Anam *et al*., 2006; Rahaman *et al*., 2008). Using various plant extracts, attempts have been made to save such crops as potato (Rajagopal and Trivedi, 1989), brinjal (Sreedevi *et al*., 1993; Ghatak and Mondal, 2008), oilseed (Ahmed, 2007; Ahmed *et al*., 2010), cucumber (Mondal and

Ghatak, 2007; 2009) and bitter gourd (Rahaman *et al.*, 2008) against the attacks of the beetle.

In spite of being one of the most common and important pest of solanaceae crops in our country no detailed accounts of the mutagenesis, ecological, cytological, genetical and biochemical investigation have been made on *Epilachna*. So, it should be of more importance to study their mutagenecity, genetics, developmental period and life history with reference to various mutagenic chemicals and antibiotics. In this current project effects of two chemical mutagens MMS, EMS and an antibiotic ACM-D was determined on various developmental stages and generations of *Epilachna*. Moreover, an important lesson to be learned from this kind of study is the fundamental research on the mechanisms of producing mutation by particular chemical structure (Sobels, 1982).

As very little information is available concerning the combined effect of different doses of methyl methanesulfonate and ethyl methanesulfonate with actinomycin-D on the pre-meiotic and post-meiotic male germ cells of *Epilachna* to the induction of dominant lethal mutation, number of larvae, number of pupae, sex-ratio, incubation period, larval and pupal period, and longevities of adult male and female it was deemed to assess it. Our increasing knowledge of these colourful characters, their beneficial nature and the ease with which many species can be observed in nature and cultured in captivity means that their study is likely to contribute to other spheres of research in the future.

BRIEF LITERATURE SURVEY

2.1 Mutagenic effects of methyl methanesulfonate (MMS):

Methyl methanesulfonate (MMS) is a monofunctional alkylating agent that has been found to be mutagenic in a wide variety of genetic test systems from viruses to mammals. The *in vitro* induction of mutations in bacteriophages by alkylating agents was first demonstrated by Loveless (1958, 1959), who observed the production of r mutations in bacteriophage T_2 by ethyl methanesulfonate (EMS) but not by methyl methanesulfonate (MMS). Bautz and Freese (1960) studied the mutagenicity of ethyl ethanesulfonate (EES) by scoring the induced reversion of T4r11 mutations but observed only very small (and statistically insignificant) increases in r mutant and $r11^+$ revertant frequencies after MMS treatments. Strauss (1961) also reported no r mutation induction by MMS. As a result, it has sometimes been assumed (Krieg, 1963) that MMS does not induce mutations in bacteriophage T_4, even though it is mutagenic in many cellular systems (Loveless, 1966).

Methyl methanesulfonate (MMS) is an extraordinarily poor mutagen compared to ethylnitrosourea (ENU) or even X-rays (Tao K. S. *et al,* 1993). In lung fibroblasts *in vivo,* MMS has been shown to induce many micronuclei but few, if any, mutations at the hpt locus. Methyl methanesulfonate (MMS) induces specific-locus and dominant lethal mutations in spermotozoa and spermatids of mice. A dose of 15 mg/kg b.w. of MMS induces 9% dominant lethal mutations in the most sensitive germ-cell stages, corresponding to the mating intervals 5–8 and 9–12 days post treatment (Ehling U.H. and Neuhäuser-Klaus A, 1990). Mutagenesis induced by methyl methanesulfonate (MMS), a germ cell mutagen, in the testis and the sperm isolated from epididymis and vas deferens have been investigated using lac Z transgenic mice (Muta™ Mouse). Male Muta™ Mice were injected intraperitoneally with MMS at a dose of 80 mg/kg, a potent dominant lethal dose (Itoh *et al,* 1997). Methyl methanesulfonate (MMS), and ethyl methanesulfonate (EMS) were used as model compounds for assessing DNA damage (Mouchet F. *et al,* 2005). *Drosophila* ATM/ATR homolog and the use of it to document a semi dominant effect on a larval mitotic checkpoint and methyl methanesulfonate (MMS) sensitivity (Anne L 2003).

In *Salmonella typhimurium,* MMS induced backward mutations to histidine requirement *in vitro* and in the host mediated assay. Forward mutations were induced in either prokaryotes, eukaryotes or mammalian cells both *in vitro* and in the host mediated assay. However, the host mediated assay with *Escherichia coli* which measured mutations to ampicillin resistance was negative at 165 mg/kg in rats and 25-100 mg/kg in mice. Higher concentrations of MMS reduced the viability of the bacteria drastically, so that no mutation rate could be established.

In mouse lymphoma cells there is a noticeable difference of sensitivity to MMS for the different markers. Suppressor mutations, recessive lethals, somatic segregation and non-disjunction tests with *Aspergillus nidulans* gave a positive response to MMS (Gualandi *et al.,* 1979; Ettakafer and Mayor, 1986). Induction of p-fluorophenylalanine resistance in a diploid strain of *Aspergillus* heterozygous for the fpa A1 mutation was positive in the spot test and the plate test but negative in the liquid test. In the recessive marker yA2 nonselective system which tests for both somatic segregation and non-disjunction, MMS was also positive. The concentrations listed for the induction of mitotic gene conversion and mitotic recombination in yeasts seem rather high but for some of the tests were the only concentrations used so far. There is one exception, however, in that mitotic recombination in *Schizosaccharomyces pompe* was negative at concentrations below 200 mM and no positive response was obtained in the host-mediated assay with mice at doses from 300 to 1200 mg/kg.

Drosophila offers numerous advantages for the detection and characterization of mutagenic agents (Vogel and Sobels, 1976; Wurgler *et al.,* 1977). Powerful genetic tools capable of identifying a total spectrum of genetic damage in germinal as well as in somatic cell types are well developed. MMS induces recessive lethal mutations (Fahmy and Fahmy, 1957, 1961a; Khan, 1969; Vogel and Leigh, 1975; Vogel and Natarajan, 1979a, Ryo *et al.,* 1981; Zimmering, 1982; Eeken and Sobels, 1983; Vogel *et al.,* 1982; Cooper *et al.,* 1983; Vogel *et al.,* 1985),

Ryo *et al.* (1981) observed that the frequencies of sex-linked recessive lethal mutations in F_1 males after feeding adult male *Drosophila melanogaster* with 0.25 and 0.5 mM methyl methanesulfonate (MMS) orally for 24 h increased approximately linearly with storage of treated spermatozoa in females, whereas the number of hits of dominant lethals in the sperm after feeding 0.3 and 0.5 mM MMS increased approximately with the square of the storage time. Chromosome losses and mosaics in F_1 males also increased with the dose of MMS to males, but their yields were too low to be analyzed quantitatively, only indicating a slight increase of chromosome losses and a slight decrease of mosaics with the time of storage of sperm. Maternal non-disjunctions (or chromosome losses), detected in F_1 males, decreased with the dose of MMS to spermatozoa and their yield decreased with the time of storage of sperm of both MMS-treated and the control groups.

Comparisons of the efficiencies of the sex-linked recessive lethal test and the rosy single gene mutagenesis test for mutation induction by methyl methanesulfonate in *Drosophila* has been studied (Cooper *et al.,* 1983). It has been shown that sex-linked recessives lethal test monitors mutation induction at approximately 700 x-chromosomal loci (Abrahamson *et al.,* 1980).

A note on the utility of repair deficient st mus 302 *Drosophila* females in detecting chromosome loss and sex-linked recessive lethals induced in the male genome by methyl methanesulfonate (MMS) was given by Zimmering (1982). Zimmering (1981a, b) has shown that when *Drosophila* males carrying a ring-x and a doubly marked y chromosome were treated with MMS, and mated with mei-9^a, st mus 302, or repair proficient females, the frequencies of induced chromosome loss acored in F_1 progeny decreased in the order st mus 302 > mei-9^a> repair-proficient females. Loss with st mus 302 was accompanied by a reduction in fertility of the crosses (modest to severe depending on the dose used) likely due to the production of dominant lethals.

Concentration-effect studies with MMS on the induction of dominant and recessive lethal mutations, chromosome loss and translocations were shown. It was found that at low concentrations there was no direct concentration-frequency relationship (Vogel and Leigh, 1975). *Drosophila melanogaster* tests for recessive lethal mutations showed that

spermatozoa and spermatids were the stages most susceptible to MMS while cell killing occurred in spermatocytes. In spermatozoa the recessive lethal yield increased with concentration with a lowest effective concentration (LEC) between 0.01 and 0.05 mM. MMS is very efficient in the induction of chromosome aberrations in *Drosophila.* It causes the spermatozoa and spermatids II-III translocations, dominant lethals, entire chromosome loss and y-rearrangements. The performance of storage experiments proved to be an absolute necessity for the detection of II-III translocations induced by monofunctional alkylating agents (Vogel and Nataraja, 1979a). There is a remarkable difference between the LEC: LD_{50} ratio for recessive lethals and the different categories of genetic damage; the ratios are 1:100 for recessive lethals, 1:10 for dominant lethals, translocations and entire chromosome loss, and 1:5 for y-rearrangements. The genetic damage associated with structural chromosome changes is not observed in non-toxic concentrations below 1/100 of the LD_{50} while mutations are still detected (Vogel and Leigh, 1975).

The somatic system can be used as a screening test for potential mutagens (Rasmuson *et al.,* 1983). This survey is an attempt to correlate the size of the mutated area of the eyes of *Drosophila* with the age of the larvae at mutagen treatment. Rasmuson (1985) used X-rays and MMS to give an indication of the mechanism of the instability, according to the different kinds of DNA damage induced. The results showed that the mean size of red spot decreased with increasing age of larvae at treatment, while the mutation frequencies were increased because of the multiplication of the cells in the eye susceptible to the mutagens. This is contradictory to the hypothesis maintained by Fahmy and Fahmy (1980) that the somatic shifts are not mutagenic but epigenetic events, due to altered regulation of the gene expression.

The dominant lethal test with the small fresh-water fish *Dryzas latipes* is a useful tool for detecting mutagenic factors in an aquatic environment (Egami *et al.,* 1983). Shimada and Egami (1984) treated males of the fish *Dryzian latipes* with MMS and mitomycin C and then mated with normal females. The fertility and hatchability of the eggs laid by the parents were examinated, and the dominant lethal effects were estimated. It was found that MMS induced dominant lethals in spermatozoa and spermatids after the injection of

200 and 400 mg/kg. Preston and Gooch (1981) demonstrated the induction of chromosome-type aberrations in G_1 by MMS in human lymphocytes. It was observed that MMS is capable of inducing this type of aberrations. Sandhu and Singh (1987) also studied the effect of MMS on human lymphocytes.

Methyl methanesulfonate (MMS) is an anti-carcinogenic drug and its toxicity has been reported in various experimental models (Vineet K.*et al.,* 2011). The mutagenicity of the rodent carcinogen methyl methanesulphonate (MMS) to the liver and bone marrow of Muta Mouse lac-Z transgenic mice was evaluated (Tinwell H *et al.,* 1998). induced by methyl methanesulphonate (MMS) as genotoxic end points both in the presence as well as absence of rat liver microsomal activation system (S9 mix) in cultured human lymphocytes (Siddique YH, and Afzal M, 2005).The consequences of a defect for nucleotide excision repair (NER) in oocytes for alkylation-induced mutagenesis in different germ-cell stages of Drosophila males (Vogel EW and Nivard MJ.,1997). Treatment of the yeast Saccharomyces cerevisiae growing on a glucose-rich medium with the DNA alkylating agent methyl methanesulphonate (MMS) triggers a rapid inhibition of respiration and enhances reactive oxygen species (ROS) production (Kitanovic A *et al.,* 2009).

Chromosomal damage by MMS was observed in mammalian cells in culture at concentrations about 10 times higher than those required for the induction of sister-chromatid exchanges (SCE). In mouse bone-marrow chromatid breaks were still significantly elevated above control level at 5 mg/kg, while micronuclei could only be detected at 10 mg/kg. For the induction of SCE in mouse and Chinese hamster bone narrow a dose of 1 mg/kg gave results that were significantly different from the control (Marquardt and Bayer, 1977; Renault *et al.,* 1978). The heritable translocation test in mice was positive with 40 mg/kg at the spermatid stage (Lang and Adler., 1977). This was the only dose tested so far.

Dominant lethal mutations by methyl methanesulfonate (MMS) in the germ cells of male mice were studied (Ehling *et al.,* 1968; Eling, 1977; Arnold *et al.,* 1976; Shimada *et al.,* 1985; Generoso *et al.,* 1971; Lang and Adler, 1977; Generoso 1969; Sega and Owens,

1983). In the dominant lethal assay spermatozoa and spermatids gave a positive response. With 40 male mice treated and mated to untreated female mice at a ratio of 1:1 a dose of 10 mg/kg of MMS significantly increased the frequency of dominant lethal 9-12 days after treatment (Ehling, 1977).

DNA-methylating agents of the S (N) 2 type target DNA mostly at ring nitrogens, producing predominantly N-methylated purines (Nikolova T *et al.,* 2010). In eukaryotes, together with the Mre11/Rad50/Xrs2 (or Nbs1) complex, a family of related protein kinases (the ATM family) is involved in checkpoint activation in response to DNA double-strand breaks (Suetomi K *et al.,* 2010). Methyl methanesulphonate (MMS) is a DNA damaging agent, which induces oxidative stress, ATP depletion, and consequently, cell death, in HL-60 and K562 cells (Mlejnek P *et al.,* 2007). The time course of development of thymic lymphoma, which occurs spontaneously in mice of the AKR strain, is accelerated by the methylating agents N-methyl-N-nitrosourea (MNU) and methyl methanesulphonate (MMS) (Warren W *et al.,* 1990).

Gene mutations in mammals were observed in the mammalian spot test which assesses the rate of presumed somatic mutations. Here, the lowest tested dose of 50 mg/kg gave a positive response. In the specific locus test MMS induced mutations only in post-spermatogonial stages and not in spermotogonia. To test the differences in the mutation rates of various post-spermatogonial stages the treated male mice were sequentially mated in 4-day intervals with untreated test-stock females. The so far limited data indicate that the peak mutation frequency corresponds well with the most sensitive stage for the induction of dominant lethal mutations. The mutation rate with 40 mg/kg of MMS was significantly higher than the control value (Ehling, 1978).

2.2 Mutagenic effects of ethyl methanesulfonate (EMS):

Ethyl methanesulfonate (EMS) is a representative of the large class of chemical alkylating agents. The mutagenic effects of this monofunctional alkylating agent are extensively documented in a variety of species (Loveless, 1966; Fishbein *et al.,* 1970).

EMS was discovered by Loveless (1958) to induce mutations in bacteriophage T_2 treated *in vitro.* Green and Krieg (1961) performed similar studies with EMS using T_4 phase. Lethality was found to increase with time after the end of the EMS exposure in T_7 phase (Verly and Brakier, 1969; Brakier and Verly, 1970; Verly *et al.,* 1974; Karska-Wysock *et al.,* 1976).

The high mutagenic activity of EMS has been demonstrated in a wide variety of bacterial genera, species and strains including *Escherichia coli* (Kondo *et al.,* 1970; Hill, 1972; Schwartz, 1963; Strauss, 1962; Turtoczky and Ehrenberg, 1969; Howell-Saxton *et al.,* 1973; Mohn *et al.,* 1974; Ishii and Kondo, 1975; Nestmann, 1975; Green *et al.,* 1977; Quillardet *et al.,* 1982, 1985; Otsuka *et al.,* 1985), Salmonella (McCann *et al.,* 1975a, b; Skopek *et al.,* 1978; Mc Mohon *et al.,* 1979; Haworth *et al.,* 1983; Maron and Ames, 1983, Rasool *et al.,* 1986), *Proteus mirabilis* (Bohme, 1968; Adler *et al.,* 1976), *Haemophilu influenzai* (Kimball and Hirsch, 1975; Notani *et al.,* 1975), *Staphylococcus aureus* (Van der Vijver *et al.,* 1975) Mycobacterium (Konickova-Radochova and Malek, 1969; Mac Naughton and Winder, 1977; Kolman and Ehrenberg, 1978), *Micrococcus glutamicus* (Shanthamma *et al.,* 1972), *Rhizobium trifolii* (Kauahik and Venkataraman, 1972), *Paeudomonas aeruginosa* (Chandler and Krishnapillai, 1974) and *Dictyostelium discoideum* (Payez and Dearing, 1972). EMS has also been shown to be mutagenic in *Micrococcus radiodurans* (Sweet and Moseley, 1976) although considerably less so than in *E. coli* and *Phaseolus vulgaris* (Prasad and Prasad, 1981). Evidence for the mutagenic activity of chemicals as well as for ionizing radiation was established with experiments using *Drosophila melanogaster* as the test system. Most of the mutagenicity studies of EMS in insects have been done using this species.

The response of *Drosophila melanogaster* germ cells to the induction of mutation by EMS has been studied. EMS is found to act primarily on post-meiotic stages. Meiotic and pre-meiotic stages are relatively immune to its effects. Browing (1970) reported a 4-5 fold higher yield of recessive lethals in post-meiotic cells compared with the yield in meiotic and pre-meiotic cells. Fahmy and Fahmy (1961a) reported more than an order of magnitude difference in recessive lethals produced in post-meiotic stages as compared to premeiotic stages. A similarly low mutagenic response of earlier germ cell stages to EMS

was reported by Jenkins (1967a, b, 1969). He found that 90% of all EMS-induced dumpy mutations occurred in broods from postmeiotic germ cells. All of these observations were consistent with the findings of Aaron *et al.*, (1973) that retained levels of DNA ethylation were much higher in germ cells treated as mature sperm and late spermatids than in those treated as early spermatids or spermatogonia.

Besides producing complete mutants or recessive lethals in *Drosophila* EMS is also able to produce 'mosaics', in which the F_1 progeny from treated male parents may contain both mutant and nonmutant cells. In the case of sex-linked lethals, for example, it is possible to detect F_1 females which are germinally mosaic for a lethal by sampling several heterozygous virgin females from a non-lethal culture, and mating these to males which have inversions on their X chromosome to prevent crossing over with the X-chromosome under test. Since, each F_2 female receives only one paternal x-chromosome from the F_1 female (which can be either normal or lethally mutated when it arises from an F_1 female germinally mosaic for a lethal). F_1 lethal mosaicism is detected by the presence of both non-lethal and lethal cultures in the F_3 sample from a non-lethal F_2 culture.

Both Alderson (1965) and Epler (1966) found the frequency of complete F_2 sex linked lethals to be greater than the frequency of F_3 mosaic sex-linked lethals. Epler (1966) also found that the frequency of complete sex-linked lethals induced by EMS increased in a linear fashion with exposures, while the frequency of mosaic sex-linked lethlas increased at the lower concentrations of EMS but decreased at the higher concentrations. However, Jenkins (1967b) using the autosomal visible mutant dumpy, found a difference in the frequencies of mosaic and complete mutations which was the reverse of that reported by Epler (1966). As pointed out by Jenkins (1967b) the difference may have resulted from the fact that the sex-linked lethal test involves an entire chromosome and would produce a much greater number of double mosaic mutants than would a test involving a single gene (A double mosaic is defined as two independently induced mutations at different sites along the two DNA stands of a post meiotic chromosome with one mutation in each strand). These double mosaic sex-linked lethals would be classified as complete mutations, and their frequency would increase with exposure.

An experimental model of rat mammary carcinoma induced by oral administration of ethyl methanesulphonate (EMS) was characterized with reference to estrogen receptors (ER) and hormone dependency. (Ueo H *et al.,* 1990). The adaptive response to alkylating agents was studied in *Drosophila* assays under various treatment procedures. Pre-treatment of males as well as treatment of females with low doses of EMS (0.05–0.1 mM) did not affect sex-linked recessive lethal (SLRL) rates induced by high doses of this mutagen (10 mM, various feeding duration) in mature sperm cells (Nataliya S *et al.,* 2003). Effects of ethyl methanesulfonate (EMS) that caused mutagenicity in a variety of organisms were tried to resolve by the methanol and chloroform extract of Echium amoenum (EAmet and EAchl) Fisch. & C.A. Mey. from the family of Boraginaceae, which is an endemic plant, and is used as an alternative treatment among public in Iran. Somatic mutation and recombination test with *Drosophila* wing was used to determine the genotoxic and antigenotoxic effects in our investigations (Handan U *et al*., 2012). Prior to having performed in depth toxicological, genotoxicological and DMPK studies on ethyl methanesulfonate (EMS) providing solid evidence for a thresholded dose response relationship, we had prepared and shared with regulatory authorities a preliminary risk estimate based on standard linear dose-effect projections (Elmar Gocke *et al.,* 2009).

Most of the genetic effects produced by EMS in *Drosophila* appear to arise from intralocus mutations. Bishop and Lee (1969) studies 83 mutations on the X chromosome at the yellow and white locus and found all to be intragenic changes. Hochman (1971) found that 74 out of 75 lethals on chromosome 4 affect only a single completementation unit. Lim and Snyder (1974) found that all 83 EMS-induced lethals in Zeste-white region of the x chromosome affected only a single complementation unit.

A few EMS-induced lethals affecting more than one complementation group have been observed (Lifschytz and Falk, 1969; Schalet and Lefevre, 1973; Wright *et al.,* 1976). The wing spot test in Drosophila melanogaster was used (Mostapha Rizki *et al* 2002) to investigate the genotoxicity of arsenic and its effects on the action of two clearly genotoxic agents: potassium dichromate (PDC) and ethyl methanesulfonate (EMS). This assay is based on the principle that the loss of heterozygosity of the suitable recessive markers

multiple wing hairs (mwh) and flare-3 (flr3) can lead to the formation of mutant clones of larval cells, which are then expressed as spots on the wings of adult flies. In order to assess the risk of patients being exposed to an anti-AIDS medication contaminated with EMS we have performed in depth genotoxicity, general toxicity and DMPK investigations (Elmar Gocke *et al.,* 2009). There are numerous published in vivo genotoxicity studies on EMS, with generally 50 mg/kg-or higher-being the minimal dose used.

The lowest EMS concentration renorted to induce translocations in *Drosophila* is 10 mM. When injected, this EMS concentration produces a 0.2% translocation frequency (Lim and Snyder, 1968, 1973) and when fed for 18h the frequency was 0.38% (Watson, 1972. A higher translocation frequency of 2.5% was observed after feeding adult males for 24h with a 25 mM EMS solution (Hotchkiss and Lim, 1968). This same treatment resulted in a 54% sex-linked recessive-lethal frequency. EMS-induced dominant lethals in *Drosophila* are also relatively rare unless the exposures are very high. Sram (1970) was able to prouce a 60% dominant-lethal frequency in *Drosophila,* but this required the injection of a 50% mM EMS solution.

In rat mammary carcinoma induced by oral administration of ethyl methanesulphonate (EMS) was characterized with reference to estrogen receptors (ER) and hormone dependency (Ueo H 1990). After the administration of EMS, the mammary carcinomas developed more rapidly in the 4-week old rats than in the 16-week old rats. DNA ethylations were measured in four mammalian cell lines, Chinese hamster ovary CHO, mouse lymphoma L5178Y t k+/-, human lymphoblastoid TK6 and Chinese hamster V79, following exposure to [3H]ethyl methanesulphonate. Concurrent estimates of cytotoxicity and gene mutation were also carried out (Waters R 1990). Lymphocytes were isolated from both blood types and examined in the alkaline comet assay (Wyatt NP *et al.,* 2007) using the monofunctional alkylating agent ethyl methanesulphonate (EMS). Chinese hamster V79 cells were exposed to ethyl methanesulphonate (EMS) and the incidence of mutant cells resistant to 8-azaguanine (8AZG), 6-thioguanine (6TG) or ouabain (OUA) was determined both by the respreading and the in situ techniques (Mirzayans F, Parry JM and Mirzayans R 1992).

Drosophila females are capable of storing viable sperm for periods of at least 1-2 weeks. Storage can also be enhanced by maintaining the females on sugar agar, which inhibits egg laying and shows the rate at which the sperms are used in fertilization. After storage of EMS-treated *Drosophila* sperm, Khan (1969) found no significant reduction in the frequency of F_1 germinally complete and F_1 germinally mosaic sex-linked recessive-lethal mutations. However, Sram (1970), using dominant lethals, Abrahamson and Lewis (1971), Using translocations, and Schalet (1977), using sex-chromosome marker loss, found increased damage after storage.

Vogel and Natarajan (1979a, b) studied the mutagenic activity of a number of chemical agents, including EMS, in *Drosophila.*They found that; in general, agents with the lowest Swain-Scott a values (ENU and DEN) did not produce translocations although they did produce recessive lethals. The highest chromosome breaking activity (Translocations and sex-chromosome loss) was found with agents having high values (MMS and DMS). EMS was found to be somewhat unique, in that it was slightly less effective in the translocation test, and also less cytotoxic but more mutagenic in the recessive lethal test than what would have been expected from it's a value. The authors took this as an indication of the influence on biological effectiveness of factors other than a value, such as methylation versus ethylation and the lipid/water partition ratio. The overall conclusion of these workers was that two factors were very important in determining the genetic damage produced by alkylating agents in *Drosophila.* These two factors were dose (intensity of alkylation) and reaction pattern (site of alkylation).

La Chance *et al.,* (1969) showed that dominant lethal mutations were induced in *Musea domestica* (house fly) when it was treated with EMS. An injection of 20 μg of EMS resulted in nearly 100% lethality. However, 2 weeks after the EMS treatment, spermatogenic activity in the testes had returned to near normal levels. McDonald and Overland (1974) also were able to recover some dominant, heat-sensitive lethal mutations after treatment of house fly sperm with EMS.

La Chance and Leverich (1969) fed EMS solutions containing 10% sucrose to Habrobrancon males. Even starved males would not drink solutions containing 2.5% or more EMS, but after drinking 0.5 and 1.0% solution of EMS the wasps were completely sterilized. On the basis of hatchability, it was estimated that 9-18% of the sperm were inactivated, and the remainder of the sterility was apparently caused by dominant lethal mutations in the sperm. Using Habrobracon females, Lobbecke and Von Borstel (1962) found that after aerosol exposures to EMS, dominant lethals were induced in oocytes in first meiotic metaphase and prophase. However, the metaphase oocytes were about 20 times more sensitive than those in prophase. Hoffman and Grosch (1971) also using Habrobaracon females, found that there was a dose-related reduction in hatchability and egg production when EMS was fed or injected, but not when it was applied topically or as an aerosol. Also fewer eggs were deposited and move embryonic deaths resulted during cleavage when starved wasps were injected. The authors suggested that the EMS dose to the germ cells may have been higher in the starved insects since the fat bodies, which would normally absorb part of the EMS dose, were greatly reduced in the starved wasps.

Administration of chemical mutagens to the female rodent can induce dominant lethal mutations in oocytes and affect embryo development after fertilization (Mohr KLand Working PK 1990). Pre- and post-implantation development in culture was monitored in embryos recovered at the two-cell stage from females dosed ip, 30-32 hr before ovulation, with 125 or 250 mg/kg ethyl methanesulphonate (EMS)/kg body weight. In vitro zygote development progressed from two-cell to trophectoderm outgrowth and inner cell mass formation. All stages of development were affected by the EMS treatment. Large doses of ethyl methanesulphonate (EMS) greatly increased the induction of auxotrophic mutants in Candida tropicalis (Mahmoud YA 1999). The maximum yield of biomass and protein was recorded in some mutants isolated after treatment with 60, 80 and 100 ppm EMS.

Sensitivity of male germ cells in the mulberry silkworm, *Bombyx mori*, to EMS was studied by Datta *et al.*, (1978). Larvae and pupae were injected with 0.05, 0.1 and 0.15% solutions of EMS. Dominant lethals were induced in spermatocytes, spermatids and

spermatozoan stages, with the741 later two stages being most sensitive. Tazima (1974), using the specific locus mutants pe and re for egg color, also found that postmeiotic germ cells of *Bombyx* are highly sensitive to EMS. Larvae and pupae of *Bombyx* females were injected with EMS by Murakami (1975). Using the specific locus mutants pe and re Murakami was able to show a large mutagenic effect in late pupal prophase I oocytes but not in larval growth stage oocytes (stage prior to prophase I).

The induction of mutations at both the thymidine kinase (tk) locus and the hypoxanthine guanine phosphoribosyl transferase (hprt) locus was determined following exposure to ethyl methanesulphonate (EMS), benzidine (BZD) and benzo[a]pyrene (B[a]P). EMS was tested in the absence of a metabolic activation system (S9 mix), BZD was tested in the presence and absence of S9 mix and B[a]P was tested only in the presence of S9 mix (O'Brien KA, Gatehouse DG, Tiley M 1990). The alkylating agents ethyl methanesulphonate (EMS) and N-ethyl-N-nitrosourea (ENU), and the plant growth regulator and herbicide maleic hydrazide (MH) were compared in tobacco seedlings for their ability to induce DNA damage measured by the Comet assay, and recombination activity measured by the GUS gene reactivation assay, and by the somatic twin sectors assay. While EMS and ENU induced a dose-dependent increase in DNA damage in leaf nuclei, MH had no significant effect. By contrast, MH induced a 6-fold higher frequency of homologous recombination as expressed by the GUS assay and a 2.8-fold higher frequency of somatic twin sectors than after EMS treatments (Gichner T 2003).

Investigate the formation and rate of hydrolysis of ethyl methanesulphonate (EMS) in BMS-214662 mesylate drug substance and parenteral formulation by a gas chromatographic/mass spectrometric (GC/MS) method. EMS levels in the drug substance ranged between 0.3 microg/g and 0.8 microg/g. The parenteral formulation contains ethanol and the reaction between residual free methane sulphonic acid and ethanol may lead to the formation of EMS. Given that EMS is a potent mutagen, it is therefore of vital importance to eliminate or reduce the risk of human exposure (Nassar MN, *et al.*, 2009). Induced mutations in Drosophila melanogaster males by treating them with 21.2 mm ethyl methanesulfonate (EMS) Kondrashov A S *et al.*, 2001.Mutations at the tk locus of

mouse-lymphoma L5178Y cells were induced by treatment with ethyl methanesulphonate (EMS). Davies MJ *et al.,* 1993. Developmental studies on wing colour patterns have been performed in nymphalid butterflies, but efficient genetic manipulations, including mutagenesis, have not been well established. Here, we have performed mutagenesis experiments in a lycaenid butterfly, the pale grass blue Zizeeria maha, to produce colour-pattern mutants. We fed the P-generation larvae an artificial diet containing the mutagen ethyl methane sulfonate (Joji M. Otaki *et al.,* 2013).

In the mouse, the frequency of EMS-induced dominant lethal mutations reaches a peak in early spermatozoa to late spermatid stages sampled 7-10 days post-treatment. The dominant-lethal effect diminishes in earlier germ cell stages so that with a 200 mg/kg treatment with EMS, no dominant lethals above control levels are detected beyond about 16 days (CAttanach *et al.,* 1968; Ehling *et al.,* 1968; Generoso and Russell, 1969; Generoso *et al.,* 1974). The induction of dominant lethals by EMS in germ cells of male mice appears to be independent of the mouse strain used (Generoso and Russell, 1969; Favor and Crenshaw, 1978). However, when female mice were treated with EMS, Generoso and Russell (1969) found that random bred T-stock females were highly sensitive to dominant lethal induction in late oocyte stages the last few days prior to ovulation but (SEC × C5781) F_1 and (101×C3H) F_1 females were much less sensitive.

2.3 Effects of actinomycin-D (ACM-D)

Actinomycin-D (ACM-D) is an antibiotic and claim their significance in being important as antineoplastics to varying degrees. Bio-chemically, their significance resides primarily in the fact that ACM-D is capable of interacting with DNA and can inhibit synthesis of macromolecules of genetic importance. At high concentrations, DNA, RNA and protein synthesis are all inhibited by this antibiotic.

Actinomycins were the first of the series of peptide containing, pigmented, antibiotics isolated in 1940. from Actinomycetes by Waksman and Woodruff (1940). The effects of ACM-D on various cellular and sub-cellular systems have been extensively reviewed (Fishbein *et al.,* 1970; Goldberg, 1965; Kann and Kohn, 1972; Kirk, 1960; Reich, 1963;

Sasaki, 1975; Yasminch and Yunis, 1970). This attention given to ACMs is impressive considering that the drug is highly toxic, has low therapeutic index, and that serious studies on biochemical effects of any of these compounds were not started till the lates 50's.

Foley (1956) noted that ACM-D acted as competitive inhibitor of pentathenate and that protein synthesis was the initial biochemical phenomenon to be inhibited by the antibiotic. The first indications that ACM-D could inhibit nucleic acid synthesis (Kirk, 1960; Shafer and Worton, 1973; Pallak *et al.,* 1982) appeared as late as 20 years after the isolation of the chemical. However, in spite of the reports of Guidice and Novelli (1963) that ACM-D inhibits the synthesis of DNA polymerse, moderate concentrations of the drug were found to have little effect either on DNA polymerase (Epifanova *et al.,* 1975) or DNA synthesis per se. The kinetics of these inhibitors are such that a 1:1 molar ratio of ACM to DNA guanine is necessary to give 100% inhibition (Samuels, 1964), and this amount of the drug is far in excess of that usually used to elicit its effects.

Actinomycin-D and cycloheximide were administered to rats under a marginally zinc-deficient condition (Norii T 2012). At high cytotoxic concentrations, actinomycin-D (Act D) blocks transcription, decreasing levels of MDM2 and thus causing p53 stabilization (Chen CS *et al.,* 2014). a chemotherapy protocol using dexamethasone, melphalan, actinomycin-D, and cytosine arabinoside (DMAC) was evaluated for efficacy and adverse event profile as a first line rescue protocol in 86 client-owned dogs previously treated with a CHOP-based protocol (Parsons-Doherty M *et al.,* 2014). Actinomycin-D (Act D), an oncogenic c-Myc promoter binder, interferes with the action of RNA polymerase. There is great demand for high-throughput technology able to monitor the activity of DNA-binding drugs (Gholami S and Kompany-Zareh M 2013). The effect of actinomycin-D on HeLa cells was studied by live fluorescence and transmission-through-dye microscopy-a recently developed technique that permits volume measurements in live cells (Kasim NR *et al.,* 2013). Oligonucleotide-functionalized gold nanoparticles (AuNP) were designed and synthesized to be delivery vehicles for the clinically used anticancer drugs doxorubicin (DOX) and actinomycin D (Act D) (Alexander CM *et al.,* 2012).

It has been shown that ACM-D completely suppresses RNA synthesis in the nucleolus, but partially on the chromosomes (Perry, 1962, 1963; Sirlin *et al.*, 1963). Such selective inhibition of only some species of RNA (Rickenson, 1970; Tsubol and Terasima, 1970) was explained by Perry (1962, 1963) who proposed that inbibiting effects of ACM-D on nucleolar RNA is due to ACM-D blocking DNA-dependent RNA synthesis selectively; the nucleolar organizing regions of DNA may have specifically high affinity for ACM-D. Additionally, t has been suggested that RNA content of ACM-D. Additionally, it has been suggested that RNA content of ACM-D treated lymphocytes is a reflective of above normal synthesis of ACM-D treated lymphocytes is a reflective of above normal synthesis of ACM-D resistant RNA species. In these studies (Martines *et al.,* 1969), the influence of ACM-D on transfer of RNA from chromosomes to nucleoplasm, or on to cytoplasm, is not affected uniformly, and church (1973) has now presented evience of stage specific RNA-transfer sensitivity in a liliaceous plant *Ornithogalum virens.*

Related to the inhibition of RNA synthesis is the inhibition of DNA-polymerases (Goldberg *et al.*, 1962; Honikel and Santo, 1972; Reich, 1964) and RNA-polymerases (Honikel and Santo, 1972; Waqar and Burgoyne, 1971). Based on the observation that ACM-DNA complex *in vitro* could inhibit the activity of DNA-dependent RNA-polymerase and DNA-polymerase. Reich (1964) has suggested that the inhibition of DNA polymerase may be an indirect effect of ACM-D. This effect could be caused by change in the physical properties of the template. The idea is supported by the observation that low concentrations of ACM-D, which have no effect on DNA-polymerase, can inhibit RNA synthesis (Reich, 1964). The slow rate of dissociation of ACM-D from DNA is the primary factor responsible for its inhibition of the progress of RNA-polymerase. The inhibition of DNA synthesis, consequently, thus appears to be related to one or more of the effects discussed above. Like RNA synthesis inhibition as discussed (Goldberg *et al.,* 1962), this inhibition of DNA synthesis is also reversible upon addition of more dye-free DNA (Waqar and Burgoyne, 1971). It is also important, in thins connection, that ACM-D can not dissociate RNA-polymerase DNA complex in spite of the ability of the former to bind to DNA (Beabealashvilly *et al.,* 1973). This

observation may reflect important structural differences between free DNA and DNA complexed with RNA polymerase.

Actinomycin-D was identified from 1496 substances and shown to induce apoptosis in primary CLL cells derived from high-risk patients including those with aberrant p53, revealing a novel p53-independent mechanism of action (Merkel O *et al.,* 2012). A combination of 5-fluorouracil plus actinomycin-D (5FU plus Act D) is the regimen that has been commonly administered to Chinese and Japanese gestational trophoblastic neoplasia patients as the first or second line of treatment with an excellent outcome (Manopunya M and Suprasert P 2012). A resonance light scattering (RLS) quenching system for DNA sequence recognition studies of actinomycin-D (ACTD) was developed in this contribution (Chen Z *et al.,* 2012). They screened a small library of compounds and identified actinomycin-D (ActD) as a weak inhibitor of pantothenate synthetase (Yang Y *et al.,* 2011). Here they chose actinomycin-D (ACTD), a genotoxic chemotherapeutic drug, to investigate whether it could cause bystander effect in Chinese hamster V79 cells (Jin C *et al.,* 2011).

Since inhibition of DNA synthesis by ACM-D is dependent upon concurrent inhibition of synthesis of RNA and proteins, the process involving initiation of DNA synthesis is said to be more sensitive than that for continuation of the synthesis (Kim *et al.,* 1968). Umeda *et al.,* (1973) however, have provided convincing evidence that inhibition is due to reduction in the rate of chain elongation and not due to loss of ability to start new chains. Also, there is no evidence that ACM-D induced RNA synthesis inhibition (i.e. inhibition of trasncription) is concomitant with nuclear DNA replication.

Seminiferous tubules (SeT) from Tg-RGN and their wild-type (Wt) counterparts were cultured ex vivo in presence or absence of apoptosis inducers thapsigargin (Thap, 10(-7) and 10(-6) m) and actinomycin-D (Act D, 0.5 and 1 μg/mL) (Correia S *et al.,* 2014). A literature search was conducted according to the guidelines for systematic reviews in order to select eligible papers reporting limb toxicity and response rates following ILI using melphalan and actinomycin-D to treat limb melanoma (Kroon HM *et al.,* 2014). Actinomycin-D (Act D) is a general transcriptional inhibitor that is approved for the

treatment of sarcomas, and Wilms, germ cell and trophoblastic tumors. Little is known about the molecular mechanisms that dictate the sensitivity of cancer cells to Act D (Ma W *et al.,* 2013). The potent anticancer drug actinomycin-D (Act D) functions by intercalating into DNA at Gp C sites, thereby interrupting essential biological processes including replication and transcription (Lo YS *et al.,* 2013). Actinomycin D plays a key role in the successful treatment of Wilms tumour (Hill CR *et al.,* 2013). To determine outcomes and factors associated with failure of 5-day actinomycin D for treatment of methotrexate-failed low-risk gestational trophoblastic neoplasia (GTN) (Lurain JR *et al.,* 2012).

Several studies are available on effect of ACM-D on cell cycle traverse. The generalities which emerge indicate that G_1 or G_2 blockage of the cell cycle by ACM-D is more uniform than blockage at S or mitosis. The lack of strong effect on S phase is understandable in view of the fact that high concentrations of the drug are required to inhibit DNA synthesis. Yet there are reports of inhibition of cell cycle progression starting in early S (Bal and Ste-Maric, 1969). It has been reported that application of ACM-D to early G_1 cells can cause a delay in onset of DNA synthesis but does not effect late S cells (Epstein *et al.,* 1972). This is in conformity with the observation that some late G_1 cells are not at all, or only slightly, effected by the presence of ACM-D at physiologically important doses (Doida and Okada, 1972). The concentrations of ACM-D which block nucleolar specific RNA synthesis are capable of blocking cells G_1 and G_2 (Baxter and Byyoet, 1975; Rickenson, 1970) and DNA synthesis, once initiated, appears to continue at uninterrupted rate (Rickenson, 1970). Also the cells treated in mitosis progress, without delay, into G_1 (Doida and Okada, 1972). ACM-D has been found to induce genetic damage including structural aberrations of chromosomes. One common feature of this drug is her ability to effect the s phase more drastically than other phases of the cell cycle. This effect may not depend entirely on DNA synthesis inhibition. The chromosomal proteins synthesize in much larger quantities in this s phase than in G_1. The concentrations of this chemical required to induce aberrations are generally very low (Fishbein *et al.,* 1970) and this chemical appears to be capable of expressing delayed type effect to greater or lesser degree.

ACM-D has no effect on the association pattern of human acrocentric chromosomes (Hochn *et al.*, 1971) and early studies with ACM-D treated human leukocytes showed a lack of chromosome breaking effect (Howell, 1964). Since then several studies with cells, for example from humans (Miles, 1970; Ostertag and Kersten, 1965; Voorhees *et al.*, 1969), *Vicia faba* (Ward and Glover, 1969), mouse (Manna and Mitra, 1971; Jain and Singh, 1967), Indian muntjack (Pathak *et al.*, 1975) and locust (Jain and Singh, 1967) have exhibited the chromosome breaking ability of the antibiotic. The ACM-D induced aberrations are primarily of chromatid-type (Ostertag and Kersten, 1965; Pathak *et al.*, 1975). These aberrations, even though distributed over the entire genome, show a preponderance of localization in the heterochromatic regions. Thus breaks have been observed in the pericentromeric regions of human genome (Ostertag and Kersten, 1965), in the nucleolar organizing constriction of Idnain Munjack (Pathak *et al.*, 1975), and secondary constrictions of *Vicia faba* (Ward and Glover, 1969). Miles (1970), on the other hand, has been unable to support the earlier findings of Ostertag and Kersten (1965) that human chromosomes show preferential breakage near the centromere. Similarly, Manna and Mitra (1971) found that 90% of ACM-D induced breaks in mouse were localized in regions other than the proximal heterochromation. In studies dealing with chromosome aberrations induced by ACM-D, production of chromatid and even subchromatid exchanges (Kihlman, 1960) seems to be that rule; one reported exception being the effect on meiotic cells of locust in which case the broken ends of chromosomes appeared to have lost the capacity to reunite or restitute (Jain and Singh, 1967). This lack of repair in locust does not seem to be a common feature of meiotic cells, since the chromosomes in mouse eggs treated with the chemical did show exchanges of chromatid type (Jagiello, 1969).

Aberrations induced by ACM-D are found to originate in the S phase of the cell cycle (Miles, 1970; Ostertag and Kersten, 1965) as well as G_2 (Miles, 1970; Ostertag and Kersten, 1965; Manna and Mitra, 1971) even though aberration frequency in the G_2 cells is only a slight fraction of that found in the S phase. Lozzio (1967) has been able to induce chromosome-type aberrations in G_1 phase of human leukocytes treated with the

drug. However, in the G_2 cells, ACM-D not only induces chromatid breaks but also alters the morphology of chromosomes, like production of bands and interbands, as these pass on into ensuing mitosis (Shafer and Worton, 1973). In Chinese hamster cells (Hsu *et al.,* 1975), treated with as high as 5 μg/ml for 2.8h, most chromosomes were decondensed or stretched leaving intact small metacentric chromosomes and mostly heterochromatic y and the x (Arrighi and Tsu, 1965). Such decondensing effect of ACM-D has been attributed to the competition (and failure) of the histone molecules with ACM molecule for the binding sites. The knowledge that ACM-D binds to constitute heterochromatin only somewhat preferentially, or at the same rate as, to enchromatin (Sieger *et al.,* 1971) may not permit such a definite conclusion. Also, the breaks observed in the extended part of the chromosome may not mean that the molecule binds at these sites since Miles (1970) has shown a lack of good correspondence between ACM-D induced breaks and the sites of (^{3}H) ACM-D label in human chromosomes.

The marine-derived strain of Streptomyces sp. MS449 produced notably higher quantities of actinomycin X(2) (1.92 mg/ml) and actinomycin-D (1.77 mg/ml) than previously reported actinomycins producing strains (Chen C *et al.,* 2012). Actinomycin-D (Act D) is a small molecule with strong antibiotic and anticancer activity (Paramanathan T *et al.,* 2012). They have recently shown in T. cruzi that a group of RNA Binding Proteins (RBPs), involved in mRNA metabolism, are accumulated into the nucleolus in response to Actinomycin-D (Act D) treatment (Názer E and Sánchez DO 2011). p53-Based cyclotherapy is proving to be a promising approach to palliate undesired effects of chemotherapy in patients with tumours carrying p53 mutations (Rao B *et al.,* 2010). Actinomycin-D-induced hepatopathy-thrombocytopenia syndrome (HTS) is characterized as a rare syndrome (Baskin Y *et al.,* 2014). Non-ribosomal peptides are bio synthesized using a range of enzymes that allow much more structural variability compared with "normal" peptides (Wills RH and O'Connor PB 2014). The interaction between the anticancer drug Actinomycin-D (Act D) and the DNA molecule by performing single molecule stretching experiments and atomic force microscopy (AFM) imaging (Cesconetto EC *et al.,* 2013).

Evidence has been available that ACM-D can induce effects which are not true mutations. Such epigenetic effects induced by ACM-D have been observed on the development of sea urchin embryos (Gross and Cousineau, 1964; Infante and Nemer, 1967), and embryos of amphibians and Chicks (Brachet and Denis, 1963).

The genetic effects of occupational, therapeutic, environmental and sometimes, voluntary exposures to chemical mutagens may be drastically potentiated or reduced by the simultaneous or near simultaneous exposure to other agents, some of which may be non-mutagenic in themselves. This assessment of co-mutagenicity appears to be critical for the safeguard of our genetic material, and is limited by data available on the subject. The problem is complicated when a given chemical has contrasting effect on different genetic parameters. For example, on the one hand ACM-D induces chromosome aberrations but, on the other, inhibits the occurrence of spontaneous mutations (Puglisi, 1968).

In this regard ACM-D has been studied more extensively because of its antimutagenic effects discovered against radiation induced mutations. ACM-D was found capable of reducing the frequency of X-ray induced mutations (Burdette, 1961) in *Droophila.* Proust *et al.*, (1972) showed that ACM-D, besides reducing the frequency of X-ray induced recessive lethals also reduced the frequency of translocations. Similar reduction by ACM-D of recessive mutations induced by X-rays has also been reported (Muller, 1975).

The antimutagenic property of ACM-D could not be confirmed in all cases. Treatment of *Drosophila* with (^{3}H) uridine and ACM-D increased the frequency of recessive lethals significantly over that induced by the nucleoside alone, and similar effects were observed when (^{3}H) thymine was used instead of (^{3}H) uridine (Olivieri and Dlivieri, 1965). Also, there are reports of an increase induced by the antibiotic in the frequency of dominant lethal mutations induced by X-rays in *Drosophila melanogaster* (Proust *et al.*, 1972), utilizing different experimental protocols.

Synergistic effects of ACM-D with other radiomimetic chemicals snd radiations have been reported in relation to induced chromosome breakage. In one of the early studies

Ward and Glover (1969), hoping to find that ACM-D would reduce the breakage effect of EMS, observed that the antibiotic did not block the action of the alkyting agent and that the combination effects of the two chemicals were not explainable by simple additive effects. Several fold increase in the frequency of ethyleneimine induced chromosome breakage was observed by Seleznev and Selezneva (1971) when human leukocytes and Chinese hamster cells in culture were treated, additionally, with ACM-D. Although a lack of such synergism with ACM-D has been reported in hamster fibroblast chromosomes treated with an inhibitor of DNA synthesis, 1, β arabinofuranosylcytosine (Benedict *et al.*, 1975), presumably because at concentrations tried (0.1 μg/ml) ACM-D did not interfere with DNA synthesis, yet ACM-D educes the frequency at aberrations induced by FUDR in human lymphocytes (Akifjev and Aingorn, 1972). Also, a lack of synergistic effect of ACM-D with radiations had been reported (Prempree and Merz, 1969), and it has been interpreted that the inhibition of transcription by ACM-D fails to produce any effect on x-ray induced aberrations because of the constant presence of repair enzymes in these cells (Generalova and Idemin, 1973). The information that ACM-D enhances double strand breakage induced in DNA by some radiomimetic chemicals e.g. blemomycin (Bearden and Haidle, 1975) may have a bearing on producing synergistic effect observable as chromosome aberrations in some cases, but not in those in which such enhancement effect would be found lacking.

MATERIALS AND METHODS

For conducting the experiment ladybird beetle (*Epilachna vigintioctopucntata)* was used as the experimental tool. The study was conducted in the Insect Genetics Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh during the year of 2008-2013.

3.1 Collection of *Epilachna vigintioctopunctata*

Adult or larvae of *Epilachna vigintioctopucntata* were collected from Rajshahi University Campus and its adjacent areas and were reared in the laboratory at the room temperature 25°C and at relative humidity (60-70%) during the month of April to October, 2009. To maintain stock culture in transparent plastic case (2.5″ diameter and 3″ in height) male and female beetles were reared and supplied daily with fresh middle-aged leaves of *Solanum nigrum.* Piece of plastic sponge moistened with water were kept at the bottom of the pots to keep the leaves fresh. The plastic cases were kept covered with pieces of fine muslin cloths and tied with rubber bands. The plastic pots were cleaned up regularly to ensure their certain temperature.

3.1.1 Mating and egg collection

One pair of newly emerged beetle was allowed for mating in five large size plastic pots. It was regularly and thoroughly checked whenever mating happened. In case of death of a male or female of a pair, a new one was replaced. The egg laying habit was observed and the numbers of eggs were counted.

As the eggs were usually layed on the surface of the leaves or on the surface of the pots, immediately after laying the fertile eggs were taken out separately and incubation period was recorded. The eggs were checked regularly and time of hatching was recorded. The number of newly hatched larva and unhatched eggs were counted and finally, the percentages of unhatched eggs were calculated.

3.1.2 Collection of 3rd instar larvae for experiments

At room temperature larvae were hatched after 10-14 days of egg laying. The newly hatched larvae were collected with a needle and transferred to a fresh food medium in plastic cases. The cases were checked daily for appropriate inster and at 3rd stage they were collected for experiment.

3.1.3 Collection of pupae and adults for experiment

After 3 to 6 days, the mature larvae were converted to pupae. The pupa is a quiescent, non-feeding and non-motile stage but the internal organs undergo gradual marked changes through which they attain adult structure. Pupae were collected and kept in a separate plastic case and given newly formed adults approximately of same age were used in the experiment. One pair of newly emerged beetles were allotted to mate in 1 large size plastic case and used fresh middle aged leaves of the host and provided with a pair of beetle (male and female) and 5 pairs of beetles and their time of emergence was noted. plant covered by a fine muslin cloth. In same way 5 pairs of newly emerged beetles were also followed to mate in 5 plastic cases. In case of death of a pairing male, a new one was released for the female. The egg laying habit was observed and the numbers of eggs were counted.

As the eggs were usually layed on the surface of the leaves or on the sides of pots, immediately after laying, the fertilized eggs were taken out separately and incubation periods were recorded. The number of newly hatched larva and unhatched eggs were also counted and finally, the percentages of hatching were calculated.

The newly hatched larvae transferred to another plastic case (80mm x 90mm) were reared. The larval stages were checked regularly and the time of moulting was recorded in each stage. All the larvae, hatched the same day, were collected from different cases with labels showing the date of hatching. The interval between two successive moults gave the duration of each instar. The non-feeding stage of the last instar larval period was considered as pre-pupal period, which was transferred to another non-feeding and inactive stage, the pupa. During the rearing, pupal periods were also recorded. The

number of adults emerged from the pupae were counted and the percentage of emergence was thus calculated. After emergence, the imago were sexed and reared separately to maintain a stock culture. Throughout the experiment, whenever a male or female beetle died it was replaced immediately to fulfill the experimental conditions. The method applied in the experiment was in accordance with A. Ahmed (1983).

3.1.4 Determination of sex

It was not possible to determine the sex at the larval and pupal stages. In adult stage the sex was determined by the external features like examining their abdominal segment and size under microscope. In the male, the hind margin of the sixth visible sternite was concave and in the female it was a deep median split. The sexed adult thus placed in a separate Petri dish for the experiment.

3.2 Chemical agents

For the purpose of the present experiment following chemical agents were used:

1. Methyl methanesulfonate (MMS): It is a colourless liquid with a density of 1.29434. MMS has the molecular formula C_2-H_6O_3-S with a molecular weight of 110.12. It is soluble in water. MMS is one of the potent monofunctional alkylating agents which is mutagenic in higher eukaryotic systems (Vogel *et al.,* 1985).

2. Ethyl methanesulfonate (EMS): It is a colourless liquid at room temperature. It has the molecular formula C_3-H_8-O_3-S with a molecular weight of 124.2 (Sax, 1979). EMS is a monofunctional ethylating agent that has been found to be mutagenic in a wide variety of genetic test systems from viruses to mammals (Sega, 1984).

3. Actinomycin-D (ACM-D): It is an antibiotic isolated in 1940 from Actinomycetes by Waksman and Woodruff (1940). Its molecular formula is C_{62}-H_{84}-N_2-O_6 and is found to inhibit DNA, RNA and protein synthesis (Kirk, 1960; Shafer and Worton, 1973; Epifanova *et al.,* 1975; Fenwick, 1977; Lone *et al.,* 1986) and also to induce genetic damage including structural aberrations of chromosomes (Ostertag and Kersten, 1965; Pathak *et al.,* 1975).

3.2.1 Treatment procedure

1) Treatment with methyl methanesulfonate (MMS): 3rd instar larvae of *E. vigintioctopunctata* was treated by four different doses of MMS which was made by taking 1.0ml,2.0ml,3.0ml and 4.0ml of MMS and 99.0ml, 98.0ml, 97.0ml and 96.0ml of distilled water, respectively. Each solution of MMS was taken in beakers and some leaves of *Solanum nigram* were drenched into each solution for 5 minutes. Then the leaves were placed in different plastic cases and 10 larvae of *E. vigintioctopunctata* Fab. were released into these cases. The experiment was continued for three generations (parental, F_1, and F_2). In each generation, there were ten observations and five pairs were observed for each generation. But in developmental period and sex-ratio there were five replications under which single pair and four doses were used.

2) Treatment with ethyl methanesulfonate (EMS): 3rd instar larvae of *E. vigintioctopunctata* Fab. were treated by four different doses of EMS which were made by taking 1.0ml, 2.0ml, 3.0ml, and 4.0ml of EMS in 99.0ml, 98.0ml, 97.0ml and 96.0ml of distilled water respectively. Each solution of EMS was taken in beakers and some leaves of *Solanum melongena* were drenched into each solution for 5 minutes. Then the leaves were placed in different plastic cases and 10 larvae of *E. vigintioctopunctata* were released into these cases. The experiment was continued for three generations (parental, F_1, and F_2). In each generation, there were ten observations and each observation had five pairs. But in developmental period, there were five replications and each replication posed single pair and four different doses were used.

3) Treatment with actinomycin-D (ACM-D): 3rd instar larvae of *E. vigintioctopunctata.* were treated by the 2.0ml actinomycin-D and 98.0ml of distilled water. Solution of ACM-D was taken in beakers and some leaves of *Solanum nigram* were drenched into each solution for 5 minutes. Then the leaves were placed in different plastic cases and 10 larvae of *E. vigintioctopunctata were* released into these cases.

3.2.2 Mating scheme

Experimental beetles were allowed to cross immediately after treatment according to the following scheme:

Batches	Mating scheme	
	Male	Female
1	Control	Control
2	MMS/EMS	Control
3	ACM-D	Control
4	MMS/EMS	ACM-D
5	ACM-D	MMS/EMS
6	MMS/EMS + ACM-D	Control

3.3 Collection of data for genetic tests

In this study the data for genetic tests were collected under the following experimental heads:

3.3.1 Dominant lethal mutations

A number of tests were devised to measure the effectiveness of a chemical in inducing dominant lethal mutation. One of the tests was to compare the percentage of unhatched eggs in a control with the treated series (Abrahamson *et al.*, 1971).

Dominant lethal mutation frequencies were calculated by using Abbott's formula (Abbott, 1925, cited in Proust *et al.*, 1972): $Px = \frac{Pt-Pc}{1-Pc}$

Where, Px = percentage of dominant lethal mutation

Pt = percentage of unhatched eggs in the treated crosses

Pc = percentage of unhatched eggs in control crosses

3.3.2 Number of eggs

The numbers of eggs were counted at each laying under the treatment of different doses and for different crosses of three generations in *E. vigintioctopunctata.*

3.3.3 Number of larvae

At room temperature larvae were hatched after 10-14 days of egg laying. After treating with different doses of mutagens and antibiotic the numbers of larvae were counted for different crosses from three generations of *E. vigintioctopunctata.*

3.3.4 Number of pupae

After mature larvae pupated the numbers of pupae were counted from every generation. The pupae are a quiescent, non-feeding and non-motile stage but the internal organs undergo gradual marked changes through which they attain adult structure.

3.3.5 Sex –ratio

Total numbers of the adults were counted at each laying. The relative proportion of the number of males and females in a population is referred to as sex-ratio (Sardar, 1978). It is usually expressed as number of males and females per hundred births by the following formula:

$$\text{Percentage of sex-ratio} = \frac{\text{No. of Males/Females}}{\text{No. of Total adults}} \times 100$$

3.3.6 Incubation period

The insect eggs pass through a period (incubation period) in which the deposited eggs are developed into 1st instar larvae. The environmental factors have pronounced effect on the incubation period of all insects. The incubation period may greatly vary depending upon the environmental factor. In *E. vigintioctopunctata* it is also variable in different season. In this experiment the fertilized eggs immediately after layed by the females were taken out separately. Proper facilities were offered during the whole period of incubation of the eggs. The chorion membrane of the eggs is transparent and the 1st instar larvae could be clearly seen through the chorion. After hatching, the chorion appeared as transparent white empty shells. Finally, the incubation periods were recorded from control and treated insects of different crosses and every generation.

3.3.7 Larval period

The total larval period, which includes number of larval stages *i.e.* larval inster, is the major part of the post embryonic development of any endopterygote insect. Just after hatching from the eggs, the larvae were treated as in the 1st instar larvae moulted to 2nd inster and thus each moult gave corresponding larval inster. During the continuous study of the post-embryonic development of *E. vigintioctopunctata* it was observed that there were four larval inster. The grubs were checked regularly and the times of each moulting were recorded by following their ecdysis in each stage. The time taken from hatching to first ecdysis was the duration of 1st instar stage. The duration of the successive instars was calculated from the date of previous ecdysis to the date of next ecdysis. The interval between two successive moults gave the duration of each inster. The average duration of single instar and total larval period were recorded from three generations.

3.3.8 Pupal period

The pupa is a quiescent non-feeding stage but the internal organs undergo gradual marked changes through which they attain their adult structure. In *E. vigintioctopunctata* whenever the pupation start, the front side of the pupa shed off and the body become to some extent spherical. Black spots start appearing on the dorsal whitish yellow surface of the pupae. The time was carefully recorded whenever the pre-pupa entered the pupal stages. The coloration of pupae was much influenced by the environmental conditions. Several factors like food, temperature and relative humidity influenced the pupal period of insects. The pupal period was determined from different generations and different crosses of treated and control insects.

3.3.9 Longevity of adult males and females

Every organism in the living world is subjected to natural death. The normal life span of insects varies considerably from a few days to several years depending upon the species and the length of life is correlated with fecundity and mating. The longevity of adult beetles varied in different species and in different sexes of same species. The present investigation on the longevity of the adult beetles, both male and female of *E. vigintioctopunctata* (treated and control) was recorded from different crosses and different generations.

3.4 Statistical analysis

Collected data were analyzed preliminary and the ANOVA and Chi-square tests were done as follows:

3.4.1 Preliminary analysis of data

For preliminary processing of the raw data obtained in each treatment groups, mean, standard deviation (SD±) and standard error (SE±) were calculated. These values were used for other statistical analysis and interpretations thereafter.

3.4.2 ANOVA

Analysis of variance (ANOVA) is a technique for testing the statistical significance of differences among average responses due to controlled variables, after suitable adjustment is made for influences on response originated from uncontrolled variables. This technique is not used to test for significant differences among variances, rather is used to test for significant differences among means. ANOVA, shown as F-values at certain degrees of freedom, therefore, is a statistical apparatus for testing the significance of various experimental 'treatment' effects. It is used as a test of variance among means of two or more universes (Mian & Miyan, 1984).

3.4.3 Chi-square test

It was necessary to determine a value of χ^2 to test for homogencity of the data. The value of χ^2 is compared with the tabulated value for n-1 degrees of freedom when n is the number of doses tested. Heterogenecity is indicated if the calculation is done by the following formula:

$$\chi^2 = \sum_{i=1}^{N} \frac{(O_i - E_i)^2}{E_i} \sim \chi^2$$

Where,

$O_{i=}$ observed value

$E_{i=}$ expected value

The number of degree of freedom (df) = n-1

Number of observation–Number of independent restriction

Value of χ^2= greater than the tabulated value at 5% level of significance

RESULTS AND DISCUSSION

4.1 Dominant lethal mutation

Effects of actinomycin-D (ACM-D) on the methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) induced dominant lethal mutations (DLM) have been studied in *Epilachna vigintioctopunctata* and are shown in tables 1-12, figures 1-20 and appendix tables I-XX. The frequencies of dominant lethal mutations were obtained from various crosses and generations and from the treatment of different doses of MMS and EMS. The calculated frequency of induced dominant lethal mutations included any increase, over the control value, in unhatched eggs (Ehling *et al.,* 1968).

4.1.1. Induction of dominant lethal mutations (DLM) by methyl methanesulfonate (MMS) and actinomycin-D (ACM-D)

Effects of different doses of MMS with ACM-D on the induction of dominant lethal mutations were determined at different crosses and are shown in tables 1-6, figures 1-10 and appendix tables I-X.

Table-1 summarized the data following the effect of 1.0ml dose of MMS and ACM-D. The analysis of variance within crosses showed highly significant ($F=55.27$, $P<0.001$, appendix table-I) differences with LSD values as 3.31 at 5% and 7.27 at 0.1% probability levels.The observed mean percentage of induced DLM in the cross ACM-D♂ x Control♀ differed significantly with other crosses such as MMS♂ x Control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS +ACM-D♂ x control♀.

Considering the case of generations it was found that the analysis of variance within generations showed significant differences ($F=8.31$, $P<0.05$, appendix table-I) with LSD value as 4.28 at 5 % and 7.24 at 0.1% levels of significance. It demonstrated that the percentage of dominant lethal mutations observed in parental generation was always higher and differed significantly with all other generations irrespective of crosses (table-1 and figure-1).

At 2.0ml dose of MMS the F value has been calculated within crosses as 776.53 ($P<0.001$, appendix table -II) with LSD values as 1.21 at 5% and 2.65 at 0.1% levels of significance which revealed that the mean percentages of DLM observed in MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀ differed significantly with other crosses and the mean percentages of DLM found in MMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly among themselves (table-2).

The analysis of variance also showed significant differences ($F=8.30, P<0.05$,appendix table-II) within generations with LSD value as 1.57 at 5% level of significance. It was observed that in case of MMS♂ x control♀ the percentage of DLM in parental and F_1 generations differed significantly with F_2 generation. In case of ACM-D♂ x control♀ no significant differences were observed within generations. For MMS♂ x ACM-D♂ it appeared that percentage of DLM in parental generation differed significantly with F_2 generation. In case of ACM-D♂ x MMS♀ it was observed that DLM percentage in parental generation differed significantly with F_1 and F_2 generations. For MMS +ACM-D♂ x control♀ the DLM percentage recovered from parental generation differed significantly with F_2 generation but no significant differences were found with F_1 generation (table-2 and figure-2)

Table-1: Effects of 1.0ml MMS and ACM-D on percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2715	329	12.12	
	F_1	2758	342	12.40	
	F_2	2763	366	13.25	
MMS♂ x control♀	Parental	2474	428	17.30	5.90
	F_1	2602	409	15.72	3.79
	F_2	2620	390	14.89	1.89
Mean					3.86
ACM-D♂ x control♀	Parental	2679	360	13.44	1.50
	F_1	2685	366	13.63	1.41
	F_2	2702	385	14.25	1.16
Mean					1.36
MMS♂ x ACM-D♀	Parental	2598	846	32.58	23.26
	F_1	2600	748	28.79	18.71
	F_2	2614	732	28.02	18.06
Mean					20.19
ACM-D♂ x MMS♀	Parental	2519	924	36.7	27.94
	F_1	2535	763	30.35	20.49
	F_2	2530	776	30.64	21.04
Mean					23.16
MMS+ACM-D♂ x control♀	Parental	2577	1706	33.77	24.61
	F_1	2637	1983	24.81	14.17
	F_2	2648	1995	24.66	14.24
Mean					17.67

Table-2: Effects of 2.0ml MMS and ACM-D on percentage of DLMi n *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2791	346	12.55	
	F_1	2783	353	12.7	
	F_2	2789	361	12.96	
MMS♂ x control♀	Parental	2593	545	21.04	9.7
	F_1	2615	542	20.76	9.21
	F_2	2627	527	20.07	3.12
Mean					7.34
ACM-D♂ x control♀	Parental	2694	373	13.87	1.5
	F_1	2699	389	14.43	1.98
	F_2	2715	395	14.55	1.83
Mean					1.77
MMS♂ x ACM-D♀	Parental	2465	929	37.31	28.3
	F_1	2517	913	36.29	27.02
	F_2	2535	884	34.9	25.21
Mean					52.23
ACM-D♂ x MMS♀	Parental	2413	1021	42.32	34.04
	F_1	2431	994	40.9	32.3
	F_2	2445	956	39.09	30.02
Mean					32.12
MMS+ACM-D♂ x control♀	Parental	2399	918	38.29	29.43
	F_1	2407	916	38.06	29.05
	F_2	2429	890	36.65	27.22
Mean					28.57

Results of 3.0ml doses of MMS with ACM-D, showed significant differences among the crosses (F=1169.32, P<0.001, appendix table -III) with LSD values as 1.41 at 5% and as 3.07 at 0.1% levels of significance.The percentages of DLM induced by ACM-D♂ x MMS♀ differed significantly with other treated crosses like MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed no significant differences ((F=2.58, P>0.05, appendix table-III). In cross of MMS♂ x control♀ the percentage of DLM in parental generation differed significantly with F_1 and F_2 generations. In case of ACM-D♂ x control♀, the F_1 and F_2 generations showed significant differences with parental generation. In cross of ACM-D♂ x MMS♀, parental generation differed significantly with other generations. When MMS+ACM-D treated males were crossed with control female the percentages of DLM observed in parental generation differed significantly with F_1 and F_2 generations (table-3 and figure-3).

Table-4 summarized the data following the effects of 4.0ml dose of MMS with ACM-D. The analysis of variance within crosses showed highly significant (F=841.01, P<0.001, appendix table -IV) differences with LSD values as 1.79 at 5% and 3.90 at 0.1% probability levels of significance. By comparing the observed mean percentage of DLM with the above value it was found that the mean percentage of DLM induced by ACM-D♂ x MMS♀ differed significantly with MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀, and MMS+ACM-D♂ x control♀.

Considering the case of generations it was found that the analysis of variance within generations showed no significant differences (F=2.08, P>0.05, appendix table -IV). In cross MMS♂ x control♀, the percentages of DLM in parental and F_1 generations differed significantly with F_2 generation. In case of ACM-D♂ X Control ♀, no significant differences were found among generations. In case of MMS♂ x ACM-D♂, parental generation differed significantly with F_1 and F_2 generations. In cross of ACM-D♂ x MMS♀ parental generation differed significantly with F_1 and F_2 generations. When MMS+ACM-D treated males were crossed with control females they showed that the percentages of DLM observed in parental generation differed significantly with F_1 and F_2 generations as in table-4 and figure-4.

Table-3: Effects of 3.0ml MMS and ACM-D on percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2791	350	12.56	
	F_1	2796	351	12.57	
	F_2	2803	351	12.53	
MMS♂ x control ♀	Parental	2719	687	25.27	14.53
	F_1	2742	656	23.94	13.01
	F_2	2752	629	22.86	11.82
Mean					13.12
ACM-D♂ x control♀	Parental	2694	386	14.35	2.05
	F_1	2705	393	14.55	1.87
	F_2	2703	384	14.2	1.91
				mean	1.93
MMS♂ x ACM-D♀	Parental	2563	1215	47.41	39.86
	F_1	2573	1194	46.41	38.46
	F_2	2596	1189	45.81	38.05
				mean	38.79
ACM-D♂ x MMS♀	Parental	2466	1256	50.94	43.89
	F_1	2463	1310	53.18	46.23
	F_2	2478	1273	51.38	44.42
				mean	44.85
MMS+ACM-D♂ x control♀	Parental	2484	1218	40.06	41.74
	F_1	2492	1198	48.07	40.36
	F_2	2505	1169	46.69	39.05
Mean					40.38

Table-4: Effects of 4.0ml MMS and ACM-D on percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2786	350	12.59	
	F_1	2794	359	12.85	
	F_2	2801	349	12.46	
MMS♂ x control♀	Parental	2728	735	26.97	16.45
	F_1	2733	739	27.03	16.27
	F_2	2744	683	24.89	14.19
				Mean	15.63
ACM-D♂ x control♀	Parental	2695	381	14.15	1.79
	F_1	2688	388	14.44	1.82
	F_2	2688	349	14.02	1.78
				Mean	1.79
MMS♂ x ACM-D♀	Parental	2469	1177	47.69	40.16
	F_1	2474	1208	48.82	41.27
	F_2	2484	1211	48.77	41.48
				Mean	40.97
ACM-D♂ x MMS♀	Parental	2392	1265	52.91	46.13
	F_1	2386	1328	55.67	49.14
	F_2	2399	1332	55.52	49.2
				Mean	48.16
MMS+ACM-D♂ x control♀	Parental	2387	1195	50.09	42.9
	F_1	2392	1281	53.57	46.73
	F_2	2416	1268	52.49	45.73
Mean					45.12

Table-5 summarized the data showing the effects on DLM following treatment with differend doses of MMS and ACM-D. Results demonstrated that the percentages of induced DLM were increased linearly with the increase doses of MMS and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix table V-IX. In case of MMS♂ x control♀ , the F value within generations has been calculated as 29.70 ($P<0.001$) with LSD values as 0.84 at 5% and 2.06 at 0.1%, the F value within doses has been calculated as 412.28 ($P<0.001$, appendix table-V) with LSD values as 0.74 at 5% and 1.79 at 0.1% level of significance respectively. The mean value of DLM in parental generation differed significntly with those of F_1 and F_2 generations. In case of doses it was observed that means of the all doses differ significantly with one another and this kind of differences were also observed in other crosses in the table-5 and figure-5.

When the ACM-D treated males were crossed with control females the analysis of variances has been calculated within generations and doses, and no significant differences were observed (appendix table-VI and figure-6) .In cross of MMS♂ x ACM-D♂ the analysis of variances has been calculated no significant differences within generations (appendix table-VII). But the F values within doses has been calculated as 197.04 ($P<0.001$) with LSD values as 3.0 at 5% and as 7.31 at 0.1% respectively showed significant differences as presented in appendix table-VII and figure-7.

When the ACM-D treated males were crossed with MMS treated females the analysis of variances has been calculated and no significant differences within generations were found (appendix table-VIII). The F values within doses has been calculated and showed highly significant differences ($F=53.14$, $P<0.001$, appendix table-VIII and figure-8) with LSD values as 4.76 at 5% and as 9.79 at 0.1%, respectively. In cross of MMS+ACM-D♂ x control♀, the F value found among generations has been calculated with non significant differences, but within doses it was found as ($F= 41.22$ $P<0.001$) with LSD values 5.76 at 5%, and 14.01 at 0.1% level of significant differences (appendix table-IX and figure-9).

Effects of different doses of MMS and ACM-D on the percentage of DLM in different crosses are shown in table-6. It was observed that mean percentages of DLM found highly differed significantly within crosses ($F=29.45$, $P<0.001$, appendix table-X) with LSD values as 7.83 at 5% and as 15.52 at 0.1% probability level. By comparing the observed mean percentage of DLM it was found that the mean percentage of DLM induced by ACM-D♂ x MMS♀ differed significantly with MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀, and MMS+ACM-D♂ x control♀. The percentages of DLM differed significantly within doses ($F=10.59$, $P<0.01$) with LSD values as 8.75 at 5% and as 17.35 at 1% probability level (appendix table-X). The percentages of DLM induced by 1.0ml MMS doses differed significantly with those of the higher doses of 2.0ml, 3.0ml and 4.0ml (table-6 and figure-10).

Table-5: Effects of different doses of MMS and ACM-D on the DLM in different generations of *Epilachna vigintioctopunctata*

Dose of MMS						
Cross	genaration	1.0 ml	2.0 ml	3.0 ml	4.0 ml	Mean ± SE
MMS♂ x control♀	Parental	5.9	9.7	14.53	16.45	11.645±2.38
	F_1	3.82	9.23	13.01	16.27	10.5825±2.67
	F_2	3.12	8.17	11.82	14.19	9.325±2.41
	Mean ± SE	4.28±0.83	9.03±0.45	13.12±0.78	15.64±0.73	
ACM-D♂ x control♀	Parental	1.89	1.5	2.05	1.79	1.8075±0.12
	F_1	1.41	1.98	1.87	1.82	1.77±0.12
	F_2	1.43	1.83	1.91	1.78	1.7375±0.11
	Mean ± SE	1.58±0.16	1.77±0.14	1.94±0.05	1.80±0.01	
MMS♂ x ACM-D♀	Parental	23.26	28.3	39.86	40.16	32.895±4.24
	F_1	18.71	27.02	38.46	41.27	31.365±5.22
	F_2	18.06	25.21	38.05	41.48	30.7±5.48
	Mean ± SE	18.39±0.27	26.12±0.74	38.26±0.17	41.38±0.09	
ACM-D♂ x MMS♀	Parental	27.94	34.04	43.83	46.13	37.985±4.25
	F_1	20.49	32.3	46.23	49.14	37.04±6.63
	F_2	21.04	30.02	44.42	49.2	36.17±6.48
	Mean ± SE	23.16±2.40	32.12±1.16	44.83±0.72	48.16±1.01	
MMS+ACM-D♂ x control♀	Parental	24.61	29.43	41.74	42.9	34.67±4.53
	F_1	14.17	29.05	40.36	46.73	32.5775±7.14
	F_2	14.24	27.22	39.05	45.73	31.56±6.93
	Mean ± SE	14.21±0.03	28.14±0.75	39.71±0.53	46.23±0.41	

EFFECTS OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF 'LADYBIRD' BEETLE *Epilachna vigintioctopunctata* (Fab.)

Table-6: Effects of different doses of MMS and ACM-D on the DLM in different crosses of *Epilachna vigintioctopunctata*.

Crosses	Dose of MMS				Mean±SE
	1.0ml	2.0ml	3.0ml	4.0ml	
MMS♂ x control♀	4.28	9.03	13.12	15.64	10.52±2.48
ACM-D♂ x control♀	1.58	1.77	1.94	1.80	1.77±0.08
MMS♂ x ACM-D♀	18.39	26.12	38.26	41.38	31.03±5.35
ACM-D♂ x MMS♀	23.16	32.12	44.83	48.16	37.07±5.78
MMS+ACM-D♂ x control♀	14.21	28.14	39.71	46.23	32.07±7.03
Mean± SE	12.32±4.11	19.43±5.92	27.57±8.44	30.64±9.28	

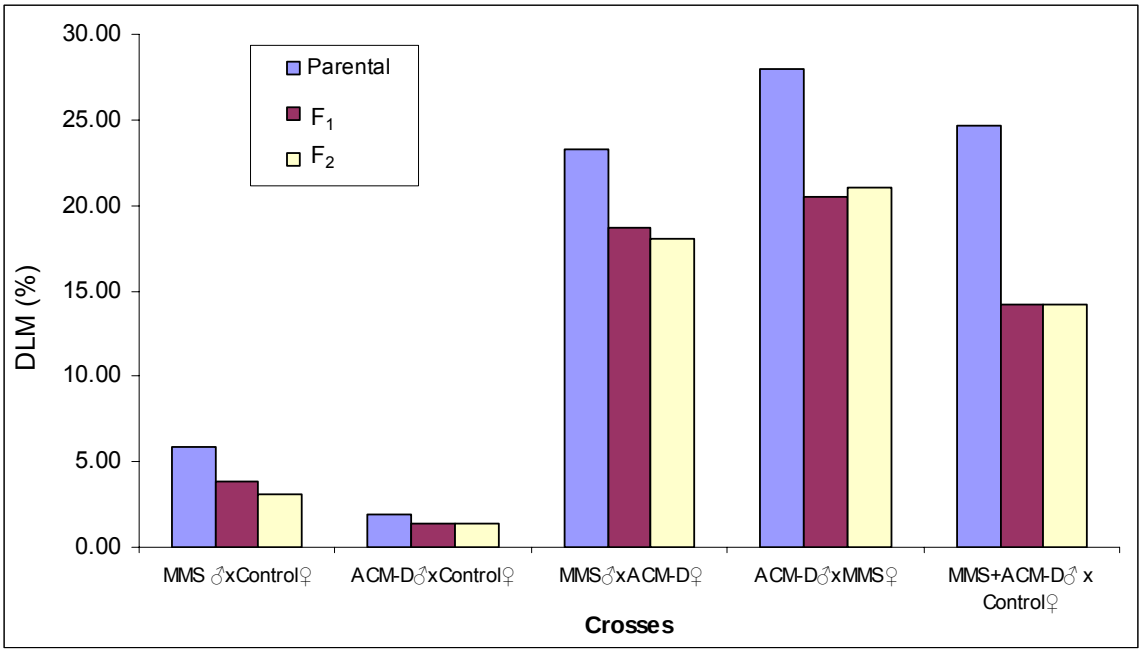

Figure-1: Effects of 1.0ml MMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

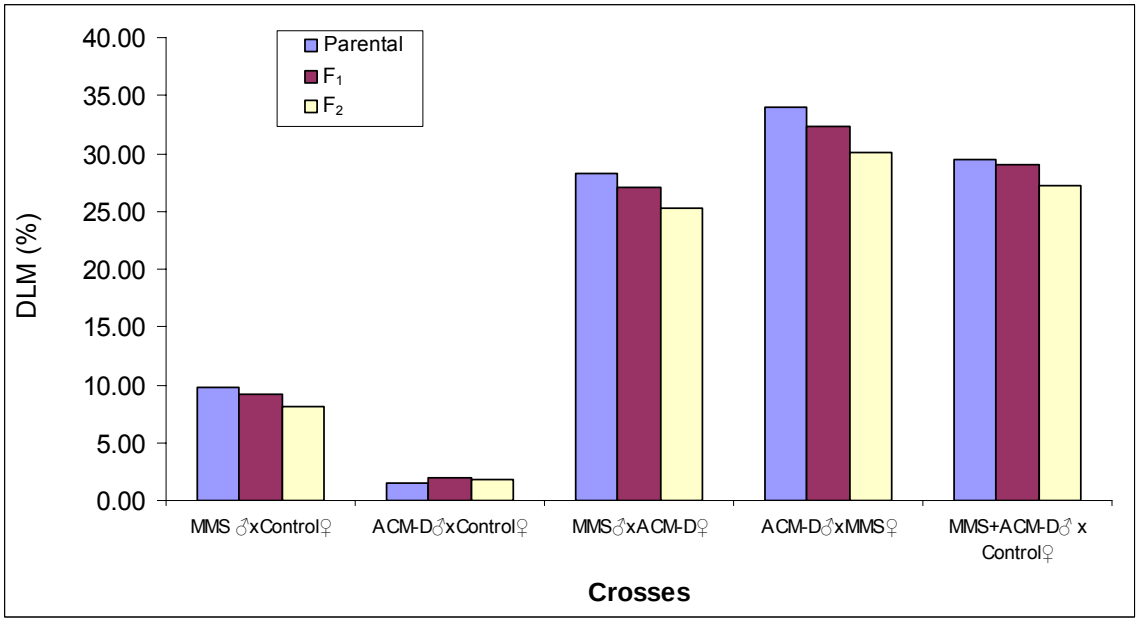

Figure-2: Effects of 2.0ml MMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

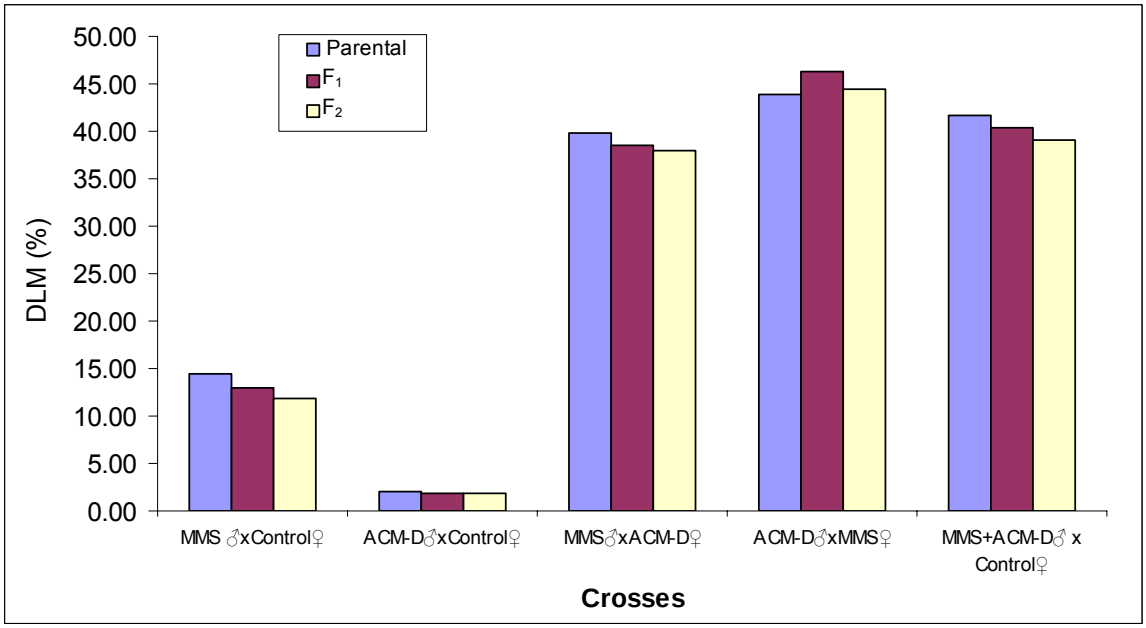

Figure-3: Effects of 3.0ml MMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

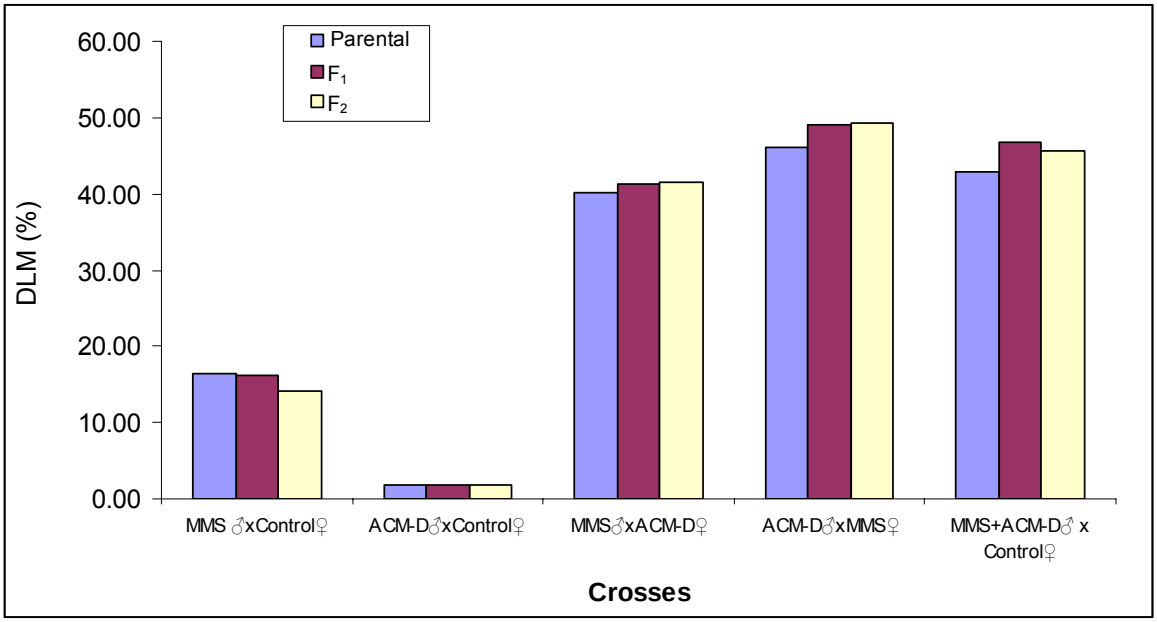

Figure-4: Effects of 4.0ml MMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

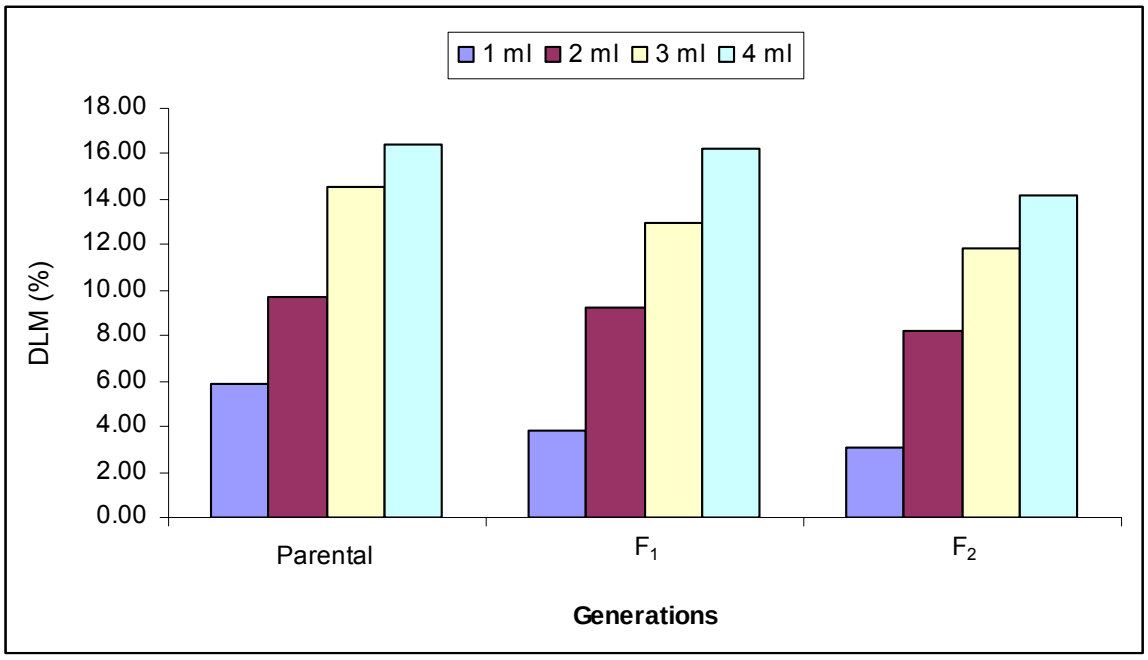

Figure-5: Effects of different doses of MMS on DLM% following cross MMS♂ x control♀ in different generations of *Epilachna vigintioctopunctata*

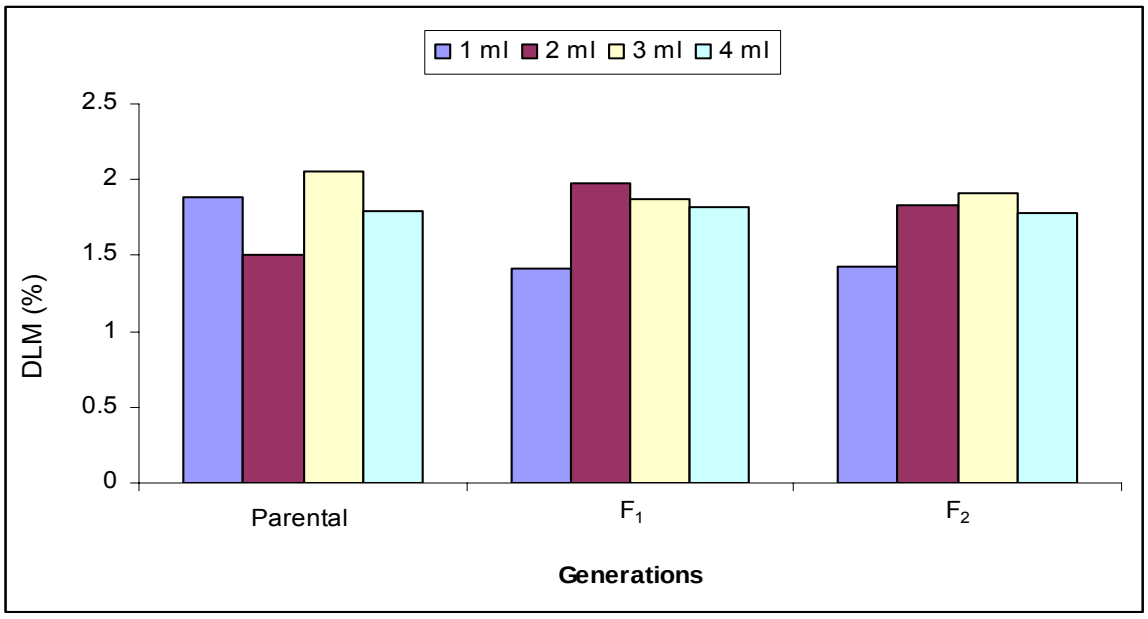

Figure-6: Effects of different doses of MMS on DLM following cross ACM-D♂ x control♀ in different generations of *Epilachna vigintioctopunctata*

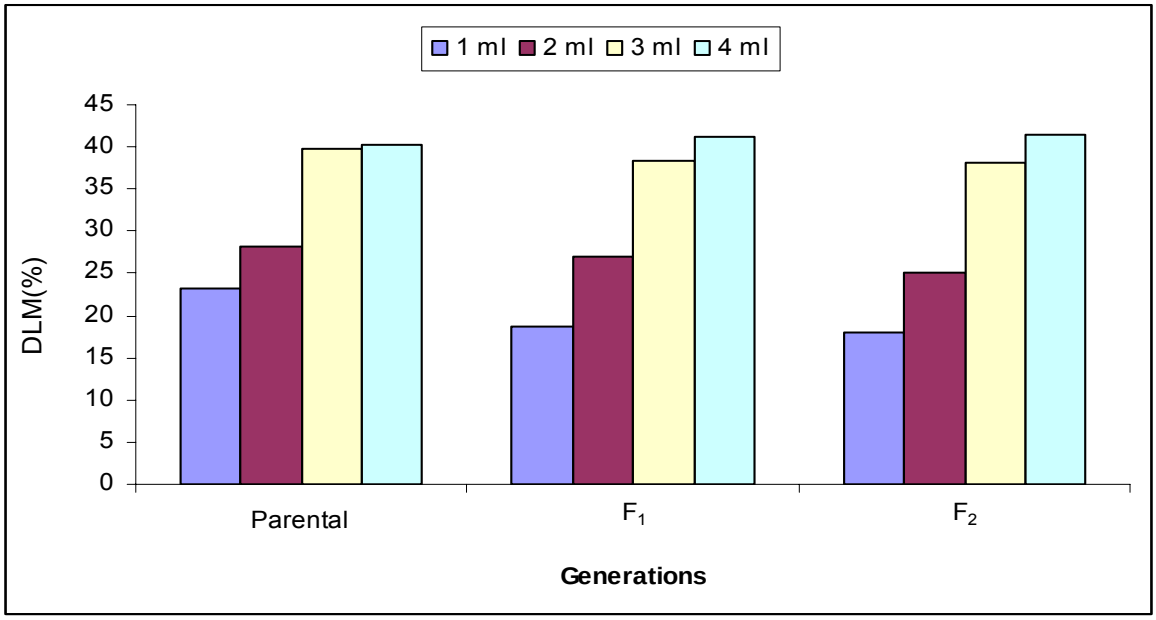

Figure-7: Effects of different doses of MMS on DLM% following cross MMS♂ x ACM-D♀ in different generations of *Epilachna vigintioctopunctata*

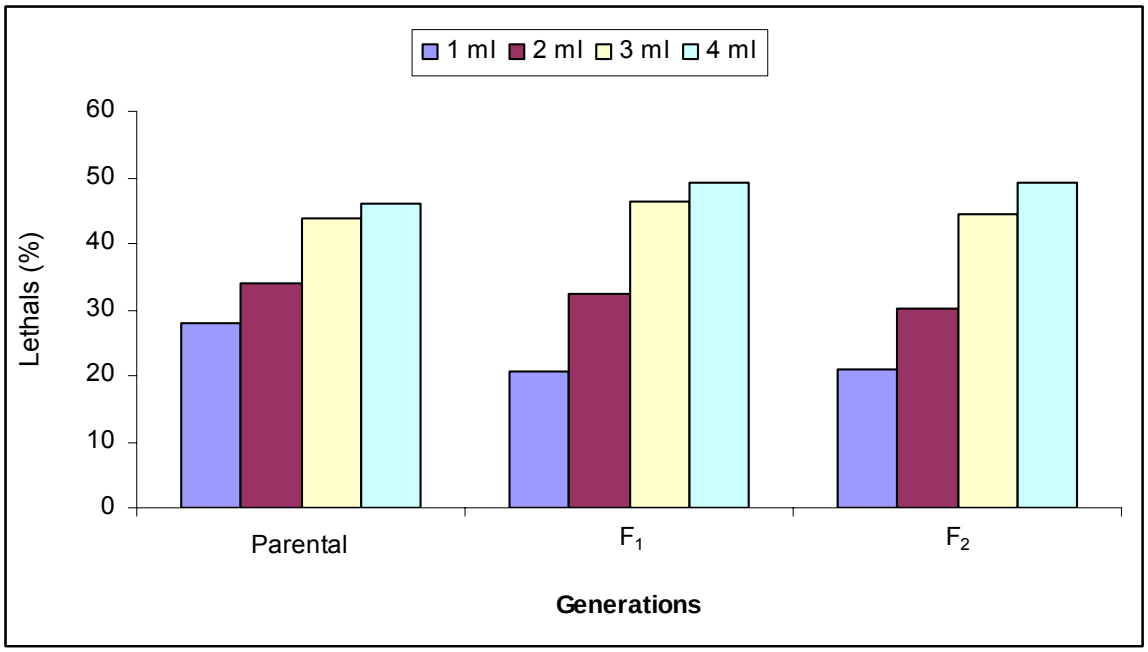

Figure-8: Effects of different doses of MMS on DLM% following cross ACM-D♂ x MMS♀ in different generations of *Epilachna vigintioctopunctata*

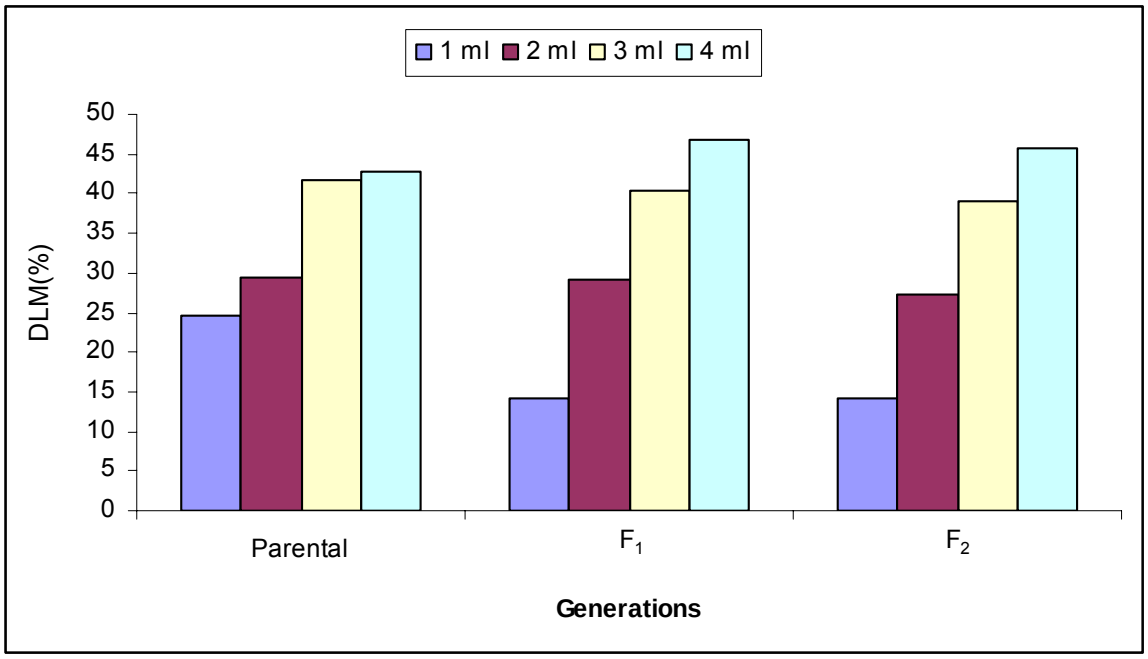

Figure-9: Effects of different doses of MMS on DLM% following cross MMS+ACM-D♂ x control♀ in different generations of *Epilachna vigintioctopunctata*

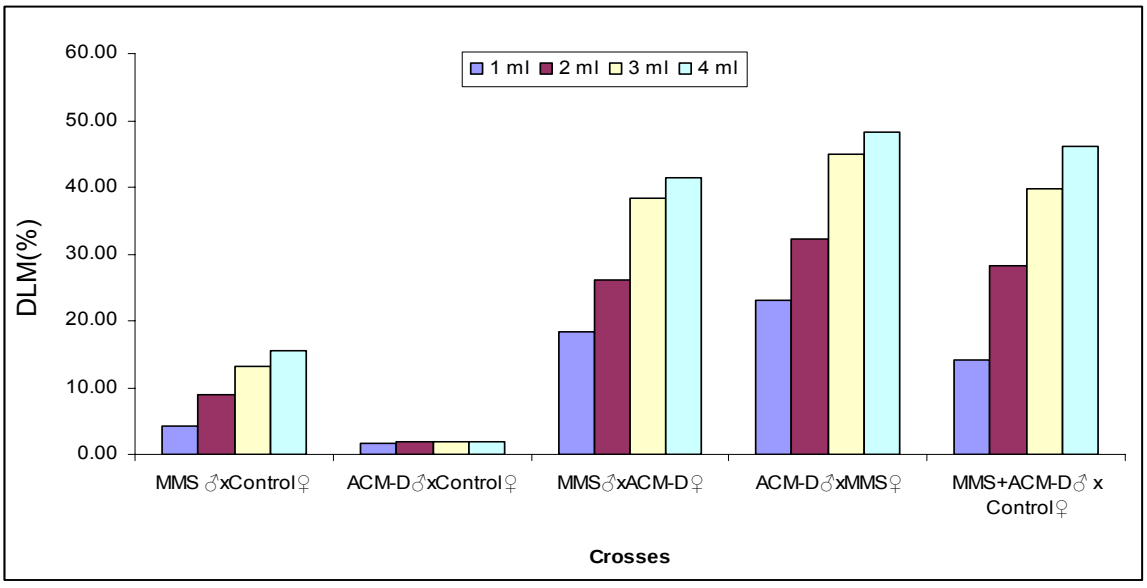

Figure-10: Effects of different doses of MMS and ACM-D on DLM % in different crossess of *Epilachna vigintioctopunctata*

4.1.2. Induction of dominant lethal mutations (DLM) by ethyl methanesulfonate (EMS) and actinomycin-D (ACM-D)

The data of dominanted lethal mutation frequencies for the three generations of *Epilachna vigintioctopunctata* in different crosses and different doses of EMS with ACM-D are presented in tables 7-12, figures 11-20 and appendix tables XI-XX.

Results obtained from 1.0ml dose of EMS (Table-7) showed that ACM-D increased the rate of EMS induced dominant lethal mutations. The analysis of variance within crosses showed highly significant differences ($F=1096$, $P<0.001$, appendix table-XI) with LSD values as 0.67 at 5% and as 1.46 at 0.1%, probability level.The observed mean percentages of DLM induced by EMS♂ x Control♀ and ACM-D♂ x control♀ differed significantly with all other crosses. The mean value of DLM % for EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀ also differed significantly with one another.

The analysis of variance within generations showed significance differences ($F=20.62$, $P<0.001$, appendix table-XI) with LSD values as 0.86 at 5% and as 1.89 at 0.1% level of significance. It reported that in case of EMS♂ x control♀ the mean percentage of DLM obtained from parental generation differed significantly with F_2 generation, but not for F_1 generation. Cross of ACM-D♂ x control♀ did not differed significantly with any other generations. In cross of EMS♂ x ACM-D♀ F_1 and F_2 generations differed significantly with parental generation but F_1 and F_2 generations showed non significant difference with each other. When ACM-D treated male's were crossed with EMS treated females and in cross of EMS+ACM-D♂ x Control♀ the mean percentages of DLM observed in parental generation differed significantly with F_1 and F_2 generations (Table-7, figure-11).

Table-7: Effects of 1.0ml EMS and ACM-D on the percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2706	326	12.06	
	F_1	2757	335	12.15	
	F_2	2761	335	12.12	
EMS♂ x control♀	Parental	2600	402	15.48	3.88
	F_1	2611	386	14.79	3
	F_2	2620	374	14.28	2.45
Mean					3.11
ACM-D♂ x control♀	Parental	2691	366	13.61	1.77
	F_1	2688	358	13.34	1.35
	F_2	2700	357	13.23	1.23
Mean					1.45
EMS♂ x ACM-D♀	Parental	2592	765	29.52	19.86
	F_1	2604	723	27.76	17.76
	F_2	2618	710	27.13	17.05
Mean					18.22
ACM-D♂ x EMS♀	Parental	2521	794	31.49	22.09
	F_1	2537	762	30.05	20.37
	F_2	2537	756	29.83	20.13
Mean					21.20
EMS+ACM-D♂ × control♀	Parental	2620	649	24.77	14.45
	F_1	2638	621	23.55	12.98
	F_2	2652	606	22.87	12.21
Mean					13.21

Data obtained from table-8 following the effect of 2.0ml doses of EMS, the F value has been calculated within crosses as 1236 and differed significanty ($P<0.001$, appendix table-XII) with LSD values as 0.92 at 5% and 2.02 at 0.1%, probability level. The mean percentages of DLM observed in EMS♂ x control♀ differed significantly with other crosses and the means of DLM of EMS♂ x ACM-D♀ and EMS+ACM-D♂ x control♀ did not show any significant differences between themselves but they differed significantly with all other crosses.

At the above doses the analysis of variance within generations also showed significant differences ($F=16.05$, $P<0.01$, appendix table-XII) with LSD values as 1.19 at 5% and as 0.1.73 at 1% level of significance. It explains that when EMS treated males were crossed with control females the percentages of DLM obtained from parental generation differed significantly with F_1 and F_2 generations. In cross of EMS♂ x ACM-D♀, parental generation differed significantly with F_1 and F_2 generations but F_1 and F_2 generations also differed significantly between themselves. When ACM-D treated males were crossed with EMS treated females, parental generation differed significantly with all other generations. In cross of EMS+ACM-D♂ x control♀, the percentages of DLM observed in parental generation differed significantly with F_1 and F_2 generations (Figure-12).

Table-8: Effects of 2.0ml EMS and ACM-D on the percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2757	327	11.85	
	F_1	2784	359	12.92	
	F_2	2786	358	12.87	
EMS♂ x control♀	Parental	2604	544	20.92	10.28
	F_1	2625	537	20.47	8.67
	F_2	2641	513	19.43	7.53
Mean					8.83
ACM-D♂ x control♀	Parental	2691	365	13.58	1.96
	F_1	2704	396	14.65	1.99
	F_2	2718	393	14.45	1.82
Mean					1.92
EMS♂ x ACM-D♀	Parental	2500	909	36.38	27.82
	F_1	2520	892	35.41	25.82
	F_2	2525	867	34.34	24.64
Mean					26.09
ACM-D♂ x EMS♀	Parental	2420	989	40.89	32.94
	F_1	2435	975	40.04	31.14
	F_2	2450	954	38.96	29.95
Mean					31.34
EMS+ACM-D♂ × control♀	Parental	2409	879	36.5	27.96
	F_1	2409	874	36.27	26.81
	F_2	2431	858	35.3	25.74
Mean					26.83

The frequencies of dominant lethal mutations induced by 3.0ml doses of EMS and ACM-D are shown in table-9. The analysis of variance within crosses showed highly significant differences (F=2548.31, P<0.001, appendix table-XIII) with LSD values as 0.95 at 5% and as 2.07 at 0.1% level of significance, which shows that the mean percentages of DLM obtained from EMS♂ x control♀ differed significantly with other crosses. Mean percentages of DLM observed in EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀ differed significantly with one another.

Considering the case of generations the analysis of variance showed significant differences (F=15.74, P<0.01, appendix table-XIII) with LSD values as 1.22 at 5% and as 1.78 at 1% probability level, in cross of EMS♂ x control♀ the percentages of DLM in parental generation differed significantly with F_1 and F_2 generations. In case of EMS♂ x ACM-D♀and ACM-D♂ x EMS♀ the percentages of DLM observed in parental generation showed significant differences with F_1 and F_2 generations. Significant differences were also observed between F_1 and F_2 generations. When EMS+ACM-D treated males were crossed with control females they showed that the percentages of DLM observed in parental generation differed significantly with F_1 and F_2 generations (table-9 and figure-13).

Table-9: Effects of 3.0ml EMS and ACM-D on the percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2782	353	12.70	
	F_1	2792	336	12.94	
	F_2	2799	350	12.53	
EMS♂ x control♀	Parental	2745	641	23.34	12.19
	F_1	2749	636	23.14	11.71
	F_2	2754	593	21.53	10.29
Mean					11.40
ACM-D ♂ x control ♀	Parental	2692	388	14.42	1.97
	F_1	2707	395	14.62	1.93
	F_2	2716	379	13.96	1.63
Mean					1.84
EMS♂ x ACM-D♀	Parental	2574	1196	46.46	38.67
	F_1	2580	1174	45.5	37.4
	F_2	2596	1148	44.24	36.03
Mean					37.37
ACM-D♂ x EMS♀	Parental	2472	1307	52.9	46.05
	F_1	2461	1266	51.45	44.23
	F_2	2478	1228	49.53	42.3
Mean					44.19
EMS+ACM-D♂ × Control♀	Parental	2490	1198	48.13	40.58
	F_1	2497	1174	47.03	39.16
	F_2	2508	1142	45.55	37.74
Mean					39.16

Data obtained from table-10 following the effect of 4.0ml doses of EMS within crosses the analysis of varience has been calculated and differed significantly (F= 2060.45, P<0.001, appendix table-XIV) with LSD values as 1.11 at 5% and as 2.43 at 0.1% probability level, The mean percentages of DLM observed in EMS♂ x control♀ differed significantly with other crosses and for the cross EMS♂ x ACM-D♀,ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀ the mean DLM differed significantly with each other.

At the above doses the analysis of variance within generations also showed non significant differences (appendix table-XIV). It explains that when EMS treated males were crossed with control females the percentages of DLM obtained from parental generation differed significantly with F_1 and F_2 generations. In cross of ACM-D♂ x EMS♀ the parental generation differed significantly with F_1 and F_2 generations (figure-14).

Table-10: Effectsof 4.0ml EMS and ACM-D on the percentage of DLM in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs laid	No. of unhatched eggs	% of unhatched eggs	% of DLM
control♂ x control♀	Parental	2783	352	12.68	
	F_1	2794	359	12.88	
	F_2	2798	349	12.49	
EMS♂ x control♀	Parental	2735	711	26.02	15.27
	F_1	2733	702	25.7	14.71
	F_2	2744	669	24.37	13.58
Mean					14.52
ACM-D♂ x control♀	Parental	2693	384	14.27	1.82
	F_1	2693	397	14.75	1.98
	F_2	2707	384	14.21	1.97
Mean					1.92
EMS♂ x ACM-D♀	Parental	2477	1160	46.86	39.14
	F_1	2477	1162	46.93	38.98
	F_2	2487	1184	47.6	40.12
Mean					39.41
ACM-D♂ x EMS♀	Parental	2392	1247	52.13	45.19
	F_1	2392	1236	51.68	44.44
	F_2	2408	1213	50.37	43.3
Mean					44.31
EMS+ACM-D♂ × control♀	Parental	2395	1312	54.74	46.12
	F_1	2392	1301	54.39	47.55
	F_2	2416	1274	52.75	46.01
Mean					46.56

Results of different doses of EMS with ACM-D on the DLM percentages of different generations are shown in table-11.It evaluate the enhancement of EMS treated dominant lethal mutation by ACM-D. It also demonstrated that the higher doses of EMS induced higher percentages of DLM. The analysis of varience within generations and doses has been calculated and are presented in appendix table XV-XIX. In case of EMS♂ x control♀, the F value within generations has been calculated as 31.58 ($P<0.001$, appendix table-XV) with LSD values as 0.69 at 5% and as 1.69 as 1% level of significance, the F value within doses has been calculated which showed significant differences as 578.61 ($P<0.001$) with LSD values as 0.60 at 5% and as 1.46 at 0.1%, respectively (appendix table-XV). The mean value of DLM of parental generation significntly differed with F_1 and F_2 generations. In case of doses it was observed that means of the all doses differed significantly with one another and this kind of differences were also observed in other crosses (table-11 and figure-15).

When the ACM-D treated males were crossed with control females the analysis of variances has been calculated and no significant differences were observed within the generations and doses (appendix table-XVI and figure-16).In cross EMS♂ x ACM-D♀ the analysis of variances did not differed significantly within generations (appendix table-XVII). But, for the F values within doses there were significance differences as 287.80 ($P<0.001$) with LSD values as 1.76 at 5% and 2.15 0.1%, respectively (appendix table-XVII and figure-17).

When the ACM-D treated males were crossed with EMS treated females the analysis of variances demonstrated significance differences within generations ($F=27.15$, $P<0.001$, appendix table-XVIII) with LSD values as 1.02 at 5 % and as 2.48 at 1% level. The F values within doses showed significance differences ($F=1478$, $P<0.001$, appendix table-XVIII, figure-18) with LSD values 0.88 at 5% and as 2.15 at 0.1%, respectively. In cross EMS+ACM-D♂ x control♀, the F value within generations showed significant differences as 6.08 $P<0.05$, with LSD value as 1.52 at 5% and F value within doses has been calculated as 1106.22 ($P<0.001$) with LSD values as 1.32 at 5%, and as 3.21 at 0.1% level of significant differences (appendix table-XIX and figure-19).

 EFFECTS OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF 'LADYBIRD' BEETLE *Epilachna vigintioctopunctata* (Fab.)

The analysis of variance showing the effects of different crosses and doses following treatment with EMS and ACM-D on the yield of dominant lethal mutations (table-12) has been shown in appendix table-XX.It was found that the calculated F values within crosses and doses were 26.26 ($P<0.001$) and 9.95 ($P<0.01$) with LSD values as 8.01 at 5%, and as 15.88 at 0.1% and as 9.96 at 5%, and as 12.58 at 1% level of significant differences. It was observed that mean percentages of DLM obtained from EMS♂ x control♀ and ACM-D♂ x control♀ differed significantly with other crosses. It was revealed that the mean values obtained from 1.0ml dose differed significantly with the higher 4.0ml dose (figure-20).

Table-11: Effects of different doses of EMS and ACM-D on the DLM in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS				Mean ± SE
		1.0ml	2.0ml	3.0ml	4.0ml	
EMS♂ x control♀	Parental	3.88	10.28	12.19	15.27	10.41±2.41
	F_1	3	8.67	11.71	14.71	9.52±2.50
	F_2	2.45	7.53	10.29	13.58	8.46±2.35
	Mean ± SE	3.11±0.42	8.83±0.80	11.40±0.57	14.52±0.50	
ACM-D♂ x control♀	Parental	1.77	1.96	1.97	1.82	1.88±0.05
	F_1	1.35	1.99	1.93	1.98	1.81±0.15
	F_2	1.23	1.82	1.63	1.97	1.66±0.16
	Mean ± SE	1.45±0.16	1.92±0.05	1.84±0.11	1.92±0.05	
EMS♂ x ACM-D♀	Parental	19.86	27.82	38.67	39.14	31.37±4.64
	F_1	17.76	25.82	37.4	38.98	29.99±5.02
	F_2	17.05	24.64	36.03	40.12	29.46±5.28
	Mean ± SE	17.41±0.29	25.23±0.48	36.72±0.56	39.55±0.47	
ACM-D♂ x EMS♀	Parental	22.09	32.94	46.05	45.19	36.57±5.68
	F_1	20.37	31.14	44.23	44.44	35.05±5.80
	F_2	20.13	29.95	42.3	43.3	33.92±5.51
	Mean ± SE	20.86±0.62	31.34±0.87	44.19±1.08	44.31±0.55	
EMS+ACM-D♂ x control♀	Parental	14.45	27.96	40.58	46.12	32.28±7.05
	F1	12.98	26.81	39.16	47.55	31.63±7.53
	F2	12.21	25.74	37.74	46.03	30.43±7.36
	Mean ± SE	13.21±0.66	26.84±0.64	39.16±0.82	46.57±0.49	

Table-12: Effects of different doses of EMS and ACM-D on DLM percentage in different crosses of *Epilachna vigintioctopunctata*

Crosses	Dose of EMS				Mean±SE
	1.0ml	2.0ml	3.0ml	4.0ml	
EMS♂ x control♀	3.11	8.83	11.40	14.52	9.47±2.42
ACM-D♂ x control♀	1.45	1.92	1.84	1.92	1.78±0.11
EMS♂ x ACM-D♀	17.41	25.23	36.72	39.55	29.73±5.14
ACM-D♂ x EMS♀	20.86	31.34	44.19	44.31	35.18±5.66
EMS+ACM-D♂ x control♀	13.21	26.84	39.16	46.57	31.45±7.31
Mean ± SE	11.21±3.85	18.83±5.69	26.66±8.41	29.37±8.94	

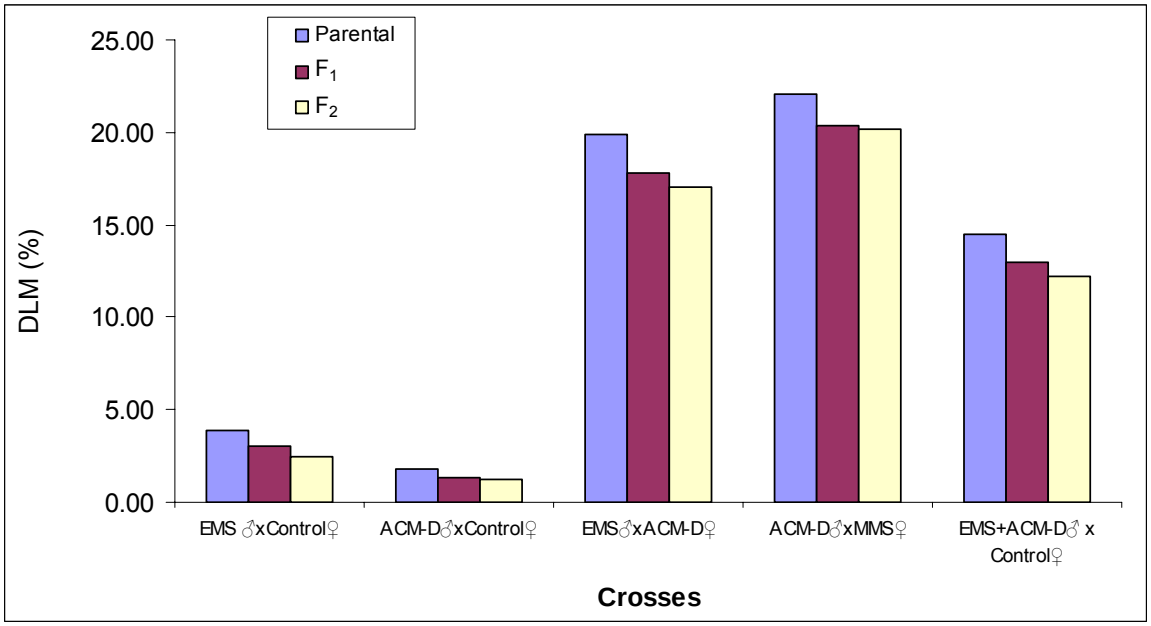

Figure-11: Effects of 1.0ml EMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

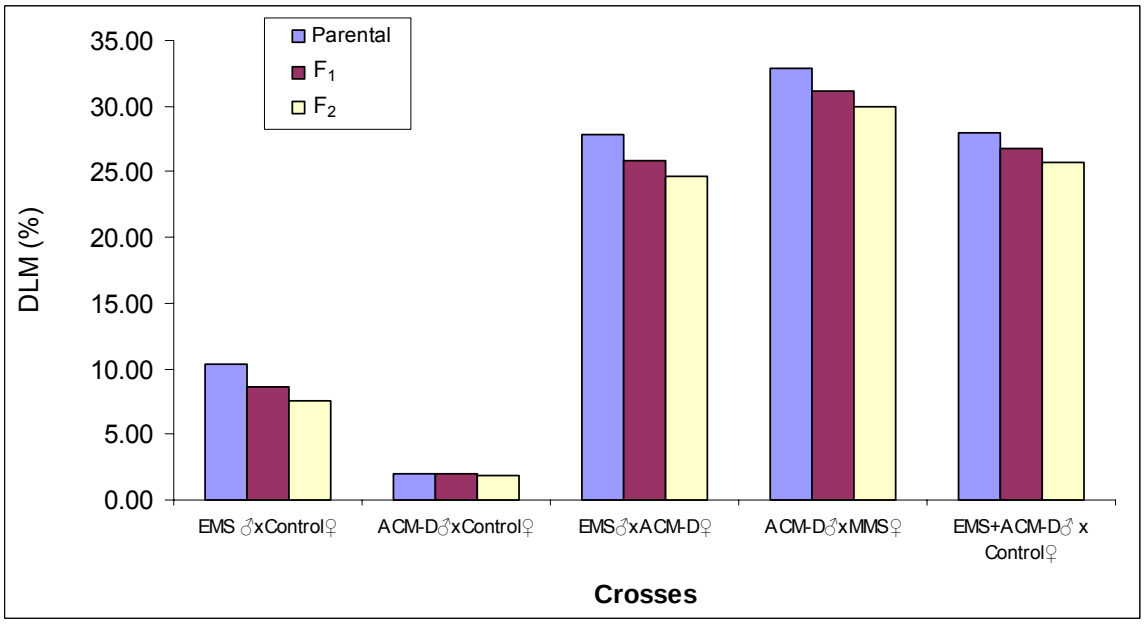

Figure-12: Effects of 2.0ml EMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

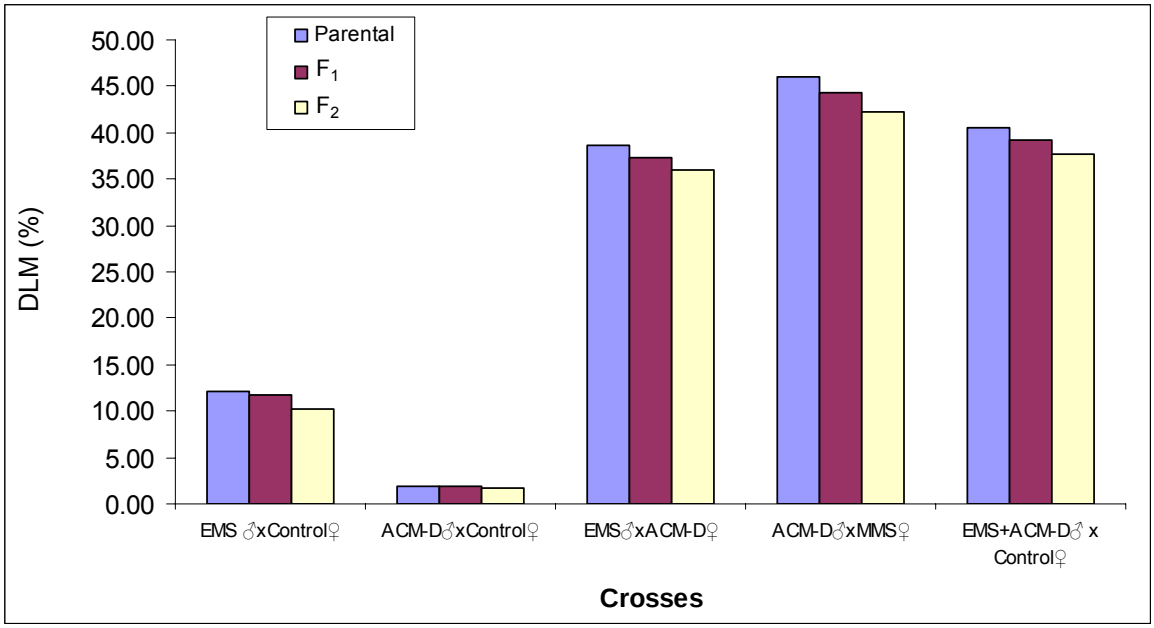

Figure-13: Effects of 3.0ml EMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

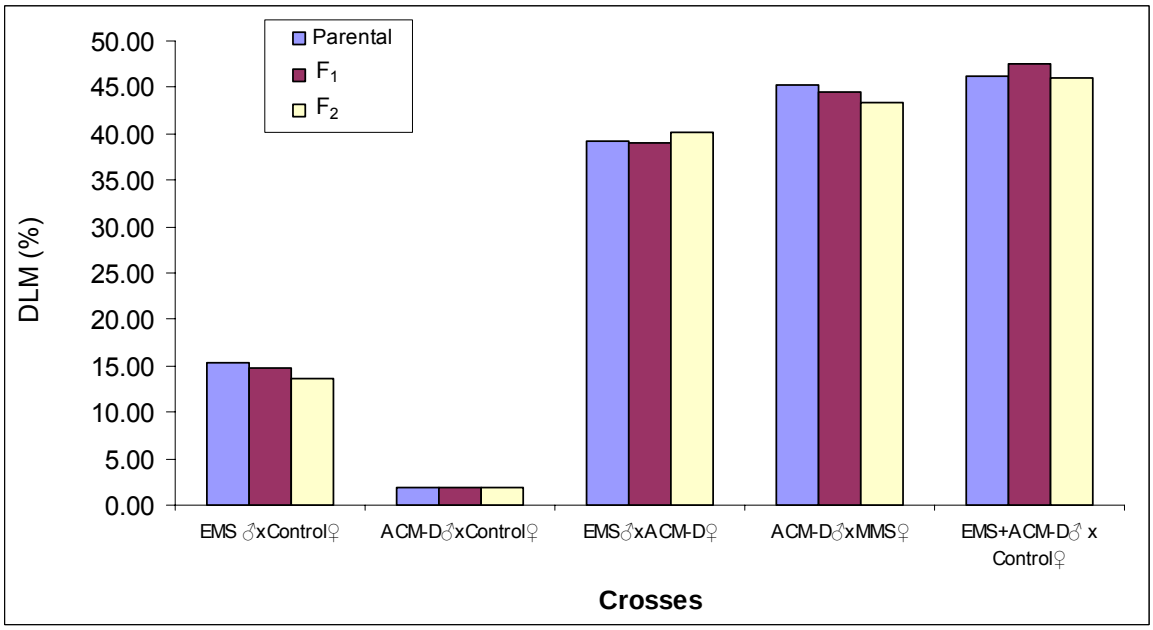

Figure-14: Effects of 4.0ml EMS and ACM-D on DLM% in different crosses and generations of *Epilachna vigintioctopunctata*

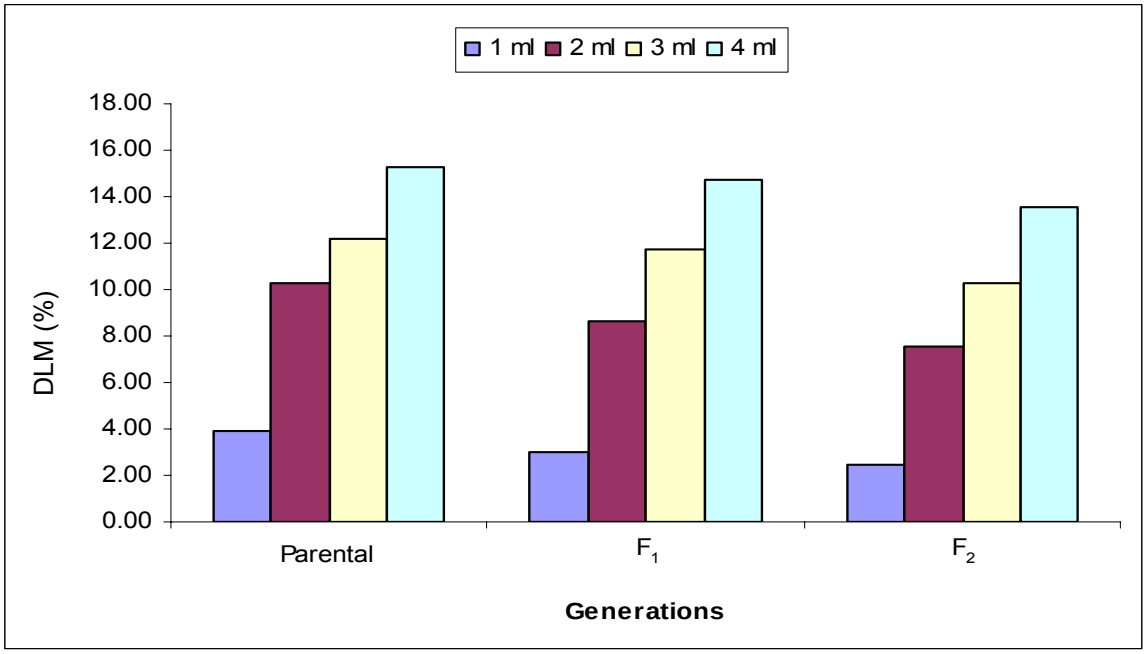

Figure-15: Effects of different doses of EMS on DLM% for the crosses EMS♂ x control♀ in different generations of *Epilachna vigintioctopunctata*

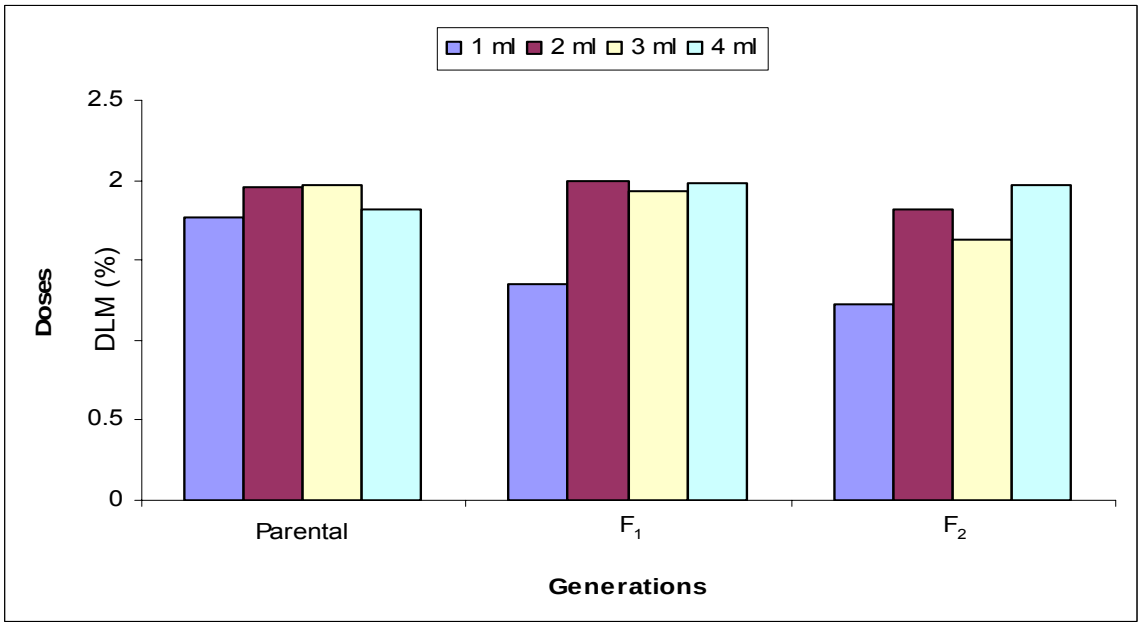

Figure-16: Effects of different doses of EMS on DLM% for the cross ACM-D♂ x control♀ in different generations of *Epilachna vigintioctopunctata*

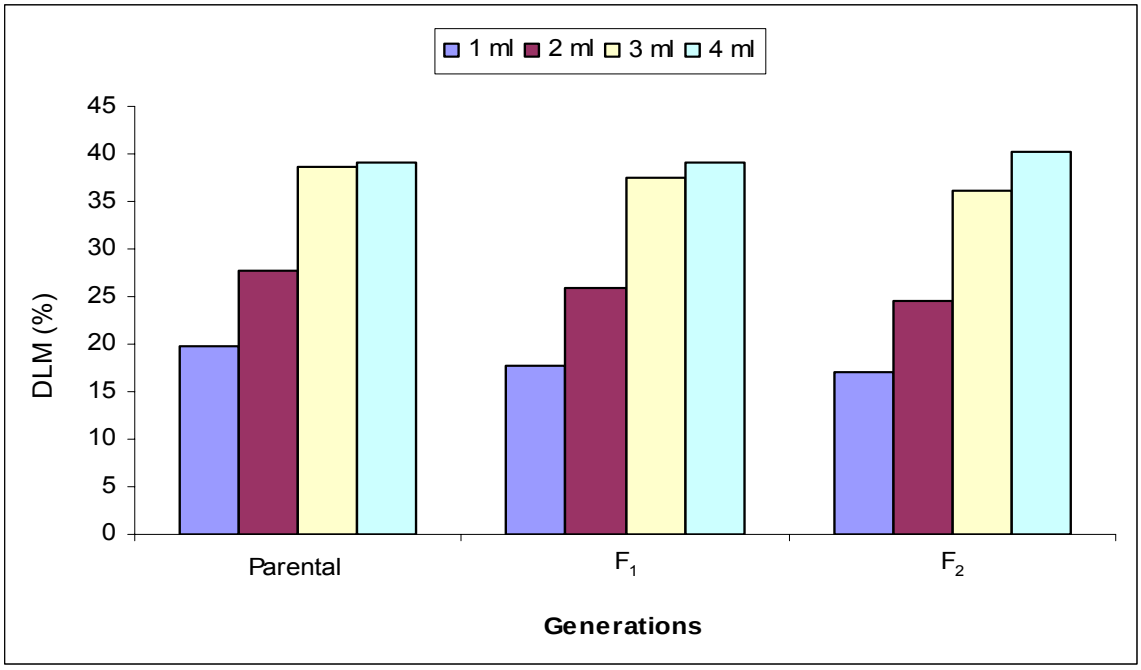

Figure-17: Effects of different doses of EMS on DLM% for the cross EMS♂ x ACM-D♀ in different generations of *Epilachna vigintioctopunctata*

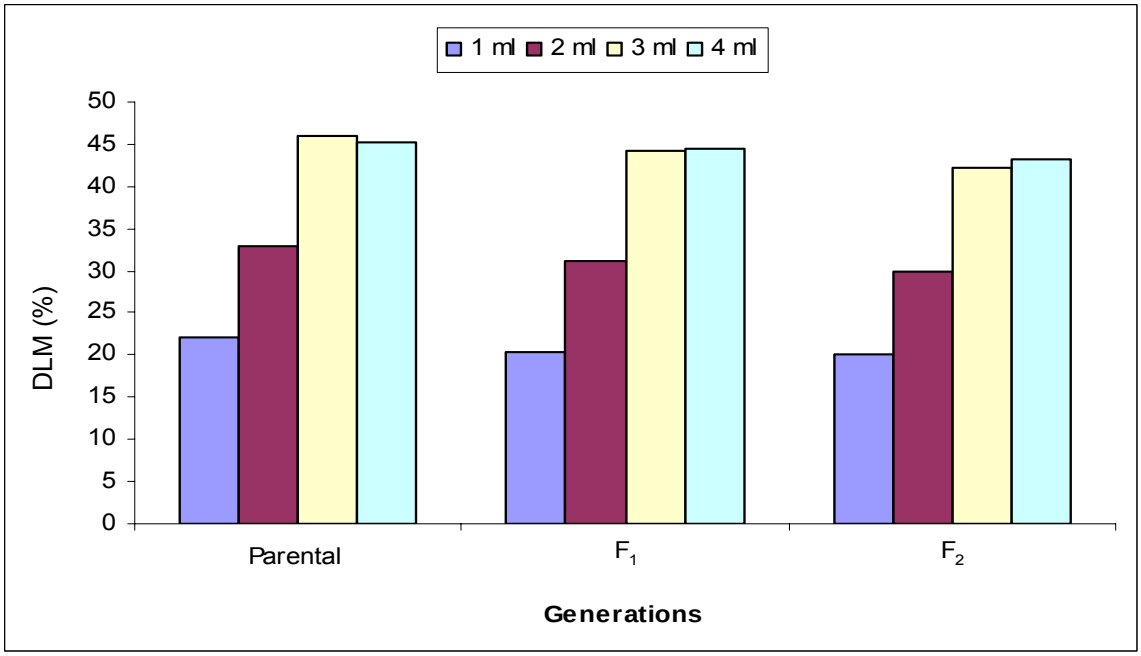

Figure-18: Effects of different doses of EMS on DLM% for the cross ACM-D♂ x EMS♀ in different generations of *Epilachna vigintioctopunctata*

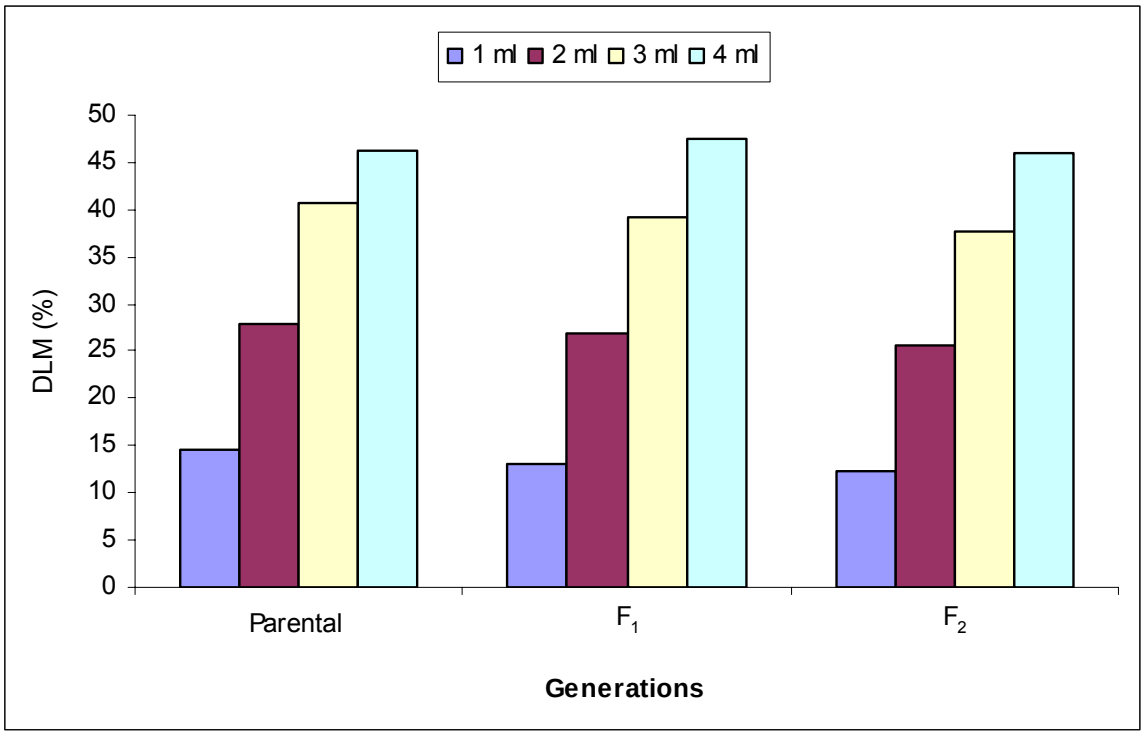

Figure-19: Effects of different doses of EMS on DLM% for the cross EMS+ACMD♂ x Control♀ in different generations of *Epilachna vigintioctopunctata*

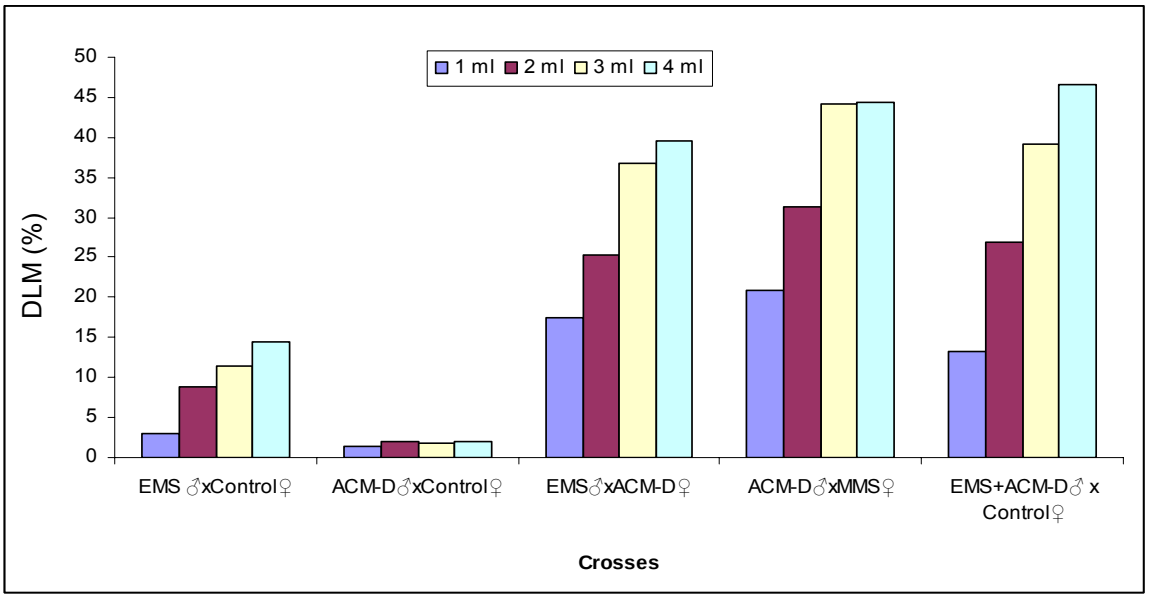

Figure-20: Effects of different doses of EMS and ACM-D on DLM% in different crosses of *Epilachna vigintioctopunctata*

4.1.3 Discussion

For evulating the mutagenic effects of methyl methanesulfornate (MMS) and ethyl methanesulfonate (EMS) with actinomycin-D (ACM-D) on different generations of *Epilachna vigintioctopunctata* dominant lethal mutation was considered as one of the parameters. A dominant lethal mutation is generally defined as a genetic event which leads to the death of the resultant heterozygotes (Shimada *et al.*, 1985). Muller (1927) noted that *Drosophila* females inseminated by irradiated males laid many eggs which failed to hatch. Afterwards Hanson (1928) published quantitative data on the induction of dominant lethal mutations by X-rays. Since almost all dominant lethals exert their effect before the egg has hatched even though a few act in larval and pupal stages, egg hatching is the usual criterion for detecting dominant lethals in *Epilachna vigintioctopunctata* (Ashburner and Novitski, 1976).

In case of inducing dominant lethal mutation in *Epilachna vigintioctopunctata* it was observed that all selective doses of MMS induced a significantly higher impact. There was found a linear relationship between an increase in mutagenic dose and an increase in the dominant lethal mutation frequency. This result was in good accordance with the findings of Vogel and Leigh (1975) who showed that MMS reduced the hatchability of eggs by increasing the percentage of dominant lethal mutations in *Epilachna vigintioctopunctata.* Haruko Ryo *et al.,* (1981) reported that MMS was sufficiently capable of inducing dominant lethal mutations. It was also in general agreement with the results of Bateman and Chandley (1964); Bateman (1956); and Fahny and Fahmy (1957) that MMS, although concentrations were dissimilar to us, induced dominant lethal mutations.

In support of the present results MMS was found (table-5 to 6 and figure 5 to 10) to induce dominant lethal mutations when an attempt was taken to establish dose-response relationships by various researchers (Parlington and Jackson, 1963; Palington and Bateman, 1964; Ehling *et al.,* 1968; Soares and Crenshaw, 1974). The results of the present investigations of inducting dominant lethal mutations by MMS were also in good agreement with the reports of Generoso (1969), Generoso *et al.* (1971), Arnold *et al.,*

(1976), Goldstein (1977), Brewen *et al.,* (1975), Shimade *et al.,* (1979, 1985), Aravindakshan *et al.,* (1987) and Adler (1980) in mice.

Dominant lethal mutations are presumed to arise mainly from non-restituted chromosome breaks, and part from aneucentric rearrangements (Mendelson and Sobels, 1974; Armstrong and Fletcher 1983; Cattanach *et al.,* 1968; Generoso *et al.,* 1974; Brewen *et al.,* 1975; and Lang and Adler, 1977). This test is based on the principle that the chemicals causing chromosome aberrtions may lead to the death of the embryo because the chromosome aberrations which produce breakage and reunion in the early cleavage stage results in bridges and micronuclei. The broken chromosomes are normally lost resulting in monosomy as well as trisomy of autosomes which are formed due to the non-disjunction, may also result in the death of the embryo. This test has been extensively used and details about its procedure etc. have recently been reviewed by Green *et al.,* (1987).

In our observation MMS (table-1 to 4 and figure 1 to 4) was found to reduce the hatchability of eggs inducing the dominant lethal mutation, but this may not be due to only genetical effect. The chromosome loss tests indicate that MMS is not very effective as a chromosome breaker at lower dose (Vogel and Leigh, 1975 and Gatti *et al.,* 1975). This interpretation is in line with the finding of Bateman and Chandley, 1964 that the sterilization of males treated with MMS was mainly due to cause other than dominant lethality. Except at the higher doses there was little reduction in egg hatchability. Thus there appears to be a threshold effect, which is evidence of a physiological rather than a genetic cause of the depression of hatching rate. Amongst the physiological cause one might expect effects such as interference with the functioning of the sperm, inadequate insemination and infrequent mating. The second and third of these possible defects would be reflected in a lowered rate of egg-laying. The effectiveness of MMS increases rapidly at higher doses. There is a slight descrease of the dominant lethal frequency with 2.0 mM (Vogel and Leigh, 1975). So it can be concluded that the induction of dominant lethal mutations at the higher concentration of MMS may not be fully due to the genetical causes but it may also be partially in addition to the defective sperm leading to laying of unfertilized eggs.

Like the previous proposals by Lee (1978) for effects on dominant lethals by MMS, our result is supported by the biochemical finding that MMS mainly produces 7-methylguanines, which are spontaneously lost by depurination (Lawley, 1974a, b), leading to accumulation of apurinic sites with time and that the apurinic sites are converted into single-strand breaks (Lindahl and Ljungquist, 1975). Therefore we can come in agreement with the proposals of Vogel and Natarajan (1979a) that the apurinic sites produced in the DNA in spermatozoa during storage of MMS-treated spermatozoa are, at least partly responsible for increasing dominant lethals. This proposal can be tested by direct analysis of apurinic sites and strand breaks. If DNA strand breaks play a major role, it is important to know whether single or double strand breaks are the cause of MMS-induced sub-lethal lesions.

In the present experiment we made an effort to investigate the effect of different doses (table-5) of MMS with ACM-D on different spermatogenic stages of *Epilachna vigintioctopunctata* for the induction of dominant lethal mutations. Considering the effect of MMS on successive stages of spermatogenesis it was observed that F_1 and F_2 generations. These results were in good agreement with Oftedal (1964) who summarized the results of most of the studies carried out on this germ cell stage and compared the rate of mutations observed in spermatogonia with that in spermatozoa. Oftedal used the earlier results of Spencer and Stern (1948) for comparison and showed that sensitivity of the spermatogonia is some three to four times lower than that estimated for sperm. Lefevre (1965) have served to provide further evidence for the higher sensitivity of mature spermatozoa relative to late spermatides with respect to the induction of dominant lethal mutations.

MMS induced dominant lethal mutations in spermatozoa and spermatids after the injection of 200 and 400 mg/kg in *Oryzias lalipes* (Shimade and Egami, 1984). In the fish *Oryzias lalipes,* however, the most sensitive stage to the induction of dominant lethals by radiation was that of the spermatozoa (Egami and Hyodo-Taguchi, 1973; Sakaizumi and Egami, 1980; Egami *et al*., 1983). Ehling *et al.,* (1968) and Ehling (1977, 1980) in their experiment on induction of dominant lethal mutations by alkylating agents observed that

MMS induced a high frequency of dominant lethal mutations in mouse spermatozoa of vas and epididymis, testicular sperm, and late spermatids, and a low frequency in early spermatids. Significant increase ($P<0.05$) in induced dominant lethal mutations and heritab le translocations were observed with MMS in post-meiotic germ stages of male mice (Lang and Adler, 1977). Our findings are in good agreement also with Adler (1980) where he summarized that in *D. melanogaster* MMS induced dominant lethal mutations in spermatozoa and spermatids.

The mechanism of action of MMS on the developing germ cells can be demonstrated in the way that the occurrence of S-methyl-L- cysteine as the major reaction product in sperm protamine after MMS exposure supports Sega and Owen's initial model of dominant lethals are induced in germ cells by this chemical. Alkylation of cysteine sulfhydryl groups contained in sperm protamine blocks normal disulfide-bond formation, preventing proper chromatin condensation in the sperm nucleus. Subsequent stresses produced in the chromating structure eventually lead to chromosome breakage, with resultant dominant lethality (Sega and Dwen, 1983). Moreover, in *D. melasogaster* chromosome breakage may also occur by the transposition of DNA element (Roeder *et al,.* 1980; Dinghom and Dudd, 1981; Fink *et al.,* 1981; Bingham *et al.* 1982; Collins and Rubin, 1982; Goldberg *et al.* 1982; Rubin *et al.,* 1982, 1983; Zachar and Bingham, 1982; Roeder and Fink, 1983; Rubin, 1983) which may also take part in lethality. Brewen *et al.* (1975) looked at chromosome breakage in the male pronuclus of mouse oocytes fertilized with sperm from MMS-treated males over a 3-week period after treatment and found an excellent correlation between dominant lethal frequency and frequency of chromosome breakage. Their data gave strong support to the idea that dominant lethality in germ cells is largely the result of chromosome (or chromatid) breakage events.

Doses effects of EMS on dominant lethal mutations were studied (table-7 to 10 and 15 to 20). It was found that there was a marked decline in the rates of the hatching eggs with increasing dose which actually increased the rate of induction of dominant lethal mutations. Our findings are in good agreement with the results of Ikebuchi and Nakao (1979) where they observed that relative dominant lethal frequency increased markedly at higher doses.

They explained it in this way that low EMS dose can potentially break chromosome which may not be converted to the true breaks due to the function of a repair system which ultimatively reduce the induction of dominant lethal mutations. EMS at high dose inhibits the function of a repair system which leads to the induction of increased dominant lethal mutations. Sega (1984) showed that EMS induced dominant lethal mutations in *Drosophila* are also relatively less unless the exposures are very high. Sram (1970) was able to produce a 60% dominant lethal frequency in *Drosophila,* but this required the injection of a 50 mM EMS solution. Mukai (1970) demonstrated the nature of EMS-induced deleterious mutations which indicates that EMS induces more lethals than detrimentals in comparison with spontaneous mutations which lend supports to our findings.

Lim and Snyder (1968) and Watson (1972) demonstrated that EMS can break chromosome in *Drosophila.* Muller (1940); Pontecorvo (1942); Demerec and Fano (1944); Sega and Dwens (1983) and Haruko *et al.* (1981) supposed that dominant lethals are induced by single breaks. It is further supposed that after breakage the two parts of the chromosomes are not absolutely immobile relative to each other. The longer it takes from break to moment of active chromosome movements the greater will the average distance be between the broken chromosome's two ends and the smaller the probability for restitution. This will lead to a higher dominant lethals.

In the case of chemical experiments, the EMS results could be explained on the premise that mono-alkylated bases in mutant C3G are not repaired, leading to more mutational events as postulated by Freese (1971), some of which will certainly be lethal. It was also postulated that if no repair occurs, then cross-links would result in greater dominant lethality due to the inhibition of subsequent DNA synthesis (Brookes and Lawley, 1961; Lawley and Brooks, 1963).

In our opinion, the results presented here support the concept that a straight interrelationship between the doses and percentage of dominant lethal mutations exists in *Epilachna vigintioctopunctata.* EMS was found most effective for the induction of

dominant lethal mutations in germ cells of medfly, *Ceratitis capitata* (Wied.), males (Busch-Petersen *et al.*, 1986).

When male axolots (*A*mbystoma mexicanum) were treated with 100 mg/liter of EMS a dominant lethal effect was obtained (Arm-strong and Fletcher, 1983).

Of the three main lesions produced in DNA by mono-functional alkylating agents base alkylation, apurinic sites and single strand breaks, base alkylation, Prakash been reported not to interfere with DNA replication and not to result in inactivation (Prakash and Strauss, 1970). Apurinic sites resulting from spontaneous depurination of alkylated bases, and single strand breaks arising eitherα, β-elimination reactions in apurinic deoxyribose 3′ phosphate residues, or from enzyme action, probably represent the major source of inactivating lesions after alkylation (Lawley, 1966; Prakash and Strauss, 1970; Strauss and Robbins, 1968). Single-strand breaks in DNA due to alkylation of the internucleotide phosphate groups, with subsequent hydrolysis of the resultant unstable phosphotriester, may also contribute to lethality (Bannon and Verly, 1972; Reiner and Zamenhof, 1957; Rhaese and Freese, 1969).

Induction of dominant lethal mutations with different doses of EMS in male germ cells of *Epilachna vigintioctopunctata* was observed. It was found that spermatozoa and spermatids were highly sensitive to EMS in the induction of dominant lethal mutations in comparison with the pre-meiotic stages. This finding is in agreement with the result of Sram (1970). He observed an increase in the frequency of dominant lethal mutations in *Drosophila* after spermatozoa, treated by monofunctional alkylating agents and formaldehyde which were stored in untreated females.

Studies on EMS mutagenicity in *Bombyx* have mainly carried out under conditions of spermatogenesis (Inagaki and Oster, 1969; Kondo and Ikenaga, 1963; Onimatru and Tazima, 1968; Tazima and Onimaru, 1968; Tazima *et al.,* 1967 and Datta *et al.,* 1978). The characteristic of EMS mutagenicity in spermatogenic cells of *Bombyx* are that post-meiotic cells are much more sensitive to the chemical than pre-meiotic cells.

In mammals, EMS induces high frequencies of dominant lethal mutations and translocations in postmeiotic male germ cells (Parlington and Jackson, 1963; 1968;

Ehling *et al.,* 1968 ;), dominant lethals in female mice (Generoso, 1969; Generoso and Russell, 1969; Generoso *et al.,* 1971) and gene mutations in postmeiotic male germ cells (Cattanach *et al.,* 1968 and Rusell, 1971).

The effect of actinomycin-D on the frequency of dominant lethal mutations has been studied. It was found that ACM-D induced some percentages of dominant lethal mutations in *Epilachna vigintioctopunctata.* This is in accord with the findings of (Proust *et al.* 1972; Aslaniyan and Salam,1984;) demonstrated that when compared to the appropriate controls the ACM-D leads to an increase of the frequency of dominant lethal mutations. In support of the present results it can be added that ACM-D has been found to induce genetic damage including structural aberrations of chromosomes. Aberrations, which may also lead to the induction of dominant lethal mutations (Green *et al.,* 1987), induced by ACM-D are found to originate in the 5 phase of the cell cycle (Miles, 1970; Ostertag and Kersten, 1965) as well as in G_2 (Manna and Mitra, 1971; Miles, 1970; Ostertag and Kersten, 1965) even though aberration frequency in the G_2 cells is only α slight fraction of that found in the 5 phase. The effects of ACM-D on the male germ cell stages on the yield of dominant lethal mutations have been studied in *Epilachna vigintioctopunctata.* It was found that ACM-D induced higher percentages of dominant lethal mutations in post-melotic (F_2generation) stages which was less in pre-meiotic stages (parental and F_2 generations). It may so happen that the post-meiotic cells do not have the biochemical machinery which can control the chromosomal aberrations which are believed to be the cause of dominant lethal mutations. Any investigation regarding the effect of ACM-D on the germ cell stages on the induction of dominant lethal mutations has not yet been seen and as such no comparison can be made.

Combined effects of ACM-D with MMS and EMS on the yield of dominant lethal mutations have been studied. The results presented here demonstrate that treatment of males or females with ACM-D prior to their mating with MMS or EMS treated males or females leads to an increment in the frequencies of dominant lethals recovered from all spermatogenic stages. The enhancements of dominant lethal frequencies in the ACM-D series are significant when the pooled data are considered. MMS and EMS with ACM-D show the same pattern of enhancement in their effect.

It may be interpreted in the way that ACM-D acts by inhibiting the restitution of breaks, thereby, increasing the frequency of MMS or EMS induced dominant lethal mutations. Our results are in accord with the findings of Proust *et al.,* (1972). They injected *Drosophila* females with ACM-D and after a day those injected females were allowed to cross with males that had been exposed to 2000 or 2500R X-irradiation. So that only mature spermatozoa were sampled. The radiation induced frequencies of dominant lethals in the paternal genome were determined. When compared to the appropriate controls, it was found that such treatment of the females with ACM-D led to an increase of the frequency of dominant lethals.

The full effect of ACM-D i.e. complete inhibition of the repair processes, obviously cannto be achieved, because such extreme effects would be incompatible with survival or fecundity. In the range of workable concentration employed, the effect is one of affecting the efficiency of the maternal repair machinery rather than one of complete inhibition. Wurgler (1971) demonstrated that the rejoining process involving chromosome fragements of the paternal set proceeds in at most 16 minutes following penetration of the sperm into the egg. Since all repair has to be completed within this highly restricted time interval, a reduction of the efficiency or rate of the repair process will obviously leave more breaks unjoined, with the concomitant increase of dominant lethal frequencies. This is evident from studies on dipteran salivary gland. Chromosomes that ACM-D inhibits DNA-mediated RNA synthesis (Laufer *et al.,* 1964). It is tempting to speculate that the effects observed in the present study are consequences of such an inhibitory action of the antibiotic.

4.2. The number of eggs, larvae, pupae, and adults in *Epilachna vigintioctopunctata*

Effects of actinomycin-D with the methyl methanesulfonate and ethyl methanesulfonate on the number of eggs, larvae, pupae, adult males and adult females have been studied in *Epilachna vigintioctopunctata* and are shown in tables (13-38), figures (21-60) and appendix tables (XXI-CCX). The frequencies of the number of eggs, larvae, pupae, adult males and adult females were obtained from various crosses and generations and from the treatment of different doses of MMS and EMS.

4.2.1 Effects of methyl methanesulfonate and actinomycin-D on the number of eggs, larvae, pupae, and adults.

Effects of different doses of MMS with ACM-D on the number of eggs, larvae, pupae and adults were determined at different crosses and are shown in tables 13-25, figures 21-40 and appendix tables XXI-LXV.

Table-13 summarized the data of 1.0ml MMS on the number of eggs in different crossess. The analysis of variance within crosses showed highly significant differences ($F=118.79$, $P<0.001$, appendix table-XXI) with LSD values as 9.81 at 5% and as 21.41 at 0.1% probability level. Considering the case of generations the analysis of variance showed nonsignificant differences ($F=3.91$, $P>0.05$ appendix table-XXI) in table-13 and figure-21.

At 2.0ml dose of MMS the F value within crosses differed significantly as 210.81 ($P<0.001$, appendix table-XXII) with LSD values as 6.83 at 5%, and as 14.89 at 0.1%, level of significance, which revealed that the number of eggs observed in MMS+ACM-D♂ x control♀ differed significantly with with those of MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses (table-14). Data obtained from table-14 at 2.0ml dose of MMS the analysis of variance also showed significant differences in different generations ($F=9.06$, $P<0.01$, appendix table-XXII) with LSD values as 8.81 at 5% and as 12.82 at 1%, probability level (figure-22)

Data of table-15, which deals with the effect of 3.0ml dose of MMS on the number of eggs showed significant differences among the crosses ($F=192.55$, $P<0.001$, appendix table -XXIII) with LSD values as 8.47 at 5% and as 18.48 at 0.1% level. In case of ACM-D♂ x MMS♀ the number of eggs differed significantly with other treated crosses i.e. MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed significant differences ($F=7.71$, $P<0.05$, appendix table-XXIII) with LSD value as 10.93 at 5% level of significance. The number of eggs in parental generation also differed significantly from F_1 and F_2 generations (figure-23).

Table-13: Effects of 1.0ml of MMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x^2
control♂ x control♀	Parental	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
MMS♂ x control♀	Parental	502	419.8	396.8	371.2	171.6	199.6	1	1.16	2.21
	F_1	508.8	454	432.4	410.8	190	220.8	1	1.16	2.48
	F_2	529.4	477	452.2	428.2	197	231.2	1	1.17	2.91
ACM-D♂ x control♀	Parental	599.8	540.6	514.8	489.6	225.4	264.2	1	1.17	3.47
	F_1	591	530.2	507.4	482	223	259	1	1.16	2.76
	F_2	594.4	538.6	513.2	491.4	224.8	266.6	1	1.19	3.68
MMS♂ x ACM-D♀	Parental	499	356.4	327.8	306.8	136.2	170.6	1	1.25	4.07
	F_1	507.2	392.2	368.2	344.4	156.4	188.2	1	1.21	3.19
	F_2	512.2	396.8	372.2	348.8	155.8	192.6	1	1.24	3.99
ACM-D♂ x MMS♀	Parental	489.4	327.4	307.8	286	130.6	155.4	1	1.19	2.25
	F_1	498.8	393.2	369.6	347.2	155.6	191.6	1	1.23	3.95
	F_2	497.2	376.4	353.2	330	150.4	179.6	1	1.2	2.69
MMS+ACM-D♂ x control♀	Parental	486.4	340.6	318.8	298.4	137	161.4	1	1.18	2.07
	F_1	494.2	393.8	369.4	347.4	158.8	188.6	1	1.19	2.86
	F_2	502.4	408.8	386.2	360.6	167.8	192.8	1	1.15	1.92

Table-14: Effects of 2.0ml of MMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x^2
control♂ x control♀	Parental	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
MMS♂ x control♀	Parental	502	372.4	349.2	325.4	148	177.4	1	1.2	2.77
	F_1	513	380	357	334.6	154.4	180.2	1	1.17	2.05
	F_2	518	436.8	392	350.6	157.2	193.4	1	1.24	3.87
ACM-D♂ x control♀	Parental	590	529.8	510	487.6	224.2	263.4	1	1.18	3.36
	F_1	585.2	536.8	514.4	489.6	225.6	264	1	1.17	3.16
	F_2	588.6	532	505.8	502.8	226.8	276	1	1.21	5.46
MMS♂ x ACM-D♀	Parental	493.6	371.4	348.2	322.2	146	176.2	1	1.21	3.01
	F_1	503.6	349.8	331.8	308	137.8	170.2	1	1.24	3.56
	F_2	512.6	363.4	341.6	318.4	145	173.4	1	1.2	2.56
ACM-D♂ x MMS♀	Parental	495.2	305.6	304	262.4	119.2	143.2	1	1.2	2.32
	F_1	494.2	316.4	293.6	270.6	123.8	146.8	1	1.19	2.1
	F_2	507	334.4	314.6	291.8	133.6	158.2	1	1.19	2.12
MMS+ACM-D♂ x control♀	Parental	487	319.4	297.8	277.6	125.8	151.8	1	1.21	2.55
	F_1	489.8	325	300.4	281.4	128.6	152.8	1	1.19	2.17
	F_2	502.8	344.6	322	338	147.2	190.8	1	1.29	5.72

Table-15: Effects of 3.0ml of MMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x^2
control♂ x control♀	Parental	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
MMS♂ x control♀	Parental	490.6	388.6	368.4	345.2	158.2	187	1	1.18	2.46
	F_1	495.2	398.4	375.4	351	160.8	190.2	1	1.18	2.66
	F_2	506.8	405.4	383.8	360.4	163	197.4	1	1.22	3.56
ACM-D♂ x control♀	Parental	590.8	531.4	506	478.6	220.4	258.2	1	1.17	3.06
	F_1	588.4	535	512	490.2	224.8	265.4	1	1.18	3.46
	F_2	596	533.6	510	484.8	220.6	264.2	1	1.18	3.59
MMS♂ x ACM-D♀	Parental	484.6	372.8	250.6	226	102.2	123.8	1	1.21	2.14
	F_1	493	294	272.2	250.6	114.2	136.4	1	1.2	2.05
	F_2	495	294.8	271.4	247.6	111.2	136.4	1	1.23	2.6
ACM-D♂ x MMS♀	Parental	474.8	281	259.2	237	108	129	1	1.2	2.09
	F_1	476.6	250.6	228.6	230.2	102.8	127.4	1	1.23	2.77
	F_2	485.6	255.2	231.8	213.2	98.8	117.4	1	1.23	2.22
MMS+ACM-D♂ x control♀	Parental	470.6	284.6	240.6	221.2	102.4	118.8	1	1.16	1.38
	F_1	494.6	286.8	263.8	240.8	110.8	130	1	1.17	1.62
	F_2	500	293.2	268.4	247.4	111.6	135.8	1	1.22	2.58

Table-16 summarized the data showing the effects of different doses of MMS and ACM-D on the number of eggs in different generations. Results demonstrated that the number of eggs changed with the increase of doses of MMS and ACM-D. The analysis of variance showing the effects within generations and doses and are presented in appendix table XXIV-XXVIII. In case of MMS♂ x control♀, the F value within generations has been calculated as 16.79 ($P<0.05$, appendix table-XXIV) with LSD value as 9.71 at 5% level of significant and the F value within doses has been calculated as 12.34 ($P<0.05$) with LSD value as 9.71 at 5% level of significant differences respectively (appendix table-XXIV). The mean value of number of eggs of parental generation significntly differed with F_1 and F_2 generations. In case of doses it was observed that means of the number of eggs differed significantly with each other (table-16).

When the ACM-D treated males were crossed with control females the analysis of variances has been calculated and no significant differences were observed between generations and doses (appendix table-XXV). In cross of MMS♂ x ACM-D♀ the analysis of variances showed significant differences within generations ($F=28.45$, $P<0.01$, appendix table-XXVI) with LSD values as 5.21 at 5% and as 8.74 at 1% level. But within doses the analysis of variences showed significant differences ($F=36.41$, $P<0.01$) with LSD values as 5.28 at 5% and as 8.74 at 1%, level of respectively (appendix table-XXVI).

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed non significant differences among generations (appendix table-XXVII). The F values within doses has been differed significantly ($F=22.44$, $P<0.01$, appendix table-XXVII) with LSD values as 8.74 at 5% and as 14.46 at 1%, level of significant. In cross of MMS+ACM-D♂ x control♀, the F value within doses showed no significant differences but in case of generations showed significant differences ($F=9.5$, $P<0.05$) with LSD value 13.5at 5% level of respectively (appendix table-XXVIII).

Effects on different crosses following the treatment with different doses of MMS and ACM-D on the number of eggs are shown in table-17. It was observed that mean number of eggs differed significantly within crosses ($F=240.66$, $P<0.001$, appendix table-XXIX) with LSD values as 6.94 at 5% and as 15.14 at 0.1% level of significance. The mean of the number of eggs induced by ACM-D♂ x MMS♀ differed significantly with other crosses MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀, and MMS+ACM-D♂ x control♀. The mean of the number of eggs differed significantly within doses ($F=8.09$, $P<0.05$) with LSD value 8.96 at 5%, level (appendix table-XXIX). The number of eggs induced by 1.0ml MMS dose differed significantly with 2.0ml and 3.0ml doses (table-17 and figure-24).

Table-16: Effect of different doses of MMS and ACM-D on the number of eggs in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	502.00	502.00	490.60	498.20±3.80
	F_1	508.90	513.00	495.20	505.70±5.39
	F_2	529.40	518.00	506.80	518.07±6.53
	Mean ± SE	513.43±8.24	511.00±4.73	497.53±4.83	
ACM-D♂ x control♀	Parental	599.80	590.00	590.80	593.53±3.15
	F_1	591.00	585.20	588.40	588.20±1.68
	F_2	594.40	588.60	596.00	593.00±2.25
	Mean ± SE	595.07±2.57	587.93±1.43	591.73±2.25	
MMS♂ x ACM-D♀	Parental	499.00	493.60	484.60	492.40±4.20
	F_1	507.20	503.60	493.00	501.27±4.27
	F_2	512.20	512.60	495.00	506.60±5.81
	Mean ± SE	506.13±3.85	503.27±5.49	490.87±3.19	
ACM-D♂ x MMS♀	Parental	489.40	495.20	474.80	486.47±6.08
	F_1	498.80	494.20	476.60	489.87±6.77
	F_2	497.20	507.00	485.60	496.60±6.19
	Mean ± SE	495.13±2.91	498.80±4.12	479.00±3.34	
MMS+ ACM-D ♂ x control♀	Parental	486.40	487.00	470.60	481.33±5.38
	F_1	494.20	489.80	494.60	492.87±1.54
	F_2	502.40	502.80	500.00	501.73±0.88
	Mean ± SE	494.33±4.62	493.20±4.87	488.40±9.05	

Table-17: Effect of different doses of MMS and ACM-D on the number of eggs in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	513.43	511.00	497.53	507.32±4.95
ACM-D♂ x control♀	595.07	587.93	591.73	591.58±2.07
MMS♂ x ACM-D♀	506.13	503.27	490.87	500.09±4.69
ACM-D♂ x EMS♀	495.13	498.8	479	490.98±6.09
EMS+ACM-D♂ x control♀	494.33	493.2	488.8	492.11±1.69
Mean ± SE	520.82±3.81	518.84±2.92	509.59±3.67	

 EFFECTS OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF 'LADYBIRD' BEETLE *Epilachna vigintioctopunctata* (Fab.)

Table-13 summarized the data on the number of larvae following the effects of 1.0ml dose of MMS and ACM-D. The analysis of variance within crosses showed significant differences (F=41.78, P<0.001, appendix table-XXX) with LSD values as 28.61 at 5% and as 62.42 at 0.1%, probability level. The number of larvae induced by ACM-D♂ x control♀ differed significantly with the other crosses *i.e.* MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀ (table-18).

Considering the case of generations it was found that the analysis of variance showed significant differences (F=5.56, P<0.05, appendix table -XXX) with LSD value as 36.94 at 5%, level of significance. It demonstrated that the observed number of larvae differed significantly with all other generations irrespective of crosses (table-13 and figure-25).

At 2.0ml dose of MMS the F value has been calculated and showed highly significant differences within crosses as 91.98 (P<0.001,appendix table-XXXI) with LSD values as 22.84 at 5% and 49.81 at 1% level of significance which revealed that the number of larvae observed in MMS+ACM-D♂ x control♀ differed significantly with the other crosses *i.e.* MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and the number of larvae found in MMS♂ x control♀, and ACM-D♂ x control♀ also differed significantly among themselves (Table-14). In case of generation the analysis of variance also showed no significant differences (appendix table–XXXI and Figure-26).

Data of table-15, which deals with the effects of 3.0ml dose of MMS, showed significant differences among the crosses (F=74.69, P<0.001, appendix table-XXXII) with LSD values as 31.97 at 5% and as 69.80 at 0.1% level. The number of larvae of the cross MMS+ACM-D♂ x control♀ differed significantly with other treated crosses *i.e* MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and ACM-D♂ x MMS♀. The analysis of variance within generations showed nonsignificant differences as shown in appendix table-XXXII and figure-27.

Table-18 summarized the data on the number of larvae following the effects of different doses of MMS and ACM-D in different generations. The number of larvae changs to dicrease with the induced of doses MMS and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are presented in

appendix tables XXXIII-XXXVII. In case of MMS♂ x control♀, the F value within generations showed nonsignificant differences (appendix table-XXXIII). The F value within doses Showed differed significantly as 11.21 ($P<0.05$, appendix table-XXXIII) with LSD value as 36.18 at 5% level ofrespectively. In case of doses it was observed that the mean number of the larvae differed significantly with one another (table-18).

When the ACM-D treated males were crossed with Control females the analysis of variances has been calculated and showed nonsignificant differences were observed between the generations and doses (appendix table-XXV).In cross of MMS♂ x ACM-D♂ the analysis of variances has been showed nonsignificant differences within generations and doses (appendix table-XXXV).

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed nonsignificant differences within generations (appendix table-XXVI). The F values within doses significantly differed ($F=11.67$, $P<0.05$, appendix table-XXXVI) With LSD value as 59.59 at 5% level, respectively. In cross of MMS+ACM-D♂ x control♀, the F value within generations showed nonsignificant differences and F value within doses has been calculated as 21.59 ($P<0.01$) with LSD values as 39.36 at 5% and as 65.14 at 1% level of significant differences (appendix table-XXXVII).

Effects of different doses on the number of larvae following the treatment with MMS and ACM-D in different crosses are shown in table-19. It was observed that mean number of larvae differed significantly within crosses ($F=51.46$, $P<0.001$, appendix table-XXXVIII) with LSD values as 31.28 at 5% and as 68.24 at 0.1% level of significance. The mean of the number of larvae induced by the cross MMS+ACM-D♂ x control♀ differed significantly with other crosses ***i.e.***MMS♂ x control, ACM-D♂ x control♀, MMS♂ x ACM-D♀, and ACM-D♂ x MMS♀. The mean of the number of larvae has been calculated and showed differed significantly within doses ($F=10.77$, $P<0.01$, appendix table-XXXVIII) with LSD values as 40.38 at 5% and as 58.78 at 1% probability level. The number of larvae induced by 2.0ml MMS dose differed significantly with 1.0ml and 3.0ml doses (table-19 and figure-28).

Table-18: Effects of different doses of MMS and ACM-D on the number of larvae in different generation of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	419.8	372.4	388.6	393.60±13.93
	F_1	454	380	398.4	410.80±22.27
	F_2	477	436.8	405.4	439.73±20.75
	Mean ± SE	450.27±16.64	396.40±20.34	397.47±4.88	
ACM-D♂ x control♀	Parental	540.6	529.8	531.4	533.93±3.37
	F_1	530.2	536.8	535	534.00±1.97
	F_2	538.6	532	533.6	534.73±1.99
	Mean ± SE	536.47±3.19	532.87±2.07	533.33±1.05	
MMS♂ x ACM-D♀	Parental	356.4	371.4	372.8	366.87±5.26
	F_1	392.2	349.8	294	345.33±28.47
	F_2	396.8	363.4	294.8	351.67±30.06
	Mean ± SE	381.80±12.78	361.53±6.31	320.53±26.17	
ACM-D♂ x EMS♀	Parental	327.4	305.6	281	304.67±13.42
	F_1	393.2	316.4	250.6	320.07±41.25
	F_2	376.4	334.4	255.2	322.00±35.57
	Mean ± SE	365.67±19.76	318.80±8.41	262.27±9.47	
EMS+ACM-D♂ x control♀	Parental	340.6	319.4	284.6	314.87±16.34
	F_1	393.8	325	286.8	335.20±31.34
	F_2	408.8	344.6	293.2	348.87±33.48
	Mean ± SE	381.07±20.72	329.67±7.65	288.20±2.58	

Table-19: Effects of different doses of MMS and ACM-D on the number of larvae in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	450.27	396.40	397.47	414.71±17.80
ACM-D♂ x control♀	536.47	532.87	533.33	534.22±1.13
MMS♂ x ACM-D♀	381.8	361.53	320.53	354.62±18.04
ACM-D♂ x EMS♀	365.67	318.8	362.27	348.91±15.11
EMS+ACM-D♂ x control♀	381.07	329.67	288.2	332.98±26.89
Mean ± SE	423.06±5.27	387.85±12.84	380.36±21.47	

Table-13 summarized the data of the number of pupae following the effect of 1.0ml dose of MMS and ACM-D. The analysis of variance within crosses showed highly significant differences (F=57.10, P<0.001, appendix table-XXXIX) with LSD values as 23.74 at 5% and as 51.8 at 0.1% probability level. The number of pupae induced by the cross ACM-D♂ x control♀ differed significantly with other crosses like MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. Considering the case of 1.0ml dose of MMS it was found that the analysis of variance within generations showed significant differences (F=9.87, P<0.01, appendix table -XXXIX) with LSD values as 30.64 at 5%,and 44.58 at 1% level of significance. Table-13 and figure-29 demonstrated that the number of pupae differed significantly with each other generations.

At 2.0ml dose of MMS the F value within crosses showed highly significant differences as 162 (P<0.001, appendix table -XXXX) with LSD values as 16.85 at 5% and 36.77 at 0.1% level of significance. Which revealed that the number of pupae observed in cross of MMS+ACM-D♂ x control♀ differed significantly with MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses .For 2.0ml doses of MMS the analysis of variance also showed nonsignificant differences within generations (F=2.71, P<0.05 appendix table-XXXX) in table-14 and figure-30.

Data obtained from table-15, which deals with the effects of 3.0 ml dose of MMS and ACM-D,showed significant differences among the crosses (F=244.07, P<0.001, appendix table -XXXXI) with LSD values as 18.42 at 5% and as 40.25 at 0.1% level. The number of pupae by the cross MMS♂ x ACM-D♀ differed significantly with other treated crossess *i.e.* MMS♂ x control♀, ACM-D♂ x control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed non significant differences (figuree-31).

Table-20 summarized the data showing the effects of different doses the following treatment with MMS and ACM-D in different generations on the number of pupae. Results demonstrated that the number of pupae changed with the increase of MMS doses and ACM-D. The analysis of variance showing the effects within generations and doses

has been calculated and are present in appendix tables XXXXII-XXXXVI. In case of MMS♂ x control♀, the analysis of variences within generations showed signinicant differences (F=7.52, P<0.05, appendix table-XXXXII) with LSD value as 23.58 at 5% probability level. The F value within doses has been calculated and showed significant differences as 22.47 (P<0.01, appendix table-XXXXII) with LSD values as 23.58 at 5% and as 39.02 at 1% respectively level of significance. The mean value the number of pupae of parental generation significntly differed with F_1 and F_2 generations. In case of doses it was observed that mean value of the larvae of all doses differed significantly with each other as shown in the table-20.When the ACM-D treated males were crossed with Control females the analysis of variances showed no significant differences between the generations and doses (appendix table-XXXXIII).

In cross of MMS♂ X ACM-D♀ the analysis of variances showed no significance differences within generations. But within doses it has been calculated showed significant differences (F=28.34, P<0.01, appendix table-XXXXIV) with LSD values as 36.09 at 5% and as 59.72 at 1% level of significance.

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed no significant differences within generations (appendix table-XXXXV). The analysis of variences within doses showed significant differences (F=12.21, P<0.05, appendix table-XXXXV) with LSD value as 58.86 at 5% level. In cross of MMS+ACM-D♂ x control♀, the F value within generation showed no significant differences(appendix table-XXXXVI) but the F value within doses showed significant differences as 36.95 (P<0.01) with LSD values as 31.81at 5% and as 52.64 at 1% level (appendix table-XXXXVI).

Effects of different doses of MMS and ACM-D on the number of pupae of different crosses are shown in table-21. It was observed that mean number of pupae differed significantly within crosses ($F=6.76$, $P<0.05$, appendix table-XXXXVII) with LSD value as 36.18 at 5% level. The mean of the number of pupae induced by ACM-D♂ x MMS♀ differed significantly with crosses like MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and MMS+ACM-D♂ x control♀. The mean of the number of pupae has been calculatted within doses and showed differed significantly ($F=10.28$, $P<0.01$, appendix table-XXXXVII) with LSD values as 46.71 at 5% and as 67.94 at 1% level.The number of pupae induced by 3.0ml of MMS dose differed significantly with that of 1.0ml and 2.0ml doses (table-21and figure-32).

Table-20: Effects of different doses of MMS and ACM-D on the number of pupae in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			
		1.0ml	2.0ml	3.0ml	mean ± SE
MMS♂ x control♀	Parental	396.8	349.2	368.4	371.47±13.84
	F_1	432.4	357	375.4	388.27±22.72
	F_2	452.2	392	383.8	409.33±21.59
	Mean ± SE	427.13±16.23	366.07±13.18	375.87±4.46	
ACM-D♂ x control♀	Parental	514.8	510	506	510.27±2.55
	F_1	507.4	514.4	512	511.27±2.06
	F_2	513.2	505.8	510	509.67±2.15
	Mean ± SE	511.80±2.25	510.07±2.49	509.33±1.77	
MMS♂ x ACM-D♀	Parental	327.8	348.2	250.6	308.87±29.76
	F_1	368.2	331.8	272.2	324.07±28.01
	F_2	372.2	341.6	271.4	328.40±29.87
	Mean ± SE	356.07±14.20	340.53±4.77	264.73±7.08	
ACM-D♂ x EMS♀	Parental	307.8	304	259.2	290.33±15.62
	F_1	369.6	293.6	228.6	297.27±40.79
	F_2	353.2	314.6	231.8	299.87±35.85
	Mean ± SE	343.53±18.51	304.07±6.07	239.87±9.72	
EMS+ACM-D♂ x control♀	Parental	318.8	297.8	240.6	285.73±23.39
	F_1	369.4	300.4	263.8	311.20±31.00
	F_2	386.2	322	268.4	325.53±34.09
	Mean ± SE	358.13±20.28	306.73±7.68	257.60±8.61	

EFFECTS OF MUTAGENS AND ANTIBIOTICS ON SOME TRAITS OF 'LADYBIRD' BEETLE *Epilachna vigintioctopunctata* (Fab.)

Table-21: Effects of different doses of MMS and ACM-D on the number of pupae in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	427.17	366.07	375.87	389.70±18.97
ACM-D♂ x control♀	315.97	313.23	308.03	312.41±2.33
MMS♂ x ACM-D♀	356.07	340.53	264.53	320.38±28.31
ACM-D♂ x EMS♀	343.53	304.07	239.87	295.82±30.24
EMS+ACM-D♂ x control♀	358.13	306.73	257.6	307.49±29.06
Mean ± SE	352.58±4.57	317.11±11.75	254.00±7.35	

Table-13 summarized the data of the number of adult males following the effect of 1.0ml dose of MMS and ACM-D. The analysis of variance within crosses showed highly significant differences (F=68.51, P<0.001, appendix table-XXXXVIII) with LSD values as 10.2 at 5% and as 22.27 at 0.1% probability level. The number of adult males induced by cross ACM-D♂ x control♀ differed significantly with other crosses like MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀.

Considering the case of generations it was observed that the analysis of variance within generations differed significantly (F=10.96, P<0.01, appendix table-XXXXVIII) with LSD values as 13.18 at 5%, and as 19.17 at 1% level of significance. Table-13 and figure-33 demonstrated that the number of adult males observed differed significantly with each other generation.

Effects of 2.0ml dose of MMS and ACM-D following on the number of adult males the F value has been calculated within crosses and showed highly significant differences (F=180.24, P<0.001,appendix table-XXXXIX) with LSD value as 7.55 at 5% and as 16.49 at 1% probability level. The number of adult males induced by the cross MMS♂ x control♀ differed significantly with other crosses *i.e.* ACM-D♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ xMMS♀ and MMS+ACM-D♂ x control♀. The anlysis of variences has been calculated within generations and showed significant differences (F=4.72, P<0.05, appendix table-XXXXIX) with LSD value as 9.76 at 5% probability level. The mean value of the number of adult males of parental generation differed significntly with F_1 and F_2 generations (table-14 and Figure-34).

Data obtained from table-15, which deals with the effects of 3.0ml dose of MMS and ACM-D, showed significant differences among the crosses (F=438.46, P<0.001, appendix table -L) with LSD values as 6.17 at 5%, and as 13.47 at 0.1% level.The number of adult males by the cross MMS♂ x ACM-D♂ differed significantly with other treated crosses like MMS♂ x control♀, ACM-D♂ x control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed no significant differences (appendix table-L and figure-35).

Effects on different doses of MMS and ACM-D in different generations following on the number of adult males. Results demonstrated that the number of adult males changes with the increase of MMS doses and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix tables LI-LV. In case of MMS♂ x control♀, the analysis of variences within generations showed non significant differences (appendix table-LI). The F value within doses showed significant differences as 27.99 ($P<0.01$, appendix table-LI) with LSD values as 12.86 at 5% and as 21.28 at 1% level. In case of doses it was observed that mean of the number of adult males of all doses differed significantly with each other as shown in the table-22.

When the ACM-D treated males were crossed with Control females the analysis of variances showed no significant differences between the generations and doses (appendix table-LII).In cross of MMS♂ x ACM-D♂ the analysis of variances has been calculated within generations and showed non significant differences but within doses showed significant differences ($F=24.14$, $P<0.01$, appendix table-LIII) with LSD values as 17.29 at 5% and as 28.61 at 1% respectively level. The mean value of the number of adult males of 3.0ml dose of MMS differed significantly with 1.0ml, 2.0ml doses of MMS.

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed nonsignificant differences within generations (appendix table-LIV). The F values within doses demonstrated significant differences ($F=14.82$, $P<0.05$, appendix table-LIV) With LSD value as 21.63 at 5% respectively. In cross of MMS+ACM-D♂ x control♀, the F value within generation has been calculated and showed no significant differences and F value within doses showed differed significantly as 33.12 ($P<0.01$, appendix table-LV) with LSD values as 15.83 5% and as 26.2 at 0.1% level of significance.

Effects of doses MMS and ACM-D on the number of adult males in different crosses are shown in table-23. It was observed that mean number of adult males showed differed significantly within crosses ($F=37.90$, $P<0.001$, appendix table-LVI) with LSD values as 16.95 at 5% and as 37 at 0.1% level.The mean value of the number of adult males

demonstrates induced by ACM-D ♂ x MMS♀ differed significantly with crosses like MMS♂ x control♂, ACM-D♂ x control♀, MMS♂x ACM-D♂, and MMS+ACM-D♂ x control♀. The mean of the number of adult males showed differed significantly within doses ($F=9.02$, $P<0.01$, appendix table-LVI) with LSD values as 31.36 at 5% and as 45.63 at 1%level of significance.The number of adult males decreases induced by MMS doses and 3.0ml of MMS doses differed significantly with 1.0ml and 2.0ml of MMS doses (table-23 and figure-36).

Table-22: Effects of different doses of MMS and ACM-D on the number of males in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS ♂x control♀	Parental	171.6	148	158.2	159.27±6.84
	F_1	190	154.4	160.8	168.40±10.97
	F_2	197	157.2	163	172.40±12.43
	Mean ± SE	186.20±7.58	153.20±2.73	160.67±1.39	
ACM-D x control	Parental	225.4	224.2	220.4	223.33±1.51
	F_1	223	225.6	224.8	224.47±0.77
	F_2	224.8	226.8	223.6	225.07±0.93
	Mean ± SE	224.40±0.72	225.53±0.75	222.93±1.31	
MMS♂ x ACM-D♀	Parental	136.2	146	102.2	128.13±13.29
	F_1	156.4	137.8	114.2	136.13±12.23
	F_2	155.8	145	111.2	137.33±13.45
	Mean ± SE	149.47±6.64	142.93±2.59	109.20±3.61	
ACM-D♂ x EMS♀	Parental	130.6	119.2	108	119.27±6.53
	F_1	155.6	123.8	102.8	127.40±15.37
	F_2	150.4	133.6	98.8	127.60±15.21
	Mean ± SE	145.53±7.63	125.53±4.25	103.20±2.67	
EMS+ACM-D♂ x control♀	Parental	137	125.8	102.4	121.73±10.21
	F_1	158.8	128.6	110.8	132.73±14.03
	F_2	167.8	147.2	111.6	142.20±16.43
	Mean ± SE	154.53±9.15	133.87±6.72	108.27±2.95	

Table-23: Effects of different doses of MMS and ACM-D on the number of males in different crosses of *Epilachna vigintioctopunctata*

cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	186.20	153.20	160.67	166.69±10.00
ACM-D♂ x control♀	224.4	225.53	222.93	224.29±0.75
MMS♂ x ACM-D♀	149.47	142.93	109.2	133.87±12.49
ACM-D♂ x EMS♀	145.53	125.53	103.2	124.75±12.24
EMS+ACM-D♂ x control♀	154.53	133.87	108.27	132.22±13.40

Table-13 summarized the data effect of 1.0ml dose of MMS and ACM-D on the number of adult females. The analysis of variance within crosses showed highly significant differences (F=53.69, P<0.001, appendix table-LVII) with LSD values as 12.73 at 5% and as 27.79 at 0.1% probability level of significance. The number of adult females induced by the cross ACM-D♂ x control♀ differed significantly with other crosses ***i.e.*** MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. Considering the case of generations the analysis of variance showed significant differences (F=9.66, P<0.01, appendix table-LVII) with LSD values as 16.44 at 5%, and as 23.92 at 1% level of significance. It demonstrated that the number of adult females showed differed significantly with all other generations on respective crosses (table-13and figure-37).

At 2.0ml dose of MMS the F value within crosses showed differed singnificance as 94.33 (P<0.001, appendix table-LVIII) with LSD values as 12.09 at 5%, and as 30.95 at 0.1% level of respectively. which revealed that the number of adult females observed in cross of MMS♂ x ACM-D♀ differed significantly with other crossess MMS+ACM-D♂ x control♀, ACM-D♂ x MMS♀ and the number of adult females found in MMS♂ x control ♀ and ACM-D♂ x control♀ also differed significantly. In case of generation the analysis of variance also showed significant differences (F=6.05, P<0.05, appendix table-LVIII) with LSD value as 15.61 at 5% level as shows in table-14 and figure-38.

Data obtained from table-15, which deals with the effects of 3.0ml dose of MMS and ACM-D, showed significant differences among the crosses (F=315.37, P<0.001, appendix table -LIX) with LSD values as 8.5 at 5% and as 18.56 at 1% level. The number of adult females by the cross MMS+ACM-D♂ x control♀ differed significantly with other treated crossess MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♂ and ACM-D♂ x MMS♀. The analysis of variance within generations showed no significant differences (appendix table-LIX and figure-39).

Table-24 summarized the data showing the effects of different doses of MMS and ACM-D on the number of adult females in different generations. Results demonstrated that the

number of adult females to changed with the increase doses of MMS and ACM-D induced. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix table LX-LXIV. In case of MMS♂ x control♀, the analysis of variences within generations showed significant differences ($F=7.01, P<0.05$, appendix table-LX) with LSD value as 14.36 at 5% probability level.The analysis of variences within doses showed differed significantly ($F=23.05$, $P<0.01$, appendix table-LX) with LSD values as 14.36 at 5% and as 23.76 at 1% level. The mean value of number of adult females of F_2 generation significntly differed with parental and F_1 generations. In case of doses it was observed that means of the number of adult females of all doses differed significantly with each another doses (table-24).

When the ACM-D treated males were crossed with Control females the analysis of variances showed non significant differences were observed between the generations and doses (appendix table-LXI).

Table-24: Effects of different doses of MMS and ACM-D on the number of females in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS ♂ x control♀	Parental	199.6	177.4	187	188.00±6.44
	F_1	220.8	180.2	190.2	197.07±12.23
	F_2	231.2	193.4	197.4	207.33±12.00
	Mean ± SE	217.20±9.31	183.67±4.94	191.53±3.08	
ACM-D♂ x control♀	Parental	264.2	263.4	258.2	261.93±1.88
	F_1	259	264	265.4	262.80±1.94
	F_2	266.6	276	264.2	268.93±3.60
	Mean ± SE	263.27±2.25	267.80±4.11	262.60±2.23	
MMS♂ x ACM-D♀	Parental	170.6	176.2	123.8	156.87±16.63
	F_1	188.2	170.2	136.4	164.93±15.20
	F_2	192.6	173.4	136.4	167.47±16.51
	Mean ± SE	183.80±6.73	173.27±1.74	132.20±4.20	
ACM-D♂ x EMS♀	Parental	155.4	143.2	129	142.53±7.64
	F_1	191.6	146.8	127.4	155.27±19.03
	F_2	179.6	158.2	117.4	151.73±18.27
	Mean ± SE	175.53±10.66	149.40±4.53	124.60±3.63	
EMS+ACM-D♂ x control♀	Parental	161.4	151.8	118.8	144.00±12.92
	F_1	188.6	152.8	130	157.13±17.07
	F_2	192.8	190.8	135.8	173.13±18.70
	Mean ± SE	180.93±9.85	165.13±12.85	128.20±5.00	

In cross of MMS♂ x ACM-D♂ the analysis of variances showed nonsignificant differences within generations (appendix table-LXII). F value has been calculated within doses showed significant differences (F=42.26, P<0.01, appendix table-LXII) with LSD values as 16.64 at 5% and as 27.28 at 1% level of significans.

When the ACM-D treated males were crossed with MMS treated females the analysis of variances has been calculated and showed no significant differences within generations (appendix table-LXIII). The F values within doses showed significant differences (F=12.51, P<0.05, appendix table-LXIII) with LSD value as 28.41 at 5% level of respectively. In cross of MMS+ACM-D♂ x Control♀, the F value within generations has been calculated and showed no significant differences and F value within doses showed significant differences as 19.89 (P<0.01, appendix table-LXIV) with LSD values as 23.85 at 5% and as 39.48 at 1% level of significant.

Effects on different doses of MMS and ACM-D on the number of adult females in dofferent crosses and are shown in table-25. Observed that mean number of adult females showed differed significantly within crosses (F=33.85, P<0.001, appendix table-LXV) with LSD values as 20.53 at 5% and as 44.74 at 0.1% level of significant. The mean of the number of adult females induced by the cross MMS +ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x control, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and ACM-D♂ x MMS♀. The mean of the number of adult females differed significantly within doses (F=8.34, P<0.05, appendix table-LXV) with LSD value as 26.51 at 5% probability level.The number of adult females induced by 3.0ml MMS dose differed significantly with other doses shown in table-25 and figure-40.

Table-25: Effects of different doses of MMS and ACM-D on the number of females in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	217.20	183.67	191.93	197.60±10.10
ACM-D♂ x control♀	263.27	267.8	262.6	264.56±1.64
MMS♂ x ACM-D♀	183.8	173.27	132.2	163.09±15.76
ACM-D♂ x EMS♀	175.53	149.4	124.6	149.84±14.72
EMS+ACM-D♂ x control♀	180.93	165.13	128.2	158.09±15.64

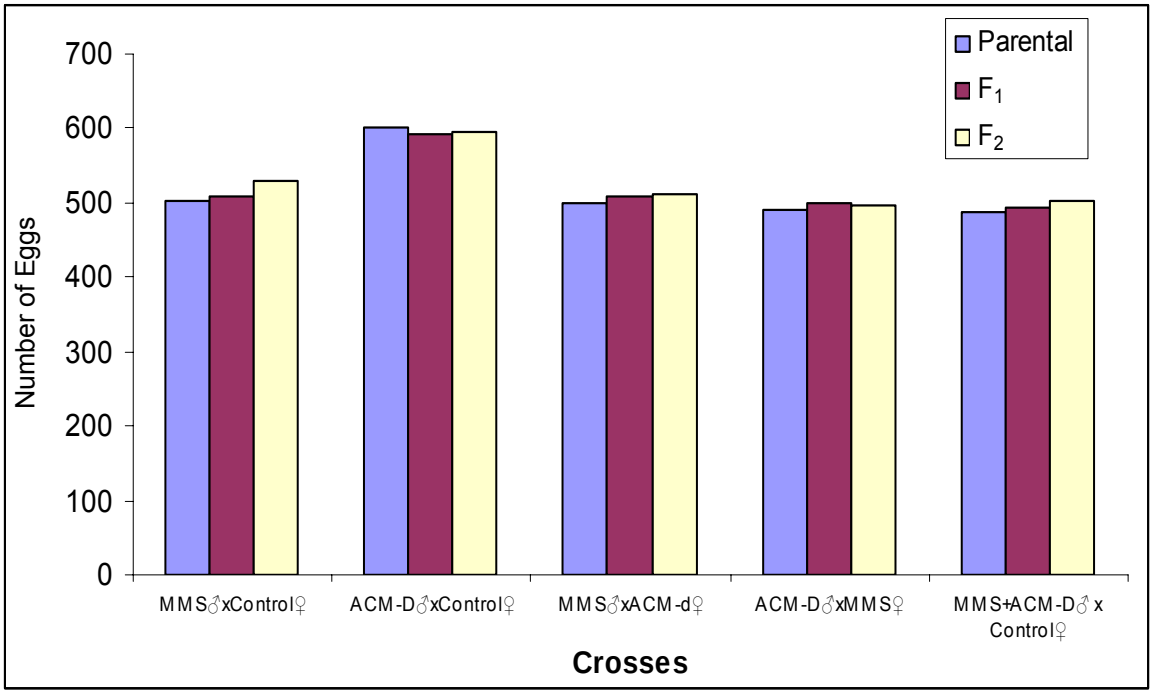

Figure-21: Effects of 1.0ml MMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*

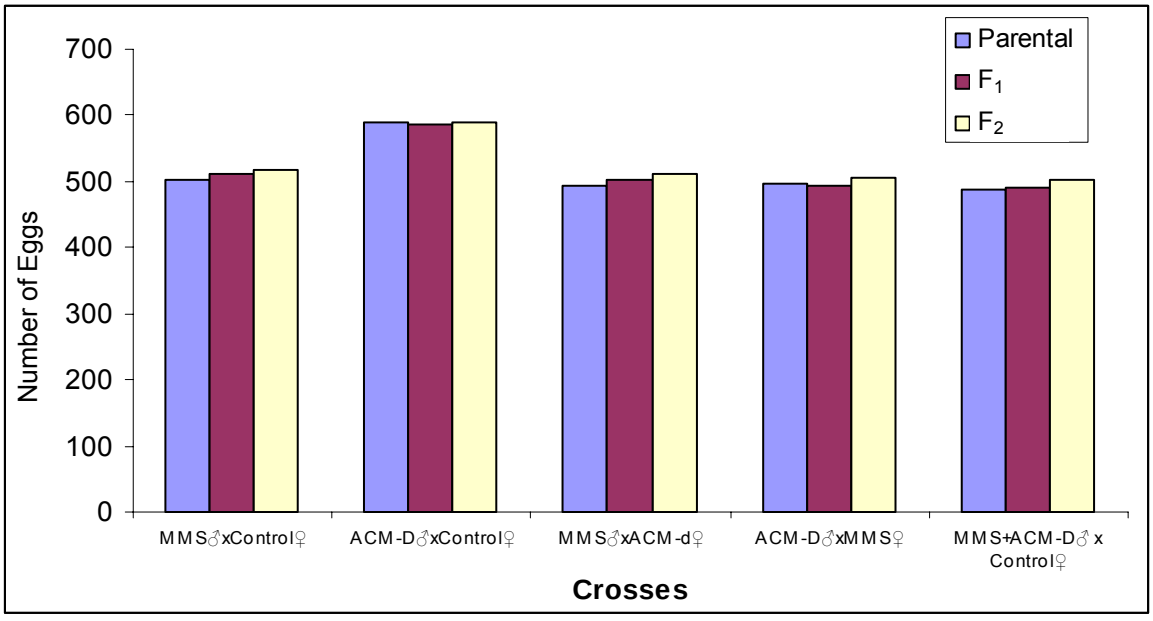

Figure-22: Effects of 2.0ml MMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*

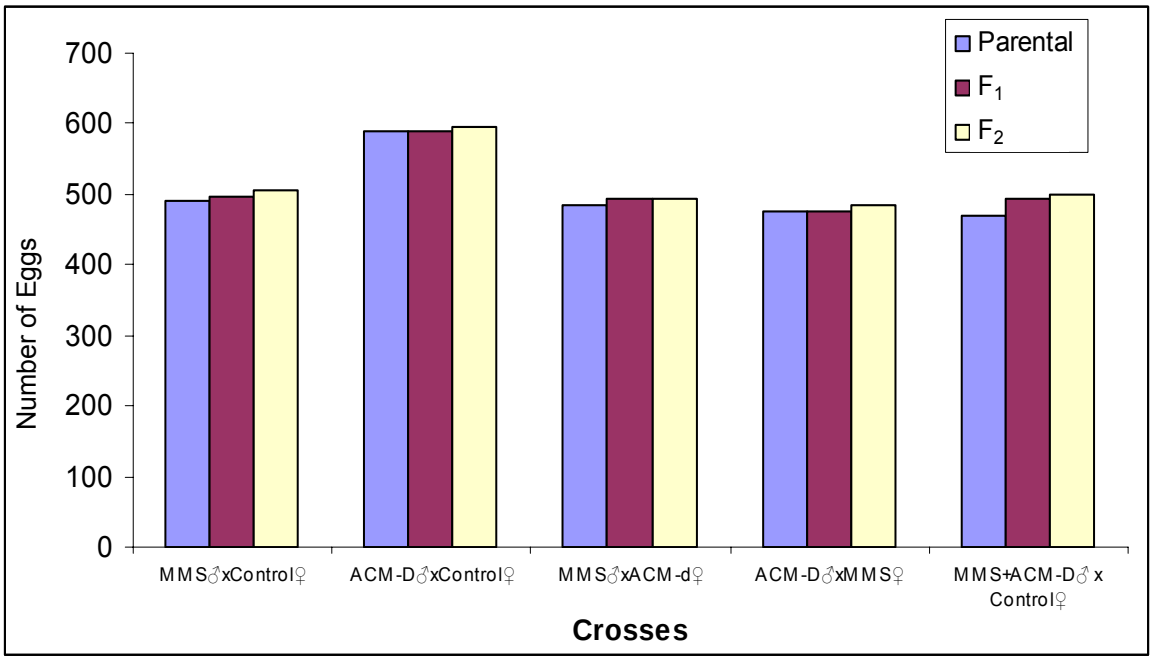

Figure-23: Effects of 3.0ml MMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*

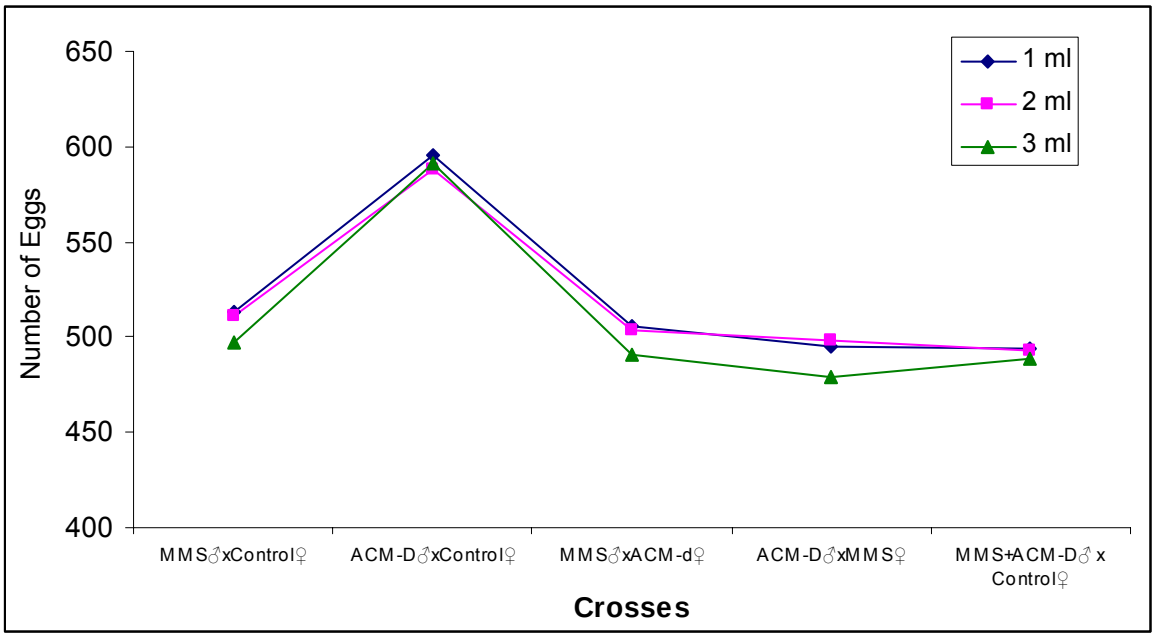

Figure-24: Effects of different doses MMS and ACM-D on the number of eggs in different crosses of *Epilachna vigintioctopunctata*

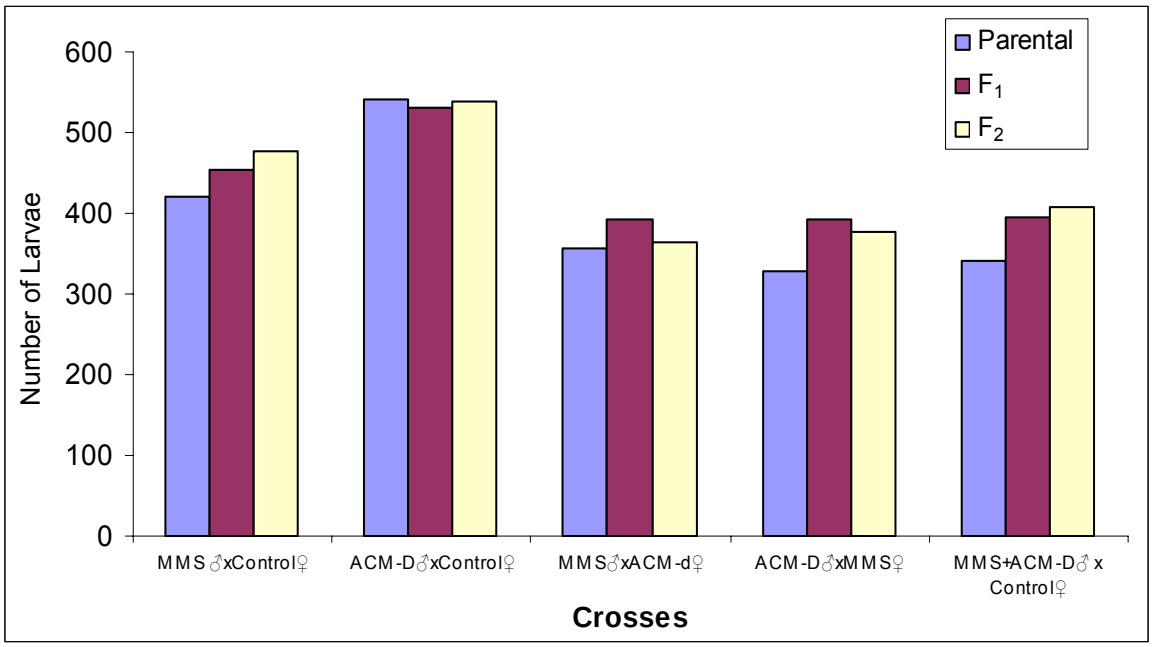

Figure-25: Effects of 1.0ml MMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

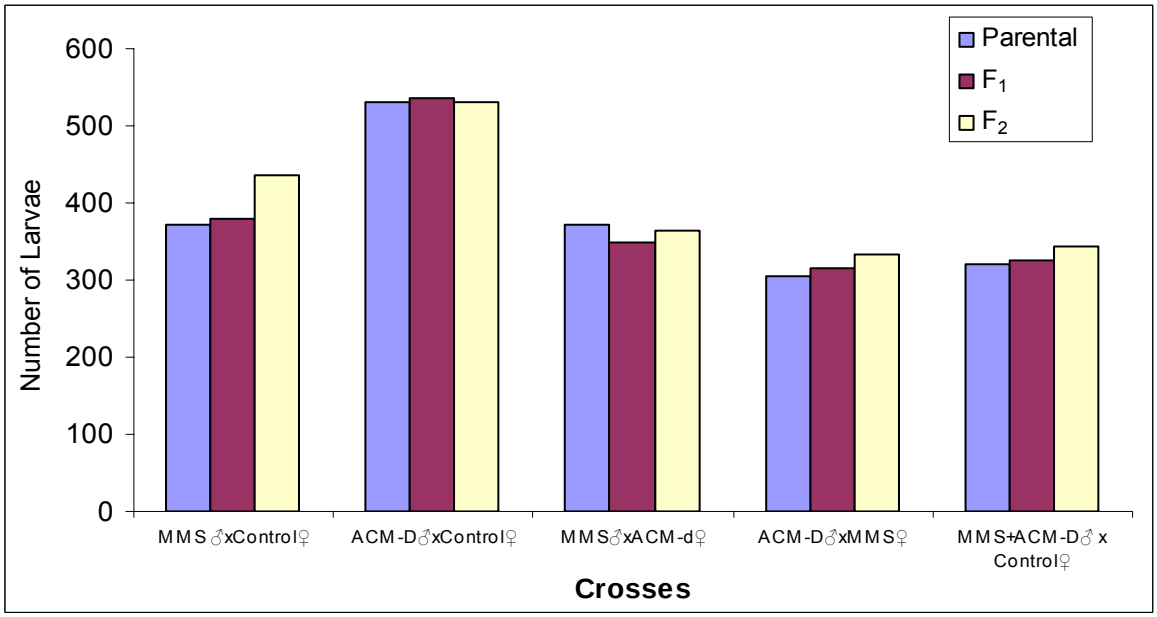

Figure-26: Effects of 2.0ml MMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

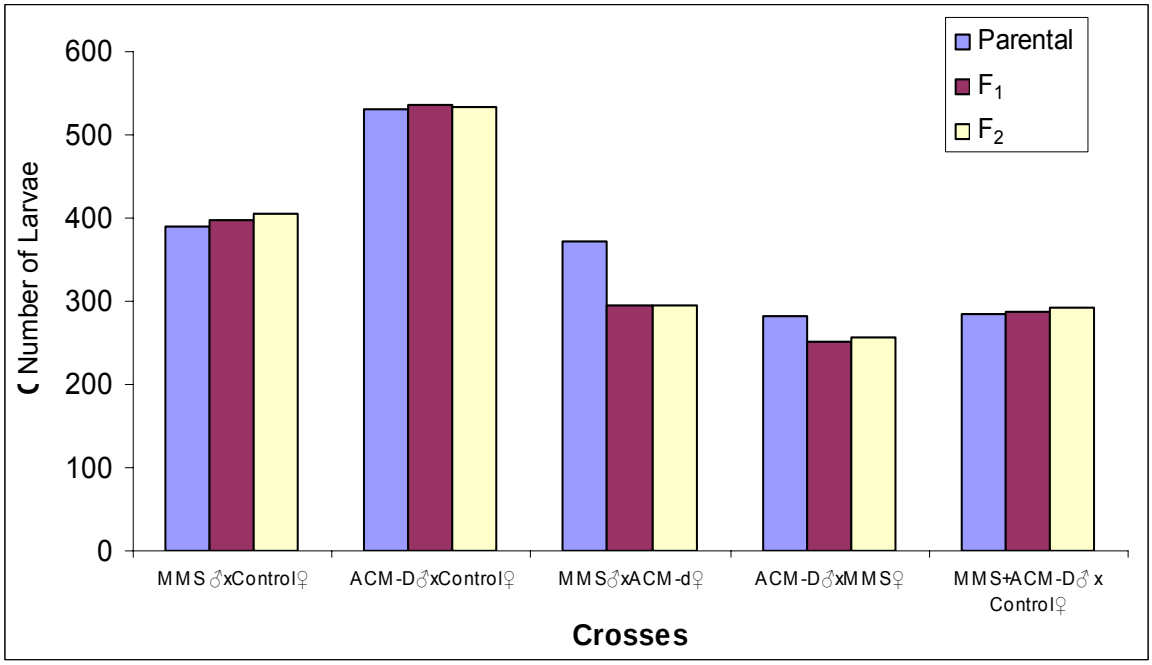

Figure-27: Effects of 3.0ml MMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

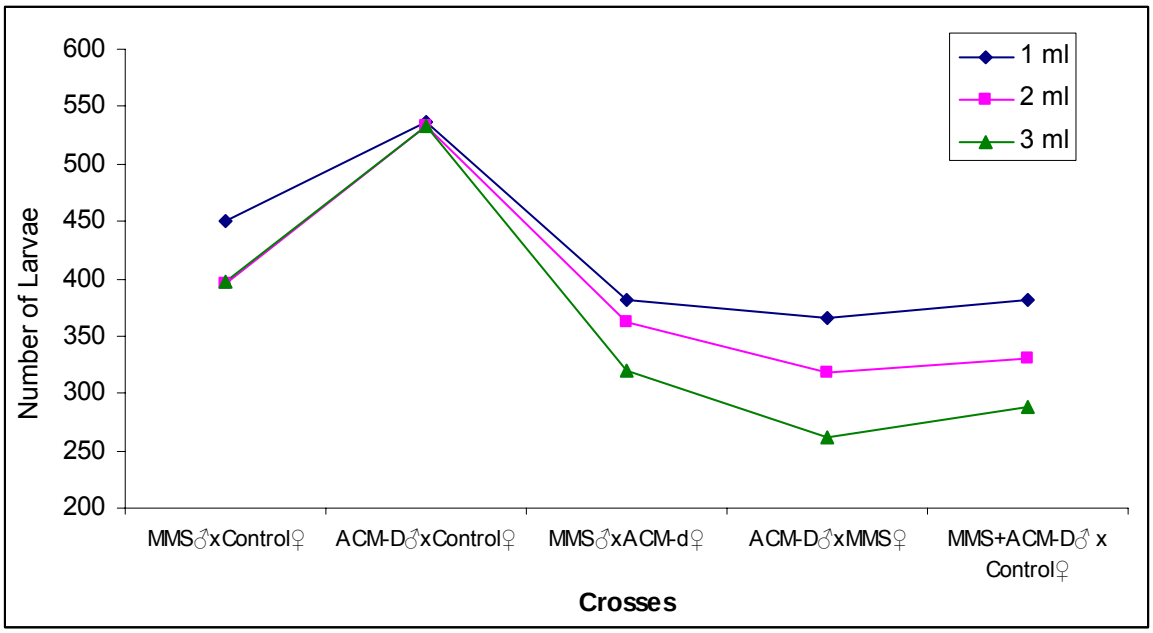

Figure-28: Effects of different doses MMS and ACM-D on the number of larvae in different crosses of *Epilachna vigintioctopunctata*

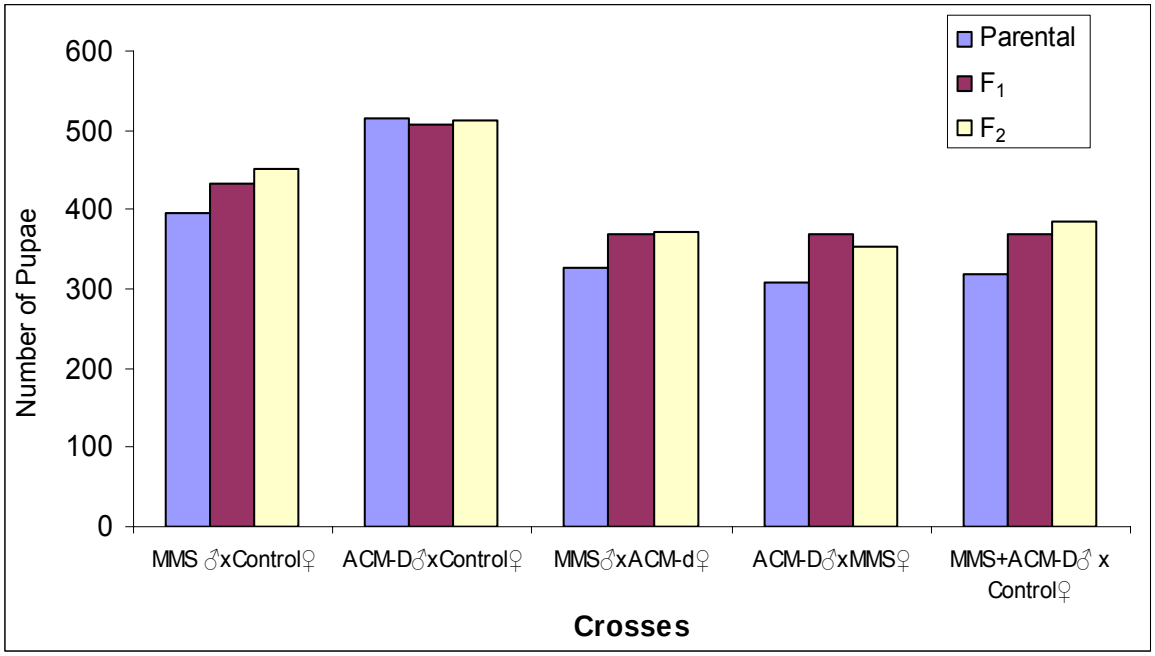

Figure-29: Effects of 1.0ml MMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

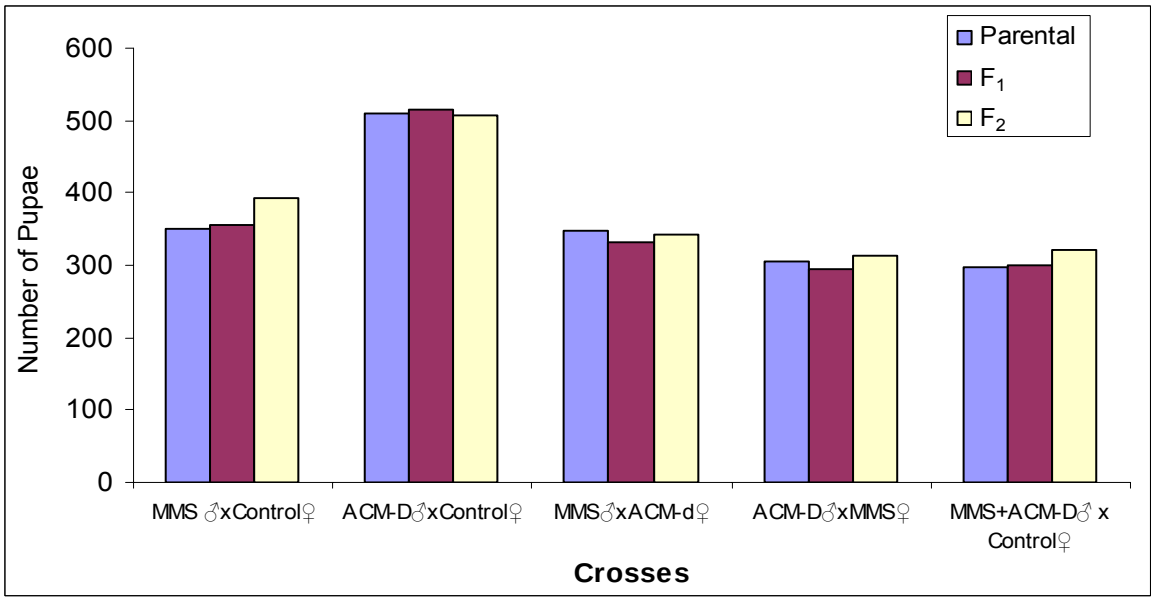

Figure-30: Effects of 2.0ml MMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

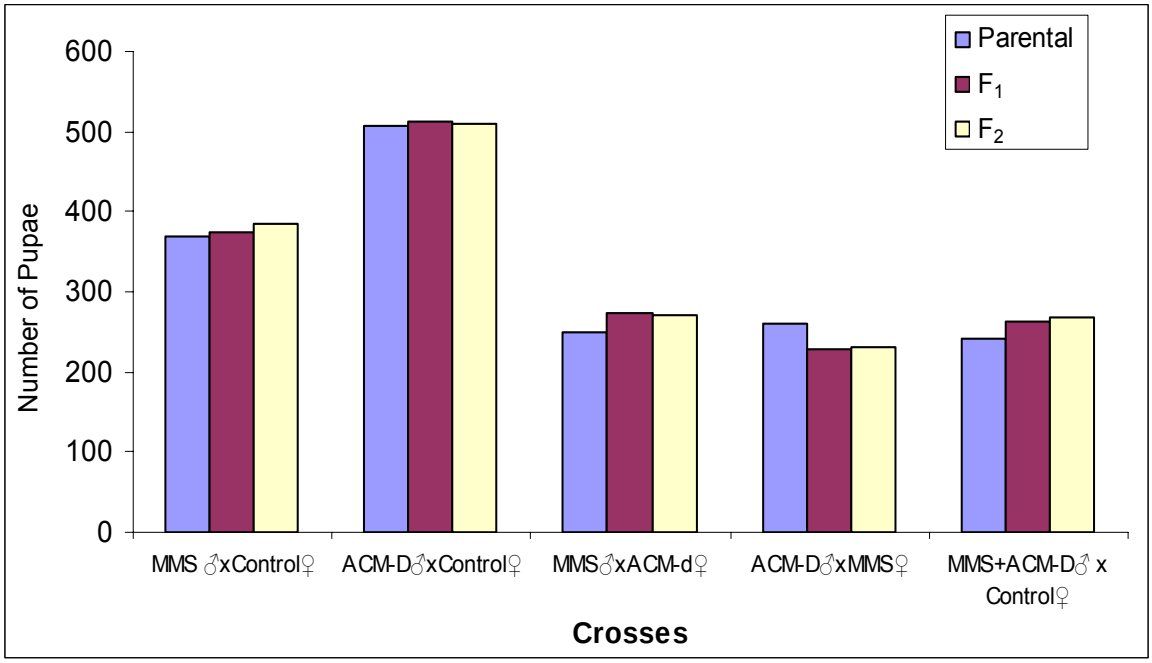

Figure-31: Effects of 3.0ml MMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

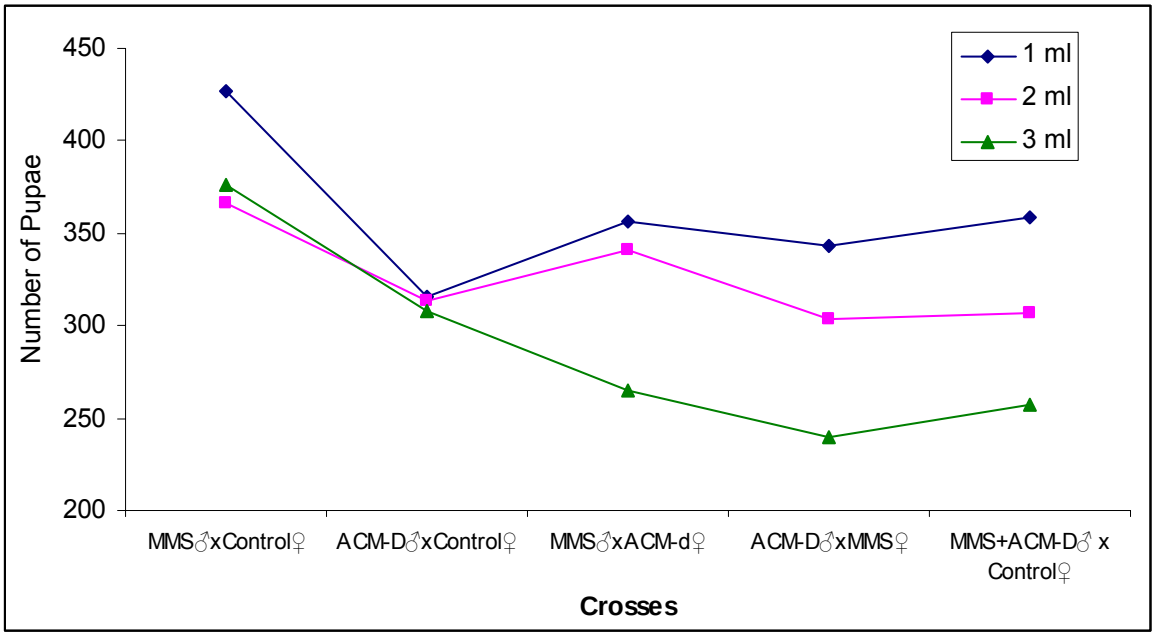

Figure-32: Effects of different doses MMS and ACM-D on the number of pupae in different crosses of *Epilachna vigintioctopunctata*

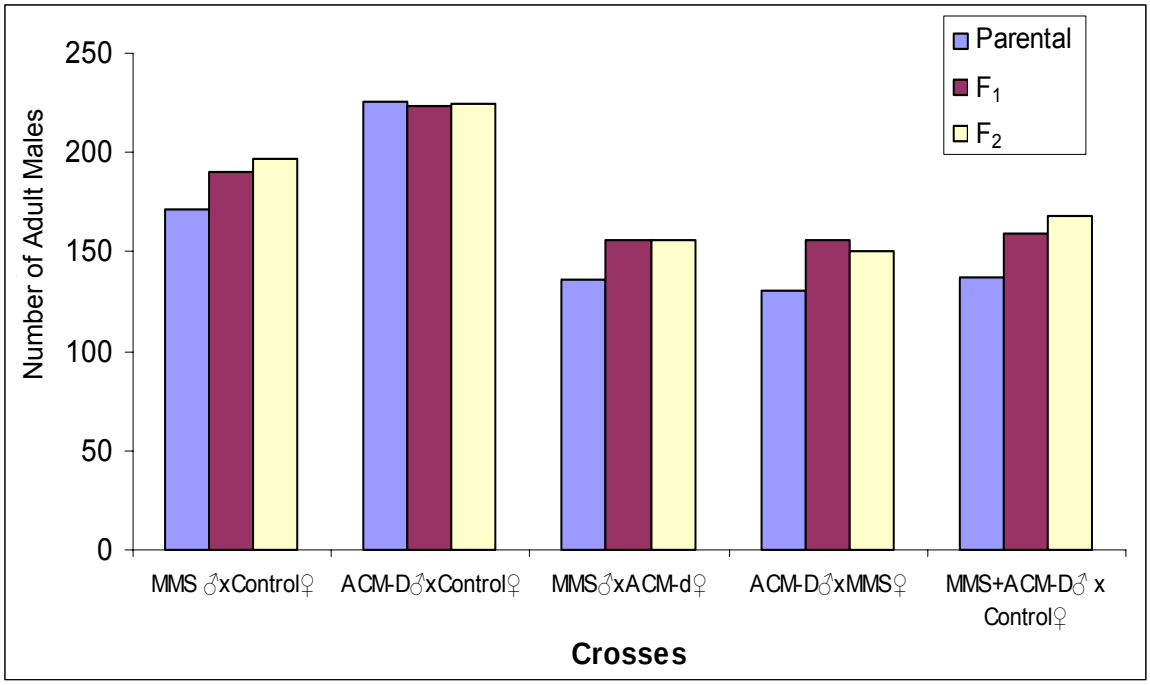

Figure-33: Effects of 1.0ml MMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

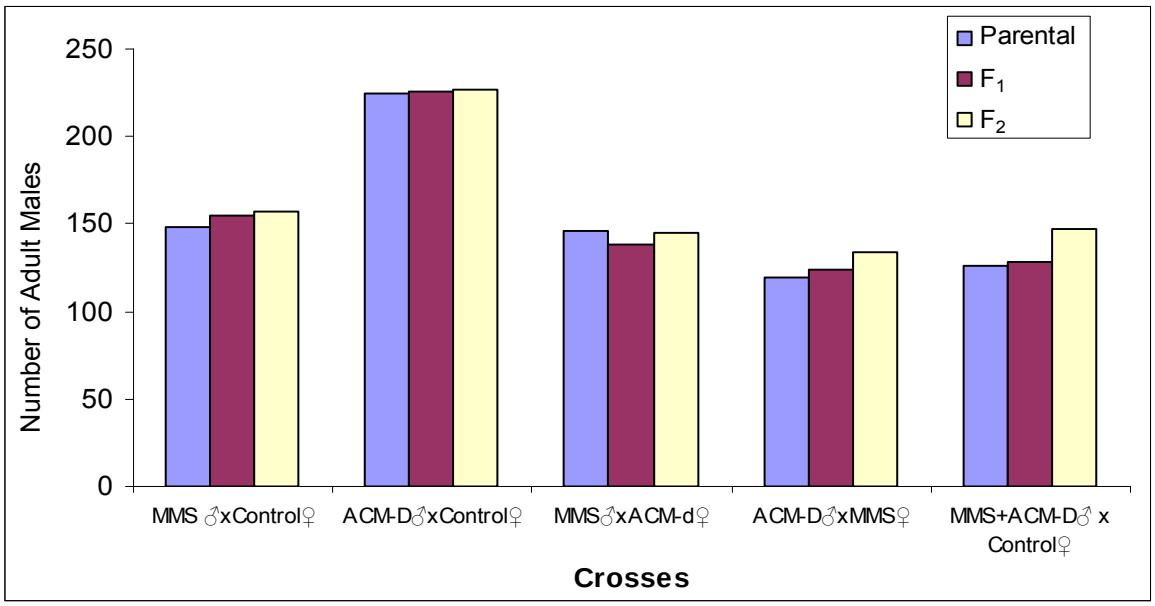

Figure-34: Effects of 2.0ml MMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

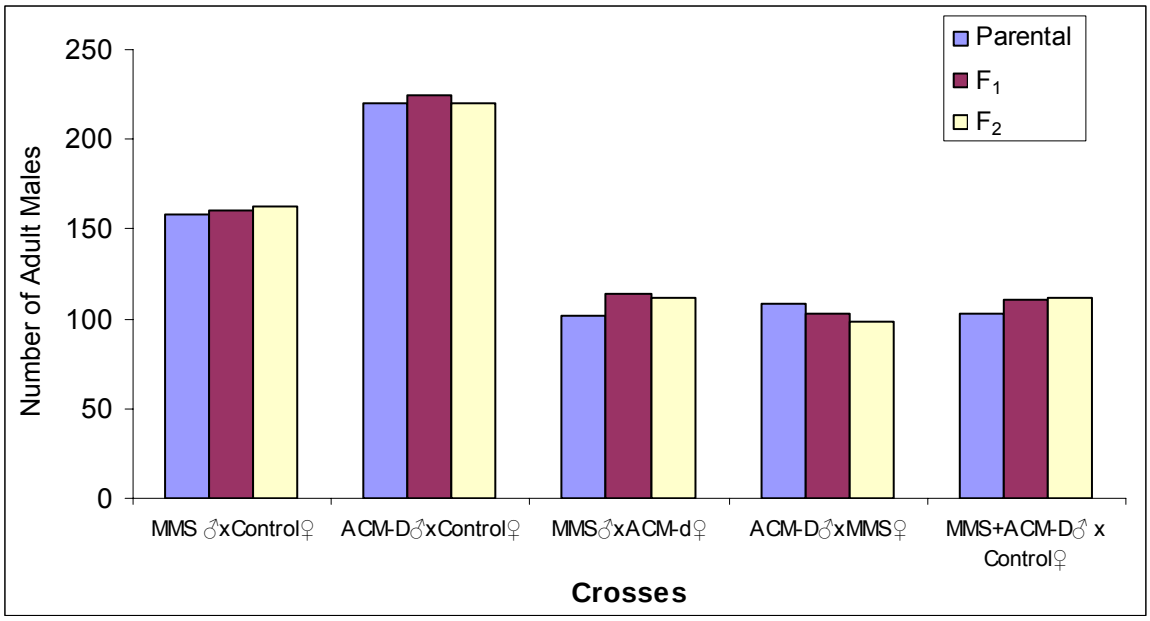

Figure-35: Effects of 3.0ml MMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

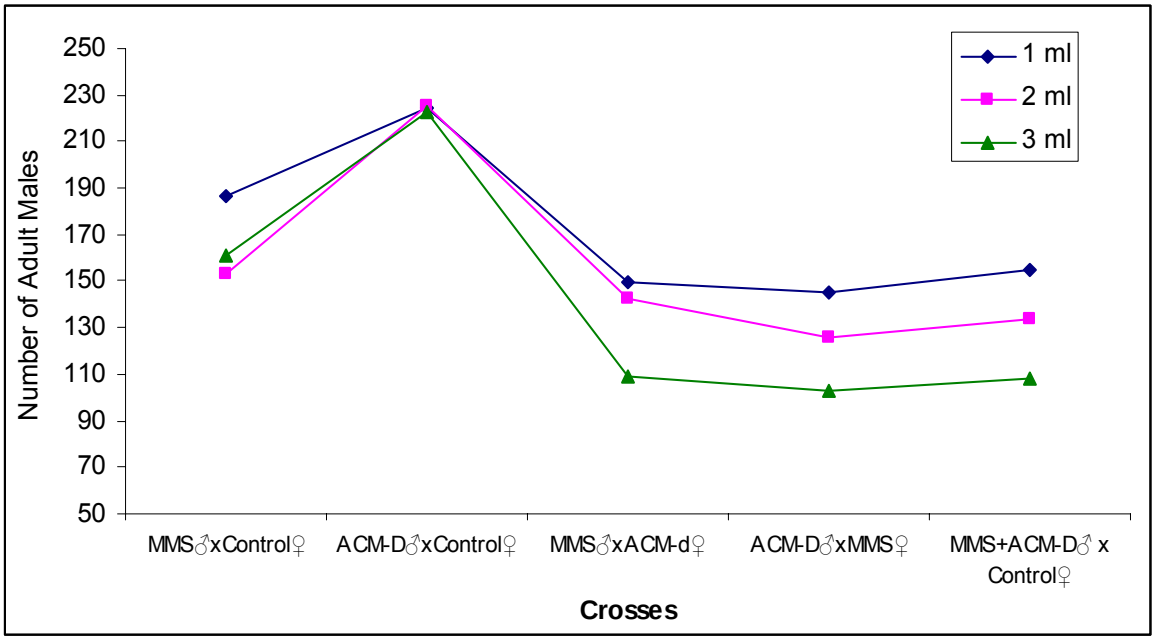

Figure-36: Effects of different doses MMS and ACM-D on the number of adult males in different crosses of *Epilachna vigintioctopunctata*

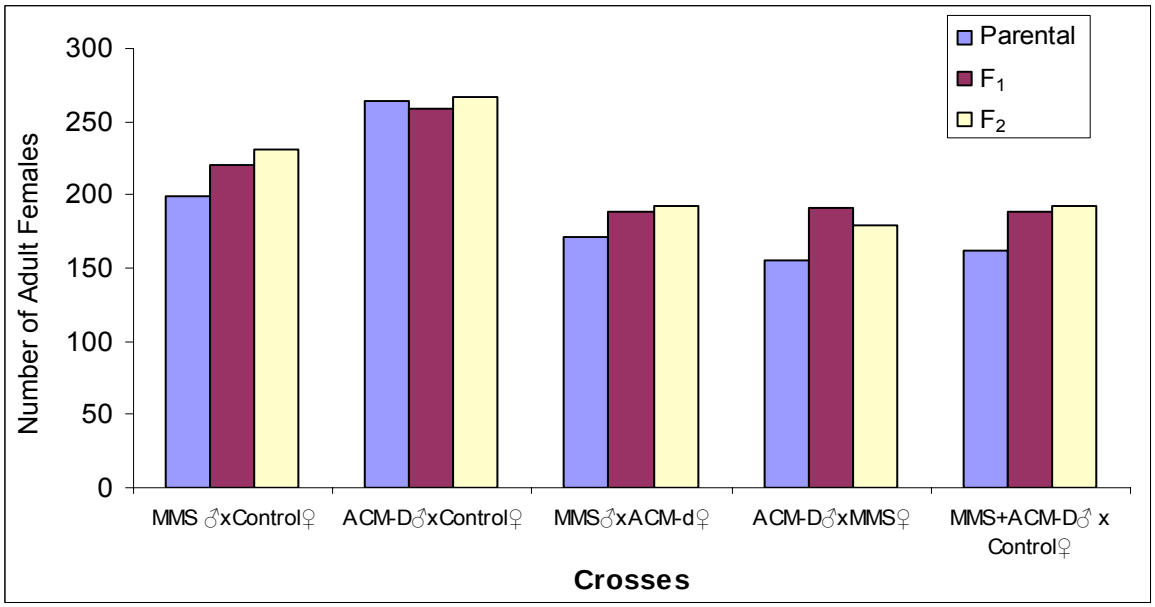

Figure-37: Effects of 1.0ml MMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

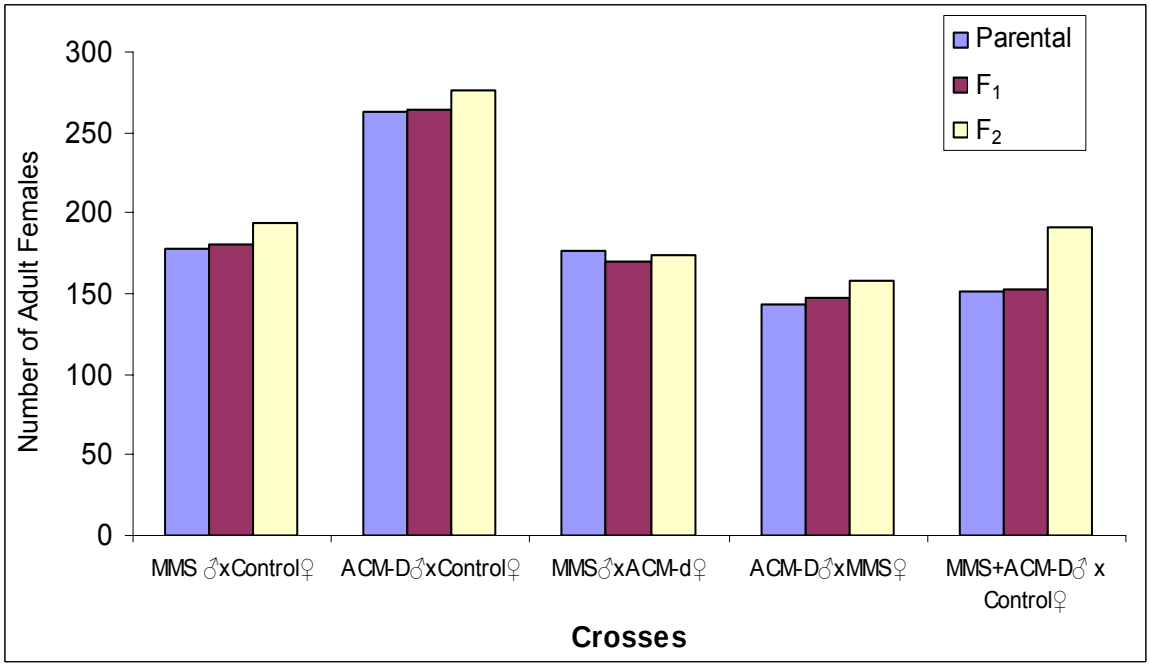

Figure 38: Effects of 2.0ml MMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

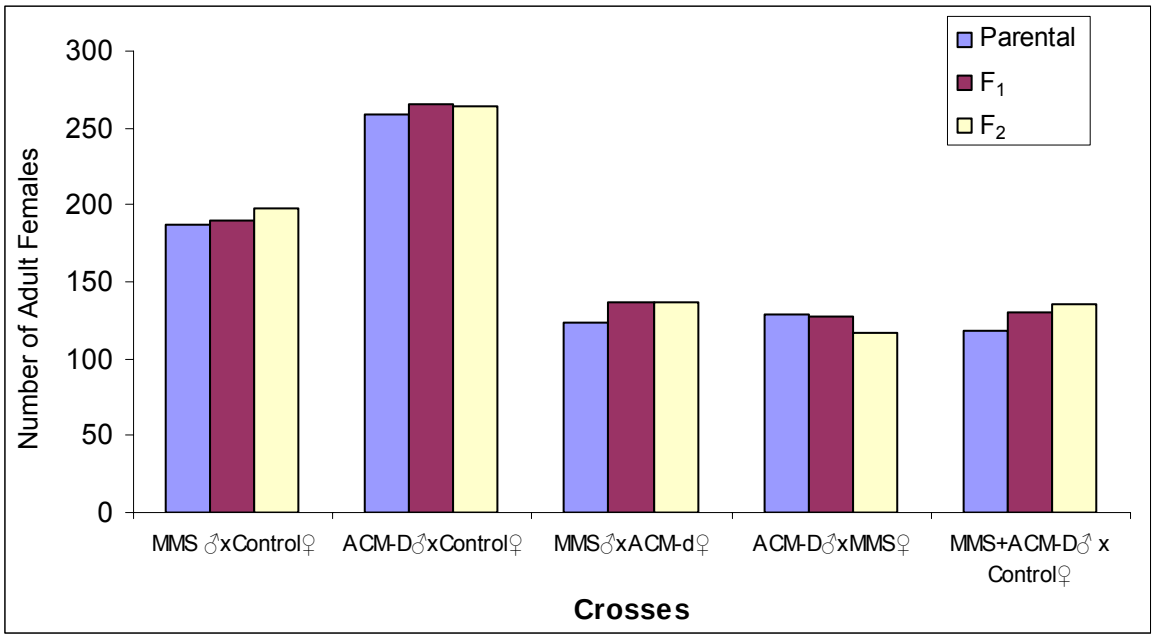

Figure 39: Effects of 3.0ml MMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*.

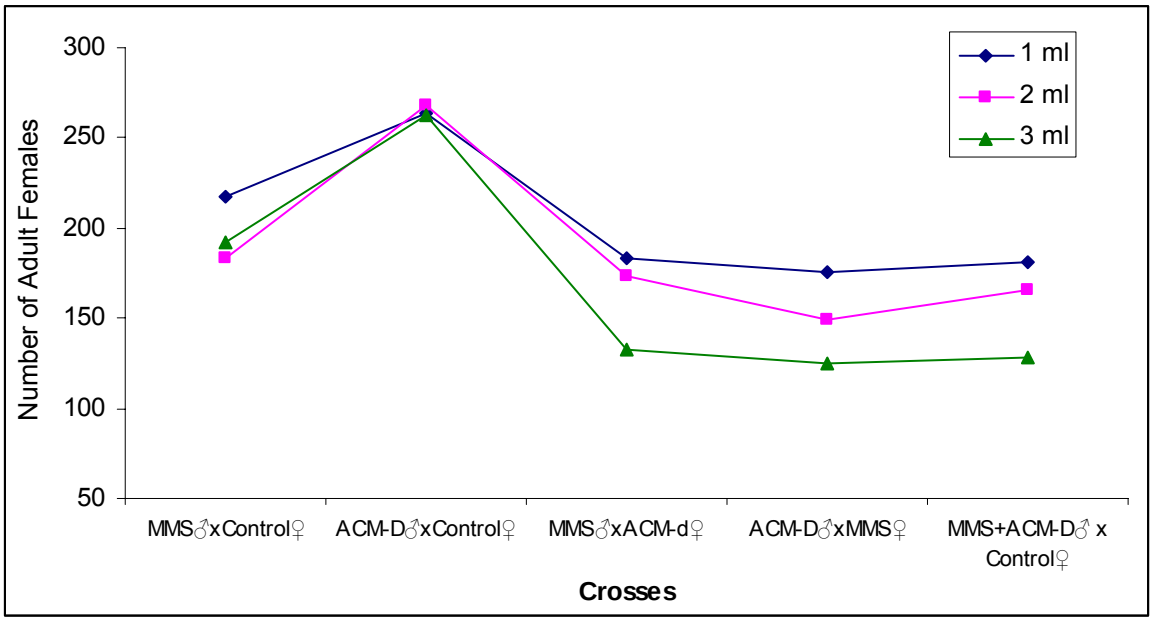

Figure 40: Effects of different doses MMS and ACM-D on the number of adult females in different crosses of *Epilachna vigintioctopunctata*

4.2.2. Effects of ethyl methanesulfonate and actinomycin-D on the number of eggs, larvae, pupae, adult males and females.

EMS dose effects with ACM-D on the number of eggs, larvae, pupae, and adult males and females were determined at different crosses and are showed in tables 26-38, figures 41-60 and appendix tables LXVI-LLX.

Table-26 summarized the data effects on 1.0ml dose of EMS and ACM-D on the number of eggs. The analysis of variance within crosses showed highly significant differences ($F=295.08$, $P<0.001$, appendix table -LXVI) with LSD values as 5.9 at 5% and as 12.86 at 0.1% probability level of significance. The number of eggs induced by the cross MMS♂ x control♀ differed significantly with other crosses MMS♂ x ACM-D♀, ACM-D♂ x control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀ (table-26).

Considering the case on the number of eggs it was found that the analysis of variance within generations differed significantly ($F=17.67$, $P<0.01$, appendix table-LXVI) with LSD values as 7.61 at 5% and as 11.07 at 1%, level of significance. Table-26 and figure 41 demonstrated that the number of eggs differed significantly with each other generations irrespective crosses.

At 2.0ml dose of EMS the F value has been calculated and showed differed significantly within crosses as 372.56 ($P<0.001$, appendix table -LXVII) with LSD values as 5.43 at 5% and as 11.85 at 0.1% level of significance.Which revealed that the number of eggs observed in cross of EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ crosses and the number of eggs founded in EMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly in table-27.

Data obtained from table-27,which deals with the effects of 2.0ml doses of EMS and ACM-D, showed significant differences within generations ($F=11.97$, $P<0.01$, appendix table-LXVII) with LSD values as 7.01 at 5% and as 10.2 at 1% probability level. It was observed that the number of eggs differed significant among the three generations as in figure 42.

Data obtained from table-28, which deals with the effect of 3.0ml doses of EMS showed significant differences among the crosses ($F=15.15$, $P<0.01$, appendix table-LXVIII) with LSD values as 25.16 at 5% and as 36.6 at 1% level.The number of eggs in cross ACM-D♂ x EMS♀ differed significantly with other treated crosses EMS♂ x Control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀ and EMS+ACM-D♂ x control ♀. The analysis of variance within generations showed no significant differences (appendix table-LXVIII and figure-43).

Table-26: Effects of 1.0ml EMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x^2
control♂ x control♀	Parental	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
EMS♂ x control♀	Parental	502.6	439.8	407	387	180	207	1	1.15	2.21
	F_1	514	456.2	433.2	413.4	190.8	222.6	1	1.17	2.54
	F_2	524.4	464.4	442.4	422.6	194.4	228.2	1	1.18	2.91
ACM-D♂ x control ♀	Parental	587.6	536.8	501	476.4	225.6	250.8	1	1.11	1.44
	F_1	591.2	538.6	438.4	417.6	191	226.6	1	1.21	3.54
	F_2	593.2	539.8	517.8	496.2	230.2	266	1	1.16	2.86
EMS♂ x ACM-D♀	Parental	496	425	384.4	365.4	171.8	193.6	1	1.13	1.38
	F_1	496	398	377	356.4	163.4	193	1	1.18	2.77
	F_2	507.4	404.4	381.6	361.2	166.6	194.6	1	1.17	2.7
ACM-D♂ x EM♀	Parental	491.6	360	340.4	319.8	148.2	171.6	1	1.16	1.98
	F_1	501.2	399.2	381.4	361	163.8	197.2	1	1.2	3.15
	F_2	513.8	387.6	365.8	343	156.2	186.8	1	1.2	2.88
EMS+ACM-D♂ x control♀	Parental	486	398	373.8	349.8	162	187.8	1	1.16	2.02
	F_1	496.8	399.8	376.4	357.6	163.6	194	1	1.19	2.61
	F_2	500.8	400.2	377.8	354.4	161.4	193	1	1.2	3.1

Table-27: Effects of 2.0ml EMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x^2
control♂ x control♀	Parantal	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
EMS♂ x control♀	Parantal	504.2	389.8	367	344.2	162	182.2	1	1.13	1.3
	F_1	513.6	420.4	399.6	376.4	171.6	204.8	1	1.19	2.95
	F_2	515.6	438.4	430.8	419.6	192	227.6	1	1.19	3.23
ACM-D♂control♀	Parantal	590	525.8	480.4	453.6	213	240.6	1	1.14	2.18
	F_1	589.2	530.4	507.6	487.4	222.6	264.8	1	1.19	3.79
	F_2	590.4	542.6	522.2	500	231.4	268.6	1	1.16	2.96
EMS♂ x ACM-D♀	Parantal	496	341.4	319.6	300.2	140.2	160	1	1.14	1.34
	F_1	499.4	364.2	342.2	319.2	146.6	172.6	1	1.18	2.43
	F_2	510.4	367.2	343.8	318	143.4	174.6	1	1.22	3.14
ACM-D♂ x EMS♀	Parantal	489.4	341.6	310.6	291	132.8	158.2	1	1.19	2.34
	F_1	492.4	317.4	296.2	279	126.6	149.4	1	1.16	1.66
	F_2	502.8	317.4	295	273.6	124.8	148.8	1	1.19	2.24
EMS+ACM-D♂ x control♀	Parantal	481	347.8	314.4	290.8	131.2	159.6	1	1.22	3.06
	F_1	486.4	334.4	312.6	289.2	133.4	155.8	1	1.17	1.79
	F_2	498.2	337.4	311.8	290.8	133	157.8	1	1.19	2.33

Table-28: Effects of 3.0ml EMS and ACM-D on the number of eggs, larvae, pupae and adults in *Epilachna vigintioctopunctata*

Cross	Generation	No. of eggs	No. of larvae	No. of pupae	No. of adults	No. of males	No. of females	Sex-ratio		
								♂	♀	x2
control♂ x control♀	Parental	590	572	532	509	230	279	1	1.21	4.72
	F_1	610	578	539	522	236	286	1	1.21	4.79
	F_2	595	560	522	511	224	287	1	1.28	7.77
EMS♂ x control♀	Parental	501.6	402.8	377.8	357.2	165	192.2	1	1.17	2.18
	F_1	495.4	393.2	369.8	345.8	158.6	187.2	1	1.18	2.68
	F_2	507	405.8	386.2	344.4	155.8	188.6	1	1.22	3.32
ACM-D♂ x control♀	Parental	592.4	531.6	501.4	477.8	222.4	255.4	1	1.15	2.35
	F_1	591.6	529.6	503	478.4	221.2	257.2	1	1.16	2.88
	F_2	598.6	540.4	518	495.2	225.6	269.6	1	1.19	4.05
EMS♂ x ACM-D♀	Parental	490	380.8	356.4	332.6	151.6	181	1	1.19	2.64
	F_1	493.4	491.8	272	253.6	116.2	137.4	1	1.18	1.85
	F_2	496.4	294	271.4	247.6	113.6	134	1	1.18	1.72
ACM-D♂ x EMS♀	Parental	479.6	350.4	321	298.8	133.2	165.6	1	1.24	3.59
	F_1	469	253.6	229.2	212.6	97.4	115	1	1.18	1.58
	F_2	473.8	255.2	236.2	217	100.2	116.8	1	1.17	1.43
EMS+ACM-D ♂ x control♀	Parental	472.6	369.6	344.2	324.8	150.6	174.2	1	1.16	2.03
	F_1	496.2	292.2	272.8	253.8	116.8	137	1	1.17	1.68
	F_2	499.2	298.4	276.4	252.8	115	137.8	1	1.2	2.14

Table-29 summarized the data showing the effects on different doses of EMS and ACM-D on the number of eggs in generations. Results demonstrated that the number of eggs changed with the increase dose of EMS and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are present in appendix tables LXIX-LXXIII. In case of EMS♂ x control♀, the analysis of variences within generations and doses has been calculated and showed no significant differences (appendix table-LXIX).

When the ACM-D treated males were crossed with control females the F value has been calculated and showed nonsignificant differences between the generations and doses (appendix table-LXX).In cross of EMS♂ x ACM-D♂ the analysis of variances has been calculated and showed significant differences within generations ($F=13.51$, $P<0.05$, appendix table-LXXI) with LSD value as 6.05 at 5% level.But within doses showed significant differences as $F=8.61$ ($P<0.5$, appendix table-LXXI) with LSD values as 6.05 at 5% respectively level.

When the ACM-D treated males were crossed with EMS treated females the analysis of variances within generations showed non significant differences (appendix table-LXXII). The F values within doses has been calculated and showed significant differences ($F=11.65$, $P<0.05$, appendix table-LXXII) with LSD value as 16.76 at 5% respectively.In cross of EMS+ACM-D♂ x control♀, the F value within generations showed significant differences as 11.98($P<0.05$, appendix, table-LXXIII) with LSD values as 13.5 at 5% level. F value within doses demonstrated no significant differences (appendix table-LXXIII).

Effects on different doses of EMS and ACM-D on the number of eggs in different crosses and are shown in table-30. It was observed that mean number of eggs differed significantly within crosses ($F=133.32$, $P<0.001$, appendix table-LXXIV) with LSD values as 9.4 at 5% and as 20.52 at 1% level. Mean the number of eggs demonstrated by the cross of ACM-D♂ x EMS♀ differed significantly with other crosses like EMS♂ x control, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and EMS+ACM-D♂ x control♀. The mean of the number of eggs analysis of varience showed within doses no significant difference (appendix table-LXXIV) in table-30 and figure-44.

Table-29: Effects of different doses of EMS and ACM-D on the number of eggs in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	502.6	504.2	501.6	502.80±0.76
	F_1	514	513.6	495.4	507.67±6.14
	F_2	524.4	515.6	507	515.67±5.03
	Mean ± SE	513.67±6.30	511.13±3.52	501.33±3.36	
ACM-D♂ x control♀	Parental	587.6	590	592.4	590.00±1.39
	F_1	591.2	589.2	591.6	590.67±0.74
	F_2	593.2	590.4	598.6	594.07±2.41
	Mean ± SE	590.67±1.64	589.87±0.35	594.20±2.21	
EMS♂ x ACM-D♀	Parental	496	496	490	494.00±2.00
	F_1	496	499.4	493.4	496.27±1.74
	F_2	507.4	510.4	496.4	504.73±4.26
	Mean ± SE	499.80±3.80	501.93±4.35	493.27±1.85	
ACM-D♂ x EMS♀	parental	491.6	489.4	479.6	486.87±3.69
	F_1	501.2	492.4	469	487.53±9.62
	F_2	513.8	502.8	473.8	496.80±11.94
	Mean ± SE	502.20±6.44	494.87±4.06	474.13±3.07	
EMS+ACM-D♂ x control♀	parental	486	481	472.6	479.87±3.91
	F_1	496.8	486.4	496.2	493.13±3.38
	F_2	500.8	498.2	499.2	499.40±0.76
	Mean ± SE	494.53±4.43	488.53±5.08	489.33±8.42	

Table-30: Effects of different doses of EMS and ACM-D on the number of eggs in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	513.67	511.13	501.33	508.71±3.77
ACM-D♂ x control♀	590.67	589.87	594.2	591.58±1.33
EMS♂ x ACM-D♀	499.8	501.93	493.27	498.33±2.61
ACM-D♂ x EMS♀	502.2	494.87	474.17	490.41±8.40
EMS+ACM-D♂ x control♀	494.53	488.53	489.33	490.80±1.88

Table-26 summarized the data on the number of larvae following the effects of 1.0ml dose of EMS and ACM-D. The analysis of variance within crosses showed highly significant differences (F=68.98, P<0.001, appendix table-LXXV) with LSD values as 19.11 at 5% and as 41.70 at 0.1% probability level. The number of larvae induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀. Considering the case of generations it was founded that the analysis of variance within generations showed nonsignificant differences (appendix table-LXXV and figure-45).

At 2.0ml dose of EMS and ACM-D the F value has been calculated within crosses and showed significant differences as 89.94 (P<0.001, appendix table-LXXVI) with LSD values as 25.04 at 5% and as 49.35 at 0.1% level. Which revealed that the number of larvae observed in cross of EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses and the number of larvae found in EMS♂ X Control ♀ and ACM-D♂ x control♀ also differed significantly among themselves in table-27. In case of generation the analysis of variance also showed no significant differences (appendix table-LXXVI and figure-46).

Data obtained from table-28, which deals with the effects of 3.0ml dose of EMS and ACM-D, analysis of varience among the crosses showed significant differences (F=9.26, P<0.01, appendix table-LXXVII) with LSD values as 79.26 at 5% and as 115.29 at 1% level. The number of larvae by the cross of EMS+ACM-D♂ x control♀differed significantly with other treated crossess EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀ and ACM-D♂ x EMS♀. The analysis of variance within generations showed nonsignificant differences (appendix table-LXXVII, figure-47).

Table-31 summarized the data, showing the effects on different doses of EMS and ACM-D on the number of larvae in different generations. Results demonstrated that the number of larvae changed with the increase of doses EMS and ACM-D induced. The analysis of variance showing the effects within generations and doses has been calculated and were presented in appendix table-LXXVIII-LXXXII. In case of EMMS♂ x control♀, the

analysis of variences has been calculated within generations no significant differences (appendix table-LXXVIII). The F value within doses has been calculated and showed differed significantly as 13.83 ($P<0.05$, appendix table-LXXVIII) with LSD values as 28.71 at 5% respectively. In case of doses it was observed that means of the number of larvae of all doses differed significantly with each other table-31.

When the ACM-D treated males were crossed with Control females the analysis of varience has been calculated and showed no significant differences between the generations and doses (appendix table-LXXIX). In cross of EMS♂ x ACM-D♂ the analysis of variances showed nonsignificant differences within generations and doses in appendix table-LXXX.

When the ACM-D treated males were crossed with EMS treated females the analysis of variances showed no significant differences within generations and doses (appendix table-LXXXI). In cross of EMS+ACM-D♂ x control♀, the F value within generations showed nonsignificant differences (appendix table-LXXXII) and the analysis of variens showed differed significantly as $F= 9.24$($P<0.05$, appendix table-LXXXII) with LSD value as 53.36 at 5% level.

Table-31: Effect of different doses of EMS and ACM-D on the number of larvae in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	439.8	389.8	402.8	410.80±15.00
	F_1	456.2	420.4	393.2	423.27±18.26
	F_2	464.4	438.4	405.8	436.20±16.97
	Mean ± SE	453.47±7.24	416.20±14.20	400.60±3.80	
ACM-D♂ x control♀	Parental	536.8	525.8	531.6	531.40±3.18
	F_1	538.6	530.4	529.6	532.87±2.88
	F_2	539.8	542.6	540.4	540.93±0.85
	Mean ± SE	538.40±0.87	532.93±5.02	533.87±3.32	
EMS♂ x ACM-D♀	Parental	425	341.4	380.8	382.40±24.18
	F_1	398	364.2	491.8	418.00±38.21
	F_2	404.4	367.2	294	355.20±32.47
	Mean ± SE	409.13±8.16	357.60±8.16	388.87±57.31	
ACM-D♂ x EMS♀	Parental	360	341.6	350.4	350.67±5.32
	F_1	399.2	317.4	253.6	323.40±42.19
	F_2	387.6	317.4	255.2	320.07±38.29
	Mean ± SE	382.27±11.64	325.47±8.08	286.40±32.04	
EMS+ACM-D♂ x control♀	Parental	398	347.8	369.6	371.80±14.55
	F_1	399.8	334.4	292.2	342.13±31.34
	F_2	400.2	337.4	298.4	345.33±29.69
	Mean ± SE				

Effects on different doses of EMS and ACM-D following on the number of larvae in different crosses are shown in table-32. It was observed that mean number of larvae showed differed significantly within crosses ($F=36.23$, $P<0.001$, appendix table-LXXXIII) with LSD values as 34.3 at 5% and as 74.83 at 0.1% level of respectively.The mean of the number of larvae induced by the cross EMS+ACM-D♂ x control♀ differed significantly with other crosses *i.e.* EMS♂ x control, ACM-D♂ x control♀, EMS♂ x ACM-D♂ and ACM-D♂ x EMS♀. The mean of the number of larvae also differed significantly within doses ($F=7.58$, $P<0.05$, appendix table-LXXXIII) with LSD value as 44.28 at 5% level.The number of larvae induced by 3.0ml EMS dose differed significantly with 1.0ml and 2.0ml doses(table-32 and figure-48).

Table-32: Effect of different doses of EMS and ACM-D on the number of larvae in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	453.47	416.20	400.60	423.42±15.70
ACM-D♂ x control♀	538.4	532.93	533.87	535.07±1.69
EMS♂ x ACM-D♀	409.13	357.6	388.87	385.20±15.01
ACM-D♂ x EMS♀	382.27	325.47	286.4	331.38±27.87
EMS+ACM-D♂ x control♀	400.2	337.4	298.4	345.33±29.69

Table-26 summarized the data on the number of pupae following the effects of 1.0ml dose of EMS and ACM-D. The analysis of variance within crosses showed highly significant differences (F=14.49, P<0.01, appendix table-LXXXIV) with LSD values as 33.61 at 5% and as 48.89 at 1% probability level. The number of pupae induced by the cross of ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMMS+ACM-D♂ x control♀ in the table-26. Considering the case of generations it was founded that the analysis of variance showed no significant differences (table-33 and figure-49).

Data obtained from table-27, which deals with the effects of 2.0ml dose of EMS the analysis of variance also showed within crosses significant differences (F=76.29, P<0.001, appendix table-LXXXV) with LSD values as 24.21 at 5% and as 52.82 at 0.1% probability level. In case of generations the analysis of variance showed no significant differences (appendix table-LXXXV and figure-50).

At 3.0ml dose of EMS and ACM-D the F value has been calculated and showed differed significantly within crosses as 30.46 (P<0.001, appendix table-LXXXVI) with LSD values as 42.83 at 5% and as 93.44 at 0.1% probability level. Which revealed that the number of pupae observed in cross of EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ crosses. The number of pupae in EMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly in Table 28. But the analysis of varience withen generations showed no significant differences (appendix table-LXXXVI and figure-51).

Table-33 summarized the data following the effects on doses of EMS and ACM-D on the number of pupae in different generations. Results demonstrated that the number of pupae changed with the increase of EMS doses and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix tables LXXXVII-LXXXXI. In case of EMS♂ x control♀, the F value has been calculated within generations and showed no significant differences (appendix table-LXXXVII). The F value within doses showed differed significantly as 8.14 (P<0.05,

appendix table-LXXXVII) with LSD value as 34.29 at 5% level of respectively. In case of doses that means of the number of pupae of all doses differed significantly with each another showed in the table-33.

When the ACM-D treated males were crossed with Control females analysis of varience showed no significant differences were observed within the generations and doses (appendix table-LXXXVIII).In cross of EMS♂ x ACM-D♀ the analysis of variances showed no significant differences within generations and doses (appendix table-LXXXIX) .

When the ACM-D treated males were crossed with EMS treated females the analysis of variances has been calculated nonsignificant differences within generations and doses (appendix table-LXXXX). In cross of EMS+ACM-D♂ x control♀, the analysis of variences has been calculated within generation and showed no significant differences(appendix table-LXXXXI) and F value within doses also showed significant differences as 9.37 ($P<0.05$) with LSD value as 53.26 at 5% prpbability level (appendix table-LXXXXI).

Table-33: Effect of different doses of EMS and ACM-D on the number of pupae in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	407	367	377.8	383.93±11.96
	F_1	433.2	399.6	369.8	400.87±18.33
	F_2	442.4	430.8	386.2	419.80±17.15
	Mean ± SE	427.53±10.62	399.13±18.44	377.93±4.74	
ACM-D♂ x control♀	Parental	501	480.4	501.4	494.27±6.94
	F_1	438.4	507.6	503	483.00±22.37
	F_2	517.8	522.2	518	519.33±1.44
	Mean ± SE	485.73±24.19	503.40±12.26	507.47±5.29	
EMS♂ x ACM-D♀	Parental	384.4	319.6	356.4	353.47±18.79
	F_1	377	342.2	272	330.40±30.92
	F_2	381.6	343.8	271.4	332.27±32.37
	Mean ± SE	381.00±2.16	335.20±7.82	299.93±28.27	
ACM-D♂ x EMS♀	Parental	340.4	310.6	321	324.00±8.74
	F_1	381.4	296.2	229.2	302.27±44.09
	F_2	365.8	295	236.2	299.00±37.51
	Mean ± SE	362.53±11.96	300.60±5.02	262.13±29.54	
EMS+ACM-D♂ x control♀	Parental	373.8	314.4	344.2	344.13±17.17
	F_1	376.4	312.6	272.8	320.60±30.21
	F_2	377.8	311.8	276.4	322.00±29.75
	Mean ± SE	376.00±1.17	312.93±0.77	297.80±23.25	

Effects on different doses of EMS and ACM-D on the number of pupae in different crosses and are shown in table-34. It was observed that mean number of pupae showed diffeedred significantly within crosses (F=29.10, P<0.001, appendix table-LXXXXII), with LSD values as 37.94 at 5% and as 82.77 at 0.1% level of significance.The mean of the number of pupae induced by the cross of ACM-D♂ x EMS♀ differed significantly with other crosses like EMS♂ x control, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and EMS+ACM-D♂ x control♀. The mean of the number of pupae showed differed significantly within doses (F=7.18, P<0.05, appendix table-LXXXXII) with LSD value as 48.97 at 5% level.The number of pupae induced by 1.0ml EMS dose differed significantly with 2.0ml and 3.0ml doses showed in table-34 and figure-52.

Table-34: Effect of different doses of EMS and ACM-D on the number of pupae in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	427.53	399.13	377.93	401.53±14.39
ACM-D♂ x control♀	485.73	503.4	507.47	498.87±6.68
EMS♂ x ACM-D♀	381	335.2	299.93	338.71±23.50
ACM-D♂ x EMS♀	362.53	300.6	262.13	308.42±29.28
EMS+ACM-D♂ x control♀	376	312.93	297.8	328.91±23.98

Table-26 summarized the data, following the effects of 1.0ml dose of EMS and ACM-D on the number of adult males. The analysis of variance within crosses showed significant differences (F=13.40, P<0.01, appendix table-LXXXXIII) with LSD values as 16.81 at 5% and as 24.44 at 1% probability level. The number of adult males induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀.The analysis of variance within generations showed no significant differences (appendix table-LXXXXIII and in figure-53).

Data obtained from table-27 effect of 2.0ml dose of EMS and ACM-D following on the number of adult males, the analysis of varience within crosses showed highly significant differences (F=75.35, P<0.001, appendix table-LXXXXIV) with LSD values as 11.44 at 5% and as 24.97 0.1% probability level. The number of adult males induced by the cross of EMS♂ x control♀ differed significantly with other crosses ACM-D♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀.In case of the number of adult males the anlysis of variences within generations showed no significant differences (appendix table-LXXXXIV and figure-54).

Data obtained from table-28, which deals with the effects of 3.0ml dose of EMS and ACM-D, showed significant differences among the crosses (F=55.93, P<0.001, appendix table-LXXXXV) with LSD values as 15.17 at 5%, and as 33.10 at 0.1% probability level. the number of adult males by the cross of EMS♂ X ACM-D♂ showed differed significantly with other treated crossess EMS♂ x control♀, ACM-D♂ x control♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀. The analysis of variance within generations showed significant difference (F=7.84, P<0.05, appendix table-LXXXXV) with LSD value as 19.59 at 5% level (figure-55).

Table-35 summarized the data showing the effects of doses EMS and ACM-D on the number of adult males in different generations. Results demonstrated that the number of adult males changed with the increase of EMS doses and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and were presented in appendix

tables LXXXXVI-C. In case of EMS♂ x control♀, the F value within generations and doses showed nonsignificant differences in appendix table-LXXXXVI.

When the ACM-D treated males were crossed with control females no significant differences were observed between the generations and doses in appendix table-LXXXXVII. In cross of EMS♂ x ACM-D♂ the analysis of variances showed no significant differences within generations.But within doses showed significant differences ($F=7.84$, $P<0.05$) with LSD value 28.35 at 5% respectively level in appendix table-LXXXXVIII.The mean number of adult males of 1.0ml dose of EMS differed significantly with 3.0ml, 2.0ml doses of EMS (table-35) .

When the ACM-D treated males were crossed with EMS treated females the analysis of variances has been calculated and showed no significant differences within generations (appendix table-LXXXXIX). The analysis of variences has been calculated within doses showed significant differences ($F=8.57$, $P<0.05$, appendix table-LXXXXIX) With LSD value as 31 at 5% respectively. In cross of MMS +ACM-D♂ x control♀, the F value within generation showed no significant differences (appendix table-C) and F value within doses showed significant differences as 7.39 ($P<0.05$) with LSD value as 27.24 at 5% probability level (appendix table-C). The mean of the number of adult males 1.0ml dose of EMS differed significantly with other doses of EMS in table -35.

Effect on different doses of EMS and ACM-D in different crosses on the number of adult males was showed in table-36. It was observed that mean number of adult males showed differed significantly within crosses ($F=31.79$, $P<0.001$, appendix table-CI) with LSD values as 16.18 at 5% and as 35.30 at 0.1% probality level. The mean the number of adult males induced by the cross of ACM-D♂ x EMS♀ differed significantly with other crosses EMS♂ x control, ACM-D♂ x control♀, EMS♂ x ACM-D♀ and EMS+ACM-D♂ x control♀. The mean of the number of adult males differed significantly within doses ($F=8.37$, $P<0.05$, appendix table-CI) with LSD value as 20.88 at 5% probability level.The number of adult males descreased induced by EMS doses and 1.0ml dose of EMS differed significantly with 2.0ml and 3.0ml EMS doses showed in table-36 and figure-56.

Table-35: Effects of different doses of EMS and ACM-D on the number of males in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	180	162	165	169.00±5.57
	F_1	190.8	171.6	158.6	173.67±9.36
	F_2	194.4	192	155.8	180.73±12.50
	Mean ± SE	188.40±4.33	175.20±8.86	159.80±2.73	
ACM-D♂ x control♀	Parental	225.6	213	222.4	220.33±3.79
	F_1	191	222.6	221.2	211.60±10.32
	F_2	230.2	231.4	225.6	229.07±1.77
	Mean ± SE	215.60±12.39	222.33±5.32	223.07±1.31	
EMS♂ x ACM-D♀	Parental	171.8	140.2	151.6	154.53±9.25
	F_1	163.4	146.6	116.2	142.07±13.83
	F_2	166.6	143.4	113.6	141.20±15.36
	Mean ± SE	167.27±2.45	143.40±1.85	127.13±12.27	
ACM-D♂ x EMS♀	Parental	148.2	132.8	133.2	138.07±5.07
	F_1	163.8	126.6	97.4	129.27±19.24
	F_2	156.2	124.8	100.2	127.07±16.22
	Mean ± SE	156.07±4.51	128.07±2.43	110.27±11.51	
EMS+ACM-D♂ x Control♀	Parental	162	131.2	150.6	147.93±9.00
	F_1	163.6	133.4	116.8	137.93±13.72
	F_2	161.4	133	115	136.47±13.52
	Mean ± SE	162.33±0.66	132.53±0.68	127.47±11.59	

Table-36: Effects of different doses of EMS and ACM-D on the number of males in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	188.40	175.20	159.80	174.47±8.27
ACM-D♂ x control♀	215.6	222.33	223.07	220.33±2.38
EMS♂ x ACM-D♀	167.27	143.4	127.13	145.93±11.67
ACM-D♂ x EMS♀	156.07	128.07	110.27	131.47±13.35
EMS+ACM-D♂ x control♀	162.33	132.53	127.47	140.78±10.89

Data obtained from table-26 following on the number of adult female's effect of 1.0ml dose of EMS and ACM-D. The analysis of variance within crosses showed significant differences (F=15.49, P<0.01, appendix table-CII) with LSD values as 16.71 at 5% and as 24.31 at 1% probability level.The number of adult female's induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x Control♀.The analysis of variance within generations showed no significant differences (appendix table-CII and figure-57).

Effects of 2.0ml dose of EMS and ACM-D the F value has been calculated within crosses and showed significant differences as 22.62 (P<0.001, appendix table-CIII) with LSD values as 21.99 at 5% as 48 at 0.1% probability level. Which revealed that the number of adult females observed in cross of EMS♂ x ACM-D♀ differed significantly with EMS+ACM-D♂ x control♀, ACM-D♂ x EMS♀ crosses and the number of adult females showed in EMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly among themselves. In case of generation the analysis of variance also showed nonsignificant differences (appendix table–CIII) in table-27 and figure-58.

Data obtained from table-28, which deals with the effects of 3.0ml dose of EMS and ACM-D,the analysis of variences showed significant differences among the crosses (F=34.25, P<0.001, appendix table-CIV) with LSD values as 22.24 at 5% and as 48.52 at 0.1% level respectively. The number of adult females by the cross EMS+ACM-D♂ x control♀ differed significantly with other treated cross *i.e*. ACM-D♂ x control♀, EMS♂ x ACM-D♀, MMS♂ x control♀ and ACM-D♂ x EMS♀. The analysis of variance within generations showed differed significantly (F=4.76, P<0.05, appendix table-CIV) with LSD value as 28.71 at 5% level in figure-59.

Table-37 summarized the data showing the effects on different doses of EMS and ACM-D on the number of adult females in different generations. Results demonstrated that the number of adult females changed with the increase of doses EMS and ACM-D induced.

The analysis of variance showing the effects within generations and doses has been calculated and were presented in appendix tables CV-CIX. In case of EMS♂ x control♀, the anlysis of variens within generations and doses showed no significant differences (appendix table-LLV).When the ACM-D treated males were crossed with control females showed nonsignificant differences were observed between the generations and doses (appendix table-LLVI).In cross of EMS♂ x ACM-D♂ the analysis of variances showed no significant differences within generations and doses (appendix table-CVII).

When the ACM-D treated males were crossed with EMS treated females the analysis of variances showed nonsignificant differences within generations and doses (appendix table-CVIII). In cross of MMS+ACM-D♂ x control ♀, the F value within generations has been calculated and showed no significant differences (appendix table-CIX) and F value within doses showed significant differences as 8.78 ($P<0.05$, appendix table-CIX) with LSD value 29.52 at 5% level.

Table-37: Effects of different doses of EMS and ACM-D on the number of females in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			
		1.0ml	2.0ml	3.0ml	Mean ± SE
EMS♂ x control♀	Parental	207	182.2	192.2	193.80±7.21
	F_1	222.6	204.8	187.2	204.87±10.23
	F_2	228.2	227.6	188.6	214.80±13.12
	Mean ± SE	219.27±6.35	204.87±13.12	189.33±1.49	
ACM-D♂ x control♀	Parental	250.8	240.6	255.4	248.93±4.38
	F_1	226.6	264.8	257.2	249.53±11.69
	F_2	266	268.6	269.6	268.07±1.07
	Mean ± SE	247.80±11.49	258.00±8.78	260.73±4.47	
EMS♂ x ACM-D♀	Parental	193.6	160	181	178.20±9.81
	F_1	193	172.6	137.4	167.67±16.26
	F_2	194.6	174.6	134	167.73±17.85
	Mean ± SE	193.73±0.47	169.07±4.58	150.80±15.15	
ACM-D♂ x EMS♀	Parental	171.6	158.2	165.6	165.13±3.88
	F_1	197.2	149.4	115	153.87±23.86
	F_2	186.8	148.8	116.8	150.80±20.26
	Mean ± SE	185.20±7.44	152.13±3.04	132.47±16.59	
EMS+ACM-D♂ x control♀	Parental	187.8	159.6	174.2	173.87±8.15
	F_1	194	155.8	137	162.27±16.79
	F_2	193	157.8	137.8	162.87±16.15
	Mean ± SE	191.60±1.92	157.73±1.10	149.67±12.28	

Effects on different doses of EMS and ACM-D in different crosses on the number of adult females are shown in table-38. It was observed that mean number of adult females showed differed significantly within crosses ($F=27.12$, $P<0.001$, appendix table-CX) with LSD values as 19.63 at 5% and as 42.83 at 0.1% level. Mean of the number of adult females induced by the cross of EMS+ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and ACM-D♂ x EMS♀. Within doses the mean of the number of adult females showed differed significantly ($F=6.75$, $P<0.05$, appendix table-CX) with LSD value 30.49 at 5% probability level.The number of adult females induced by 1.0ml EMS doses differed significantly with 2.0ml and 3.0ml doses (table-38 and figure-60).

Table-38: Effects of different doses of EMS and ACM-D on the number of females in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	219.27	204.87	189.33	204.49±8.66
ACM-D♂ x control♀	247.8	258	260.73	255.51±3.94
EMS♂ x ACM-D♀	193.73	169.07	150.8	171.20±12.45
ACM-D♂ x EMS♀	185.2	152.13	132.47	156.60±15.40
EMS+ACM-D♂ x control♀	191.6	157.73	149.67	166.33±12.86

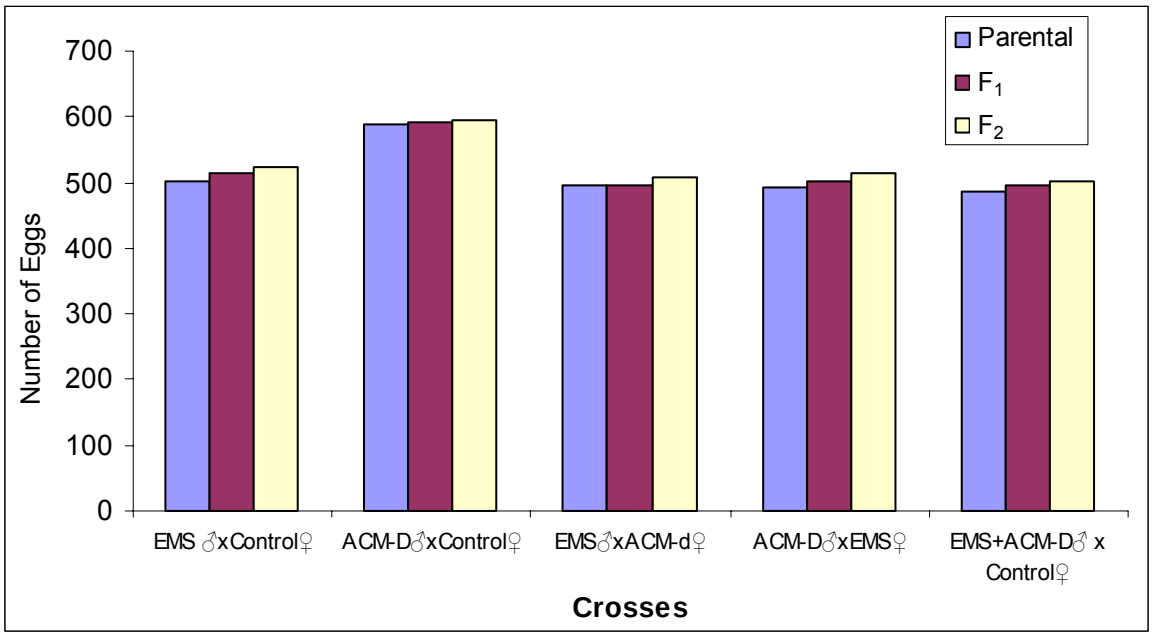

Figure-41: Effects of 1.0ml EMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*.

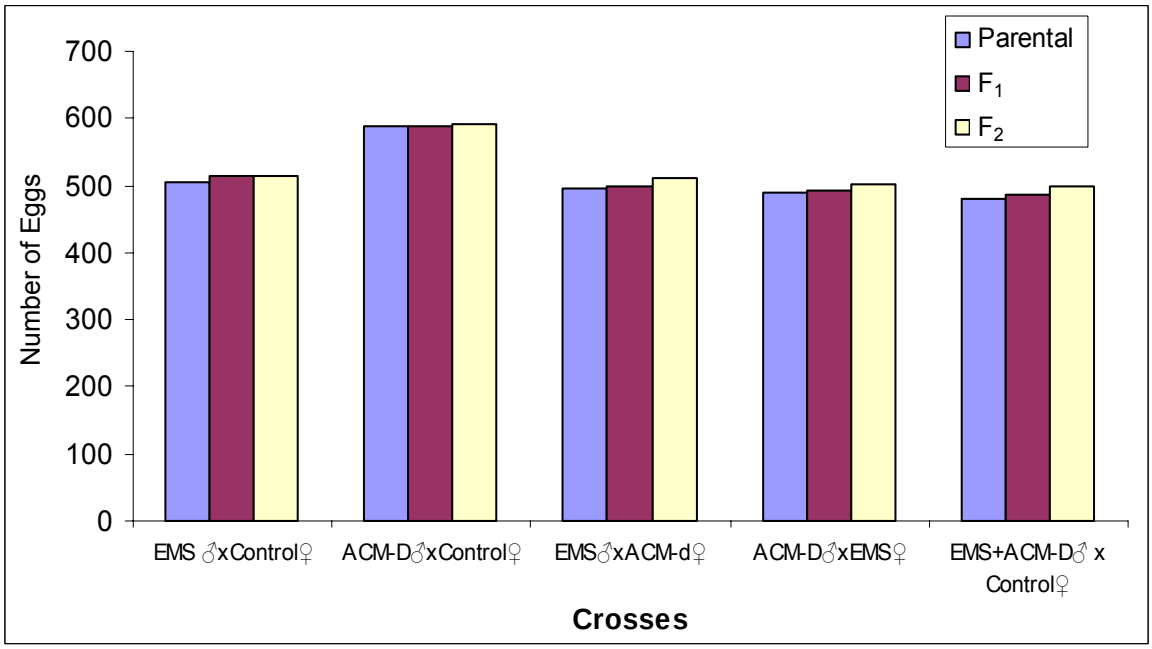

Figure-42: Effects of 2.0ml EMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*.

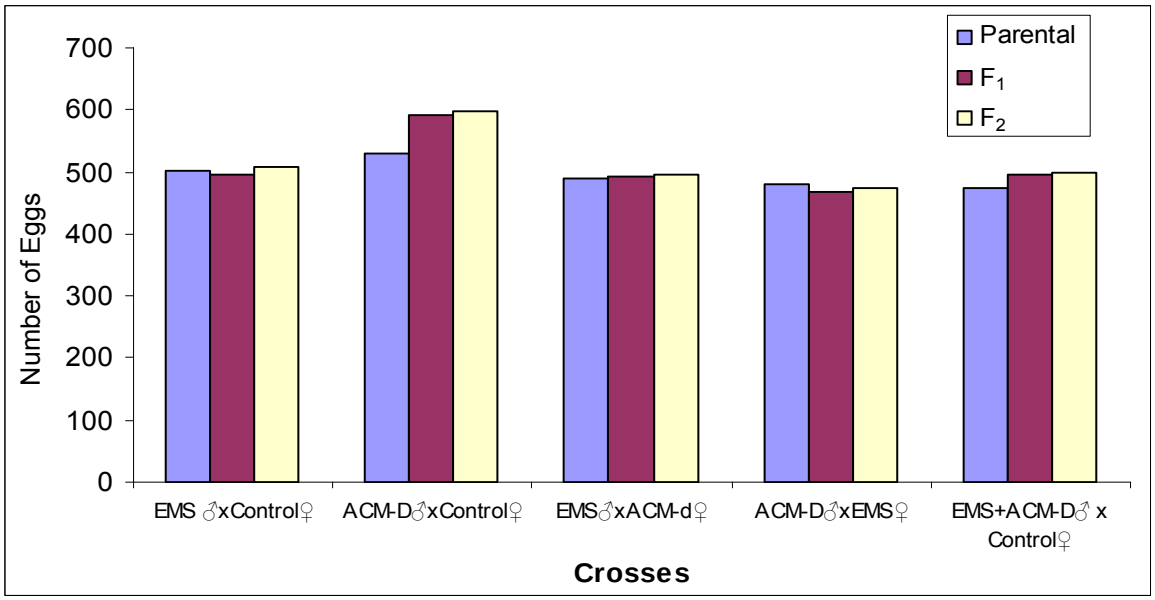

Figure-43: Effects of 3.0ml EMS and ACM-D on the number of eggs in different crosses and generations of *Epilachna vigintioctopunctata*

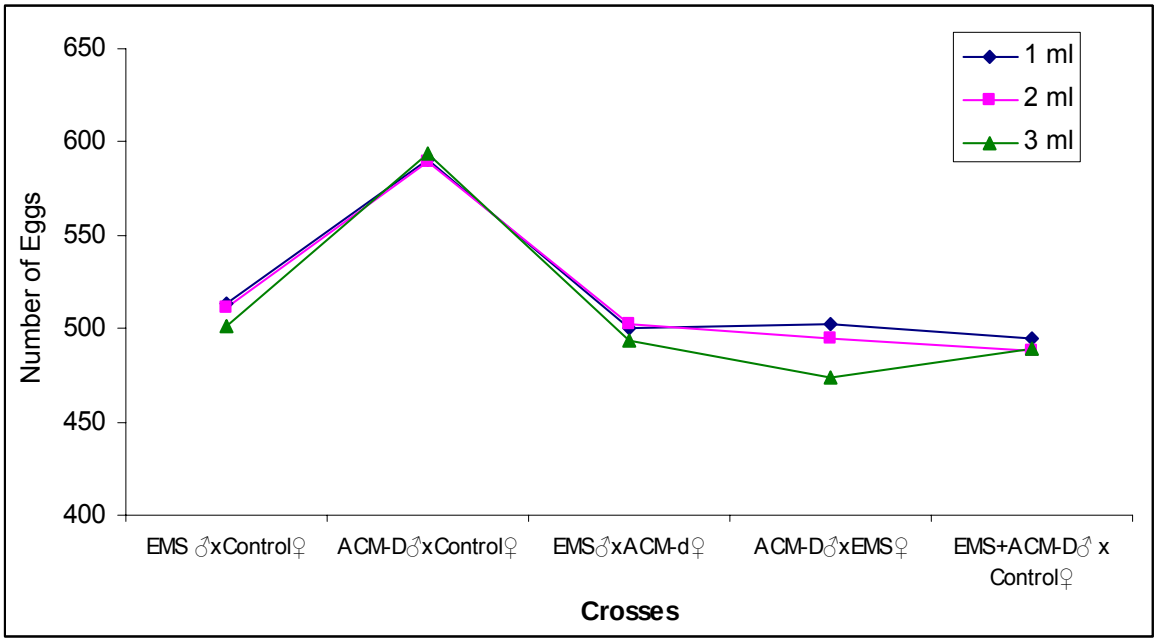

Figure-44: Effects of different doses of EMS and ACM-D on the number of eggs in different crosses of *Epilachna vigintioctopunctata*

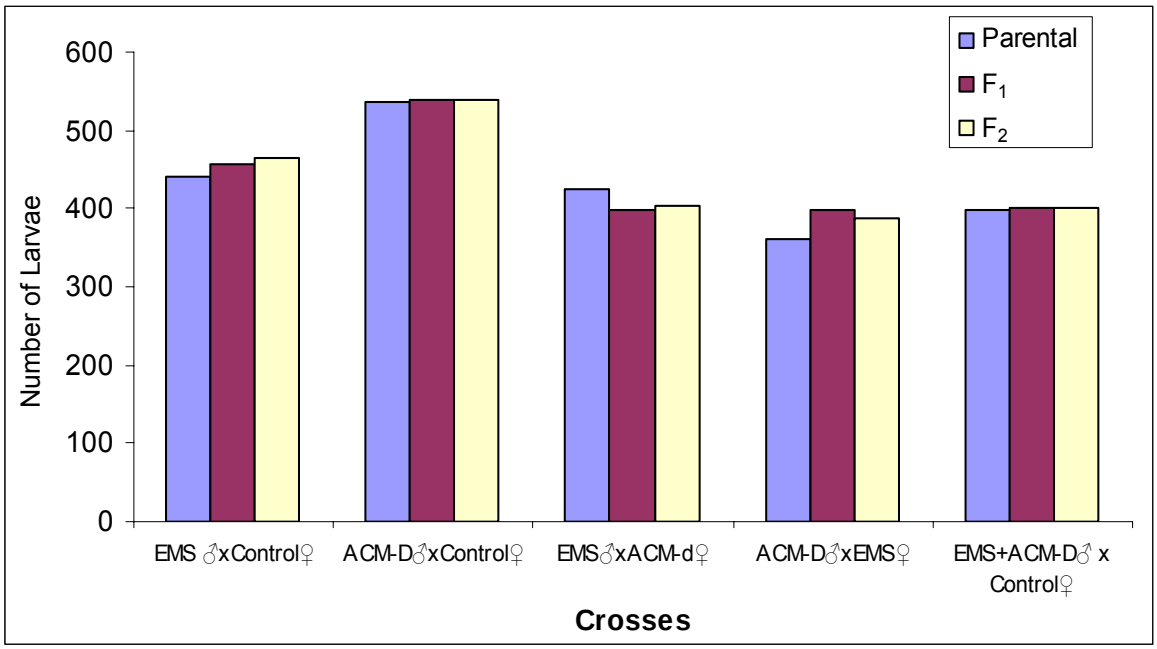

Figure-45: Effects of 1.0ml EMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

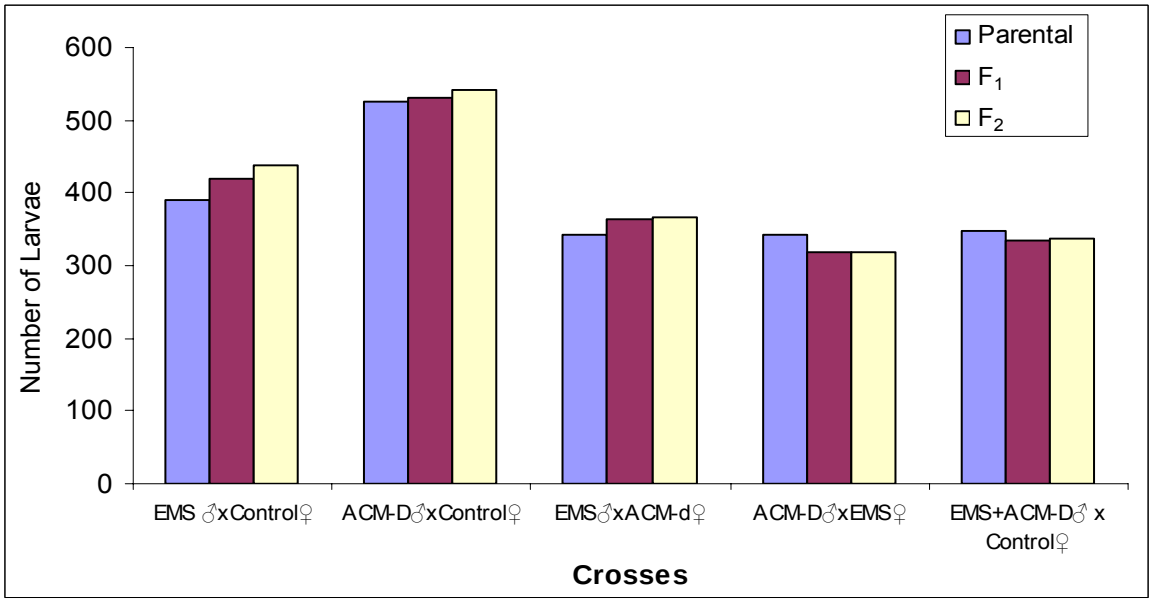

Figure-46: Effects of 2.0ml EMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

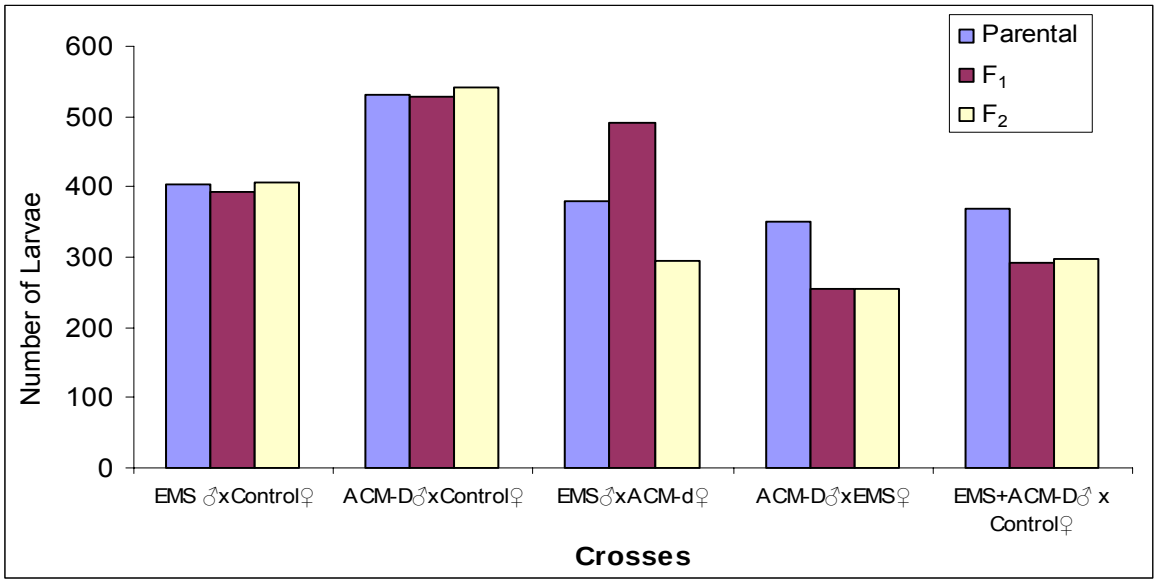

Figure-47: Effects of 3.0ml EMS and ACM-D on the number of larvae in different crosses and generations of *Epilachna vigintioctopunctata*

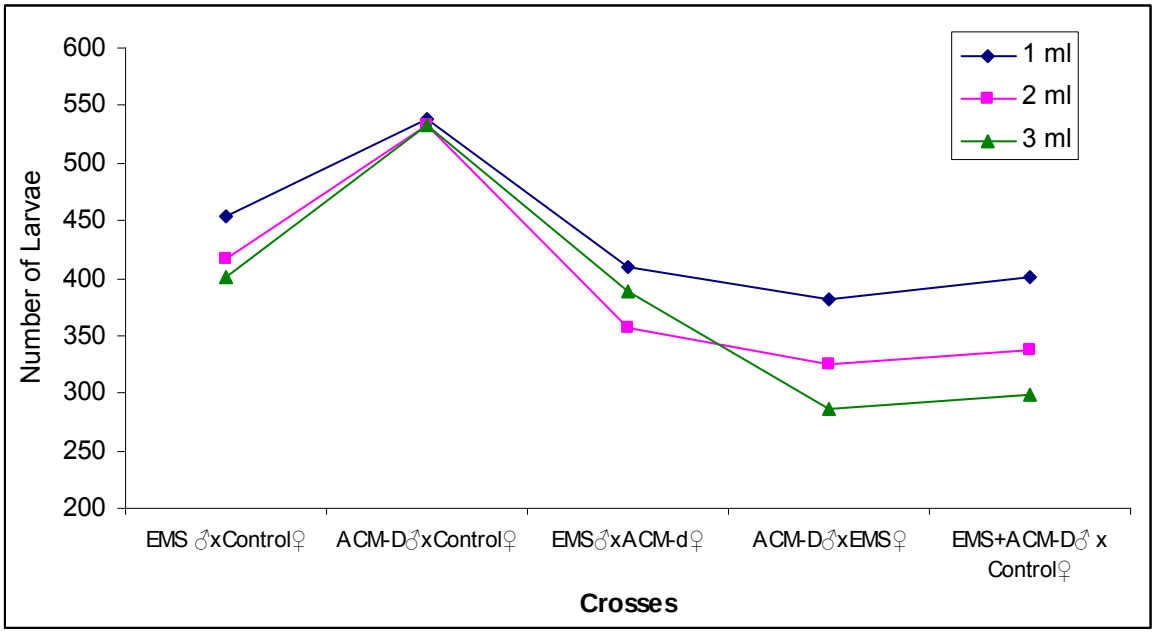

Figure-48: Effects of different doses of EMS and ACM-D on the number of larvae in different crosses of *Epilachna vigintioctopunctata*

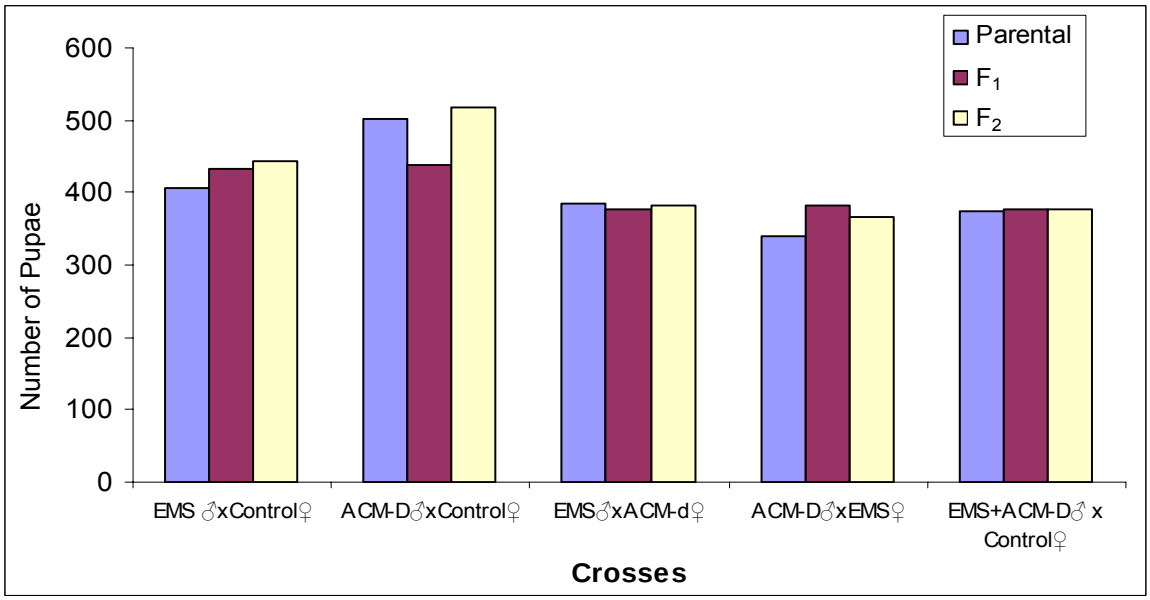

Figure-49: Effects of 1.0ml EMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

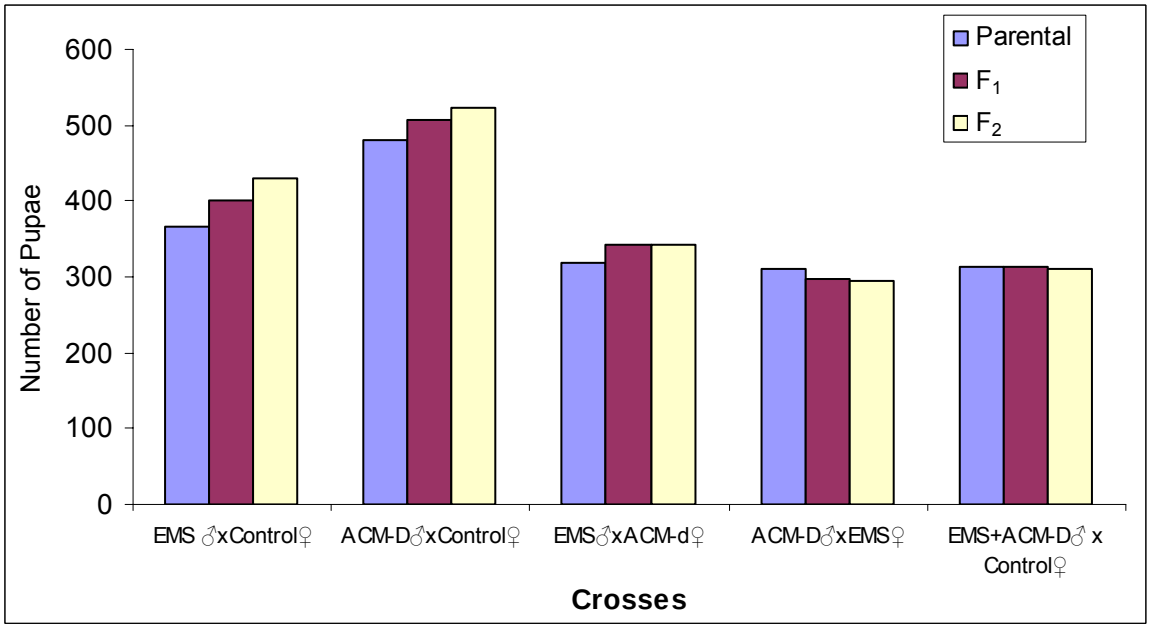

Figure-50: Effects of 2.0ml EMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

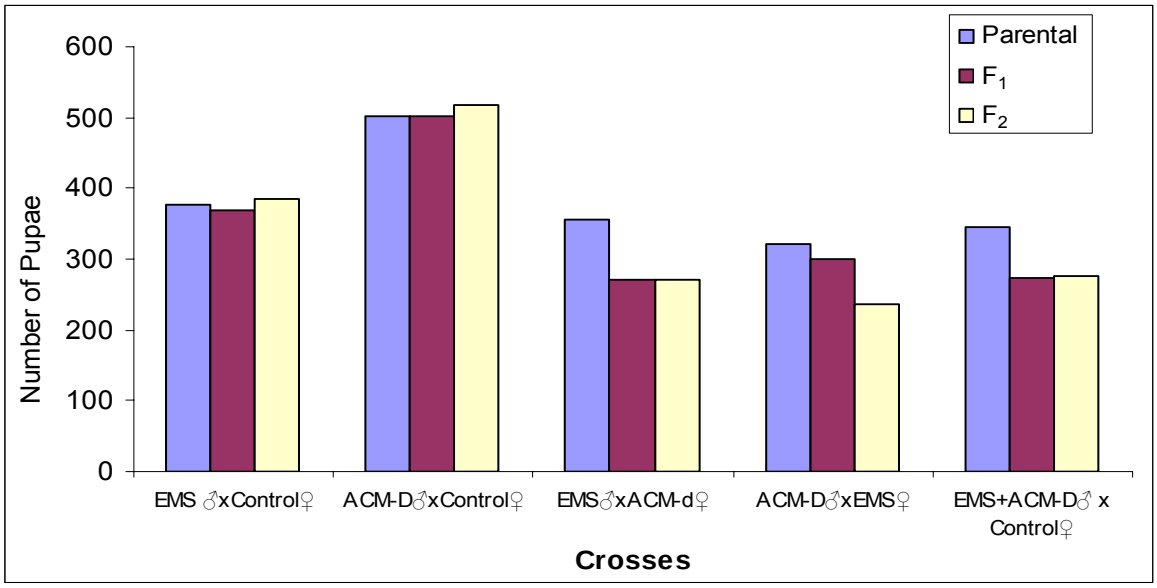

Figure-51: Effects of 1.0ml EMS and ACM-D on the number of pupae in different crosses and generations of *Epilachna vigintioctopunctata*

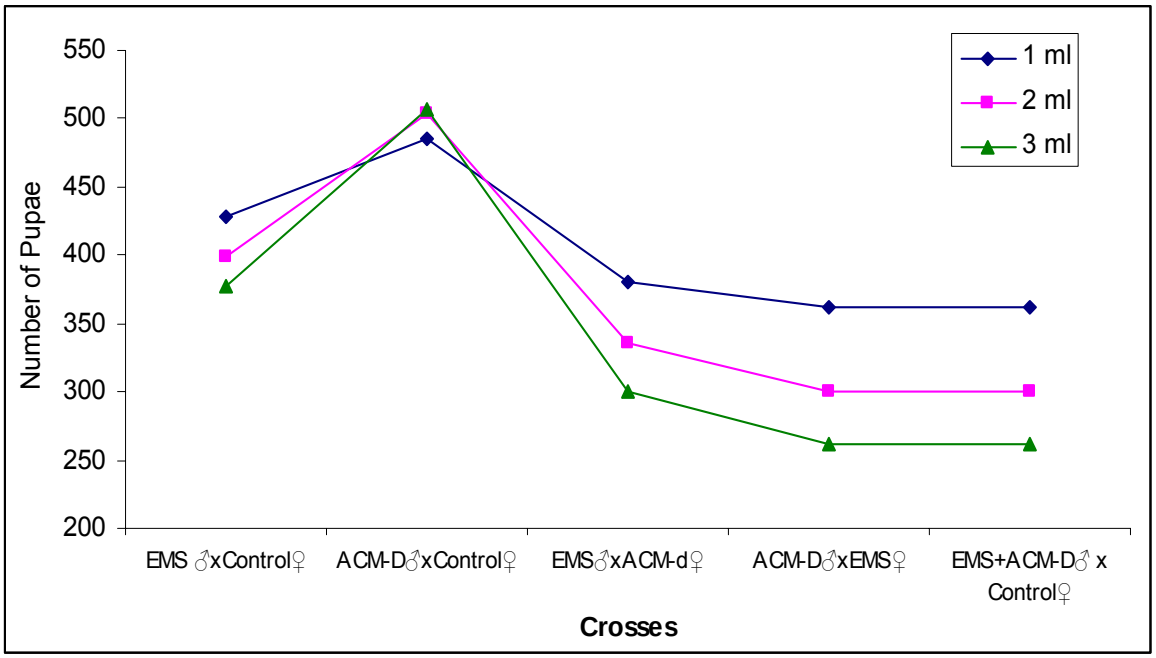

Figure-52: Effects of different doses of EMS and ACM-D on the number of pupae in different crosses of *Epilachna vigintioctopunctata*

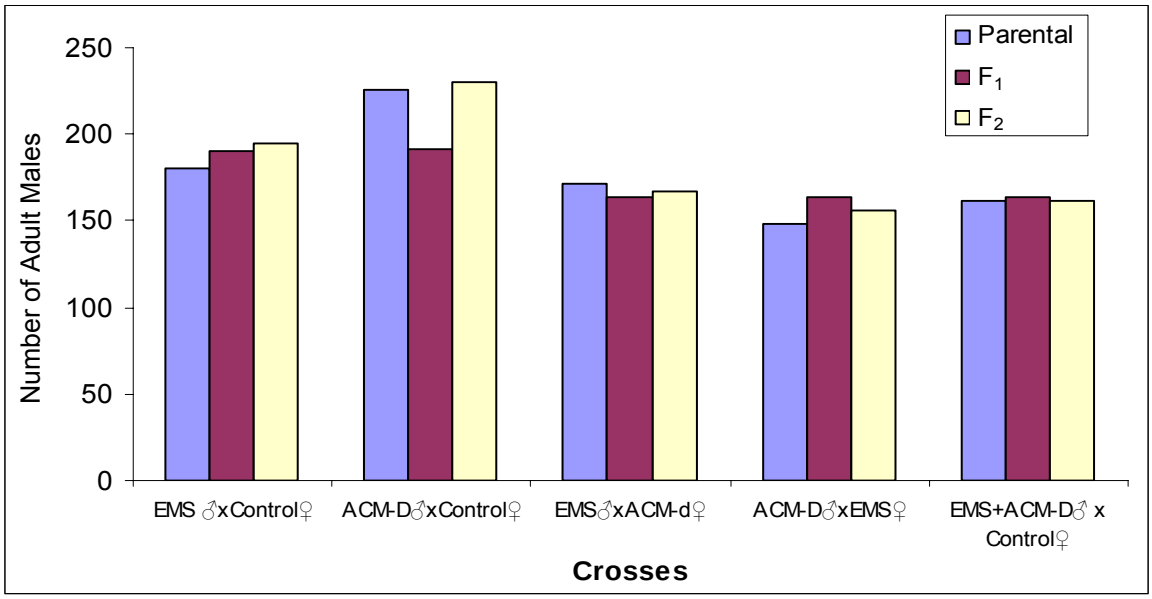

Figure-53: Effects of 1.0ml EMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

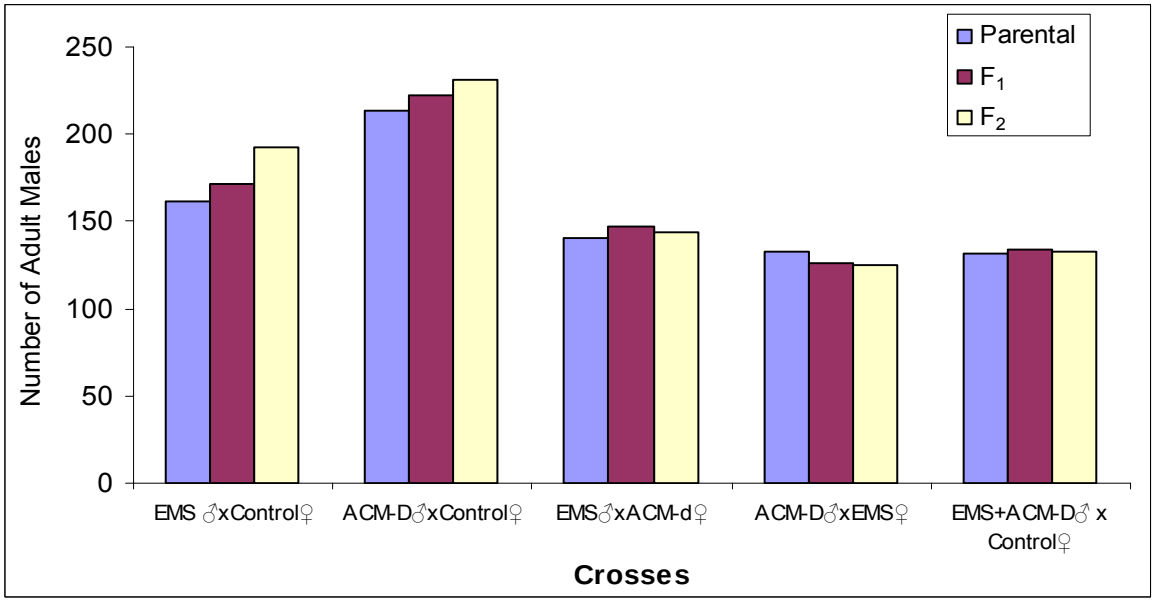

Figure-54: Effects of 2.0ml EMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

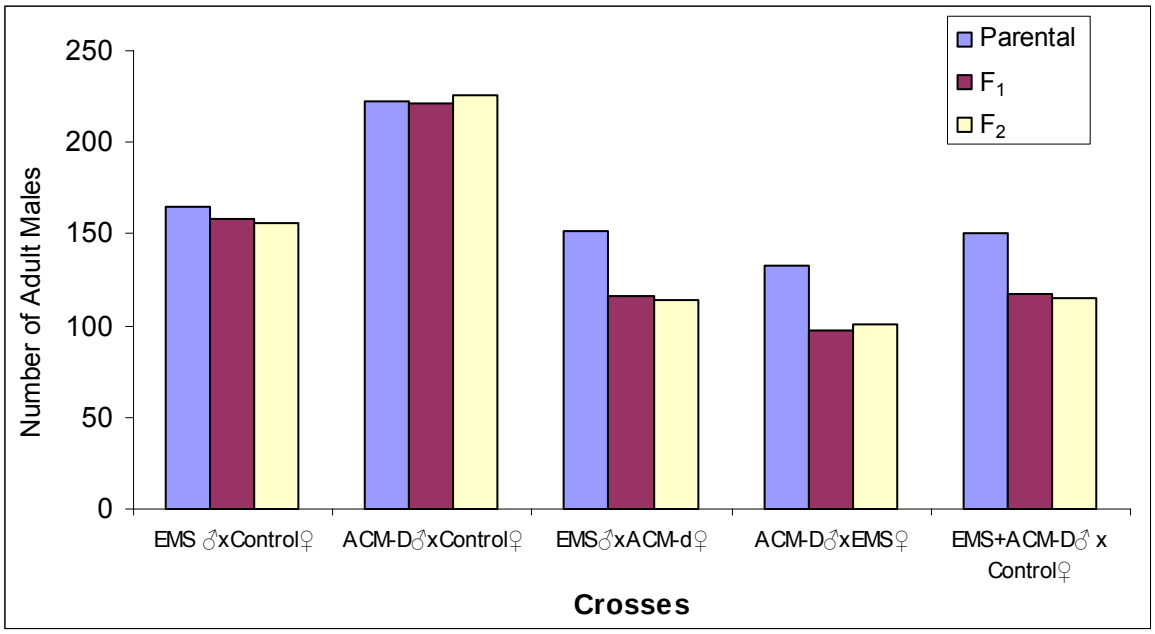

Figure-55: Effects of 3.0ml EMS and ACM-D on the number of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

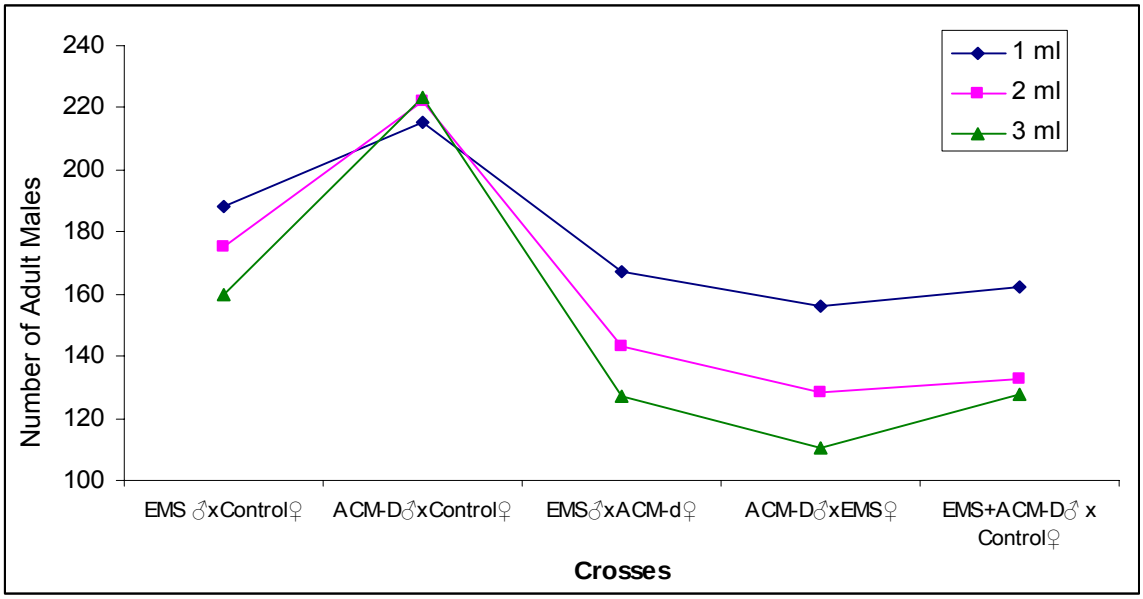

Figure-56: Effects of different doses of EMS and ACM-D on the number of adult males in different crosses of *Epilachna vigintioctopunctata*

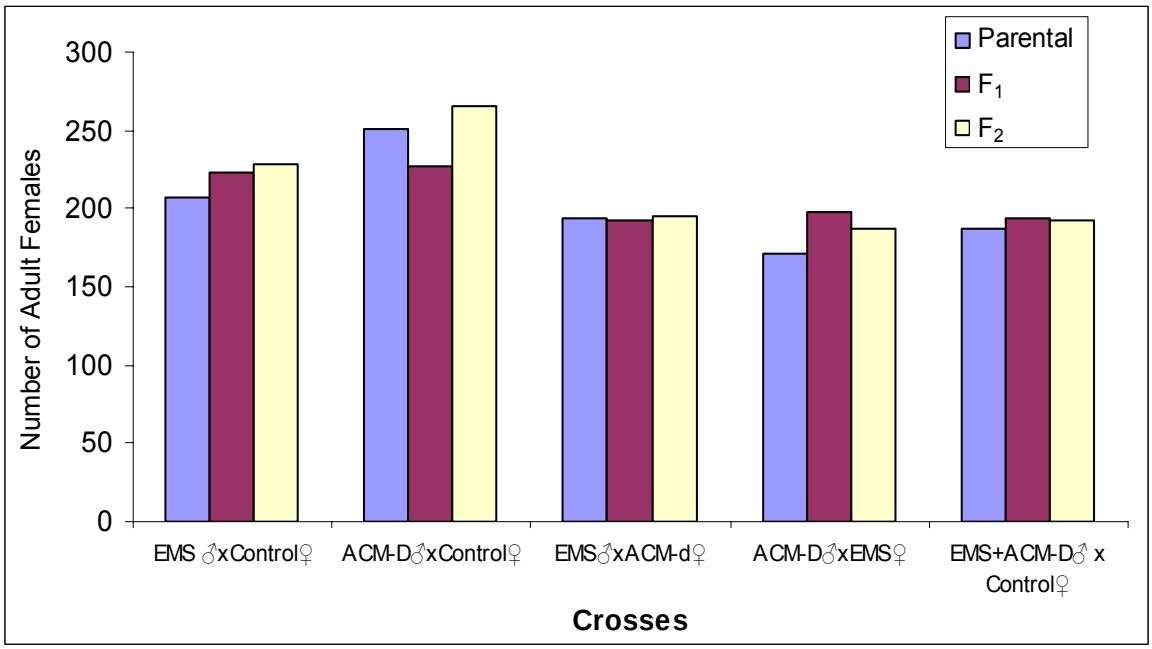

Figure-57: Effects of 1.0ml EMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

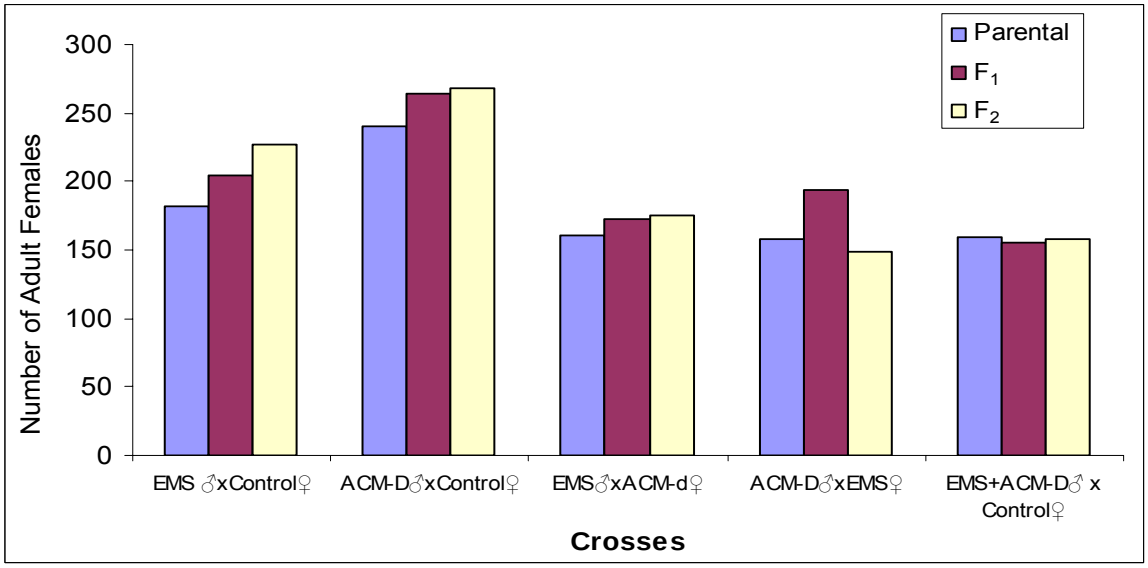

Figure-58: Effects of 2.0ml EMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

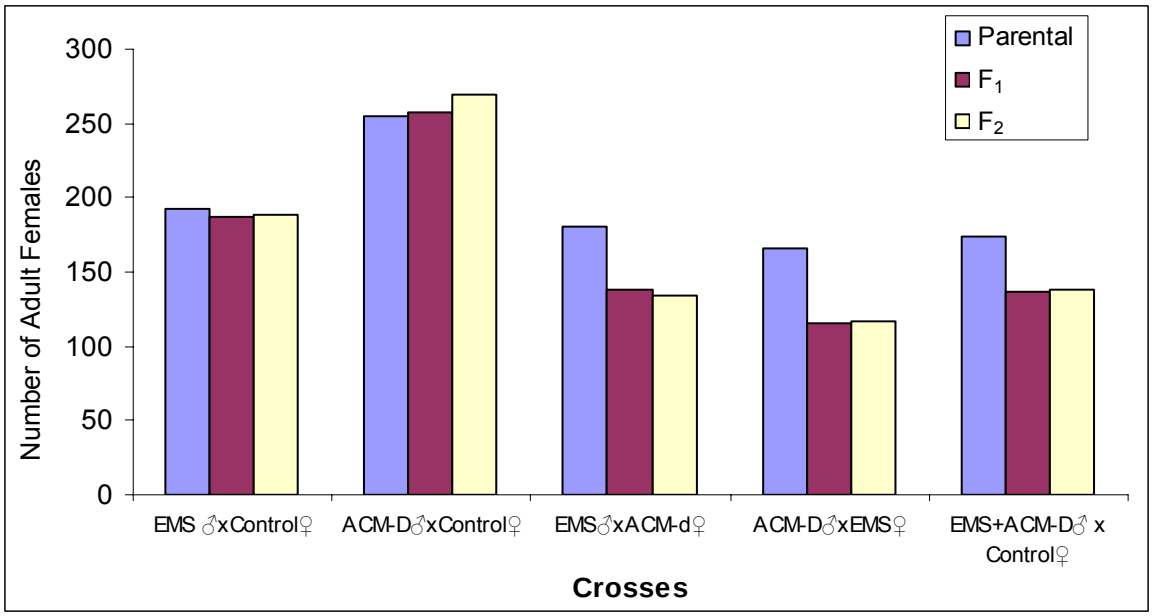

Figure-59: Effects of 3.0ml EMS and ACM-D on the number of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

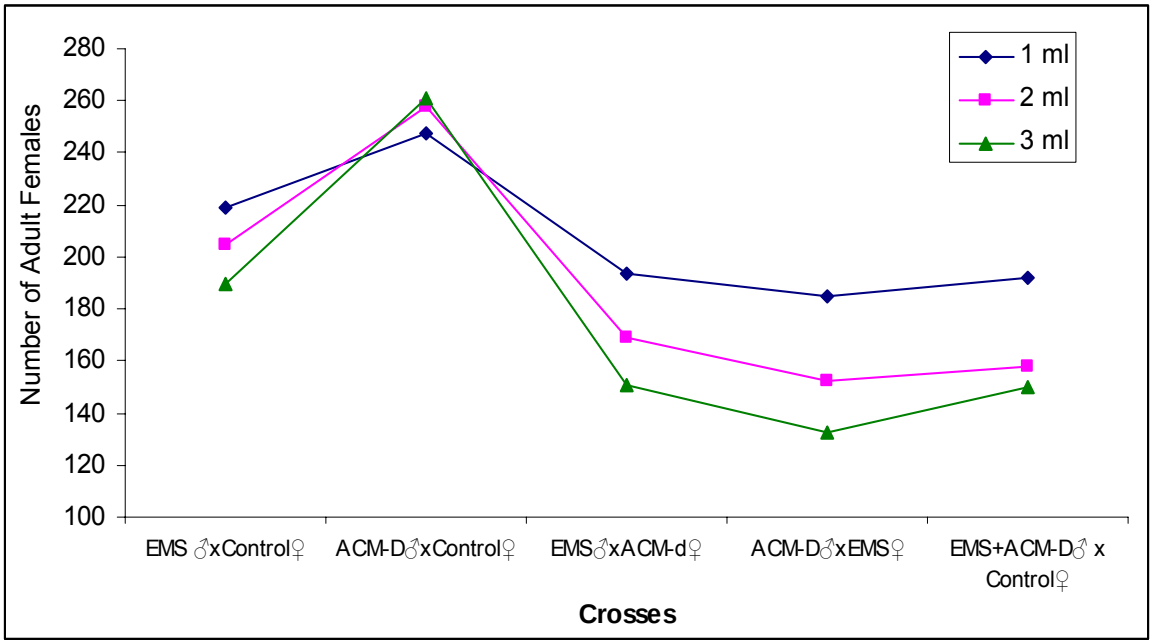

Figure-60: Effects of different doses of EMS and ACM-D on the number of adult females in different crosses of *Epilachna vigintioctopunctata*

4.2.3 Discussion

The results of the present study showed the number of eggs at all doses in the parental, F_1 and F_2 generations. The results presented here demonstrate that the treatment of males or females with ACM-D prior their mating with MMS or EMS treated males or females led to decrease the number of eggs recovered from parental, F_1 generation but nearly increase in F_2 generation. As no references are available on the effects of MMS, EMS and ACM-D on the number of eggs, but comparison can be made on the effect of other physical and chemical mutagens.The present results are in good agreements with the finding effects of pesticides diazinon and dinoseb in eyed eggs and alevins of chimook salmon showed that metabolic disorders and developmental defects occurred during early life stages Viant MR and Pincetich CA *et al*, (2006b). In a similar study the effect of α-cypermethrin on *Rana arvalis* eggs and larvae was observed, and metamorphosis was inhibited as individual and exposed to the insecticide during the early stages of life. This study also reported that hatchability decreased and growth regressed (Greulich K and Pflugmacher S 2003). Another study applied beta-cyfluthrin to *Drosophila melanogaster* and reported a decrease in hatchability Nadda G, *et al,* (2005). In a study that supports the present findings the toxic effect of 4 pesticides (carbaryl, carbofuran, malathion, and phosphamidon) on the developmental stages of *Cyprinus carpio* eggs, larvae, and tadpoles was tested, and younger embryonic phases (before gastrulation) were observed to be more sensitive to the pesticides. Moreover, morphological deformations, such as abnormal larval development and larval death were observed. (El-Toukhy *et al,* 1993). It was also reported that the effect to which the organism was exposed in the larval stage affected fecundity. In another study the effect of diazinon on various life phases of *Daphnia pulex* was examined and it was reported that first and second stages were more sensitive, and the effect decreased in subsequent stages (Stark JD and Vargas RI. (2003).]

Number of larvae and pupae

In the present findings it was observed that the MMS, EMS and ACM-D have significant effects on the number of larvae and pupae of *E. vigintioctopunctata*. In the parental generation, numbers of larvae and pupae of all the treated crossess were decreased in

comparison with the control cross F_1 and F_2 generations also showed the same result except one or two crossess. It is in good agreement with the findings of Ikebuchi and Nakao (1979) and Ratnayake (1968), who claimed that due to the storage effects of aklylating agents in *Drosophila melanogaster* the percentage of larval and pupal mortality can be increased. Das *et al.* (2007) reported that menthol extract of *Aristolochia saccata* roots was found to be most effective against. In addition, disturbed moulting, larval to pupal intermediates and malformed moth emergence/dead pupae were also observed (Malarvannan *et al.* 2008).

Chandra Sekhar and Joshi (1984) revealed that the larvae of Trichoplusia ni (Cabbage Semilooper) are susceptible to the pathogen, Bacillus cereus providing 50% mortality after 120hr.with the concentration of $2.374x10^8$ viable spores per ml in the laboratory. Similarly combinations of BKT and thuringiensin substantially increased the mortality rate, within 3 Dayes ≥ 90% mortality was observed (*Moar et al*. 1986). *Hunter et al*. (1973) observed that for larvae were individually exposed to the respective polyhedral inclusion body (PIB) concentration, mortality rate ranged from 18 to 80%, respectively.

Number of adult males and females

Effects of MMS, EMS and ACM-D on the number of adult males, females and the sex ratio of *E. vigintioctopunctata* were studied. The present investigation revealed that number of adult males, was decreased with comparison to females in the treated and control *E. vigintioctopunctata* of parental, F_1 and F_2 generation. Male, female ratio plays an important role in the maintenance of a species influence ling the number and the most species give birth to equal numbers of male and female following the typical Mendelian sex ratio (1:1) (Leigh, 1970). However, some organisms showed distortions of this typical sex-ratio. This may be attributed to the fact that environmental factors influence the physiological conditions of the animals (Charmov & Bull, 1977), reported no significant different in sex ratio of *T. castaneum* in the insecticide treated media. In the present experiment the proportion of females were found to be higher than the males in all the treatments groups.

4.3 Devlopmental period.

Effects of actinomycin-D with the methyl methanesulfonate and ethyl methanesulfonate on the incubation, larval,pupal period,longevity of adult males and adult females have been studied in *Epilachna vigintioctopunctata* and are shown in tables 39-64, figures 61-84 and appendix tables CXI-CLXIV. The frequencies of the incubation, larval, pupal period, longevity of adult males and adult females were obtained from various crosses and generations and from the treatment of different doses of MMS and EMS.

4.3.1 Effects of methyl methanesulfonate and actinomycin-D on the incubation, larval, and pupal period, and longevity of adult males, adult females

Effects of different doses of MMS with ACM-D on the incubation, larval and pupal period and longevity of adult males and female were determined at different crosses and are shown in tables 39-51, figure 61-72 and Appendix tables' CXI-CXXXVII.

Table-39 summarized the data of 1.0ml dose of MMS and ACM-D on the number of incubation period. The analysis of variance within crosses showed highly significant differences ($F=48.77$, $P<0.001$, appendix table-CXI) with LSD values as .15 at 5% and as.39 at 0.1% probability level. The incubation period induced by the cross of ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀ .Considering the case of generations it was founded that the analysis of variance has been calculated and showed significant differences ($F=8.13$, $P<0.05$, appendix table-CXI) with LSD value as 0.19 at 5%, probability level. The incubation period within generations showed differed significantly with each other generations irrespective crosses (table-39 and figure-61).

At 2.0ml dose of MMS the the analysis of variences has been calculated within crosses showed differed significantly ($F=112.81$, $P<0.001$, appendix table-CXII) with LSD values as 0.21 at 5% and as 0.42 at 0.1% level. Which revealed that the incubation period in cross of MMS+ACM-D♂ x control♀ differed significantly with MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses and the incubation period found in MMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly (Table-40). In case of generation the

analysis of variance also showed significant differences (F=13.80, P<0.01, appendix table-CXII) with LSD values as 0.27 at 5%, and as.39 at 1% probability level in figure-62.

Data of table-41, which deals with the effects of 3.0ml dose of MMS and ACM-D, showed significant differences among the crosses (F=148.06, P<0.001, appendix table-CXIII) with LSD values as 0.25 at 5% and 0.58 at 0.1% level. The incubation period by the cross MMS+ACM-D♂ x control♀ differed significantly with other treated crosses MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀ and ACM-D♂ x MMS♀. The F value has been showed significant differences within generations as 10.09 (P<0.01, appendix table-CXIII) with LSD values as 0.33 at 5% and 0.48 at 1% probability level (figure-63).

Table-39: Effects of 1.0ml MMS and ACM-D on the incubation, larval and pupal period and longevity of Adult Beetles in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
control♂ x control♀	Parental	3.65	11.21	4.05	121.22	113.25
	F1	3.25	11.02	3.98	122.24	114.22
	F2	3.32	10.58	4.11	122.56	112.54
MMS♂ x control ♀	Parental	4.68	12.25	4.19	115.59	109.16
	F1	4.54	12.11	4.15	116.03	109.84
	F2	4.34	12.11	4.08	116.41	110.53
ACM-D♂ x control♀	Parental	3.98	11.94	4.08	120.57	112.56
	F1	3.89	11.88	4.09	120.77	111.82
	F2	4.06	11.98	4.02	120.19	112.13
MMS♂ x ACM-D♀	Parental	5.54	13.58	5.22	111.9	106.75
	F1	5.28	13.2	5.05	112.47	107.2
	F2	5.11	12.95	4.92	113.28	108.67
ACM-D♂ x MMS♀	Parental	5.06	13.12	5.06	112.67	107.82
	F1	4.91	12.92	4.91	113.56	108.9
	F2	4.69	12.66	4.71	113.9	109.92
MMS+ACM-D♂ x control♀	Parental	4.92	12.47	4.8	114.76	109.68
	F1	4.73	12.16	4.42	115.49	110.27
	F2	4.43	11.94	4.12	116.21	111.31

Table-40: Effects of 2.0ml MMS and ACM-D on the incubation, larval and pupal period and longevity of Adult Beetles in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
control♂ x control♀	Parental	3.65	11.21	4.05	121.22	113.25
	F_1	3.25	11.02	3.98	122.24	114.22
	F_2	3.32	10.58	4.11	122.56	112.54
MMS♂ x control♀	Parental	5.39	13.24	5.3	112.33	106.96
	F_1	5.05	13.09	5.18	113.42	107.68
	F_2	4.74	12.72	4.97	114.21	108.6
ACM-D♂ x control♀	Parental	4.01	11.79	4.03	121.82	112.41
	F_1	3.95	11.85	4.1	120.2	112.57
	F_2	3.99	11.82	3.95	120.03	112.39
MMS♂ x ACM-D♀	Parental	6.29	14.83	6.75	107.93	103.2
	F_1	6.02	14.2	6.2	107.95	104.51
	F_2	5.85	13.99	6.02	109.84	105.49
ACM-D♂ x MMS♀	Parental	6.05	14.02	6.01	109.72	105.17
	F_1	5.85	13.89	5.74	110.81	107.94
	F_2	5.44	13.12	5.12	111.94	108.1
MMS+ACM-D♂ x control ♀	Parental	5.73	13.82	5.74	109.9	105.76
	F_1	5.45	13.21	5.29	110.76	108.04
	F_2	5.26	12.95	5.06	111.12	107.94

Table-41: Effects of 3.0ml MMS and ACM-D on the incubation, larval and pupal period and longevity of Adult Beetles in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
control♂ x control♀	Parental	3.65	11.21	4.05	121.22	113.25
	F_1	3.25	11.02	3.98	122.24	114.22
	F_2	3.32	10.58	4.11	122.56	112.54
MMS♂ x control♀	Parental	6.2	14.11	6.17	106.69	101.56
	F_1	6.06	13.9	5.96	107.54	102.34
	F_2	5.81	13.62	5.8	108.79	103.55
ACM-D♂ x control♀	Parental	3.91	11.92	4.06	120.56	112.21
	F_1	3.93	11.89	4.03	120.39	112.41
	F_2	3.95	11.91	4.06	121.2	113.05
MMS♂ x ACM-D♀	Parental	7.5	15.65	7.44	104.17	98.97
	F_1	7.15	15.2	7.16	105.72	100.25
	F_2	6.84	14.99	6.92	106.87	101.31
ACM-D♂ x MMS♀	Parental	7.2	15.24	7.35	105.53	99.17
	F_1	6.98	15.06	7.05	106.7	100.63
	F_2	6.43	14.95	6.84	108.02	101.93
MMS+ACM-D♂ x control♀	Parental	6.87	15.09	7.13	105.75	99.85
	F_1	6.38	14.53	6.69	106.24	101.1
	F_2	6.07	14.18	6.16	107.97	102.87

Table-42 summarized the data showing the effects on different doses MMS and ACM-D on the incubation period in different generations. Results demonstrate that the incubation period changed with the increased of doses MMS and ACM-D induced. The analysis of variance showing the effected within generations and doses has been calculated and is presented in appendix tables CXIV-CXVIII. In case of MMS♂ x contro♀, the F value within generations showed significant differences as 23.28 ($P<0.01$, appendix table-CXIV) with LSD values as 0.19 5% and as 0.74 at 1% level.The analysis of variences has been showed significant differences ($F=253.19$, $P<0.001$) with LSD values as 0.19 at 5% and 0.58 at 0.1% level of respectively in appendix table-CXIV. The mean value of the incubation period of F_1 generation showed significntly differed with parental and F_2 generations. In case of doses it was observed that means of the incubation period of all doses differed significantly with each other.

When the ACM-D treated males were crossed with Control females the analysis of variences has been calculated ans showed nonsignificant differences were observed between the generations and doses (appendix table-CXV).In cross of MMS♂ x ACM-D♂ the analysis of variances showed significant differences within generations ($F=40.40$, $P<0.01$, appendix table-CXVI) with LSD values as 0.16 at 5% and as 0.26 at 1% level. The analysis of variances within doses showed differed significantly ($F=527.27$, $P<0.001$, appendix table-CXVI) with LSD values as 0.16 at 5% and as 0.49 at 0.1% probability level (table-42).

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed within generations significant differences ($F=27$, $P<0.01$, appendix table-CXVII) with LSD values as 0.23 at 5% and as 0.38 at 1% level. The F values within doses has been calculated and showed significant differences as 296 ($P<0.001$, appendix table-CXVII) With LSD values 0.23 at 5% and as 0.77 at 0.1% respectively. In cross of MMS+ACM-D♂ x control♀, the analysis of variences within generations showed significant differences ($F=26$, $P<0.01$, appendix table-CXVIII) with LSD values as 0.23 at 5% and as 0.38 at 1%level.The analysis of varience within doses has been calculated and

showed significant differences (F=230, P<0.001, appendix table-CXVIII) with LSD values as 0.23 at 5% and 0.70 at 0.1% level (table-42).

Effect of different doses of MMS and ACM-D on the incubation period in different crosses and are shown in table-43. It was observed that mean of the incubation period differed significantly within crosses (F=12.11, P<0.01, appendix table-CXIX) with LSD values as 0.62 at 5% and as 0.90 at 1% probability level. The mean of the incubation period induced by the cross MMS+ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x Control♀, ACM-D♂ x Control ♀, MMS♂ x ACM-D♂, and ACM-D♂ x MMS♀. The mean of the incubation period differed significantly within doses (F=13.83, P<0.01, appendix table-CXIX) with LSD values as 0.80 at 5% and as 1.16 at 1% level of significance.The incubation period induced by 2.0ml MMS dose differed significantly with 1.0ml and 3.0ml doses (table-43 and figure-64).

Table-42: Effects of different doses of MMS and ACM-D on the incubation period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	4.68	5.39	6.2	5.42±0.44
	F_1	4.54	5.05	6.06	5.22±0.45
	F_2	4.34	4.74	5.81	4.96±0.44
	Mean ± SE	4.52±0.10	5.06±0.19	6.02±0.11	
ACM-D♂ x control♀	Parental	3.98	4.01	3.91	3.97±0.03
	F_1	3.89	3.95	3.93	3.92±0.02
	F_2	4.06	3.99	3.95	4.00±0.03
	Mean ± SE	3.98±0.05	3.98±0.02	3.93±0.01	
MMS♂ x ACM-D♀	Parental	5.54	6.29	7.5	6.44±0.57
	F_1	5.28	6.02	7.15	6.15±0.54
	F_2	5.11	5.85	6.84	5.93±0.50
	Mean ± SE	5.31±0.13	6.05±0.13	7.16±0.19	
ACM-D♂ x MMS♀	Parental	5.06	6.05	7.2	6.10±0.62
	F_1	4.91	5.85	6.98	5.91±0.60
	F_2	4.69	5.44	6.43	5.52±0.50
	Mean ± SE	4.89±0.11	5.78±0.18	6.87±0.23	
MMS+ACM-D♂ x control♀	Parental	4.92	5.73	6.87	5.84±0.57
	F_1	4.73	5.45	6.38	5.52±0.48
	F_2	4.43	5.26	6.07	5.25±0.47
	Mean ± SE	4.69±0.14	5.48±0.14	6.44±0.23	

Table-43: Effects of different doses of MMS and ACM-D on the incubation period in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	4.52	5.06	6.02	5.20±0.44
ACM-D♂ x control♀	3.98	3.98	3.93	3.96±0.02
MMS♂ x ACM-D♀	5.31	6.05	7.16	6.17±0.54
ACM-D♂ x MMS♀	4.89	5.78	6.87	5.85±0.57
MMS+ACM-D♂ x control♀	4.69	5.48	6.44	5.54±0.51

Data obtained from table-44 showing the effects of different doses of MMS and ACM-D on the larval period in different generations. Results demonstrated that the larval period changed with the increase of doses MMS and ACM-D induced. The mean value of larval period of parental generation showed significntly differed with F_2 generation. When the ACM-D treated males were crossed with Control females showed nonsignificant differences between the generations and doses. In cross of MMS♂ x ACM-D♂ the mean value of larval period of F_1 generation significntly differed with F_2 generation.The mean value of larval period among the doses,showed 1.0ml dose differ significant with 2.0ml and 3.0ml doses.

When the ACM-D treated males were crossed with MMS treated females the mean value of larval period within generations the parental generation significntly differed with F_2 generation. In case of doses it was observed that all doses differ significantly with each other doses.In cross of MMS+ACM-D♂ x control♀, larval period within generations showed nonsignificant differences.In case of doses 1.0ml, 2.0ml and 3.0ml doses differed significant with each other.

Effects on different doses of MMS and ACM-D on the larval period in different crosses are shown in table-45. It was observed that mean larval period showed differed significantly within crosses. Mean the larval period found that induced by the cross ACM-D♂ x control♀ differed significantly with MMS♂ x control♂, MMS♂ x ACM-D♂. The mean of the larval period within doses 1.0ml dose showed differed significantly with 2.0ml and 3.0ml doses (table-45).

Table-44: Effects of different doses of MMS and ACM-D on the larval period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	12.25	13.24	14.11	13.20±0.54
	F_1	12.11	13.09	13.9	13.03±0.52
	F_2	12.11	12.72	13.62	12.82±0.44
	Mean ± SE	12.16±0.05	13.02±0.15	13.88±0.14	
ACM-D♂ x control♀	Parental	11.94	11.79	11.92	11.88±0.05
	F_1	11.88	11.85	11.89	11.87±0.01
	F_2	11.98	11.82	11.91	11.90±0.05
	Mean ± SE	11.93±0.03	11.82±0.02	11.91±0.01	
MMS♂ x ACM-D♀	Parental	13.58	14.83	15.65	14.69±0.60
	F_1	13.2	14.2	15.2	14.20±0.58
	F_2	12.95	13.99	14.99	13.98±0.59
	Mean ± SE	13.24±0.18	14.34±0.25	15.28±0.19	
ACM-D♂ x MMS♀	Parental	13.12	14.02	15.24	14.13±0.62
	F_1	12.92	13.89	15.06	13.96±0.62
	F_2	12.66	13.12	14.95	13.58±0.70
	Mean ± SE	12.90±0.13	13.68±0.28	15.08±0.08	
MMS+ACM-D♂ x Control♀	Parental	12.47	13.82	15.09	13.79±0.76
	F_1	12.16	13.21	14.53	13.30±0.69
	F_2	11.94	12.95	14.18	13.02±0.65
	Mean ± SE	12.19±0.15	13.33±0.26	14.60±0.27	

Table-45: Effects of different doses of MMS and ACM-D on the larval period in different crossess of *Epilachna vigintioctopunctata*

	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	12.16	13.02	13.88	13.02±0.50
ACM-D♂ x control♀	11.93	11.82	11.91	11.89±0.03
MMS♂ x ACM-D♀	13.24	14.34	15.28	14.29±0.59
ACM-D♂ x MMS♀	12.9	13.68	15.08	13.89±0.64
MMS+ACM-D♂ x control♀	12.19	13..33	14.6	13.40±0.99

Data obtain from table-46 showing the effects of different doses of MMS and ACM-D on the pupal period in different generations. Results demonstrated that the pupal period changed with the increased of doses MMS and ACM-D induced. The mean value of pupal period of F_1 generation significntly differed with F_2 generations. In case of doses it was observed that mean of pupal period of 1.0ml dose differed significantly with 3.0ml doses. When the ACM-D treated males were crossed with control females no significant differences were observed between the generations and doses. In cross of MMS♂ x ACM-D♂ the mean value of pupal period within generations showed parental generation significntly differed with F_2 generation.Among the doses of 1.0ml dose of the pupal period differed significantly with 2.0ml and 3.0ml doses.

When the ACM-D treated males were crossed with MMS treated females the mean value of pupal period within generations showed, parental generation significntly differed with F_2 generations. In case of doses it was observed that all doses differ significantly with each other doses.

In cross of MMS+ACM-D♂ x control♀, the pupal period within generation showed no significant differences.In case of doses the mean value of pupal period of 1.0ml, 2.0ml and 3.0ml doses differed significantly among themselve.

Effects on different doses of MMS and ACM-D on the pupal period in different crosses. It was observed that the mean pupal period differed significantly within crosses. Mean the pupal period induced by the cross ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x control, MMS♂ x ACM-D♂, ACM-D♂ x MMS♀, MMS+ACM-D♂ x Control♀.The mean of the pupal period within doses 1.0ml dose differed significantly with 2.0ml and 3.0ml doses (table-47).

Table-46: Effects of different doses of MMS and ACM-D on the pupal period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	4.19	5.3	6.17	5.22±0.57
	F_1	4.15	5.18	5.96	5.10±0.52
	F_2	4.08	4.97	5.8	4.95±0.50
	Mean ± SE	4.14±0.03	5.15±0.10	5.98±0.11	
ACM-D♂ x control♀	Parental	4.08	4.03	4.06	4.06±0.01
	F_1	4.09	4.1	4.03	4.07±0.02
	F_2	4.02	3.95	4.06	4.01±0.03
	Mean ± SE	4.06±0.02	4.03±0.04	4.05±0.01	
MMS♂ x ACM-D♀	Parental	5.22	6.75	7.44	6.47±0.66
	F_1	5.05	6.2	7.16	6.14±0.61
	F_2	4.92	6.02	6.92	5.95±0.58
	Mean ± SE	5.06±0.09	6.32±0.22	7.17±0.15	
ACM-D♂ x MMS♀	Parental	5.06	6.01	7.35	6.14±0.67
	F_1	4.91	5.74	7.05	5.90±0.62
	F_2	4.71	5.12	6.84	5.56±0.65
	Mean ± SE	4.89±0.10	5.62±0.26	7.08±0.15	
MMS+ACM-D♂ x control♀	Parental	4.8	5.74	7.13	5.89±0.68
	F_1	4.42	5.29	6.69	5.47±0.66
	F_2	4.12	5.06	6.16	5.11±0.59
	Mean ± SE	4.45±0.20	5.36±0.20	6.66±0.28	

Table-47: Effects of different doses of MMS and ACM-D on the pupal period in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂x control♀	4.14	5.15	5.98	5.09±0.53
ACM-D♂ x control♀	4.06	.4.03	4.05	4.06±0.00
MMS♂ x ACM-D♀	5.06	.6.32	7.14	6.10±0.85
ACM-D♂ x MMS♀	4.89	5.62	7.08	5.86±0.64
MMS+ACM-D♂ x control♀	4.45	5.36	6.66	5.49±0.64

Table-39 summarized the data showing the effect of 1.0ml dose of MMS and ACM-D on the logevity of adult males in different crosses. The analysis of variance within crosses showed significant differences (F=180.62, P<0.001, appendix table-CXX) with LSD values as 0.58 at 5% and as 1.27 at 0.1% probability level. The logevity of adult males induced by the cross ACM-D♂ x control♀ differed significantly with other crosses ***i.e.*** MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. Considering the case of generations the analysis of variance showed significant differences (F=6.56, P<0.05, appendix table-CXX) with LSD values as 0.75 at 5% probability level. It demonstrated that the logevity of adult males showed differ significantly with each other generation irrespective crosses (Table-39 and figure-65).

At 2.0ml dose of MMS and ACM-D, the F value has been showed differed significantly within crosses as 89.63 (P<0.001, appendix table-CXXI) with LSD values as 1.23 at 5% and as 2.76 at 0.1% level. Which revealed that the logevity of adult males observed in cross of MMS+ACM-D♂ x control♀ differed significantly with MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses and the logevity of adult males showed in MMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly among themselves in table-40.The analysis of varience within generations showed nonsignificant differences (appendix table-CXXI and figure-66).

Data obtained from table-41, which deals with the effect of 3.0ml dose of MMS and ACM-D, the analysis of varience among the crosses showed highly significant differences (F=600.70, P<0.001, appendix table-CXXII) with LSD values as 0.65 at 5% and as 1.42 at 0.1% level. The longevity of adult males by the cross MMS♂ x ACM-D♂ differed significantly with other treated crossess MMS♂ x control♀, ACM-D♂ x control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed significant differences (F=26.05, P<0.001 appendix table-CXXII) with LSD value as 0.84 at 5% and as 1.84 at 0.1% probability level (figure-67).

Table-48 summarized the data showing the effects of different doses of MMS and ACM-D on the logevity of adult males in different generations. Results demonstrated that the longevity of adult males changes to increase with the dose MMS and ACM-D induced. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix tables CXXIII-CXXVII. In case of MMS♂ x control♀, the F value within generations showed differed significantly as 14.46 ($P<0.05$, appendix table- CXXIII) with LSD value as 0.82 at 5% level. The analysis of varience showed highly significant differences within doses ($F=417.17$, $P<0.001$, appendix table-CXXIII) with LSD values as 0.82 at 5% and as 2.53 at 0.1% probability level. The mean value of the longevity of adult males of parental generation also showed significntly differed with F_1 and F_2 generations.

When ACM-D treated males were crossed with control females on the longevity of adult males, the anlysis of variences between the generations and doses showed no significant differences (appendix table-CXXIV).In cross of MMS♂ x ACM-D♂ the analysis of variances showed significant differences within generations ($F=13.34$, $P<0.05$, appendix table-CXXV) with LSD value as 1.1 at 5% level of respectively.But the analysis of variens within doses showed significant differences ($F=159$, $P<0.001$, appendix table-CXXV) with LSD values as 1.1 at 5% and as 2.87 at 0.1% probability level (table-48).

When the ACM-D treated males were crossed with MMS treated females the analysis of variances showed significant differences within generations ($F=24.58$, $P<0.01$, appendix table-CXXVI) with LSD values as 0.77 at 5% and as 1.3 at 1% respectively. The analysis of variances within doses showed significant differences ($F=279$, $P<0.001$, appendix table-CXXVI) with LSD values as 0.77 at 5% and as 2.44 at 0.1% probability level. In cross of MMS+ACM-D♂ x control♀, the analysis of varience has been calculated within generation and showed significant differences ($F=14.36$, $P<0.05$, appendix table-CXXVII) With LSD value as 0.85 at 5% respectively. The analysis of variens within doses showed significant differences ($F=419.64$, $P<0.001$, appendix table-CXXVII) with LSD values as0.85 at 5% and as 2.63 at 0.1% level of significance.

Effects of different doses of MMS and ACM-D on the longevity of adult males in different crosses are shown in table-49. It was observed that mean of longevity of adult males showed differed significantly within crosses (F=18.11, P<0.01 appendix table-CXXVIII) with LSD values as 2.76 at 5% and as 4.02 at 1% probability level. The longevity of adult males induced by the cross MMS♂ x ACM-D♂ differed significantly with other crosses MMS♂ x control, ACM-D♂ x control♀, ACM-D♂ x MMS♀, and MMS+ACM-D♂ x control♀. The mean of the longevity of adult males differed significantly within doses (F=13.08, P<0.01, appendix table-XXXXVII) with LSD values as 3.57 at 5% and as 5.19 at 1% probability level. The longevity of adult males descreased induced by MMS dose and 3.0ml MMS dose differed significantly with 1.0ml and 2.0ml doses of MMS (figure-68).

Table-48: Effects of different doses of MMS and ACM-D on the longevity of adult males in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂x control♀	Parental	115.59	112.33	106.69	111.54±2.60
	F_1	116.03	113.42	107.54	112.33±2.51
	F_2	116.41	114.21	108.79	113.14±2.27
	Mean ± SE	116.01±0.24	113.32±0.55	107.67±0.61	
ACM-D♂ x control♀	Parental	120.57	121.82	120.56	120.98±0.42
	F_1	120.77	120.2	120.39	120.45±0.17
	F_2	120.19	120.03	121.2	120.47±0.37
	Mean ± SE	120.51±0.17	120.68±0.57	120.72±0.25	
MMS♂ x ACM-D♀	Parental	111.9	107.93	104.17	108.00±2.23
	F_1	112.47	107.95	105.72	108.71±1.99
	F_2	113.28	109.84	106.87	110.00±1.85
	Mean ± SE	112.55±0.40	108.57±0.63	105.59±0.78	
ACM-D♂xMMS♀	Parental	112.67	109.72	105.53	109.31±2.07
	F_1	113.56	110.81	106.7	110.36±2.00
	F_2	113.9	111.94	108.02	111.29±1.73
	Mean ± SE	113.38±0.37	110.82±0.64	106.75±0.72	
MMS+ACM-D♂ x control♀	Parental	114.76	109.9	105.75	110.14±2.61
	F_1	115.49	110.76	106.24	110.83±2.67
	F_2	116.21	111.12	107.97	111.77±2.40
	Mean ± SE	115.49±0.42	110.59±0.36	106.65±0.67	

Table-49: Effects of different doses of MMS and ACM-D on the longevity of adult males in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	116.01	113.32	107.67	112.33±2.46
ACM-D♂ x control♀	120.51	120.68	120.72	120.64±0.06
MMS♂ x ACM-D♀	112.55	108.57	105.59	108.90±2.02
ACM-D♂ x MMS♀	113.38	110.82	106.75	110.32±1.93
MMS+ACM-D♂ x Control♀	115.49	110.59	106.65	110.91±2.56

Table-39 summarized the data on the longevity of adult females following the effect of 1.0ml dose of MMS and ACM-D. The analysis of variance within crosses showed highly significant ($F=32.03$, $P<0.001$, appendix table-CXXIX) differences with LSD values as 0.77 at 5% and as 1.69 at 0.1% probability level.The longevity of adult females induced by the cross ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS +ACM-D♂ x control♀.

The analysis of variance has been calcullated within generations and showed significant differences ($F=8.14$, $P<0.05$, appendix table-CXXIX) with LSD value as 0.1 at 5%, level of significance in table-39 and figure-69.

Data obtain from table-40 effect of 2.0 ml dose of MMS and ACM-D following on the longevity of adult females the analysis of variences within crosses and showed highly significant differences ($F=56.78$, $P<0.001$,appendix table-CXXX) with LSD value as 0.99 at 5% and 2.16 at 0.1% probability level. The longevity of adult females induced by the cross ACM-D♂ x control♀ differed significantly with other crosses MMS♂ x control♀, MMS♂ x ACM-D♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. The longevity of adult females the anlysis of variences been calculated within generations and showed significant differences ($F=9.96$, $P<0.01$, appendix table-CXXX) with LSD value as 1.28 at 5% and as 1.86 at 1% probability level. The mean value of the longevity of adult females of parental generation significntly differed with F_2 generation in figure-70.

Data of table-41, which deals with the effect of 3.0ml dose of MMS and ACM-D, the analysis of variences showed significant differences among the crosses ($F=424.05$, $P<0.001$, appendix table-CXXXI) with LSD values as 0.64 at 5%, and 1.39 at 0.1% level. The longevity of adult females by the cross of MMS♂ x ACM-D♂ differed significantly with other treated cross MMS♂ x control♀, ACM-D♂ x control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. The analysis of variance within generations showed significant differences ($F=31.61$, $P<0.001$, appendix table -CXXXI) with LSD values as 0.82 at 5%, and as 1.79 at 0.1% probability level in figure-71.

Table-50 summarized the data showing the effects on different doses of MMS and ACM-D on the longevity of adult females in different generations. Results demonstrated that the longevity of adult females changes with the increase of MMS doses and ACM-D. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix tables CXXXII-CXXXVI. In case of MMS ♂ X Control ♀, the analysis of variance within generations has been calculated and showed significant differences ($F=70$, $P<0.001$, appendix table-CXXXII) with LSD values as 0.39 at 5%, and as 1.22 at 0.1% level. The analysis of varians within doses showed highly significant differences ($F=1438$, $P<0.001$, appendix table-CXXXII) with LSD values as 0.39 at 5% and as 1.22 at 0.1% level of significance. In case of doses it was observed that means of of the longevity of adult females of all doses differed significantly with each other in table-50.

When ACM-D treated males were crossed with control females the analysis of variences showed no significant differences between the generations and doses (appendix table-CXXXIII).

In cross of MMS♂ x ACM-D♂ the analysis of variances has been calculated and showed significant differences within generations ($F=59.67$, $P<0.01$, appendix table-CXXXIV) with LSD values as 0.56 at 5%, and 0.92 at 1% level.But within doses the analysis of variances showed significant differences ($F=682.66$, $P<0.001$, appendix table-CXXXIV) with LSD values as 0.56 5% and as 1.72 1% respectively level. The longevity of adult females 3.0ml doses of MMS differed significantly with other doses of MMS.

When the ACM-D treated males were crossed with MMS treated females the analysis of variances also showed significant differences within generations ($F=24$, $P<0.01$, appendix table-CXXXV) with LSD values as 1.06 at 5% and as 1.76 at 1% respectively level. The F values within doses showed highly differed significantly ($F=259.95$, $P<0.001$, appendix table-CXXXV) With LSD values as 1.06 at 5% and as 3.30 at 0.1% level of significant. In cross of MMS+ACM-D♂ x control♀, the analysis of variances within generation has been calculated and showed significant differences ($F=11.25$,

P<0.05, appendix table-CXXXVI) with LSD value 1.34 at 5% respectively level. F value within doses has been calculated and showed significant differences (F=184.88, P<0.001, appendix table-CXXXVI) with LSD values as 1.34 at 5% and as 4.16 at 0.1% level of significant.

Effects of different doses of MMS and ACM-D on the longevity of adult females in different crosses are shown in table-51. It was observed that mean longevity of adult females differed significantly within crosses (F=7.71, P<0.05, appendix table-CXXXVII), with LSD value as 2.91 at 5% level.The observed mean the longevity of adult females induced by the cross ACM-D♂ x MMS♀ differed significantly with other crosses like MMS♂ x control♀, ACM-D♂ x control♀, MMS♂ x ACM-D♀, and MMS+ACM-D♂ x control♀. The mean of the longevity of adult females differed significantly within doses (F=13.36, P<0.01, appendix table-CXXXVII) with LSD values as 3.75 at 5% and as 5.45 at 1% probability level.The longevity of adult females decreases induced by MMS doses. Also 3.0ml MMS doses differed significantly with other MMS doses (table-51 and figure-72).

Table-50: Effects of different doses of MMS and ACM-D on the longevity of adult females in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of MMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	Parental	109.16	106.96	101.56	105.89±2.26
	F_1	109.84	107.68	102.34	106.62±2.23
	F_2	110.53	108.6	103.55	107.56±2.08
	Mean ± SE	109.84±0.40	107.75±0.48	102.48±0.58	
ACM-D♂ x control♀	Parental	112.56	112.41	112.21	112.39±0.10
	F_1	111.82	112.57	112.41	112.27±0.23
	F_2	112.13	112.39	113.05	112.52±0.27
	Mean ± SE	112.17±0.21	112.46±0.06	112.56±0.25	
MMS♂ x ACM-D♀	Parental	106.75	103.2	98.97	102.97±2.25
	F_1	107.2	104.51	100.25	103.99±2.03
	F_2	108.67	105.49	101.31	105.16±2.13
	Mean ± SE	107.54±0.58	104.40±0.66	100.18±0.68	
ACM-D♂x MMS♀	Parental	107.82	105.17	99.17	104.05±2.56
	F_1	108.9	107.94	100.63	105.82±2.61
	F_2	109.92	108.1	101.93	106.65±2.42
	Mean ± SE	108.88±0.61	107.07±0.95	100.58±0.80	
MMS+ACM-D♂ x control♀	Parental	109.68	105.76	99.85	105.10±2.86
	F_1	110.27	108.04	101.1	106.47±2.76
	F_2	111.31	107.94	102.87	107.37±2.46
	Mean ± SE	110.42±0.48	107.25±0.74	101.27±0.88	

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Table-51: Effects of different doses of MMS and ACM-D on the longevity of adult females in different crosses of *Epilachna vigintioctopunctata*

	Dose of MMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
MMS♂ x control♀	109.84	107.75	102.48	106.69±2.19
ACM-D♂ x control♀	112.17	112.46	112.56	112.40±0.12
MMS♂ x ACM-D♀	107.54	104.4	100.18	104.04±2.13
ACM-D♂ x MMS♀	108.88	107.07	100.58	105.51±2.52
MMS+ACM-D♂ x control♀	110.42	107.25	101.27	106.31±2.69

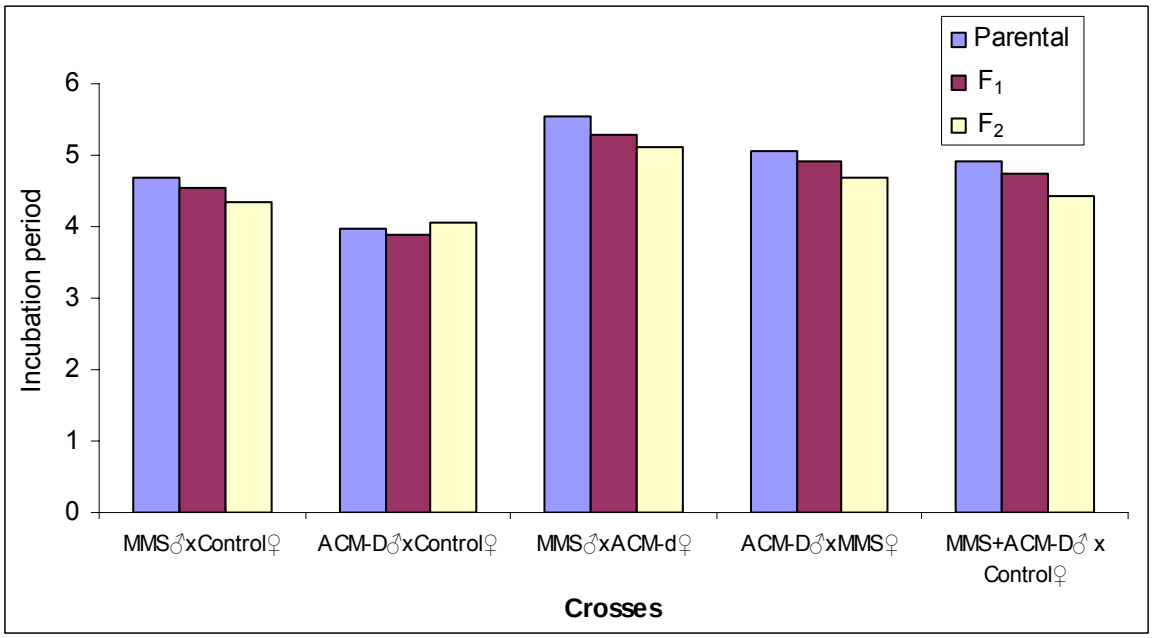


Figure 61: Effects of 1.0ml MMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

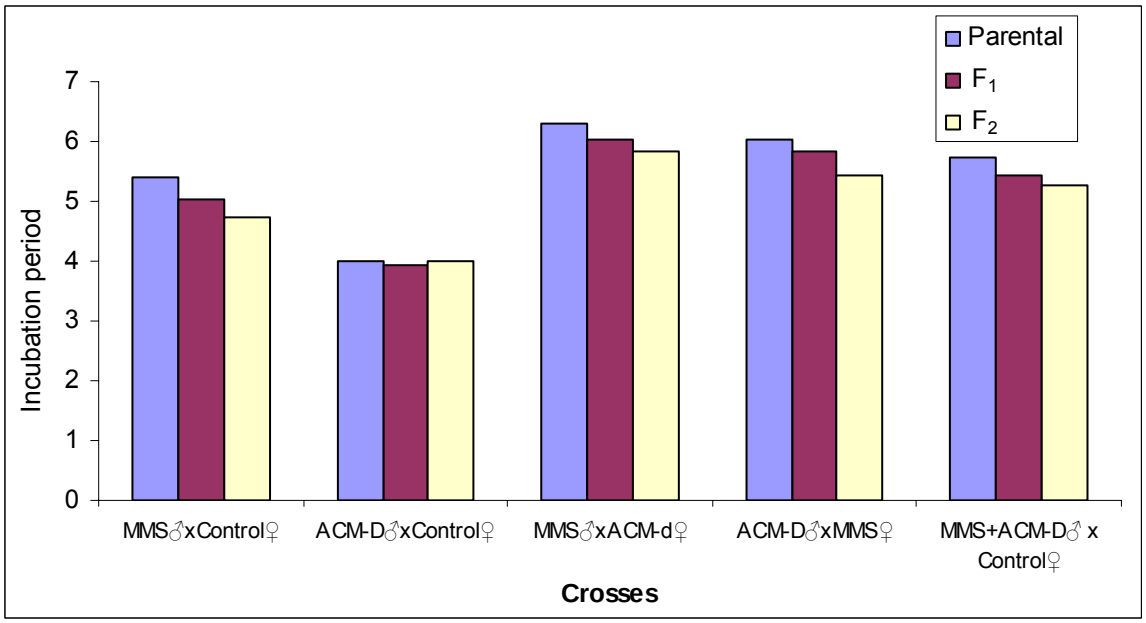


Figure 62: Effects of 2.0ml MMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

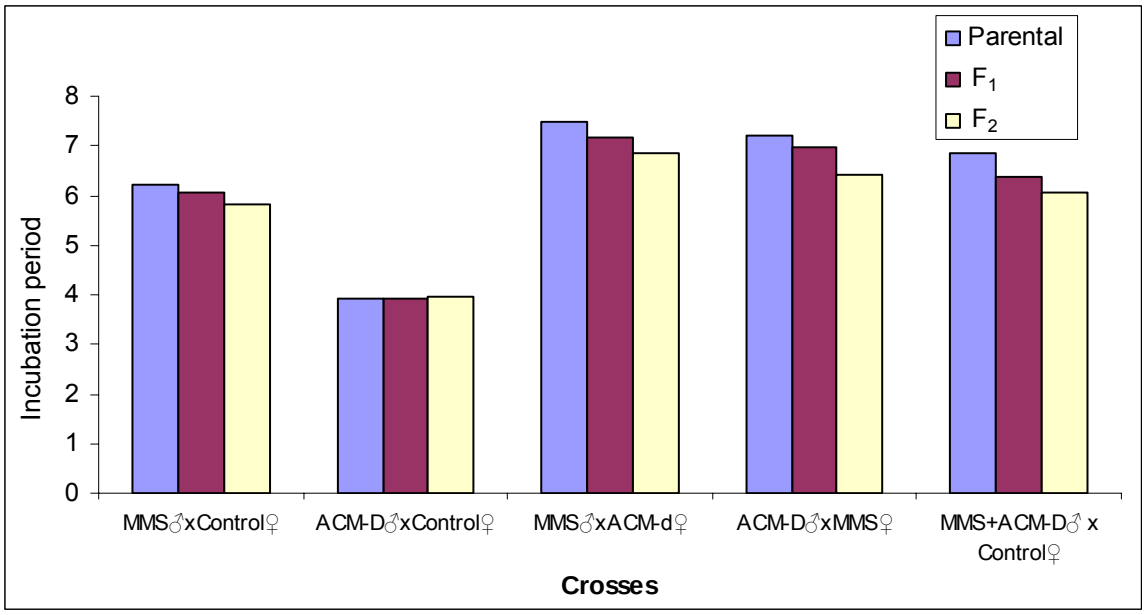


Figure 63: Effects of 3.0ml MMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

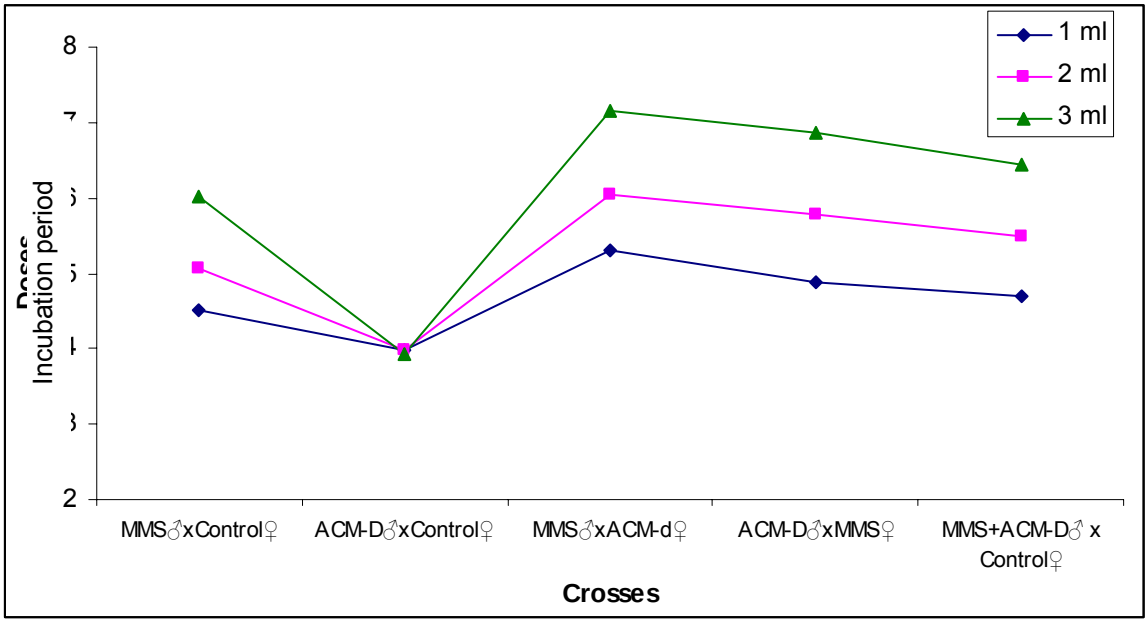


Figure 64: Effects of different doses of MMS and ACM-D on incubation period in different crosses of *Epilachna vigintioctopunctata*

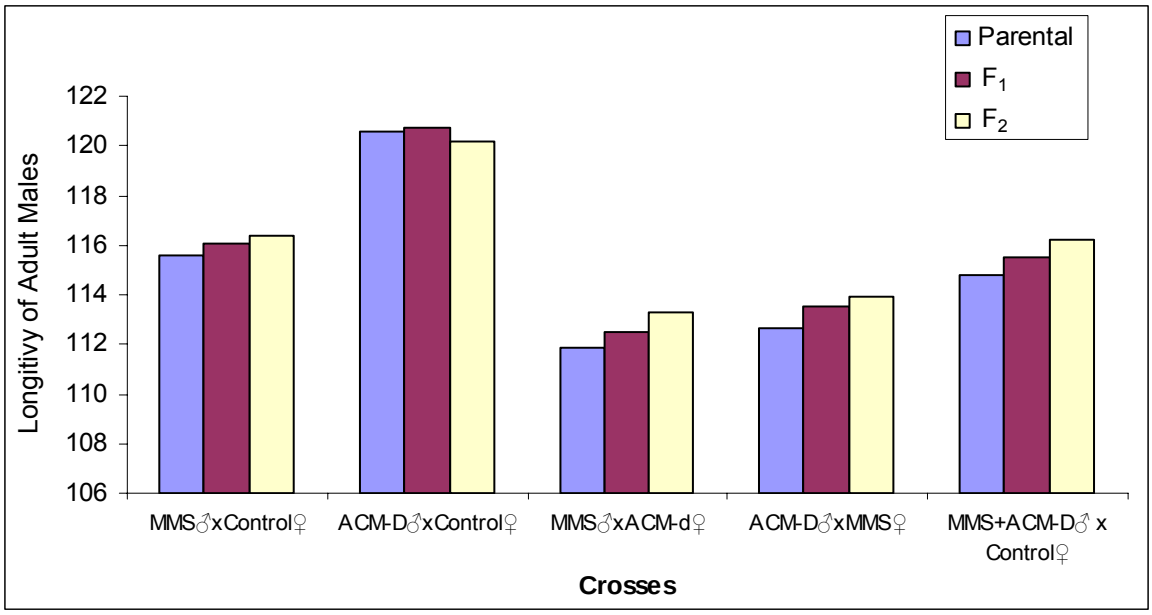


Figure 65: Effects of 1.0ml MMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

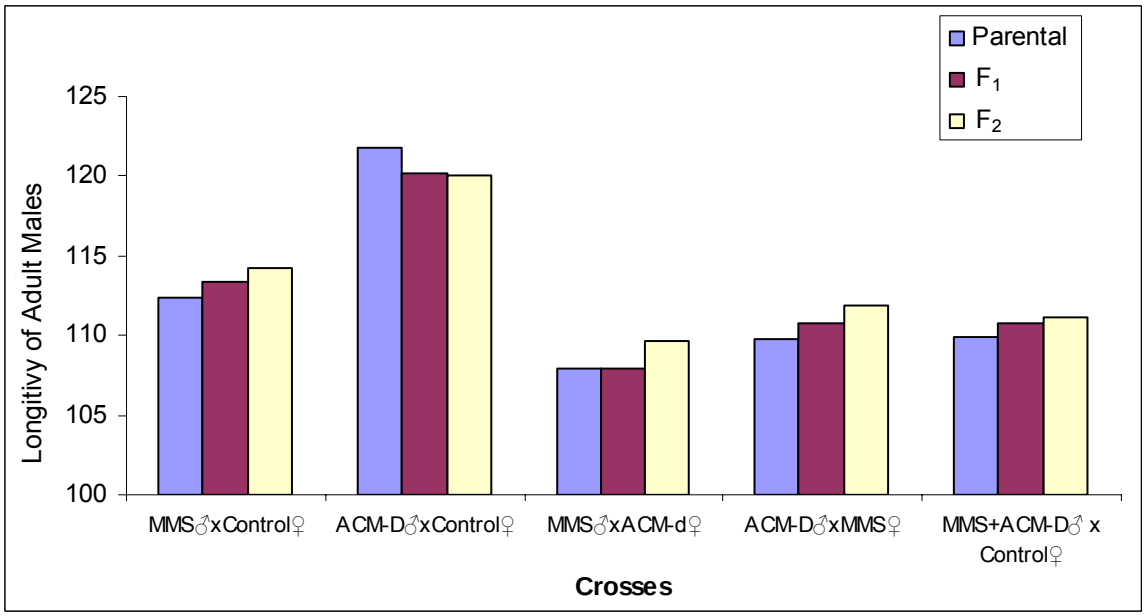


Figure 66: Effects of 2.0ml MMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

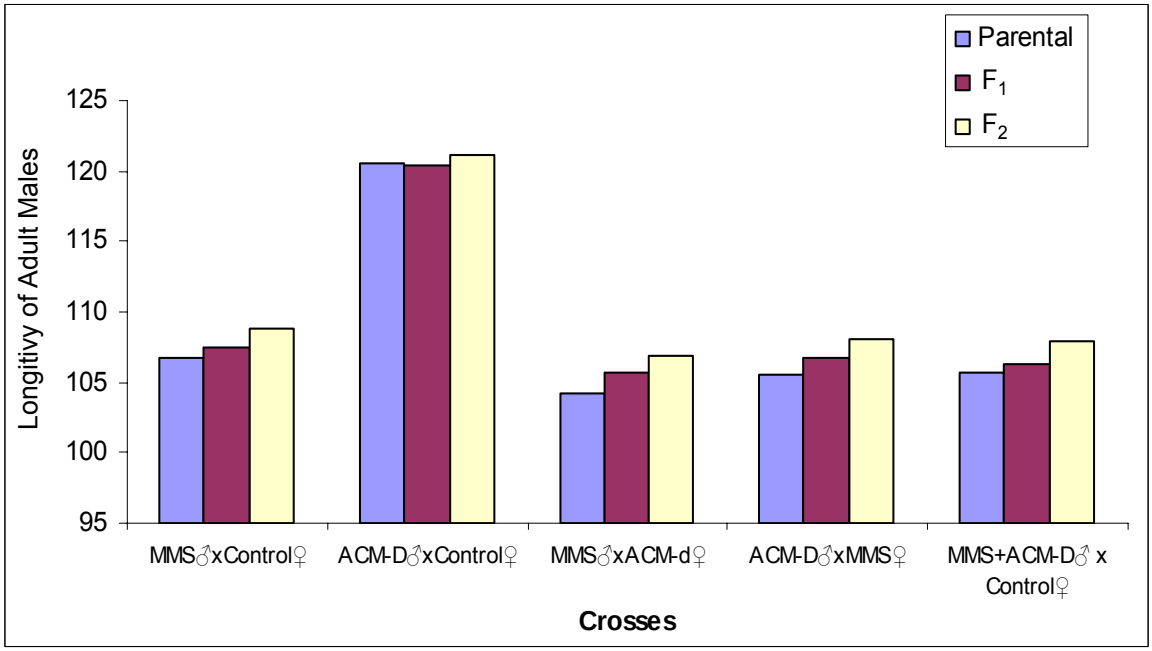


Figure 67: Effects of 3.0ml MMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

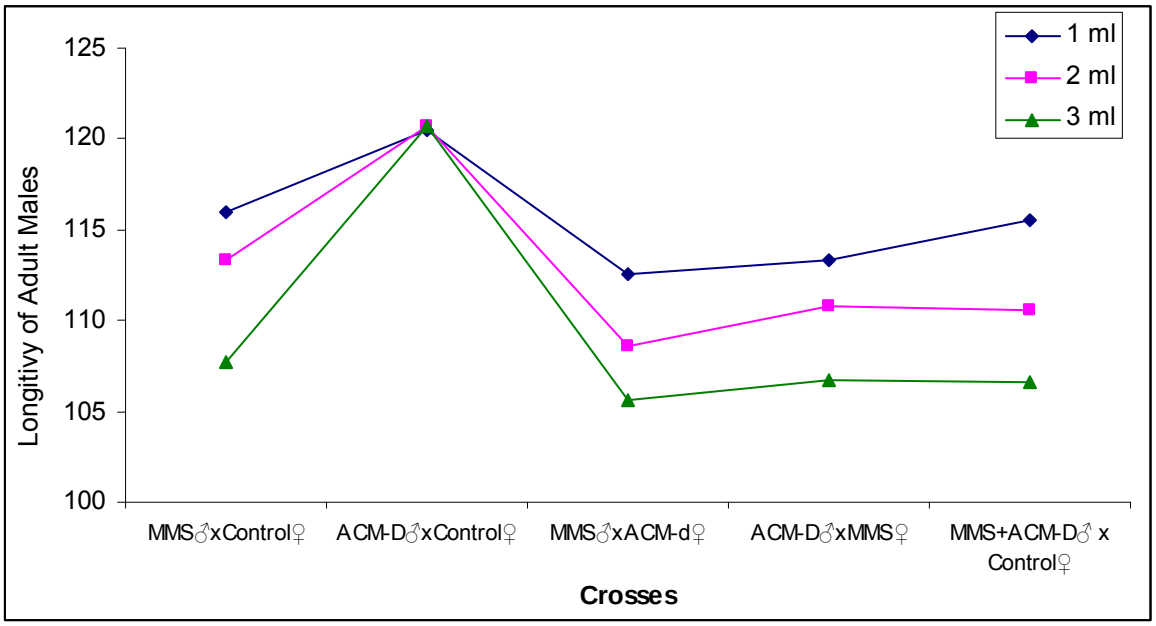


Figure 68: Effects of different doses of MMS and ACM-D on the longevity of adult males in different crosses of *Epilachna vigintioctopunctata*

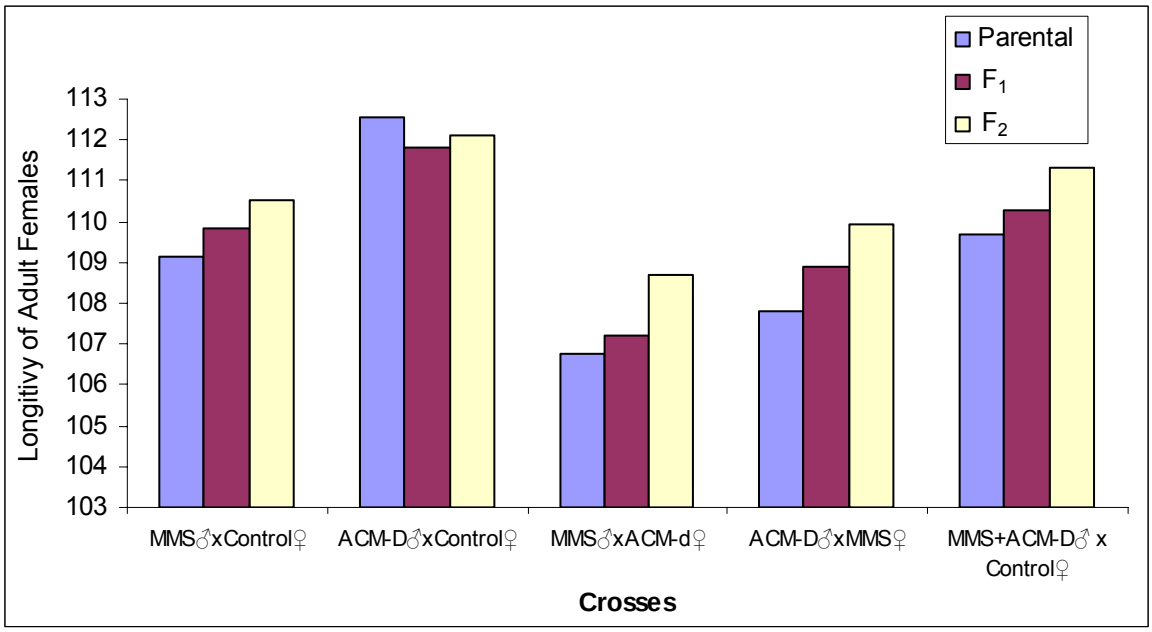
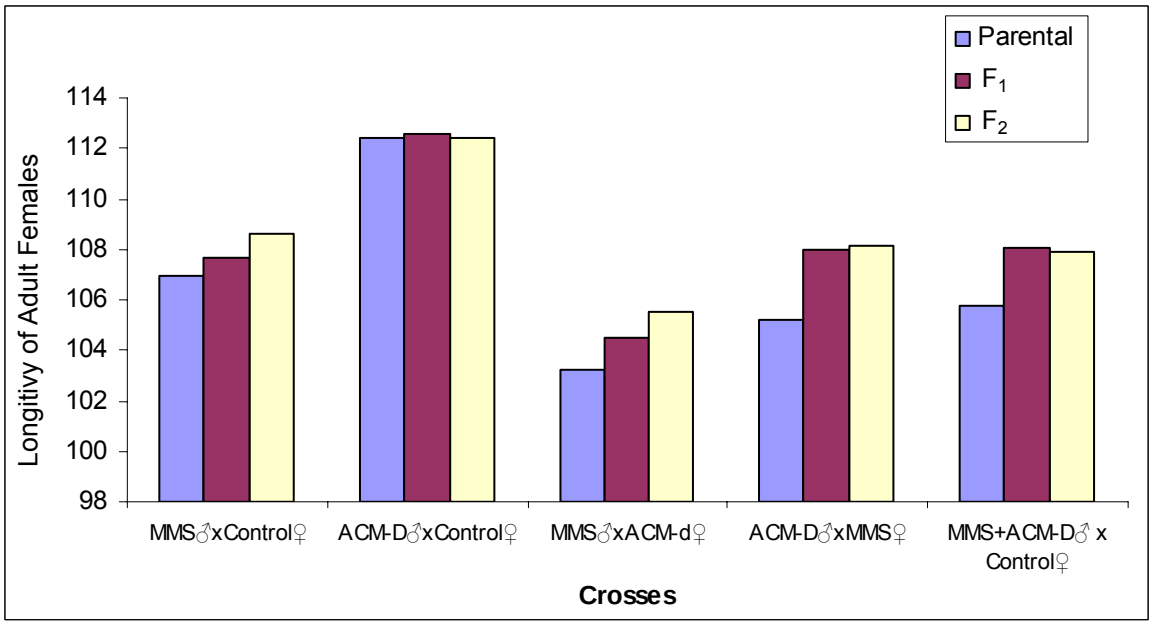


Figure 69: Effects of 1.0ml MMS and ACM-D on the longevity of adult females in different crosses and generations of *Epilachna vigintioctopunctata*



Figur-70: Effects of 2.0ml MMS and ACM-D on the longevity of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

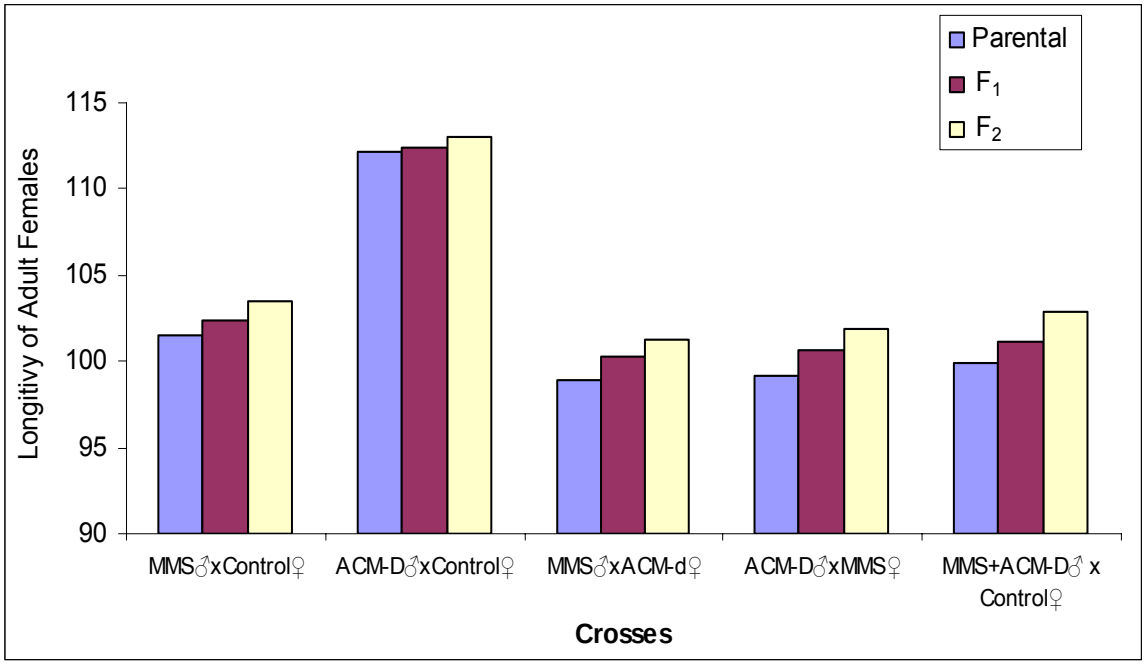
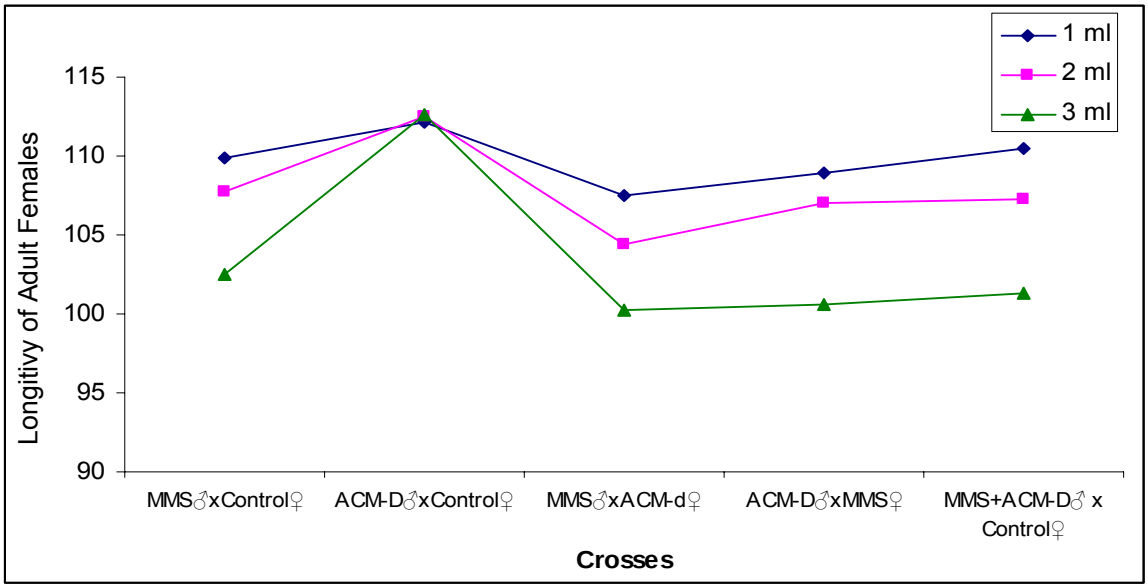


Figure-71: Effects of 3.0ml MMS and ACM-D on the longevity of adult females in different crosses and generations of *Epilachna vigintioctopunctata*



Figur-72: Effects of different doses of MMS and ACM-D on the longevity of adult females in different crosses of *Epilachna vigintioctopunctata*

4.3.2 Effects of ethyl methanesulfonate (EMS) and actinomycin-D (ACM-D) on the incubation, larval, and pupal period, and longevity of adult males female.

Effects of different dose of EMS with ACM-D on the incubation, larval, and pupal period, and longevity of adult males and females were determined at different crosses and are shown in tables 52-64, figures73-84 and appendix tables CXXXVIII-CLXIV.

Table-52 summarized the data 1.0ml dose of EMS on the incubation period in different crosses. The analysis of variance within crosses showed significant (F=24.00, P<0.001, appendix table-CXXXVIII) differences with LSD values as 0.21 at 5% and as 0.45 at 0.1% probability level. The incubation period induced by the cross MMS♂ x control♀ differed significantly with other crosses MMS♂ x ACM-D♀, ACM-D♂ x Control♀, ACM-D♂ x MMS♀ and MMS+ACM-D♂ x control♀. Considering the case of generations it was found that the analysis of variance showed significant differences (F=7.5, P<0.05, appendix table-CXXXVIII) with LSD value as 0.27 at 5% level. It demonstrated that the incubation period differed significantly with each other generations irrespective crosses (table -52 and figure-73).

Effects of 2.0ml dose of EMS and ACM-D, the F value has been showed differed significantly within crosses as 65.50 (P<0.001, appendix table-CXXXIX) with LSD values as 0.21 at 5% and as 0.45 at 0.1% level. Which revealed that the incubation period in cross of EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ crosses and the incubation period found in EMS♂ x control♀ and ACM-D♂ x control♀ also differed significantly. In case case of generations the analysis of variance showed significant diferances (F=12.00, P<0.01, appendix table-CXXXIX) with LSD values as 0.27 at 5% and 0.39 at 1%, probability level in table-53 and figure-74.

Data obtain from table-54 at 3.0ml dose of EMS and ACM-D the analysis of variance within crosses showed significant differences (F=66.80, P<0.001, appendix table-CXXXX) with LSD values as 33 at 5% and as 0.71 at 0.1% level. The mean of the incubation period by the cross ACM-D♂ x EMS♀ differed significantly with other treated crossess EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀ and EMS+ACM-D♂ x control♀. The analysis of variance within generations showed significant differences (F=6.60, P<0.05, appendix table-CXXXX) with LSD value as 0.42 at 5% level. It was observed that the incubation period differed significantly with each other generations in figure-75.

Table-52: Effects of 1.0ml EMS and ACM-D on the incubation, larval and pupal period and longevity of Adults in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
Control♂ x control♀	Parental	3.65	11.21	4.05	121.22	113.25
	F_1	3.25	11.02	3.98	122.24	114.22
	F_2	3.32	10.58	4.11	122.56	112.54
EMS♂ x control♀	Parental	4.68	12.55	4.19	115.59	109.16
	F_1	4.21	12.09	4.16	114.74	109.78
	F_2	4.31	12.07	4.04	117.1	110.61
ACM-D♂ x control♀	Parental	3.94	11.68	4.05	120.17	112.44
	F_1	3.88	11.82	4.07	120.22	112.24
	F_2	3.98	11.76	4.01	120.46	112.38
EMS♂ x ACM-D♀	Parental	5.21	13.12	5.03	112.13	107.88
	F_1	5.03	12.95	4.97	113.16	108.14
	F_2	4.89	12.6	4.63	114.31	109.08
ACM-D♂ x EMS♀	Parental	4.78	12.78	4.95	113.58	109.33
	F_1	4.49	12.46	4.71	114.13	110.19
	F_2	4.28	12.22	4.45	115.18	110.81
EMS +ACM-D ♂ X Control ♀	Parental	4.59	12.03	4.3	115.74	110.18
	F_1	4.38	12.06	4.15	116.86	111.28
	F_2	4.1	12.03	4.11	115.62	111.94

Table-53: Effects of 2.0ml EMS and ACM-D on the incubation, larval and pupal period and longevity of Adults in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
control♂ x control♀	Parental	3.65	11.21	4.05	121.22	113.25
	F_1	3.25	11.02	3.98	122.24	114.22
	F_2	3.32	10.58	4.11	122.56	112.54
EMS♂ x control♀	Parental	5.1	13.21	5.03	113.62	107.85
	F_1	4.85	12.95	4.9	112.74	108.27
	F_2	4.38	12.31	4.57	115.01	108.98
ACM-D♂ x control♀	Parental	3.9	11.72	4.06	120.28	112.19
	F_1	3.89	11.81	4.12	121.16	112.37
	F_2	3.93	11.88	4.07	120.44	112.69
EMS♂ x ACM-D♀	Parental	6.06	14.06	6.07	109.66	104.69
	F_1	5.78	13.93	5.75	110.28	105.39
	F_2	5.46	13.5	5.55	111.07	105.86
ACM-D♂ x EMS♀	Parental	5.15	13.16	5.12	110.92	106.86
	F_1	4.89	12.71	4.93	112.2	107.44
	F_2	4.6	12.41	4.69	113.22	108.21
EMS +ACM-D ♂ x control ♀	Parental	5.03	13.02	4.94	111.17	109.6
	F_1	4.95	12.94	4.81	112.34	109.92
	F_2	4.71	12.16	4.57	113.11	110.3

Table-54: Effects of 3.0ml EMS and ACM-D on the incubation, larval and pupal period and longevity of Adults in (days) in *Epilachna vigintioctopunctata*

Cross	Generation	Incubation period	Larval period	Pupal period	Longevity of Adult Beetles	
					Male	Female
control♂ x control ♀	Parental	3.65	11.21	4.05	121.22	113.25
	F_1	3.25	11.02	3.98	122.24	114.22
	F_2	3.32	10.58	4.11	122.56	112.54
EMS♂ x control♀	Parental	5.68	14.03	6.15	107.99	102.05
	F_1	5.17	13.88	5.86	108.77	104.36
	F_2	5.06	13.57	5.66	109.32	103.6
ACM-D♂ x control♀	Parental	3.87	11.82	4.08	120.59	112.01
	F_1	3.97	11.89	4.1	120.33	112.1
	F_2	4.02	11.92	4.1	121.3	112.19
EMS♂ x ACM-D♀	Parental	7.12	15.23	7.12	105.27	99.65
	F_1	6.86	14.5	6.97	106.8	100.34
	F_2	6.1	14.61	6.57	107.21	101.14
ACM-D♂ x EMS♀	Parental	6.5	14.88	6.83	107.88	100.9
	F_1	6.25	14.26	6.29	108.84	101.26
	F_2	5.98	14.08	5.95	109.11	102.07
EMS+ACM-D♂ x control♀	Parental	6.03	14.14	6.09	106.89	101.21
	F_1	5.88	13.86	5.89	109.03	102.24
	F_2	5.49	13.6	5.56	109.07	103.45

Table-55 summarized the data showing the effects on different doses EMS and ACM-D on the incubation period in different generations. Results demonstrated that the incubation period changed to decreased with the EMS doses and ACM-D induced. The analysis of variance showing the effects within generations and doses has been calculated and are presented in appendix tables CXXXXI-CXXXXV. In case of EMS♂ x control♀, the analysis of variances within generations has been calculated and showed signifinant differences ($F=13$, $P<0.05$, appendix table-CXXXXI) with LSD value as 0.32 at 5% level and F value within doses showed as 31 ($P<0.01$, appendix table-CXXXXI) significant differences with LSD values as 0.32 at 5% and as 0.53 at 1 % probability level.

When the ACM-D treated males were crossed with control females the analysis of variance were observed between the generations and doses showed nonsignificant differences (appendix table-CXXXXII).In cross of EMS♂ x ACM-D♀ the analysis of variances showed significant differences within generations as $F=8.0$ ($P<0.05$, appendix table-CXXXIII) with LSD value 0.45 at 5% level.But F values has been calculated within doses showed significant differences as 51.25 ($P<0.01$, appendix table-CXXXXIII) with LSD values as 0.45 at 5% and as 0.75 at 1% respectively level.

When the ACM-D treated males were crossed with EMS treated females the analysis of variances showed significance differences within generations as ($F=210$, $P<0.001$, appendix table-CXXXXIV) With LSD values as 0.072 at 5% and as 0.24 at 0.1% probability level. The F values within doses showed significant differences ($F=2490$, $P<0.001$, appendix table-CXXXXIV) With LSD values as 0.072 at 5% and as 0.24 at 0.1% respectively. In cross of EMS+ACM-D♂ X control♀, the analysis of variances within generations showed significant differences ($F=53$, $P<0.01$, appendix table-CXXXXV) with LSD values 0.12 at 5%, and as 0.21 at 1 % level. F value within doses has been calculated and showed significant differences as 533 ($P<0.001$, appendix table-CXXXXV) with LSD values as 0.12 at 5%, and as 0.39 at 0.1 % probability level.

Effect on different doses of EMS and ACM-D on the incubation period in different crosses and are shown in table-56. It was observed that mean incubation period showed

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differed significantly within crosses (F=10.21, P<0.01, appendix table-CXXXXVl) with LSD values as 0.55 at 5% and as 0.79 at 1% level. The observed mean the incubation period induced by the cross ACM-D♂ x EMS♀ differed significantly with other crosses *i.e.*EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and EMS+ACM-D♂ x control♀. The mean of the incubation period showed within doses differed significantly (F=12.14, P<0.01, appendix table-CXXXXVl) with LSD values as 0.71 at 5% and as 1.03 at 1% probability level (table-56 and figure-76).

Table-55: Effects of different doses of EMS and ACM-D on the incubation period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	4.68	5.1	5.68	5.15±0.29
	F_1	4.21	4.85	5.17	4.74±0.28
	F_2	4.31	4.38	5.06	4.58±0.24
	Mean ± SE	4.40±0.14	4.78±0.21	5.30±0.19	
ACM-D♂ x control♀	Parental	3.94	3.9	3.87	3.90±0.02
	F_1	3.88	3.89	3.97	3.91±0.03
	F_2	3.98	3.93	4.02	3.98±0.03
	Mean ± SE	3.93±0.03	3.91±0.01	3.95±0.04	
EMS♂ x ACM-D♀	Parental	5.21	6.06	7.12	6.13±0.55
	F_1	5.03	5.78	6.86	5.89±0.53
	F_2	4.89	5.46	6.1	5.48±0.35
	Mean ± SE	5.04±0.09	5.77±0.17	6.69±0.31	
ACM-D♂ x EMS♀	Parental	4.78	5.15	6.5	5.48±0.52
	F_1	4.49	4.89	6.25	5.21±0.53
	F_2	4.28	4.6	5.98	4.95±0.52
	Mean ± SE	4.52±0.15	4.88±0.16	6.24±0.15	
EMS+ACM-D♂ x control♀	Parental	4.59	5.03	6.03	5.22±0.43
	F_1	4.38	4.95	5.88	5.07±0.44
	F_2	4.1	4.71	5.49	4.77±0.40
	Mean ± SE	4.36±0.14	4.90±0.10	5.80±0.16	

Table-56: Effect of different doses of EMS and ACM-D on the incubation period in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	4.40	4.78	5.30	4.83±0.26
ACM-D♂ x control♀	3.93	3.91	3.95	3.93±0.01
EMS♂ x ACM-D♀	5.04	5.77	6.69	5.83±0.48
ACM-D♂ x EMS♀	4.52	4.88	6.24	5.21±0.52
EMS+ACM-D♂ x control♀	4.36	4.9	5.8	5.02±0.42

Data obtain from table-57 showing the effects on different doses of EMS and ACM-D on the larval period in different generations. Results demonstrate that the larval period changs to increase with the doses of EMS and ACM-D induced. The mean value of larval period in parantal generation significntly differed with F_1 and F_2 generations but F_1 and F_2 generation no significant differences. In case of doses it was observed that means larval period of 1.0ml dose differed significantly with 2.0ml dose. When the ACM-D treated males were crossed with control females non significant differences were observed between the generations and doses. In cross of EMS♂ x ACM-D♂ the mean value of larval period within parental generation showed significntly differs with F_2 generations.The mean value of larval period within doses of 1.0ml dose differed significant with other doses.

When the ACM-D treated males were crossed with EMS treated females the mean value of larval period within generation F_1 generation significntly differed with F_2 generations. In case of doses it was observed that 2.0ml doses differed significantly with 3.0ml doses.In cross of EMS+ACM-D♂ x control♀, within generation showed parental generation significantly differed with F_2 generation.In case of doses 1.0ml dose differed significantly with 3.0ml dose.

Effects of different doses of EMS and ACM-D on the larvl period in different crosses are shown in table-58. It was observed that mean larval period showed differ significantly within crosses. The observed mean the larval period induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀, EMS+ACM-D♂ x control♀.The mean of the larval period within doses shows 1.0ml dose differed significantly with 3.0ml doses (table-58).

Table-57: Effect of different doses of EMS and ACM-D on the larval period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	12.55	13.21	14.03	13.26±0.43
	F_1	12.09	12.95	13.88	12.97±0.52
	F_2	12.07	12.31	13.57	12.65±0.47
	Mean ± SE	12.24±0.16	12.82±0.27	13.83±0.14	
ACM-D♂ x control♀	Parental	11.68	11.72	11.82	11.74±0.04
	F_1	11.82	11.81	11.89	11.84±0.03
	F_2	11.76	11.88	11.92	11.85±0.05
	Mean ± SE	11.75±0.04	11.80±0.05	11.88±0.03	
EMS♂ x ACM-D♀	Parental	13.12	14.06	15.23	14.14±0.61
	F_1	12.95	13.93	14.5	13.79±0.45
	F_2	12.6	13.5	14.61	13.57±0.58
	Mean ± SE	12.89±0.15	13.83±0.17	14.78±0.23	
ACM-D♂ x EMS♀	Parental	12.78	13.16	14.88	13.61±0.65
	F_1	12.46	12.71	14.26	13.14±0.56
	F_2	12.22	12.41	14.08	12.90±0.59
	Mean ± SE	12.49±0.16	12.76±0.22	14.41±0.24	
EMS+ACM-D♂ x control♀	Parental	12.03	13.02	14.14	13.06±0.61
	F_1	12.06	12.94	13.86	12.95±0.52
	F_2	12.03	12.16	13.6	12.60±0.50
	Mean ± SE	12.04±0.01	12.71±0.27	13.87±0.16	

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Table-58: Effect of different doses of EMS and ACM-D on the larval period in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	12.24	12.82	13.83	12.96±0.47
ACM-D♂ x control♀	11.75	11.8	11.88	11.81±0.04
EMS♂ x ACM-D♀	12.89	13.83	14.78	13.83±0.55
ACM-D♂ x EMS♀	12.49	12.76	14.41	13.22±0.60
EMS+ACM-D♂ x control♀	12.04	12.71	13.87	12.87±0.54

Data obtained from table-59 showing the effects of different doses of EMS and ACM-D on the pupal period in different generations. Results demonstrated that the pupal period changs to increase with the doses of EMS and ACM-D induced. The mean value of pupal period of F_2 generation significntly differed with parantal generation. In case of doses it was observed that means of pupal period of 1.0 ml dose differed significantly with 3.0ml dose. When the ACM-D treated males were crossed with control females showed nonsignificant differences between the generations and doses. In cross of EMS♂ x ACM-D♂ the mean value of pupal period within generations showed parental generation significntly differed with F_2 generation.In case of doses the pupal period of 1.0 ml dose differed significant with 2.0ml and 3.0ml doses.

When the ACM-D treated males were crossed with EMS treated females the mean value of pupal period within generations showed nonsignificnt difference. In case of doses it was observed that 1.0ml dose differed significantly with 2.0ml dose.In cross of EMS+ACM-D♂ x control♀, the pupal period within generations showed parental generation significantly differed with F_2 generations.In case of doses it was observed that 1.0ml dose EMS differed significant with 3.0ml dose.

Effects on different doses of EMS and ACM-D on the pupal period in different crosses are shown in table-60. It was observed that mean pupal period differed significantly within crosses. The observed mean the pupal period induced by the cross ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, but EMS♂ x control♀ showed nonsignificant differences with EMS+ACM-D♂ x control♀.The mean of the pupal period within doses it was observed that 1.0ml dose of EMS differed significantly with 3.0ml dose of EMS in table-60.

Table-59: Effect of different doses of EMS and ACM-D on the pupal period in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	4.19	5.03	6.15	5.12±0.57
	F_1	4.16	4.9	5.86	4.97±0.49
	F_2	4.04	4.57	5.66	4.76±0.48
	Mean ± SE	4.13±0.05	4.83±0.14	5.89±0.14	
ACM-D♂ x control♀	Parental	4.05	4.06	4.08	4.06±0.01
	F_1	4.07	4.12	4.1	4.10±0.01
	F_2	4.01	4.07	4.1	4.06±0.03
	Mean ± SE	4.04±0.02	4.08±0.02	4.09±0.01	
EMS♂ x ACM-D♀	Parental	5.03	6.07	7.12	6.07±0.60
	F_1	4.97	5.75	6.97	5.90±0.58
	F_2	4.63	5.55	6.57	5.58±0.56
	Mean ± SE	4.88±0.12	5.79±0.15	6.89±0.16	
ACM-D♂ x EMS♀	Parental	4.95	5.12	6.83	5.63±0.60
	F_1	4.71	4.93	6.29	5.31±0.49
	F_2	4.45	4.69	5.95	5.03±0.47
	Mean ± SE	4.70±0.14	4.91±0.12	6.36±0.26	
EMS+ACM-D♂ x control♀	Parental	4.3	4.94	6.09	5.11±0.52
	F_1	4.15	4.81	5.89	4.95±0.51
	F_2	4.11	4.57	5.56	4.75±0.43
	Mean ± SE	4.19±0.06	4.77±0.11	5.85±0.15	

Table-60: Effect of different doses of EMS and ACM-D on the pupal period in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	4.13	4.83	5.89	4.95±0.51
ACM-D♂ x control♀	4.04	4.08	4.09	4.07±0.02
EMS♂ x ACM-D♀	4.88	5.79	6.89	5.85±0.58
ACM-D♂ x EMS♀	4.7	4.91	6.36	5.32±0.52
EMS+ACM-D♂ x control♀	4.19	4.77	5.85	4.94±0.49

Table-52 summarized the data effects of 1.0ml dose of EMS and ACM-D on the longevity of adult males in different crosses. The analysis of variance within crosses showed highly significant differences (F=38.98, P<0.001, appendix table-CXXXXVII) with LSD values as 1.09 at 5% and 2.38 at 0.1% probability level. The longevity of adult males induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMS+ACM-D♂ x control♀.Considering the case of generations it was found that the analysis of variance showed no significant differences (appendix table-CXXXXVII and figure-77).

At 2.0ml dose of EMS the analysis of varience within crosses showed significant differences (F=111.60, P<0.001, appendix table-CXXXXVIII) with LSD values as 0.96 at 5% and as 2.09 at 0.1% probability level.Which revealed that the longevity of adult males observed in cross of EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x MMS♀ crosses and the longevity of adult males in cross of EMS♂ x control♀ also differed significantly with ACM-D♂ x control♀ cross. In case of generation the analysis of variance also showed significant differences (F=6.07, P<0.05, appendix table–CXXXXVIII) with LSD value as 1.24 at 5% level (table-53 and figure-78).

Data obtain from table-54, which deals with the effects of 3.0ml dose of EMS and ACM-D, showed significant differences among the crosses (F=474.90, P<0.001, appendix table -CXXXXIX) with LSD values as 0.67 at 5%, and as 1.46 at 0.1% level. The longevity of adult males by the cross EMS+ACM-D♂ x control♀ differed significantly with other treated crosses EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀ and ACM-D♂ x EMS♀. The analysis of variance within generations showed significant differences (F=13.66, P<0.01, appendix table-CXXXXIX) with LSD values as 0.86 at 5% and 1.26 at 1% level of significance in table-54 and figure-79.

Table-61 summarized the data showing the effects of different doses of EMS and ACM-D on the longevity of adult males in different generations. Results demonstrated that the longevity of adult males changed to decrease with the doses of EMS and ACM-D

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induced. The analysis of variance showing the effects within generations and doses has been calculated and are present in appendix tablesCL-CLIV. In case of EMMS♂ x control♀, the analysis of variance within generations showed significant differences ($F=7.69$, $P<0.05$, appendix table-CL) with LSD values as 1.30 at 5% probability of level. The analysis of variance within doses also showed differed significantly ($F=122.27$, $P<0.001$, appendix table-CL) with LSD values as 1.30 at 5% and as 4.04 at 0.1% level of significant.In case of doses it was observed that means longevity of adult males of all doses differed significantly with each other in the table-61.

When the ACM-D treated males were crossed with Control females the F value has been calculated and showed nonsignificant differences were observed between the generations and doses (appendix table-CLI).

When the EMS treated males were crossed with ACM-D treated females the analysis of variances has been calculated and showed significant differences within generations ($F=32.12$, $P<0.01$, appendix table-CLII) with LSD values as 0.64 at 5% and as 1.06 at 1% respectively.The longevity of adult males within doses has been calculated and showed significant differences ($F=433.50$, $P<0.001$, appendix table-CLII) with LSD values as 0.64 at 5% and as 1.99 at 0.1% probability level.In cross of ACM-D♂ x EMS♀, the analysis of varience within generations showed differed significantly ($F=22.00$, $P<0.01$, appendix table-CLIII) with LSD values as 0.71 at 5% and as 1.19 at 1% probability level. The analysis of variances within doses has been calculated and showed significant differences ($F=2469$, $P<0.001$, appendix table-CLIII) with LSD values as 0.71 at 5% and 2.22 at 1% level.

In cross of EMS+ACM-D treated male with crossed control female, the analysis of variances has been calculated within generation showed nonsignificant differences (appendix table-CLIV). The longevity of adult males within doses has been calculated and also showed significant differences ($F=89.94$, $P<0.001$, appendix table-CLIV) with LSD values as 1.61 at 5% and 4.97 at 1% probability level in table-61.

Effects on different doses of EMS and ACM-D on the longevity of adult males in different crosses and are shown in table-62. It was observed that mean longevity of adult males differed significantly within crosses (F=17.37, P<0.01, appendix table-CLV) with LSD values as 2.49 at 5% and as 3.63 at 1% level. The observed mean the longevity of adult males induced by the cross of EMS+ACM-D♂ x control♀ differs significantly with other crossess EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and ACM-D♂ x EMS♀. The mean of the longevity of adult males showed differed significantly within doses (F=12.55, P<0.01, appendix table-CLV) with LSD values 3.22 at 5% and as 4.69 at 1% probability level.The longevity of adult males induced by 3.0ml EMS dose differed significantly with other doses (table-62 and figure-80).

Table-61: Effects of different doses of EMS and ACM-D on the longevity of adult males in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	115.59	113.62	107.99	112.40±2.28
	F_1	114.74	112.74	108.77	112.08±1.76
	F_2	117.1	115.01	109.32	113.81±2.33
	Mean ± SE	115.81±0.69	113.79±0.66	108.69±0.39	
ACM-D♂ x control♀	Parental	120.17	120.28	120.59	120.35±0.13
	F_1	120.22	121.16	120.33	120.57±0.30
	F_2	120.46	120.44	121.3	120.73±0.28
	Mean ± SE	120.28±0.09	120.63±0.27	120.74±0.29	
EMS♂ x ACM-D♀	Parental	112.13	109.66	105.27	109.02±2.01
	F_1	113.16	110.28	106.8	110.08±1.84
	F_2	114.31	111.07	107.21	110.86±2.05
	Mean ± SE	113.20±0.63	110.34±0.41	106.43±0.59	
ACM-D♂ x EMS♀	Parental	113.58	110.92	107.88	110.79±1.65
	F_1	114.13	112.2	108.84	111.72±1.55
	F_2	115.18	113.22	109.11	112.50±1.79
	Mean ± SE	114.30±0.47	112.11±0.67	108.61±0.37	
EMS+ACM-D♂ x control♀	Parental	115.74	111.17	106.89	111.27±2.56
	F_1	116.86	112.34	109.03	112.74±2.27
	F_2	115.62	113.11	109.07	112.60±1.91
	Mean ± SE	116.07±0.40	112.21±0.56	108.33±0.72	

Table-62: Effect of different doses of EMS and ACM-D on the longevity of adult males in different crossess of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	115.81	113.79	108.69	112.76±2.12
ACM-D♂ x control♀	120.28	120.63	120.74	120.55±0.14
EMS♂ x ACM-D♀	113.2	110.34	106.43	109.99±1.96
ACM-D♂ x EMS♀	114.3	112.11	108.61	111.67±1.66
EMS+ACM-D♂ x control♀	116.07	112.21	108.33	112.20±2.24

Table-52 summarized the data effects of 1.0ml dose of EMS and ACM-D on the longevity of adult females in different crosses. The analysis of variance within crosses showed highly significant ($F=47.50$, $P<0.001$, appendix table-CLVI) differences with LSD values as 0.54 at 5% and as 1.19 at 0.1% probability level. The longevity of adult females induced by the cross ACM-D♂ x control♀ differed significantly with other crosses EMS♂ x control♀, EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ and EMMS+ACM-D♂ x control♀. Considering the case of generations the analysis of variance showed significant differences ($F=12.21$, $P<0.01$, appendix table-CLVI) with LSD values as 0.71 at 5% and as 1.03 at 1% probability level (table-52 and figure-81).

Data obtained from table-53 at 2.0ml dose of EMS and ACM-D the analysis of variance also showed within crosses highly significant differences ($F=709$, $P<0.001$, appendix table-CLVII) with LSD values as 0.25 at 5% and as 0.55 at 0.1% probability level. In case of generations the analysis of variance showed significant differences ($F=39.33$, $P<0.001$, appendix table-CLVII) with LSD values as 0.33 at 5% and as 0.71 at 0.1% level in figure-82.

At 3.0ml dose of EMS and ACM-D, the F value showed differed significantly within crosses as 207.97 ($P<0.001$, appendix table-CLVIII) with LSD values as 0.83 at 5% and as 1.80 at 0.1% level of significant which revealed that the longevity of adult females in cross EMS+ACM-D♂ x control♀ differed significantly with EMS♂ x ACM-D♀, ACM-D♂ x EMS♀ crosses and the longevity of adult females found in EMS♂ x control♀ also differed significantly ACM-D♂ x control♀ in table-54. The analysis of variance within generations showed ($F=7.16$, $P<0.05$, appendix table-CLVIII) significant differences with LSD value as 1.07 at 5%, level in figure-83.

Table-63 summarized the data showing the effects of different doses of EMS and ACM-D on the longevity of adult females in generations. Results demonstrated that the longevity of adult females changes to decrease with the EMS doses and ACM-D induced. The analysis of variance showing the effects within generations and doses has been calculated and are present in appendix table CLIX-CLXIV. In case of EMS♂ x control♀, the analysis of variance within generations showed nonsignificant differences (appendix table-CLIX). The

analysis of variance within doses showed significant differences (F=109.25, P<0.001, appendix table-CLIX) with LSD values as 1.28 at 5% and 3.98 at 0.1% probability level. In case of doses it was observed that means of the longevity of adult females for all doses differed significantly with each other in the table-63.

When the ACM-D treated males were crossed with control females showed no significant differences were observed within the generations and doses (appendix table-CLX).In cross of EMS♂ x ACM-D♂ the analysis of variances has been calculated and showed within generation significant differences (F=62.50, P<0.001, appendix table-CLXI) with LSD values as 0.32 at 5% and as 0.99 at 0.1% level. The analysis of variances within doses showed highly significant differences (F=2438.5, P<0.001, appendix table-CLXI) with LSD values as 0.32 at 5% and 0.99 at 0.1% probability level.

When the ACM-D treated males were crossed with EMS treated females the analysis of variances has been calculated and showed significant differences within generations (F=67, P<0.001, appendix table-CLXII) with LSD values as 0.32 at 5% and as 0.99 at 0.1% level. The analysis of variances within doses showed highly significant differences (F=2953.50, P<0.001, appendix table-CLXII) with LSD values as 0.32 at 5% and as 0.99 at 0.1% probability level. In cross of EMS+ACM-D♂ x control♀, the F value within generation showed significant differences as 10.82 (P<0.05, appendix table-CLXIII) with LSD value as 0.94 at 5%, probability level and the analysis of variances within doses showed differed significantly (F=405.35, P<0.05, appendix table-CLXIII) with LSD values as 0.94 at 5% and as 2.90 at 0.1% level in table-63.

Effects on different doses of EMS and ACM-D on the longevity of adult males in different crosses and are shown in table-64. It was observed that mean longevity of adult females differed significantly within crosses (F=6.63, P<0.05 appendix table-CLXIV), with LSD value 2.79 at 5% level. The observed mean the longevity of adult females induced by the cross ACM-D♂ x EMS♀ differed significantly with other crosses EMS♂ x control♀, ACM-D♂ x control♀, EMS♂ x ACM-D♀, and EMS+ACM-D♂ x control♀. The mean of the longevity of adult females differed significantly within generations (F=15.42, P<0.01,

appendix table-CLXIV) with LSD values as 3.60 at 5% and as 5.24 at 1% level. The number of longevity of adult females induced by 1.0ml EMS doses differed significantly with other doses (table-64 and figure-84).

Table-63: Effect of different doses of EMS and ACM-D on the longevity of adult females in different generations of *Epilachna vigintioctopunctata*

Cross	Generation	Dose of EMS			Mean ± SE
		1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	Parental	109.16	107.85	102.05	106.35±2.19
	F_1	109.78	108.27	104.36	107.47±1.62
	F_2	110.61	108.98	103.6	107.73±2.12
	Mean ± SE	109.85±0.42	108.37±0.33	103.34±0.68	
ACM-D♂ x control♀	Parental	112.44	112.19	112.01	112.21±0.12
	F_1	112.24	112.37	112.1	112.24±0.08
	F_2	112.38	112.69	112.19	112.42±0.15
	Mean ± SE	112.35±0.06	112.42±0.15	112.10±0.05	
EMS♂ x ACM-D♀	Parental	107.88	104.69	99.65	104.07±2.40
	F_1	108.14	105.39	100.34	104.62±2.29
	F_2	109.08	105.86	101.14	105.36±2.31
	Mean ± SE	108.37±0.36	105.31±0.34	100.38±0.43	
ACM-D♂ x EMS♀	Parental	109.33	106.86	100.9	105.70±2.51
	F_1	110.19	107.44	101.26	106.30±2.64
	F_2	110.81	108.21	102.07	107.03±2.59
	Mean ± SE	110.11±0.43	107.50±0.39	101.41±0.35	
EMS+ACM-D♂ x control♀	Parental	110.18	109.6	101.21	107.00±2.90
	F_1	111.28	109.92	102.24	107.81±2.82
	F_2	111.94	110.3	103.45	108.56±2.60
	Mean ± SE	111.13±0.51	109.94±0.20	102.30±0.65	

Table-64: Effect of different doses of EMS and ACM-D on the longevity of adult females in different crosses of *Epilachna vigintioctopunctata*

Cross	Dose of EMS			Mean ± SE
	1.0ml	2.0ml	3.0ml	
EMS♂ x control♀	109.85	108.37	103.34	107.19±1.97
ACM-D♂ x control♀	112.35	112.42	112.1	112.29±0.10
EMS♂ x ACM-D♀	108.37	105.31	100.38	104.69±2.33
ACM-D♂ x EMS♀	110.11	107.5	101.41	106.34±2.58
EMS+ACM-D♂ x control♀	111.13	109.94	102.3	107.79±2.77

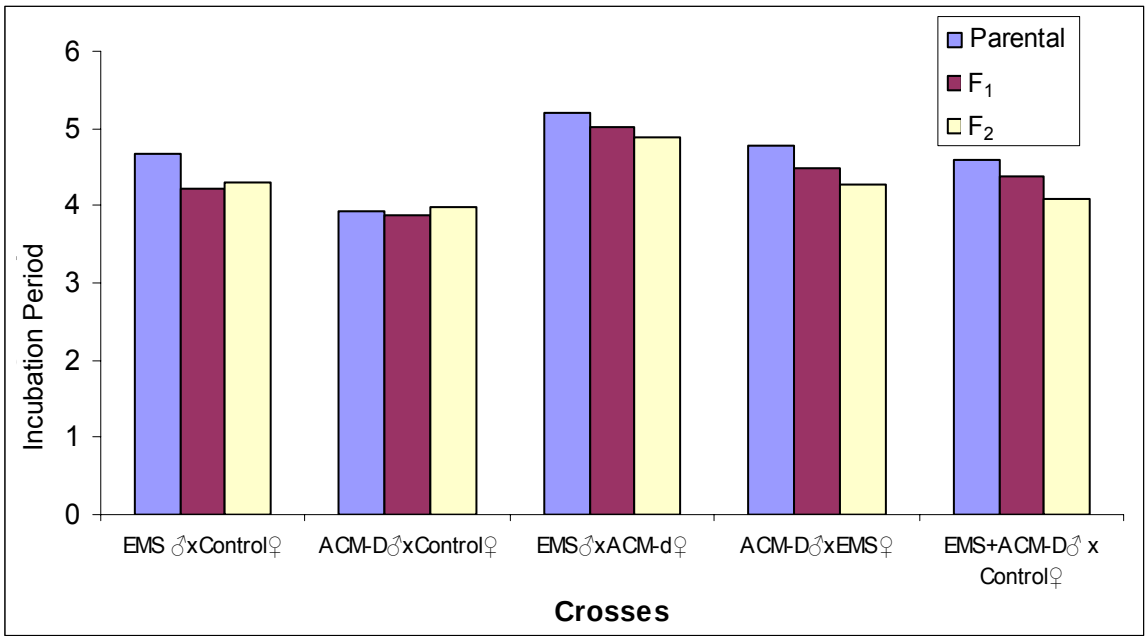

Figure-73: Effects of 1.0ml EMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

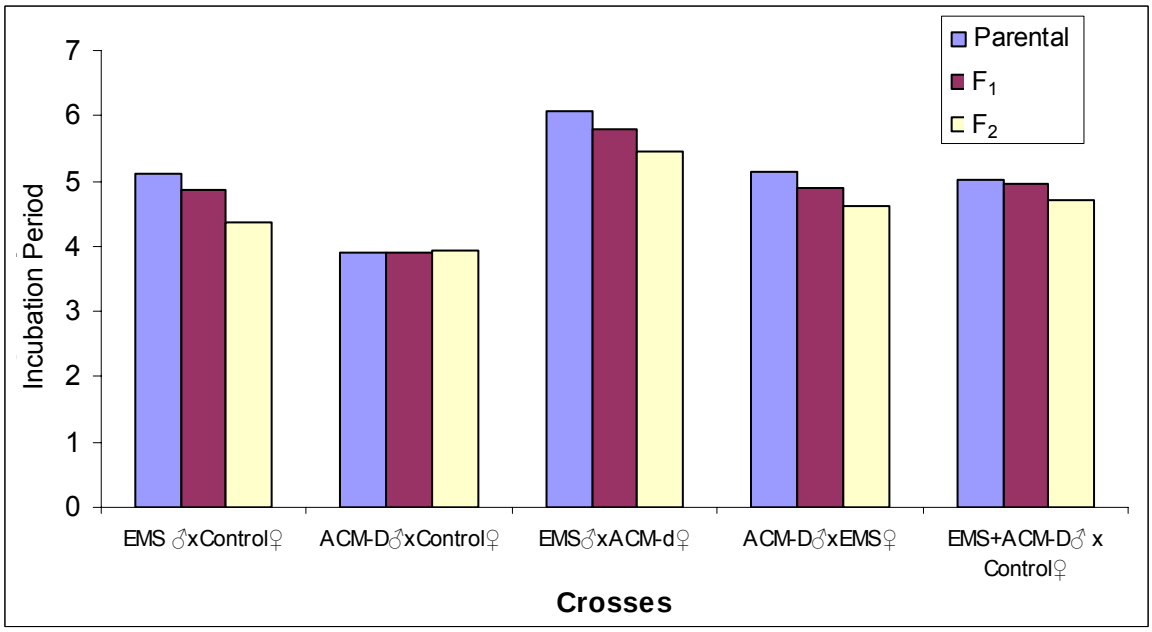

Figure-74: Effects of 2.0ml EMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

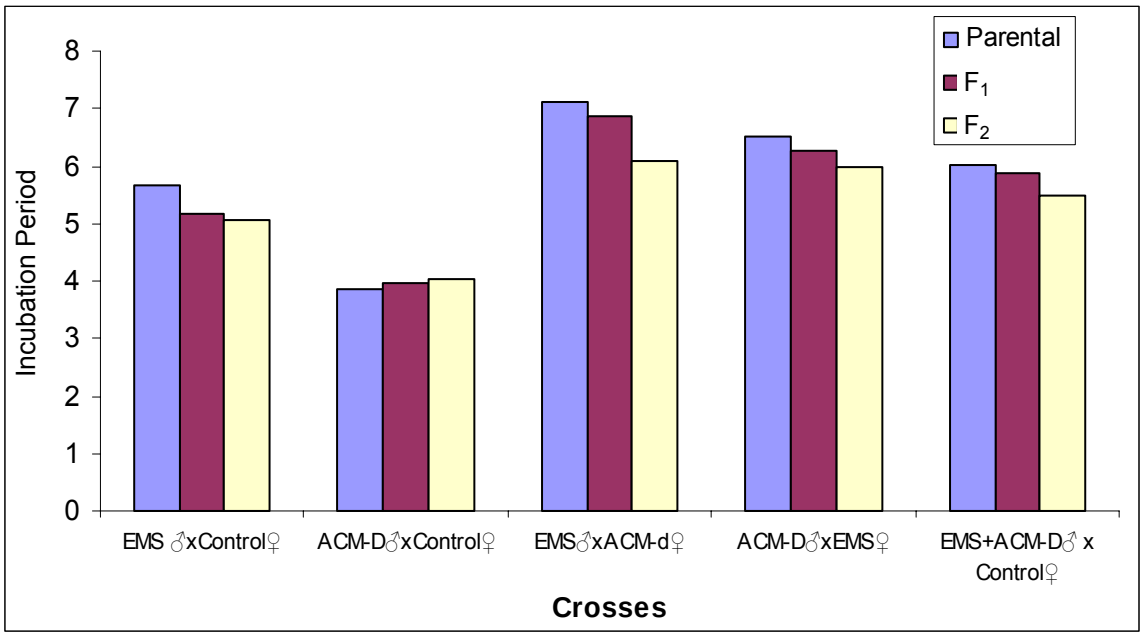

Figure-75: Effects of 3.0ml EMS and ACM-D on the incubation period in different crosses and generations of *Epilachna vigintioctopunctata*

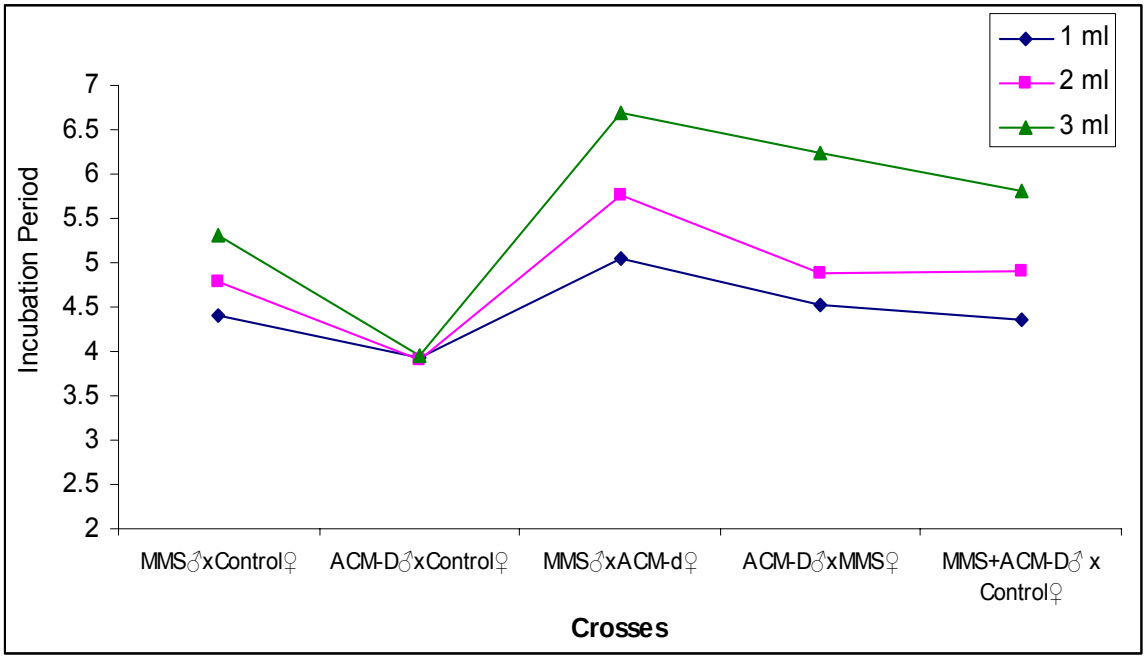

Figure-76: Effects of different doses of EMS and ACM-D on the incubation periodin in different crossess of *Epilachna vigintioctopunctata*

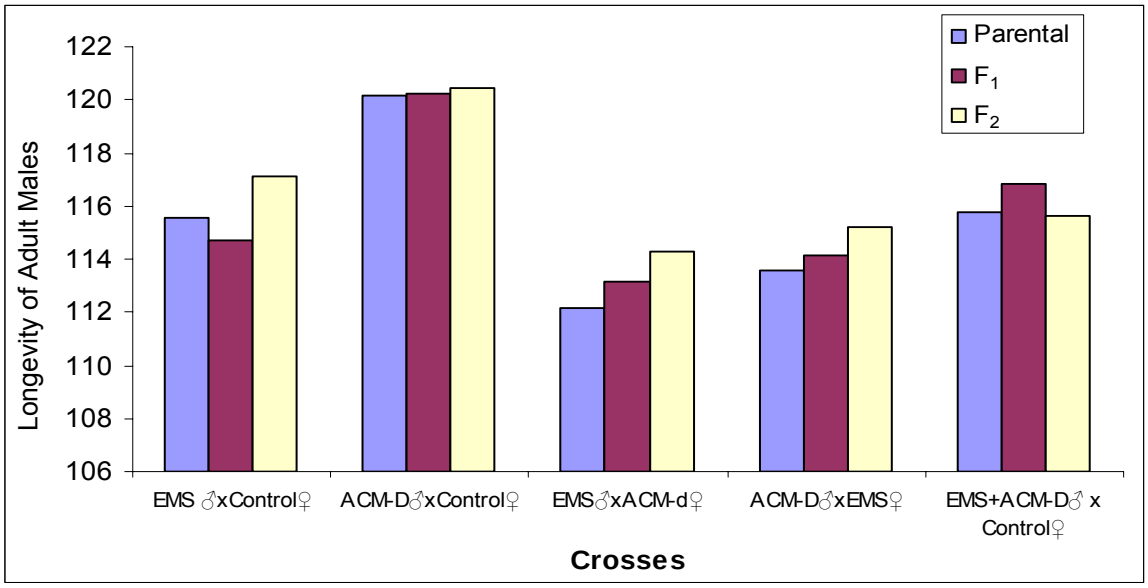

Figure-77: Effects of 1.0ml EMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

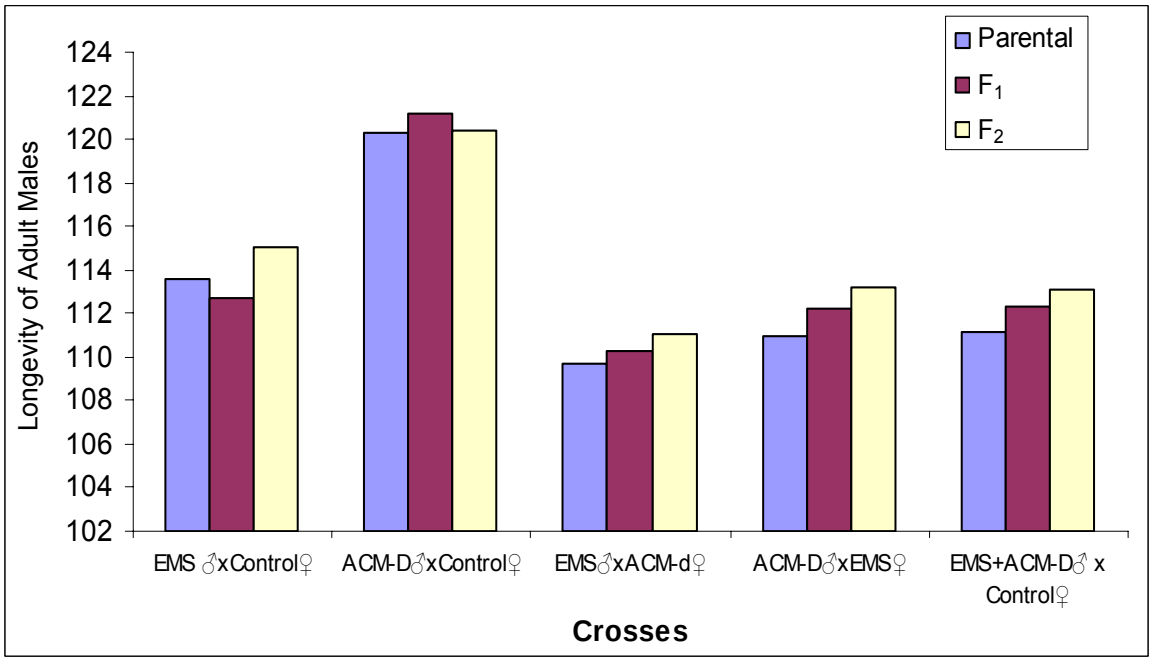

Figure-78: Effects of 2.0ml EMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

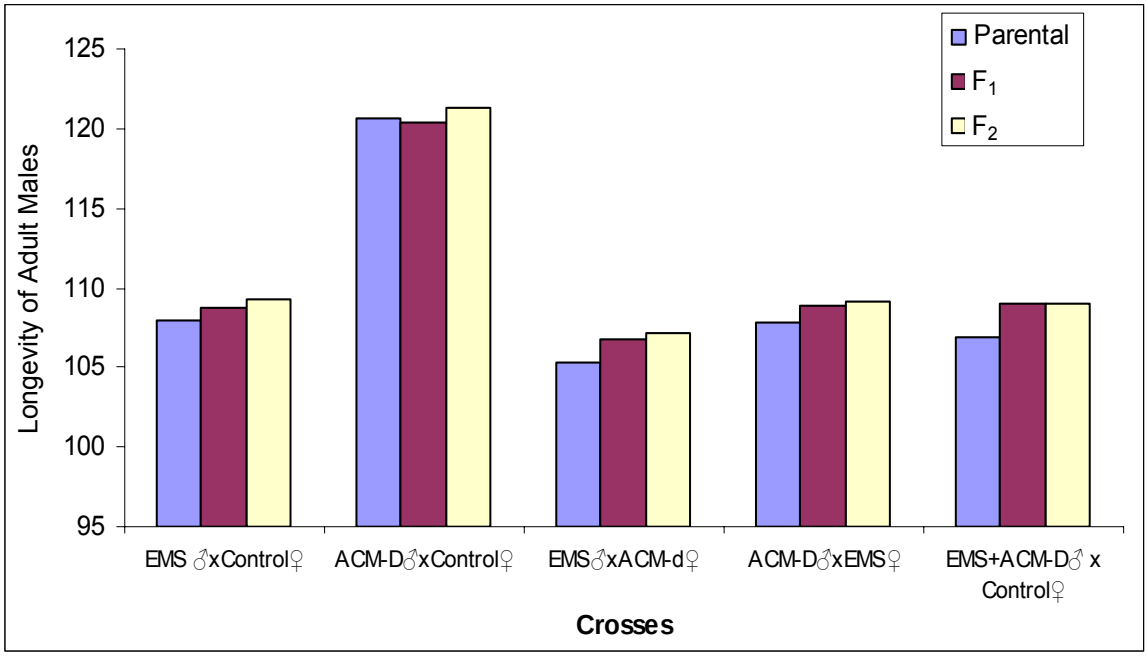

Figure-79: Effects of 3.0ml EMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

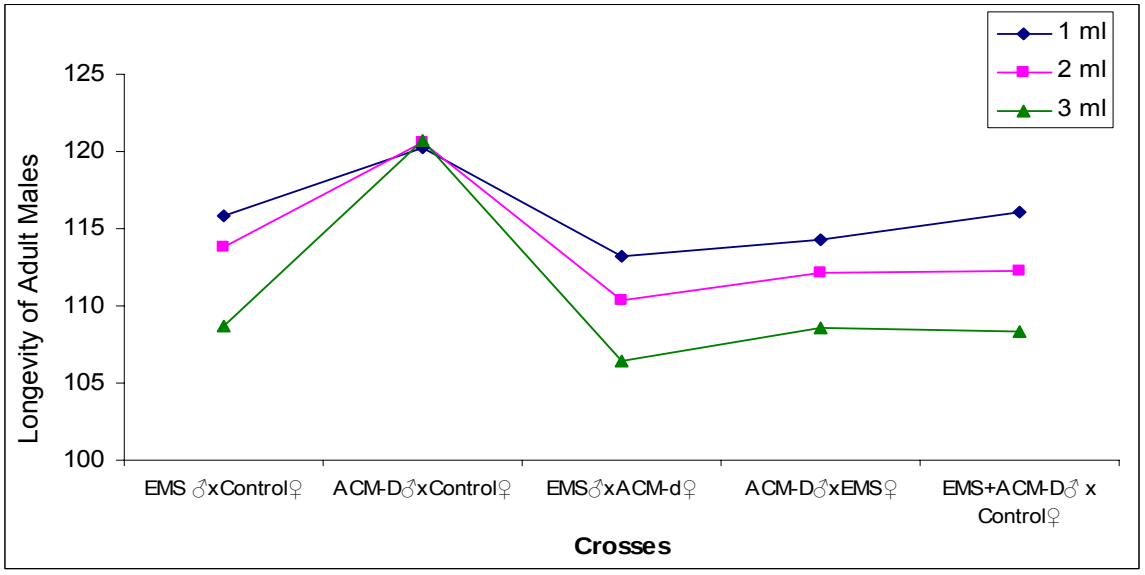

Figure-80: Effects of different doses of EMS and ACM-D on the longevity of adult males in different crossess of *Epilachna vigintioctopunctata*

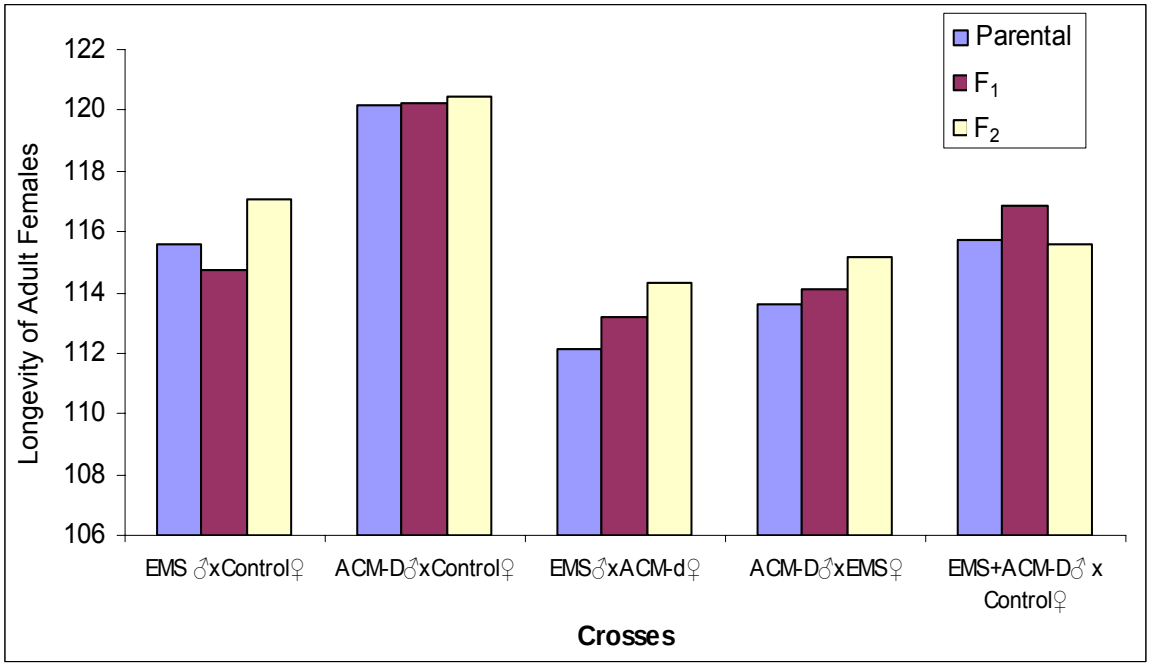

Figure-81: Effects of 1.0ml EMS and ACM-D on the longevity of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

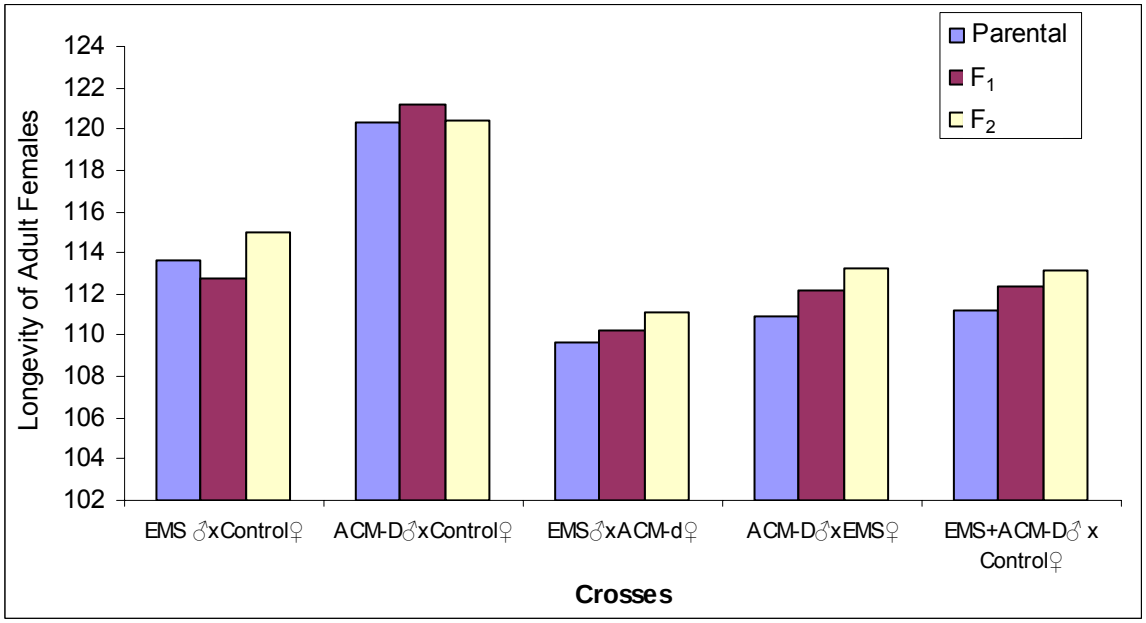

Figure-82: Effects of 2.0ml EMS and ACM-D on the longevity of adult females in different crosses and generations of *Epilachna vigintioctopunctata*

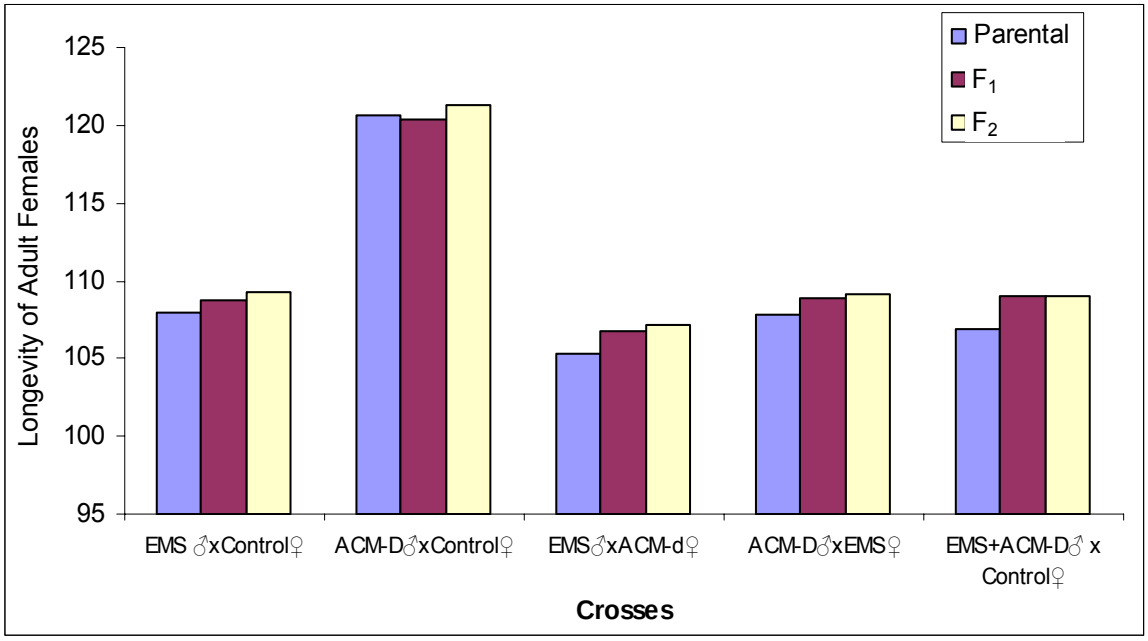

Figure-83: Effects of 3.0ml EMS and ACM-D on the longevity of adult males in different crosses and generations of *Epilachna vigintioctopunctata*

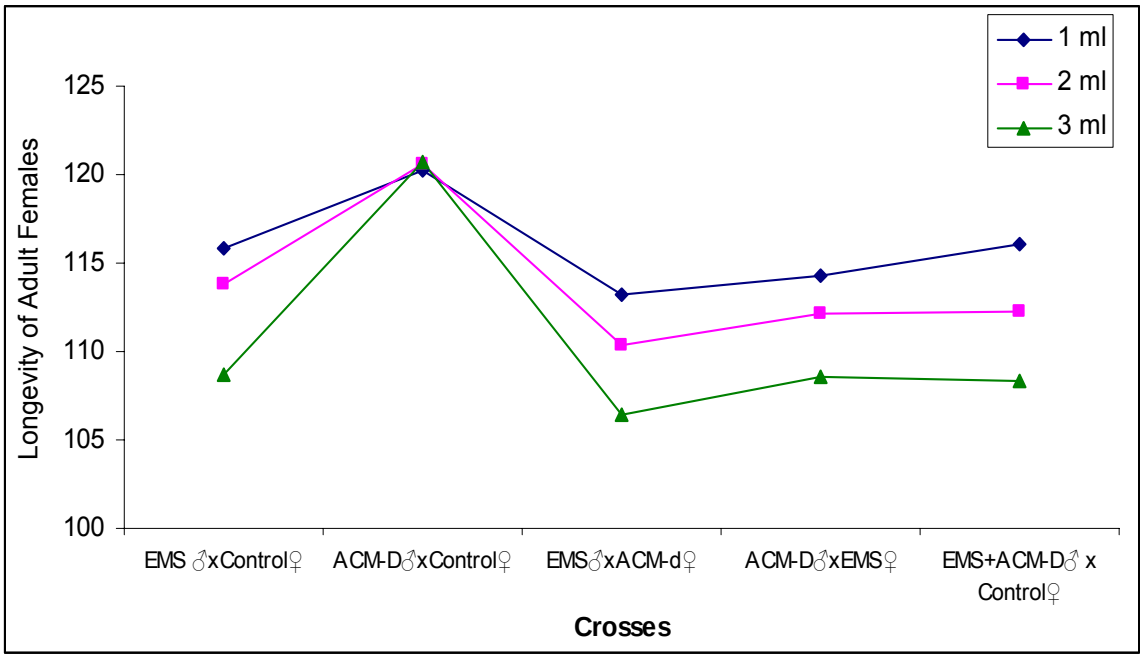

Figure: 84. Effects of different doses of EMS and ACM-D on the longevity of adult females in different crossess of *Epilachna vigintioctopunctata*

4.3.3 Discussion

In addition to the incubation period, larval period, pupal period and longevity of adult male and female for the evaluation of genetic effects caused by various doses of methyl methanesulfonates and ethyl methanesulfonates with actinomycin-D on different generations of *Epilachna vigintioctopunctata* was observed that in the parental, F_1 and F_2 generations.

The incubation period for *E. vigintioctopunctata* was recorded as 4 to 9 days (Ahmed and Alam 1964), 4.60±0.24 to 7.00±0.00 days (Anon, 1976), 4.26±0.15 days (Ahmed, 1983) and 4.01±0.13 (Islam, 2001), but the present investigation of incubation period showed highly increased value in parental generation but gradually decreased in F_1 and F_2 generations. It was observed that the incubation period was increased in all crosses exect the cross ACM-D♂ × control♀.

The total larval period of *E. vigintioctopunctata* was recorded 12-15 days (Khatun, 2006). The pupal duration of *E. vigintioctopunctata* was recorded as 3.5 days (Banu, 2004) and 3.66±0.23 day (Islam, 2001). In the present finding, it was observed that the larval period was increased in all crosses exect the cross ACM-D♂ × control♀. But for F_1 and F_2 generations it was gradually dicreased in comparison with the parental generation. In the present experiment the pupal period was increased in all crosses and in parental generation exect the cross ACM-D♂ × control♀. Although, the pupal period of F_1 and F_2 generations gradually decreased from parental generation.

As no references are available on the effects of MMS, EMS and ACM-D on the incubation period, larval period, pupal period and no compare could be made. But comparison can be made on the effect of other physical and chemical mutagens. The present findings are in good agreements with the finding of Molner *et al.* (1964) and Shigematsu *et al.* (1968) who reported that higher doses of gamma irradiation on the egg stage of *B. mori* decreased the silk contents of the cocoons and length of the spinnable filament.

The longevity of adults of *E. vigintioctopunctata* was recorded (Ahmed and Alam, 1964). Liotta (1965) the described that winter males and females live for 170 to 200 days, and female 200 to 240 days, respectively. Ahmed (1983) reported that the longevity of males of this species were longer than that of females. In the present investigation the effects of MMS, EMS and ACM-D on different generations of *E. vigintioctopunctata* on the longevity of adult males and female were increased in all crosses exect the cross ACM-D♂ × control♀.

SUMMARY

The final section provides a summary of the major findings of the mutagenic effects of methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) and actinomycin-D (ACM-D) on the quantitative traits of *Epilachna vigintioctopunctata*. The chemicals used for the experiment were of two types: MMS and EMS were monofunctional alkylating agents and ACM-D was an antibiotic. These two types of chemicals differed to their performance in the dominant lethal test. Experimental evidence provided information that at all doses both the alkylating agents induced dominant lethal mutations and affected the number of eggs, number of larvae, number of pupae, sex-ratio and developmental biology (incubation period,larval period,pupal period, longivity efficiently. There was a clear linear relationship between an increase in mutagen doses with the lethal frequency. Attention had also been paid to the mutagenic effects produced in different male germ cell stages which revealed that the first two generations which comprised post-meiotic stage was much more pronounced in comparison to the last two stages which constituted meiotic and pre-meiotic stages in response to the lethal mutations.

In the assessment of the mutagenic action of the antibiotic, ACM-D on the induction of the aforesaid lethal mutation, it was observed that although it induced some percentages of dominant lethal mutations but it showed weak effects on sex-ratio and developmental biology in *Epilachna vigintioctopunctata* and it also showed some differences in relations to generation which was something higher in post-meiotic stages.

Combined effects of MMS or EMS with ACM-D showed similar pattern of action on the induction of lethal mutations. ACM-D with either of the alkylating agents MMS/EMS increased the frequency of dominant lethal mutations and decreased the frequency of life cycle, sex-ratio and developmental biology irrespective of the spermatogenic stages or doses of MMS or EMS.

Research findings proved that testing for the induction of dominant lethal mutation, sex-ratio and developmental biology are the most effective ways of screening for mutagenic activity in *Epilachna vigintioctopunctata*. So, it can be concluded that more detailed characterization can be obtained by using other parameters of genetic damage with this organism and a wide range of direct, indirect, water-soluble, water-insoluble, stable, unstable, chromosome-breaking, non-chromosome-breaking mutagens can be detected by treating adult males, sampling male germ cells and testing for dominant lethal mutations.

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APPENDICES

Appendix table-I: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	1140.28	4	285.07	55.27***
Within generations	85.77	2	42.89	8.31*
Residual	41.2639	8	5.16	

LSD value crosses=3.31 (5%), 6.23 (1%)
LSD value generations=4.28 (5%),

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-II: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	2157.48	4	539.37	776.53***
Within generations	11.53	2	5.77	8.30*
Residual	5.5567	8	0.69	

LSD value crosses=1.21 (5%), 1.76 (1%)
LSD value generations=1.57 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-III: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	4359.29	4	1089.82	1169.32***
Within generations	4.80	2	2.40	2.58^{NS}
Residual	7.4561	8	0.93	

LSD value crosses=1.41 (5%), 2.05 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-IV: Analysis of variance showing differences within crosses and generations following treatment with 4.0ml MMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	5039.37	4	1259.84	841.01***
Within generations	6.23	2	3.12	2.08^{NS}
Residual	11.9842	8	1.50	

LSD value crosses=1.79 (5%), 2.6 (1%) *** Highly significant (P<0.001)
NS = non Significant

Appendix table-V: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the DLM

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	10.79	2	5.40	29.70***
Within doses	222.64	3	74.21	408.58^{NS}
Residual	1.0898	6	0.18	

LSD value generations=0.55 (5%), 0.79 (1%) *** Highly significant (P<0.001)
NS=non significant

Appendix table-VI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.01	2	0.00	0.00^{NS}
Within doses	86.01	3	28.67	-0.01^{NS}
Residual	-85.5286	6	-14.25	

NS=non significant

Appendix table-VII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	10.14	3	3.38	1.50^{NS}
Within doses	889.31	2	444.66	197.04***
Residual	13.5403	6	2.26	

LSD value generations=2.45 (5%), 3.57 (1%)

NS = non significant
*** Highly significant (P<0.001)

Appendix table-VIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	6.60	2	3.30	0.44^{NS}
Within doses	1203.49	3	401.16	53.14***
Residual	45.2913	6	7.55	

LSD value generations=5.49 (5%), 8.31 (1%)

NS = non significant
*** Highly significant (P<0.001)

Appendix table-IX: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	20.11	2	10.06	0.91^{NS}
Within doses	1367.86	3	455.95	41.22***
Residual	66.3705	6	11.06	

LSD value generations=6.65 (5%), 10.07 (1%)

NS=non significant
*** Highly significant (P<0.001)

Appendix table-X: Analysis of variance showing the effects of different crosses and doses following treatment with MMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	3799.19	4.00	949.80	29.45***
Within doses	1024.45	3.00	341.48	10.59***
Residual	387.07	12.00	32.26	

LSD value crosses=7.83 (5%), 10.99 (1%) *** Highly significant (P<0.001)
LSD value doses = 8.75 (5%), 12.29 (1%)

Appendix table-XI: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	921.37	4	230.34	1085.22***
Within generations	8.65	2	4.33	20.38***
Residual	1.6980	8		

LSD value crosses=0.67 (5%), 0.97 (1%) *** Highly significant (P<0.001)
LSD value generations=0.86 (5%), 1.26 (1%)

Appendix table-XII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	1977.59	4	494.40	1248.73***
Within generations	12.83	2	6.42	16.20**
Residual	3.1674	8	0.40	

LSD value crosses=0.92 (5%), 1.34 (1%) *** Highly significant (P<0.001)
LSD value generations=1.19 (5%), 1.73 (1%) ** Significant (P<0.01)

Appendix table-XIII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	4281.15	4	1070.29	2553.75***
Within generations	13.22	2	6.61	15.77**
Residual	3.3528	8	0.42	

LSD value crosses=0.95 (5%), 1.38 (1%) *** Highly significant (P<0.001) LSD value generations=1.22 (5%), 1.78 (1%) ** Significant (P<0.01)

Appendix table-XIV: Analysis of variance showing differences within crosses and generations following treatment with 4.0ml EMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	4780.22	4	1195.06	2597.95***
Within generations	0.92	2	0.46	0.80^{NS}
Residual	4.6274	8	0.58	

LSD value crosses=1.11 (5%), 1.62 (1%) *** Highly significant (P<0.001)
NS = non significant

Appendix table-XV: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	7.57	2	3.79	31.25***
Within doses	210.23	3	70.08	578.61***
Residual	0.7267	6	0.12	

LSD value crosses=2.73 (5%), 4.14 (1%) *** Highly significant (P<0.001)
LSD value doses=0.60 (5%), 0.91 (1%)

Appendix table-XVI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.01	2	0.00	0.00^{NS}
Within doses	93.16	3	31.05	-2.02^{NS}
Residual	-92.4457	6	-15.41	

NS=non significant

Appendix table-XVII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	7.80	2	3.90	3.79^{NS}
Within doses	889.31	3	296.44	288.44***
Residual	6.1663	6	1.03	

LSD value doses=1.76 (5%), 2.66 (1%)

Non Significant
*** Highly significant (P<0.001)

Appendix table-XVIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	14.12	2	7.06	27.42***
Within doses	1152.85	3	384.28	1492.72***
Residual	1.5446	6	0.26	

LSD value generations=1.02 (5%), 1.54 (1%)
LSD value doses=0.88 (5%), 1.12 (1%)

*** Highly significant (P<0.001)

Appendix table-XIX: Analysis of variance showing the effects on different generations and do ses following cross EMS+ACM-D treated males and control females on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	7.06	2	3.53	6.04*
Within doses	1924.84	3	641.61	1097.02**
Residual	3.5092	6	0.58	

LSD value generations=1.52 (5%) * Significant (P<0.05)
LSD value doses=1.32 (5%), 2.00 (1%) *** Highly significant (P<0.001)

Appendix table-XX: Analysis of variance showing the effects on different crosses and doses following treatment with EMS and ACM-D on the DLM.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	variance ratio (F)
Within crosses	3548.88	4	887.22	26.26***
Within doses	1008.44	3	336.15	9.95**
Residual	405.47	12	33.79	

LSD value crosses=8.01 (5%), 11.24 (1%) *** Highly significant (P<0.001)
LSD value doses=9.96 (5%), 12.58 (1%) ** Significant (P<0.01)

Appendix table-XXI: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	21434.01	4	5358.50	118.79***
Within generations	353.06	2	176.53	3.91 NS
Residual	360.8873	8	45.11	

LSD value crosses=9.81 (5%), 14.27 (1%) *** Highly significant (P<0.001)
N=non significant

Appendix table-XXII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	18410.68	4	4602.67	210.81***
Within generations	395.71	2	197.86	9.06**
Residual	174.67	8	21.83	

LSD value crosses=6.83 (5%), 9.93 (1%) *** Highly significant (P<0.001)
LSD value generations=8.81 (5%), 12.82 (1%) ** significant (P<0.01)

Appendix table-XXIII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	25884.55	4	6471.14	192.55***
Within generations	518.42	2	259.21	7.71*
Residual	268.8593	8	33.61	

LSD value crosses=8.47 (5%), 12.31 (1%) *** Highly significant (P<0.001)
LSD value generations=10.93 (5%), * Significant (P<0.05)

Appendix table-XXIV: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	614.89	2	307.45	16.79*
Within doses	451.91	2	225.96	12.34*
Residual	73.2622	4	18.32	

LSD value generations=9.71 (5%), * Significant (P<0.05)
LSD value doses=9.71 (5%),

Appendix table-XXV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	51.77	2	25.89	3.45^{NS}
Within doses	76.44	2	38.22	5.10^{NS}
Residual	29.9856	4	7.50	

NS=non significant

Appendix table-XXVI: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	308.70	2	154.35	28.45**
Within doses	395.05	2	197.53	36.41**
Residual	21.6989	4	5.42	

LSD value generations=5.21 (5%), 8.74 (1%) ** Significant (P<0.01)
LSD value doses=5.28 (5%), 8.74 (1%)

Appendix table-XXVII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	159.58	2	79.79	5.38^{NS}
Within doses	665.77	2	332.89	22.44**
Residual	59.3256	4	14.83	

LSD value doses=8.74 (5%), 14.46 (1%)

NS=non Significant
** Significant (P<0.01)

Appendix table-XXVIII: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	627.79	2	313.90	9.50*
Within doses	59.53	2	29.77	0.90^{NS}
Residual	132.2356	4	33.06	

LSD value generations=13.5 (5%),

* Significant (P<0.05)
NS=non Significant

Appendix table-XXIX: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	21728.51	4	5432.13	240.66***
Within doses	365.00	2	182.50	8.09*
Residual	180.57	8	22.57	

LSD value crosses=6.94 (5%), 10.09 (1%)
LSD value doses=8.96 (5%),

*** Highly significant (P<0.001)
** Significant (P<0.05)

Appendix table-XXX: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM -D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	64096.41	4	16024.10	41.78***
Within generations	4267.46	2	2133.73	5.56*
Residual	3068.1673	8	383.52	

LSD value crosses=28.61 (5%), 41.62 (1%)
LSD value generations=36.94 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-XXXI: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	89846.12	4	22461.53	91.98***
Within generations	1561.16	2	780.58	3.20^{NS}
Residual	1953.64	8	244.20	

LSD value cross=22.84 (5%), 33.21 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-XXXII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	143136.63	4	35784.16	74.69***
Within generations	991.34	2	495.67	1.03^{NS}
Residual	3832.6060	8	479.08	

LSD value cross=31.97 (5%), 46.50 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-XXXIII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	3261.26	2	1630.63	6.43^{NS}
Within doses	5690.60	2	2845.30	11.21*
Residual	1015.1089	4	253.78	

LSD value doses=36.18 (5%)

NS=non significant
* Significant (P<0.05)

Appendix table-XXXIV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1.18	2	0.59	0.03^{NS}
Within doses	23.00	2	11.50	0.50^{NS}
Residual	91.9356	4	22.98	

NS=non Significant

Appendix table-XXXV: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generation	734.84	2	367.42	0.32^{NS}
Within doses	5845.32	2	2922.66	2.55^{NS}
Residual	4580.0356	4	1145.01	

NS=non Significant

Appendix table-XXXVI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	541.34	2	270.67	0.39^{NS}
Within doses	16084.06	2	8042.03	11.67*
Residual	2756.6356	4	689.16	

NS=non Significant

LSD value doses=59.59 (5%)

* Significant (P<0.05)

Appendix table-XXXVII: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1756.22	2	878.11	2.92^{NS}
Within doses	12985.66	2	6492.83	21.59**
Residual	1202.7156	4	300.68	

LSD value doses=39.36 (5%), 65.14 (1%)

NS=non significant
** Significant (P<0.01)

Appendix table-XXXVIII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	94354.76	4	23588.69	51.46***
Within doses	9875.63	2	4937.82	10.77**
Residual	3667.25	8	458.41	

LSD value cross=31.28 (5%), 45.5 (1%)
LSD value doses=40.38 (5%), 58.74 (1%)

*** Highly significant (P<0.001)
** significant (P<0.01)

Appendix table-XXXIX: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	60314.03	4	15078.51	57.10***
Within generations	5212.13	2	2606.07	9.87**
Residual	2112.4533	8	264.06	

LSD value crosses=23.74 (5%), 34.53 (1%)
LSD value doses=30.64 (5%), 44.58 (1%)

*** Highly significant (P<0.001)
** significant (P<0.01)

Appendix table-XXXX: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	86252.26	4	21563.07	162.00***
Within generations	721.05	2	360.53	2.71^{NS}
Residual	1064.84	8	133.10	

LSD value crosses=16.85 (5%), 24.51 (1%)

*** Highly significant (P<0.001)
NS=non significant

Appendix table-XXXXI: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	155665.08	4	38916.27	244.07***
Within generations	171.80	2	85.90	0.54^{NS}
Residual	1275.5867	8	159.45	

LSD value crosses=18.42 (5%), 26.79 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-XXXXII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	2159.93	2	1079.97	7.52*
Within doses	6453.45	2	3226.73	22.47**
Residual	574.3889	4	143.60	

LSD value cross=23.58 (5%)
LSD value doses=23.58 (5%), 39.02 (1%)

* Significant (P<0.05)
** Significant (P<0.01)

Appendix table-XXXXIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generation	3.92	2	1.96	0.10^{NS}
Within doses	9.63	2	4.82	0.23^{NS}
Residual	82.0500	4	20.51	

NS=non Significant

Appendix table-XXXXIV: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	631.37	2	315.69	1.25^{NS}
Within doses	14328.70	2	7164.35	28.34**
Residual	1011.2722	4	252.82	

LSD value doses=36.09 (5%), 59.72 (1%)

NS=non significant
** significant (P<0.01)

Appendix table-XXXXV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	145.72	2	72.86	0.11^{NS}
Within doses	16426.04	2	8213.02	12.21*
Residual	2690.3556	4	672.59	

LSD value doses=58.86 (5%)

NS=non significant
* Significant (P<0.05)

Appendix table-XXXXVI: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	2438.04	2	1219.02	5.94^{NS}
Within doses	15163.00	2	7581.50	36.95**
Residual	820.8089	4	205.20	

LSD value doses=31.81 (5%), 52.64 (1%)

NS=non significant
** Significant (P<0.01)

Appendix table-XXXXVII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	16572.81	4	4143.20	6.76*
Within doses	12607.37	2	6303.69	10.28**
Residual	4906.54	8	613.32	

LSD value crosses=36.18 (5%)
LSD value doses=46.71 (5%), 67.94 (1%)

* Significant (P<0.05)
** Significant (P<0.01)

Appendix table-XXXXVIII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	13382.15	4	3345.54	68.51***
Within generations	1070.53	2	535.27	10.96**
Residual	390.6693	8	48.83	

LSD value crosses=10.2 (5%), 14.85 (1%)
LSD value generations=13.18 (5%), 19.17 (1%)

*** Highly significant (P<0.001)
** Significant (P<0.01)

Appendix table-XXXXIX: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the yield of number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	19294.01	4	4823.50	180.24***
Within generations	252.58	2	126.29	4.72*
Residual	214.09	8	26.76	

LSD value crosses=7.55 (5%), 10.99 (1%)
LSD value generations=9.76 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-L: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	31346.80	4	7836.70	438.46***
Within generations	50.41	2	25.21	1.41NS
Residual	142.9873	8	17.87	

LSD value crosses=6.17 (5%), 8.98 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LI: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	271.90	2	135.95	4.24^{NS}
Within doses	1796.70	2	898.35	27.99**
Residual	128.3689	4	32.09	

LSD value doses=12.86 (5%), 21.28 (1%)

NS=non Significant
** Significant (P<0.001)

Appendix table-LII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	4.65	2	2.33	0.76^{NS}
Within doses	10.20	2	5.10	1.67^{NS}
Residual	12.1989	4	3.05	

NS=non Significant

Appendix table-LIII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	150.08	2	75.04	1.29^{NS}
Within doses	2802.03	2	1401.02	24.14**
Residual	232.1300	4	58.03	

LSD value doses=17.29 (5%), 28.61 (1%)

NS=non significant
** significant (P<0.01)

Appendix table-LIV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	135.64	2	67.82	0.75^{NS}
Within doses	2690.89	2	1345.45	14.82*
Residual	363.1322	4	90.78	

LSD value doses=21.63 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-LV: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	629.50	2	314.75	6.47^{NS}
Within doses	3223.08	2	1611.54	33.12**
Residual	194.6556	4	48.66	

LSD value doses=15.83 (5%), 26.2 (1%) ** Significant (P<0.01)

Appendix table-LVI: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the yield of number of adults male.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	20424.75	4	5106.19	37.90***
Within doses	2429.41	2	1214.71	9.02**
Residual	1077.79	8	134.72	

LSD value crosses=16.95 (5%), 24.66 (1%) *** Highly significant (P<0.001)
LSD value doses=31.36 (5%), 45.63 (1%) ** Significant (P<0.01)

Appendix table-LVII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	16320.69	4	4080.17	53.69***
Within generations	1468.42	2	734.21	9.66**
Residual	607.9833	8	76.00	

LSD value crosses=12.73 (5%), 18.52 (1%) *** Highly significant (P<0.001)
LSD value generations=16.44 (5%), 23.92 (1%) ** Significant (P<0.01)

Appendix table-LVIII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	25849.88	4	6462.47	94.33***
Within generations	828.33	2	414.17	6.05*
Residual	548.07	8	68.51	

LSD value crosses=12.09 (5%), 17.59 (1%) *** Highly significant (P<0.001)
LSD value generations=15.61 (5%) * Significant (P<0.05)

Appendix table-LIX: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	42756.20	4	10689.05	315.37***
Within generations	149.60	2	74.80	2.21^{NS}
Residual	271.1493	8	33.89	

LSD value crosses=8.5 (5%), 12.37 (1%) *** Highly significant (P<0.001)
NS=non Significant

Appendix table-LX: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	561.39	2	280.70	7.01*
Within doses	1845.15	2	922.58	23.05**
Residual	160.1000	4	40.03	

LSD value generations=14.36 (5%) * Significant (P<0.05)
LSD value doses=14.36 (5%), 23.76 (1%) ** significant (P<0.01)

Appendix table-LXI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	87.37	2	43.69	2.37^{NS}
Within doses	48.04	2	24.02	1.31^{NS}
Residual	73.6122	4	18.40	

NS=non Significant

Appendix table-LXII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	183.84	2	91.92	1.74^{NS}
Within doses	4459.98	2	2229.99	42.26**
Residual	211.0689	4	52.77	

LSD value doses=16.64 (5%), 27.28 (1%)

NS=non Significant
** Significant (P<0.01)

Appendix table-LXIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	259.26	2	129.63	0.83^{NS}
Within doses	3892.20	2	1946.10	12.51*
Residual	622.4422	4	155.61	

LSD value doses=28.41 (5%)

NS=non significant
* Significant (P<0.05)

Appendix table-LXIV: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1277.24	2	638.62	5.78^{NS}
Within doses	4394.52	2	2197.26	19.89**
Residual	441.9289	4	110.48	

LSD value doses=23.85 (5%), 39.48 (1%)

NS=non significant
** Significant (P<0.01)

Appendix table-LXV: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	26745.10	4	6686.28	33.85***
Within doses	3294.48	2	1647.24	8.34*
Residual	1580.43	8	197.55	

LSD value generations=20.53 (5%), 29.87 (1%)
LSD value doses=26.51 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-LXVI: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	19221.51	4	4805.38	295.08***
Within generations	575.40	2	287.70	17.67**
Residual	130.2793	8	16.28	

LSD value crosses=5.9 (5%), 8.57 (1%)
LSD value generations=7.61 (5%), 11.07 (1%)

*** Highly significant (P<0.001)
** Significant (P<0.01)

Appendix table-LXVII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	20612.56	4	5153.14	372.56***
Within generations	331.16	2	165.58	11.97**
Residual	110.6533	8	13.83	

LSD value crosses=5.43 (5%), 7.9 (1%)
LSD value generations=7.01 (5%), 10.2 (1%)

*** Highly significant (P<0.001)
** Significant (P<0.01)

Appendix table-LXVIII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	17978.10	4	4494.53	15.15**
Within generations	1097.96	2	548.98	1.85^{NS}
Residual	2372.9373	8	296.62	

LSD value crosses=25.16 (5%), 36.6 (1%)

** Significant (P<0.01)
NS=non Significant

Appendix table-LXIX: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	253.24	2	126.62	4.02^{NS}
Within doses	254.57	2	127.29	4.04^{NS}
Residual	126.0389	4	31.51	

NS=non Significant

Appendix table-LXX: Analysis of variance showing the effects on different generations and doses following cross ACD-M treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	28.54	2	14.27	3.23^{NS}
Within doses	31.90	2	15.95	3.61^{NS}
Residual	17.6756	4	4.42	

NS=non Significant

Appendix table-LXXI: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control ACD-M treated females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	192.03	2	96.02	13.51*
Within doses	122.35	2	61.18	8.61*
Residual	28.4200	4	7.10	

LSD value generations =6.05 (5%),
LSD value doses=6.05 (5%) * Significant (P<0.05)

Appendix table-LXXII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	184.99	2	92.50	1.70^{NS}
Within doses	1271.39	2	635.70	11.65*
Residual	218.1800	4	54.54	

NS=non Significant
LSD value doses=16.76 (5%) * Significant (P<0.05)

Appendix table-LXXIII: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	596.83	2	298.42	11.98*
Within doses	63.68	2	31.84	1.28^{NS}
Residual	99.6500	4	24.91	

LSD value generations=11.32 (5%)

* Significant (P<0.05)
NS=non Significant

Appendix table-LXXIV: Analysis of variance showing the effects on different crosses and doses following treatment with EMS and ACM-D on the number of eggs.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	22102.41	4	5525.60	133.32***
Within doses	248.57	2	124.29	3.00^{NS}
Residual	331.56	8	41.44	

LSD value crosses=9.4 (5%), 13.68 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXV: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	47229.08	4	11807.27	68.98***
Within generations	160.82	2	80.41	0.47^{NS}
Residual	1369.3640	8	171.17	

LSD value crosses=19.11 (5%), 27.8 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXVI: Analysis of variance showing differences within crosses and generations following treatment with 2.0 EMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	86239.33	4	21559.83	89.94***
Within generations	328.67	2	164.34	0.69^{NS}
Residual	1917.6373	8	239.70	

LSD value crosses=25.04 (5%), 36.42 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXVII: Analysis of variance showing differences within crosses and generations following treatment with 3.0 EMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	109059.86	4	27264.97	9.26**
Within generations	6108.30	2	3054.15	1.04^{NS}
Residual	23549.2160	8	2943.65	

LSD value cross=79.26 (5%), 115.29 (1%)

** Significant (P<0.01)
NS=non Significant

Appendix table-LXXVIII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	967.85	2	483.93	3.02^{NS}
Within doses	4427.05	2	2213.53	13.83*
Residual	640.0156	4	160.00	

LSD value doses=28.71 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-LXXIX: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	158.11	2	79.06	5.00^{NS}
Within doses	51.31	2	25.66	1.62^{NS}
Residual	63.2200	4	15.80	

NS=non Significant

Appendix table-LXXX: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	5951.04	2	2975.52	0.82^{NS}
Within doses	4044.03	2	2022.02	0.56^{NS}
Residual	14505.2500	4	3626.31	

NS=non Significant

Appendix table-LXXXI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1690.94	2	845.47	0.60^{NS}
Within doses	13942.86	2	6971.43	4.93^{NS}
Residual	5655.7556	4	1413.94	

NS=non Significant

Appendix table-LXXXII: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1590.84	2	795.42	1.44^{NS}
Within doses	10211.53	2	5105.77	9.24*
Residual	2210.3589	4	552.59	

LSD value doses =53.36 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-LXXXIII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of larvae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	79873.76	4	19968.44	36.23***
Within doses	8354.95	2	4177.48	7.58*
Residual	4409.86	8	551.23	

LSD value crosses=34.3 (5%), 44.27 (1%)
LSD value doses=44.28 (5%),

*** Highly significant (P<0.001)
** Significant (P<0.05)

Appendix table-LXXXIV: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	30693.24	4	7673.31	14.49**
Within generations	825.83	2	412.92	0.78^{NS}
Residual	4237.4473	8	529.68	

LSD value crosses=33.61 (5%), 48.89 (1%)

** Significant (P<0.01)
NS=non Significant

Appendix table-LXXXV: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	83783.98	4	20946.00	76.29***
Within generations	1259.88	2	629.94	2.29^{NS}
Residual	2196.3373	8	274.54	

LSD value crosses=24.21 (5%), 35.21 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXXVI: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	104708.68	4	26177.17	30.46***
Within generations	5324.85	2	2662.43	3.10^{NS}
Residual	6874.6140	8	859.33	

LSD value cross=42.83 (5%), 62.29 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXXVII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1931.63	2	965.82	4.23^{NS}
Within doses	3716.16	2	1858.08	8.14*
Residual	913.1700	4	228.29	

LSD value doses=34.29 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-LXXXVIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	2075.39	2	1037.70	1.66^{NS}
Within doses	800.99	2	400.50	0.64^{NS}
Residual	2494.1800	4	623.55	

NS=non Significant

Appendix table-LXXXIX: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	984.99	2	492.50	0.47^{NS}
Within doses	9913.18	2	4956.59	4.73^{NS}
Residual	4192.1589	4	1048.04	

NS=non Significant

Appendix table-LXXXX: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1108.01	2	554.01	0.43^{NS}
Within doses	15395.58	2	7697.79	6.01^{NS}
Residual	5121.6456	4	1280.41	

NS=non Significant

Appendix table-LXXXXI: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	1045.66	2	522.83	0.95^{NS}
Within doses	10321.66	2	5160.83	9.37*
Residual	2202.0489	4	550.51	

LSD value doses=53.26 (5%)

NS=non significant
* Significant (P<0.05)

Appendix table-LXXXXII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of pupae.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	78470.83	4	19617.71	29.10***
Within doses	9680.14	2	4840.07	7.18*
Residual	5393.99	8	674.25	

LSD value crosses=37.94 (5%), 55.18 (1%)
LSD value doses=48.97 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-LXXXXIII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	7090.85	4	1772.71	13.40**
Within generations	132.33	2	66.17	0.50^{NS}
Residual	1058.5533	8	132.32	

LSD value crosses=16.81 (5%), 24.44 (1%)

** significant (P<0.01)
NS=non Significant

Appendix table-LXXXXIV: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	18497.19	4	4624.30	75.35***
Within generations	206.28	2	103.14	1.68^{NS}
Residual	490.9993	8	61.37	

LSD value crosses=11.44 (5%), 16.65 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-LXXXXV: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	24129.37	4	6032.34	55.93***
Within generations	1690.50	2	845.25	7.84*
Residual	862.8073	8	107.85	

LSD value crosses=15.17 (5%), 22.07 (1%)
LSD value generations=19.59 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-LXXXXVI: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	209.39	2	104.70	1.00^{NS}
Within doses	1229.36	2	614.68	5.90^{NS}
Residual	416.8500	4	104.21	

NS=non Significant

Appendix table-LXXXXVII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	457.63	2	228.82	1.43^{NS}
Within doses	101.63	2	50.82	0.32^{NS}
Residual	640.4200	4	160.10	

NS=non Significant

Appendix table-LXXXXVIII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	333.95	2	166.98	1.07^{NS}
Within doses	2444.91	2	1222.46	7.84*
Residual	623.7800	4	155.94	

NS=non Significant
LSD value doses=28.35 (5%) * Significant (P<0.05)

Appendix table-LXXXXIX: Analysis of variance showing the effects of different generations and doses following cross ACM-D treated males and EMS treated females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	203.28	2	101.64	0.54^{NS}
Within doses	3198.48	2	1599.24	8.57*
Residual	746.4800	4	186.62	

NS=non Significant
LSD value doses=31 (5%) * Significant (P<0.05)

Appendix table-C: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	233.64	2	116.82	0.81^{NS}
Within doses	2129.40	2	1064.70	7.39*
Residual	576.0356	4	144.01	

LSD value doses=27.24 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-CI: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	15591.20	4	3897.80	31.79***
Within doses	2053.74	2	1026.87	8.37*
Residual	980.95	8	122.62	

LSD value crosses=16.18 (5%), 23.53 (1%)
LSD value doses=20.88 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-CII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	8106.50	4	2026.63	15.49**
Within generations	339.40	2	169.70	1.30^{NS}
Residual	1046.6440	8	130.83	

LSD value crosses=16.71 (5%), 24.31 (1%)

**significant (P<0.01)
NS=non Significant

Appendix table-CIII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	20514.00	4	5128.50	22.62***
Within generations	970.00	2	485.00	2.14^{NS}
Residual	1813.8560	8	226.73	

LSD value cross=21.99 (5%), 31.99 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-CIV: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	31738.05	4	7934.51	34.25***
Within generations	2204.85	2	1102.43	4.76*
Residual	1853.3400	8	231.67	

LSD value crosses=22.24 (5%), 32.34 (1%)
LSD value generation=28.71 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-CV: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	662.14	2	331.07	2.13^{NS}
Within doses	1344.65	2	672.33	4.32^{NS}
Residual	623.1389	4	155.78	

NS=non Significant

Appendix table-CVI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	709.93	2	354.97	2.15^{NS}
Within doses	278.78	2	139.39	0.84^{NS}
Residual	660.6589	4	165.16	

NS=non Significant

Appendix table-CVII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	220.51	2	110.26	0.34^{NS}
Within doses	2785.39	2	1392.70	4.35^{NS}
Residual	1279.9400	4	319.98	

NS=non Significant

Appendix table-CVIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	341.79	2	170.90	0.40^{NS}
Within doses	4260.99	2	2130.50	5.03^{NS}
Residual	1693.4600	4	423.36	

NS=non Significant

Appendix table-CIX: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	255.92	2	127.96	0.76^{NS}
Within doses	2970.40	2	1485.20	8.78*
Residual	676.6400	4	169.16	

LSD value doses=29.52 (5%)

NS=non Significant
* Significant (P<0.05)

Appendix table-CX: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the number of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	19581.64	4	4895.41	27.12***
Within doses	2435.75	2	1217.88	6.75*
Residual	1444.15	8	180.52	

LSD value crosses=19.63 (5%), 28.55 (1%)
LSD value doses=30.49 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-CXI: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	2.88	4	0.72	48.77***
Within generations	0.24	2	0.12	8.13*
Residual	0.1181	8	0.01	

LSD value crosses=0.15 (5%), 0.21 (1%)
LSD value generations=0.19 (5%),

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-CXII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	7.85	4	1.96	112.81***
Within generations	0.48	2	0.24	13.80**
Residual	0.14	8	0.02	

LSD value crosses=0.21(5%), 0.3 (1%) *** Highly significant (P<0.001)
LSD value generations=0.27 (5%), 0.39 (1%) ** Significant (P<0.01)

Appendix table-CXIII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	19.66	4	4.92	148.06***
Within generations	0.67	2	0.34	10.09**
Residual	0.2656	8	0.03	

LSD value crosses=0.25 (5%), 0.37 (1%) *** Highly significant (P<0.001)
LSD value generations=0.33 (5%), 0.48 (1%) ** Significant (P<0.01)

Appendix table-CXIV: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.32	2	0.16	23.28**
Within doses	3.48	2	1.74	253.19***
Residual	0.0275	4	0.01	

LSD value generations=0.19 (5%), 0.74 (1%) ** Significant (P<0.01)
LSD value doses=0.19 (5%), 0.74 (1%) *** Highly significant (P<0.001)

Appendix table-CXV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.01	2	0.005	2.5^{NS}
Within doses	0.01	2	0.005	2.5^{NS}
Residual	0.0082	4	0.002	

NS=non Significant

Appendix table-CXVI: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.39	2	0.20	40.40**
Within doses	5.22	2	2.61	527.27***
Residual	0.0198	4	0.00495	

LSD value generations=0.16 (5%), 0.26 (1%) * Significant (P<0.01)
LSD value doses=0.16 (5%), 0.26 (1%) *** Highly significant (P<0.001)

Appendix table-CXVII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.53	2	0.27	27.00**
Within doses	5.92	2	2.96	296.00***
Residual	0.0470	4	0.01	

LSD value generations=0.23 (5%), 0.38 (1%) ** significant (P<0.01)
LSD value doses=0.23 (5%), 0.38 (1%) *** Highly significant (P<0.001)

Appendix table-CXVIII: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.52	2	0.26	26.00**
Within doses	4.59	2	2.30	230.00***
Residual	0.0406	4	0.01	

LSD value generations=0.23 (5%), 0.38 (1%) ** Significant (P<0.01)
LSD value doses=0.23 (5%), 0.38 (1%) *** Highly significant (P<0.001)

Appendix table - CXIX: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	8.71	4	2.18	12.11**
Within doses	4.98	2	2.49	13.83**
Residual	1.42	8	0.18	

LSD value crosses= .62 (5%), 0.90 (1%) ** Significant (P<0.01)
LSD value doses=0.80 (5%), 1.16 (1%)

Appendix table-CXX: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	115.60	4	28.90	180.62***
Within generations	2.07	2	1.04	6.56*
Residual	1.2619	8	0.16	

LSD value crosses=0.58 (5%), 0.85 (1%) *** Highly significant (P<0.001)
LSD value generations=0.75 (5%), * Significant (P<0.05)

Appendix table-CXXI: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	268.88	4	67.22	89.63***
Within generations	2.93	2	1.47	1.96^{NS}
Residual	5.98	8	0.75	

LSD value crosses=1.26 (5%), 1.84 (1%)

*** Highly significant (P<0.001)
NS=non Significant

Appendix table-CXXII: Analysis of variance showing differences within crosses and gen erations following treatment with 3.0ml MMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	480.55	4	120.14	600.70***
Within generations	10.42	2	5.21	26.05***
Residual	1.5966	8	0.20	

LSD value cross=0.65 (5%), 0.95 (1%)
LSD value generations=0.84 (5%), 1.23 (1%)

*** Highly significant (P<0.001)

Appendix table-CXXIII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	3.84	2	1.92	14.76*
Within doses	108.62	2	54.31	417.77***
Residual	0.5116	4	0.13	

LSD value generations=0.82 (5%)
LSD value doses=0.82 (5%), 1.35 (1%)

* Significant (P<0.05)
*** Highly significant (P<0.001)

Appendix table-CXXIV: Analysis of variance showing the effects of different generations and doses following cross ACM-D treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.54	2	0.27	0.55^{NS}
Within doses	0.07	2	0.04	0.08^{NS}
Residual	1.9548	4	0.49	

NS=non Significant

Appendix table-CXXV: Analysis of variance showing the effects of different generations and doses following cross MMS treated males and ACM-D treated females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	6.14	2	3.07	13.34*
Within doses	73.22	2	36.61	159.17***
Residual	0.9024	4	0.23	

LSD value generations=1.1 (5%) * Significant (P<0.05)
LSD value doses=1.1 (5%), 1.8 (1%) *** Highly significant (P<0.001)

Appendix table-CXXVI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	5.89	2	2.95	24.58**
Within doses	67.02	2	33.51	279.25***
Residual	0.4894	4	0.12	

LSD value generations=0.77 (5%), 1.3 (1%) ** Significant (P<0.01)
LSD value doses = 0.77 (5%), 1.3 (1%) *** Highly significant (P<0.001)

Appendix table-CXXVII: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	4.01	2	2.01	14.36*
Within doses	117.49	2	58.75	419.64***
Residual	0.5537	4	0.14	

LSD value generations=0.85 (5%) * Significant (P<0.05)
LSD value doses=0.85 (5%), 1.4 (1%) *** Highly significant (P<0.001)

Appendix table-CXXVIII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	259.17	4	64.79	18.11**
Within doses	93.62	2	46.81	13.08**
Residual	28.62	8	3.58	

LSD value crosses=2.76 (5%), 4.02 (1%) ** Significant (P<0.01)
LSD value doses=3.57 (5%), 5.19 (1%)

Appendix table-CXXIX: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml MMS and ACM-D on the longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	35.86	4	8.97	32.03***
Within generations	4.55	2	2.28	8.14*
Residual	2.2477	8	0.28	

LSD value crosses=0.77 (5%), 1.12 (1%) *** Highly significant (P<0.001)
LSD value generations=1.0 (5%) * Significant (P<0.05)

Appendix table-CXXX: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml MMS and ACM-D on the longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	102.21	4	25.55	56.78***
Within generations	9.15	2	4.58	9.96**
Residual	3.66	8	0.46	

LSD value crosses=0.99 (5%), 1.44 (1%) *** Highly significant (P<0.001)
LSD value generations=1.28 (5%), 1.86 (1%) ** Significant (P<0.01)

Appendix table-CXXXI: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml MMS and ACM-D on the Longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	322.28	4	80.57	424.05***
Within generations	12.01	2	6.01	31.61***
Residual	1.5200	8	0.19	

LSD value crosses=0.64 (5%), 0.93 (1%) *** Highly significant (P<0.001)
LSD value generations=0.82 (5%), 1.19 (1%)

Appendix table-CXXXII: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and control females on the number of longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	4.19	2	2.10	70.00***
Within doses	86.27	2	43.14	1438.00***
Residual	0.1091	4	0.03	

LSD value generations=0.39 (5%), 0.65 (1%) *** Highly significant (P<0.001)
LSD value doses=0.39 (5%), 0.65 (1%)

Appendix table-CXXXIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the number of longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.09	2	0.05	0.30^{NS}
Within doses	0.24	2	0.12	0.81^{NS}
Residual	0.5924	4	0.15	

NS=non significant

Appendix table-CXXXIV: Analysis of variance showing the effects on different generations and doses following cross MMS treated males and ACM-D treated females on the longevity of Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	7.16	2	3.58	59.67**
Within doses	81.91	2	40.96	682.66***
Residual	0.2475	4	0.06	

LSD value generations=0.56 (5%), 0.92 (1%) ** Significant (P<0.01)
LSD value doses=0.56 (5%), 0.92 (1%) *** Highly significant (P<0.001)

Appendix table-CXXXV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and MMS treated females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	10.56	2	5.28	24.00**
Within doses	114.38	2	57.19	259.95***
Residual	0.8913	4	0.22	

LSD value generations=1.06 (5%), 1.76 (1%) ** Significant (P<0.01)
LSD value doses=1.06 (5%), 1.76 (1%) *** Highly significant (P<0.001)

Appendix table-CXXXVI: Analysis of variance showing the effects on different generations and doses following cross MMS+ACM-D treated males and control females on the longevity Adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	7.88	2	3.94	11.25*
Within doses	129.41	2	64.71	184.88***
Residual	1.4100	4	0.35	

LSD value generations=1.34 (5%) * Significant (P<0.05)
LSD value doses=1.34 (5%), 2.22 (1%) *** Highly significant (P<0.001)

Appendix table-CXXXVII: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the longevity adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	122.02	4	30.51	7.71*
Within doses	105.75	2	52.88	13.36**
Residual	31.65	8	3.96	

LSD value crosses=2.91 (5%) * Significant (P<0.05)
LSD value doses=3.75 (5%), 5.45 (1%) ** Significant (P<0.01)

Appendix table-CXXXVIII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	1.90	4	0.48	24.00***
Within generations	0.29	2	0.15	7.50*
Residual	0.1400	8	0.02	

LSD value cross=0.21 (5%), 0.3 (1%) *** Highly significant (P<0.001)
LSD value generations=0.27 (5%) * Significant (P<0.05)

Appendix table-CXXXIX: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	5.22	4	1.31	65.50***
Within generations	0.47	2	0.24	12.00**
Residual	0.1808	8	0.02	

LSD value crosses=0.21 (5%), 0.3 (1%) *** Highly significant (P<0.001)
LSD value doses=0.27 (5%), 0.39 (1%) ** Significant (P<0.01)

Appendix table-CXXXX: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	13.35	4	3.34	66.80***
Within generations	0.66	2	0.33	6.60*
Residual	0.4194	8	0.05	

LSD value crosses=0.33 (5%), 0.48 (1%) *** Highly significant (P<0.001)
LSD value generations=0.42 (5%) * Significant (P<0.05)

Appendix able-CXXXXI: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.52	2	0.26	13.00*
Within doses	1.24	2	0.62	31.00**
Residual	0.0840	4	0.02	

LSD value generations=0.32 (5%), * Significant (P<0.05)
LSD value doses=0.32 (5%), 0.53 (1%) ** Significant (P<0.01)

Appendix table-CXXXXII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.01	2	0.005	2.35^{NS}
Within doses	0.003	2	0.0015	0.82^{NS}
Residual	0.0081	4	0.002	

NS=non Significant

Appendix table-CXXXXIII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.64	2	0.32	8.00*
Within doses	4.10	2	2.05	51.25**
Residual	0.1580	4	0.04	

LSD value generations=0.45 (5%)
LSD value doses=0.45 (5%), 0.75 (1%)

* Significant (P<0.05)
** Significant (P<0.01)

Appendix table-CXXXXIV: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.41	2	0.21	210.00***
Within doses	4.97	2	2.49	2490.00***
Residual	0.0048	4	0.0012	

LSD value generations=0.072 (5%), 0.12 (1%)
LSD value doses=0.072 (5%), 0.12 (1%)

*** Highly significant (P<0.001)

Appendix table-CXXXXV: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.32	2	0.16	53.00**
Within doses	3.19	2	1.60	533.00***
Residual	0.0126	4	0.003	

LSD value generations=0.12 (5%), 0.21 (1%) ** Significant (P<0.01)
LSD value doses = 0.12 (5%), 0.21 (1%) *** Highly significant (P<0.001)

Appendix table-CXXXXVI: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the incubation period.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	5.73	4	1.43	10.21**
Within doses	3.39	2	1.70	12.14**
Residual	1.09	8	0.14	

LSD value crosses=0.55 (5%), 0.79 (1%) ** Significant (P<0.01)
LSD value doses=0.71 (5%), 1.03 (1%)

Appendix table-CXXXXVII: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	87.32	4	21.83	38.98***
Within generations	3.07	2	1.54	2.75^{NS}
Residual	4.4725	8	0.56	

LSD value crosses=1.09 (5%), 1.59 (1%) *** Highly significant (P<0.001)
NS=non Significant

Appendix table-CXXXXVIII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	191.94	4	47.99	111.60***
Within generations	5.22	2	2.61	6.07*
Residual	3.4048	8	0.43	

LSD value crosses=0.96 (5%), 1.39 (1%) *** Highly significant (P<0.001)
LSD value generations=1.24 (5%) * Significant (P<0.05)

Appendix table-CXXXXIX: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	398.93	4	99.73	474.90***
Within generations	5.74	2	2.87	13.66**
Residual	1.6954	8	0.21	

LSD value crosses=0.67 (5%), 0.98 (1%) *** Highly significant (P<0.001)
LSD value generations=0.87 (5%), 1.26 (1%) ** Significant (P<0.01)

Appendix table-CL: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	5.07	2	2.54	7.69*
Within doses	80.70	2	40.35	122.27***
Residual	1.3038	4	0.33	

LSD value generations=1.30 (5%) * Significant (P<0.05)
LSD value doses=1.30 (5%), 2.16 (1%) *** Highly significant (P<0.001)

Appendix table-CLI: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.23	2	0.12	0.63^{NS}
Within doses	0.34	2	0.17	0.89^{NS}
Residual	0.7610	4	0.19	

NS=non Significant

Appendix table-CLII: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	5.14	2	2.57	32.12**
Within doses	69.36	2	34.68	433.50***
Residual	0.3332	4	0.08	

LSD value generations =0.64 (5%), 1.06 (1%) ** Significant (P<0.01)
LSD value doses=0.64 (5%), 1.06 (1%) *** Highly significant (P<0.001)

Appendix table-CLIII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	4.40	2	2.20	22.00**
Within doses	49.38	2	24.69	2469.00***
Residual	0.4122	4	0.10	

LSD value generations=0.71 (5%), 1.19 (1%) ** Significant (P<0.01)
LSD value doses=0.71 (5%), 1.19 (1%) *** Highly significant (P<0.001)

Appendix table-CLIV: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	3.97	2	1.99	4.00^{NS}
Within doses	89.94	2	44.97	89.94***
Residual	1.9840	4	0.50	

LSD value doses=1.61 (5%), 2.67 (1%)

NS = non significant
*** Highly significant (P<0.001)

Appendix table-CLV: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the longevity of adult males.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	202.69	4	50.67	17.37**
Within generations	73.23	2	36.62	12.55**
Residual	23.34	8	2.92	

LSD value crosses=2.49 (5%), 3.63 (1%)
LSD value generations=3.22 (5%), 4.69 (1%)

** Significant (P<0.01)

Appendix table-CLVI: Analysis of variance showing differences within crosses and generations following treatment with 1.0ml EMS and ACM-D on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	26.60	4	6.65	47.50***
Within generations	3.41	2	1.71	12.21**
Residual	1.1547	8	0.14	

LSD value crosses=0.54 (5%), 0.79 (1%)
LSD value generations=0.71 (5%), 1.03 (1%)

*** Highly significant (P<0.001)
** Significant (P<0.01)

Appendix table-CLVII: Analysis of variance showing differences within crosses and generations following treatment with 2.0ml EMS and ACM-D on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	85.09	4	21.27	709.00***
Within generations	2.36	2	1.18	39.33***
Residual	0.2774	8	0.03	

LSD value crosses=0.25 (5%), 0.37 (1%)
LSD value generations=0.33 (5%), 0.48 (1%)

*** Highly significant (P<0.001)

Appendix table-CLVIII: Analysis of variance showing differences within crosses and generations following treatment with 3.0ml EMS and ACM-D on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	266.19	4	66.55	207.97***
Within generations	4.58	2	2.29	7.16*
Residual	2.5564	8	0.32	

LSD value crosses=0.83 (5%), 1.20 (1%)
LSD value generations=1.07 (5%)

*** Highly significant (P<0.001)
* Significant (P<0.05)

Appendix table-CLIX: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and control females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	3.21	2	1.61	5.03^{NS}
Within doses	69.92	2	34.96	109.25***
Residual	1.2778	4	0.32	

LSD value doses=1.28 (5%), 2.12 (1%)

NS=non significant
*** Highly significant (P<0.001)

Appendix table-CLX: Analysis of variance showing the effects of different generations and doses following crosses ACM-D treated males and control females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	0.08	2	0.04	2.00^{NS}
Within doses	0.19	2	0.10	5.00^{NS}
Residual	0.0640	4	0.02	

NS=non significant

Appendix table-CLXI: Analysis of variance showing the effects on different generations and doses following cross EMS treated males and ACM-D treated females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	2.50	2	1.25	62.50***
Within doses	97.54	2	48.77	2438.50***
Residual	0.0960	4	0.02	

LSD value generations=0.32 (5%), 0.53 (1%) *** Highly significant (P<0.001)
LSD value doses=0.32 (5%), 0.53 (1%)

Appendix table-CLXII: Analysis of variance showing the effects on different generations and doses following cross ACM-D treated males and EMS treated females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	2.68	2	1.34	67.00***
Within doses	119.61	2	59.81	2953.50***
Residual	0.0637	4	0.02	

LSD value generations=0.32 (5%), 0.53 (1%) *** Highly significant (P<0.001)
LSD value doses=0.32 (5%), 0.53 (1%)

Appendix table-CLXIII: Analysis of variance showing the effects on different generations and doses following cross EMS+ACM-D treated males and control females on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within generations	3.68	2	1.84	10.82*
Within doses	137.82	2	68.91	405.35***
Residual	0.6623	4	0.17	

LSD value generations=0.94 (5%) * Significant (P<0.05)
LSD value doses=0.94 (5%), 1.55 (1%) *** Highly significant (P<0.001)

Appendix table-CLXIV: Analysis of variance showing the effects on different crosses and doses following treatment with MMS and ACM-D on the longevity of adult females.

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean sum of squares (MS)	Variance ratio (F)
Within crosses	96.78	4	24.20	6.63*
Within generations	112.46	2	56.23	15.42**
Residual	29.17	8	3.65	

LSD value crosses= 0.79 (5%) * Significant (P<0.05)
LSD value generations=3.60 (5%), 5.24 (1%) ** Significant (P<0.01)