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Integration of Irradiation with Cytoplasmic Incompatibility in the Management of the Pulse Beetle Callosobruchus spp. (Coleoptera: Bruchidae)

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Integration of Irradiation with Cytoplasmic Incompatibility in the Management of the Pulse Beetle *Callosobruchus* spp. (Coleoptera: Bruchidae)

A THESIS

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By

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Summary

In the thesis, the impact of radiation on successive generations and cytoplasmic incompatibility of *Callosobruchus* spp. is studied. *C. chinensis* and *C. maculatus* are considered as the study insects. Experiments of oviposition preference and mating competitiveness are also conducted. Study showed that *C. chinensis* collected either from lentil or Bengal gram preferred lentil << Bengal gram < green gram for oviposition. Regarding the adult emergence, their preferred seeds are green gram >> Bengal gram> Lentil. *C. maculatus* preferred seed according as they collected from. The highest emergences rate is found in green gram. *C. maculatus* preferred pea for oviposition but the emergence rate is nearer to zero in it. Irradiation is able to increase the development time of egg to adult in successive generations of *C. maculatus*. The dose 12Gy the maturity time in F5 generation has gone more than 100 days. In breeding of the progeny of the irradiated parent do not affect the percentage of adult emergence. For *C. chinensis* the degree CVs decreases as the treated males increased. Further increase of the number of males the CVs would be the same. For *C. maculatus* the ratio 1:1:1 showed the highest competitiveness values for both radiation doses 6Gy and 8Gy. The dose with 8Gy showed the highest competitiveness value. For 6Gy dose the competitiveness values were the maximum for the ratio 1:1:3 and 1:1:4 and the minimum number of value is attained at the ratio 1:1:2. Both in *C. chinensis* and *C. maculatus* the regional and reciprocal crosses have significant variation regarding the different components of reproductive potentials. In *C. chinensis* only the cross Chittagong ♂ X Narayangonj ♀ showed the imcompatibility. In *C. maculatus* suspected reciprocal crosses are Chittagong ♂ X Japan ♀, Chittagong ♂ X Narayangonj ♀, Narayangonj ♂ X Japan ♀ and Narayangonj ♂ X Rajshahi ♀ that are supposed to be the incompatible crosses. The CI level for these crosses are 0.597, 0.592. , 0.582 and 0.464 respectively and the relative the estimated relative fecundity level estimated and found that Chittagong ♂ X Japan ♀, Chittagong ♂ X Narayangonj ♀, and Narayangonj ♂ X Japan ♀ fulfil the condition of persistent incompatibility. The critical migration rates for the crosses Chittagong ♂X Japan ♀, Chittagong ♂ X Narayangonj ♀, and Narayangonj ♂ X Japan ♀ was 0.0040, 0.0032 and 0.0042 respectively. The only 2% of migration of incompatibility related infection happened in the crosses on an average.

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Declaration

I hereby declare that the thesis titled "**Integration of Irradiation with Cytoplasmic Incompatibility in the Management of the Pulse Beetle *Callosobruchus* spp. (Coleoptera: Bruchidae)**" is the result of my own research work undertaken by the Department of Zoology, University of Rajshahi, Bangladesh. I further declare that this thesis has not concurrently been submitted in part or in full previously for any degree or diploma in either this university or any other university.

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Certificate

This is to certify that this thesis titled "**Integration of Irradiation with Cytoplasmic Incompatibility in the Management of the Pulse Beetle *Callosobruchus* spp. (Coleoptera: Bruchidae)**" is a research work carried out by Eliza Sharmeen, for the degree of Doctor of Philosophy undertaken by the Department of Zoology, University of Rajshahi, Bangladesh. Any part or whole of this thesis has not been submitted elsewhere for any degree or award.

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Certificate

This is to certify that the thesis titled "**Integration of Irradiation with Cytoplasmic Incompatibility in the Management of the Pulse Beetles, *Callosobruchus* spp. (Coleoptera: Bruchidae)**" is a research work done by Eliza Sharmeen, for the degree Doctor of Philosophy under the department of Zoology, University of Rajshahi, Bangladesh. As far as I know, any part or whole of this thesis has not been submitted elsewhere for any other degree or award.

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Chapter 1

Introduction

1.1 Background

Pulses like peas, cowpeas and beans are grown in most part of the world as food crops (Raina, 1970). These are especially important source of protein in the tropical and sub-tropical region of the world. The seeds of these crops are very prone to infest in storage condition by different stored grain pests of the family Bruchidae. The pulse beetle, *Callosobruchus maculatus* (Fab.) is a major field-to-store pest of cowpea (*Vigna unguiculata* Walp.) and other grain legumes in the tropics and sub-tropics. *C. chinensis* (L.) is another major pest of stored pulses which causes a substantial amount of damage thus affecting national economy (Islam, 1980). *C. chinensis* is a major pest of chickpea (*Cicer arietinum*), lentil (*Lens esculenta*), green gram (*Vigna radiata*), adzuki bean (*V. unguilaris)* and cowpeas (*V. unguiculata*) (Haines, 1991). It also attacks other pulses on occasions but appears to be in capable of developing on the common beans, *Phaseolus vulgaris.*

The pest multiplies rapidly under warm humid condition and inflicts serious damage within a short time will loss be up to 100% (Borikar and Puri, 1985) *C. chinensis* (L.) is responsible for 30% losses in the shored pulses every year in Bangladesh except in blackgram, *Vigna mungo*. Both the pests cause damage only at immature stage and the adults do not have granaries (Begum *et al.*, 1985). The beetles multiply rapidly in the tropics, thus inflicting much damage to the stored pulse within short time (Hill, 1983, 1990). Adult female insects lay their eggs on the seed coat of the seed. The eggs hatch and the larvae burrows into the seed, consuming the cotyledons, through four instars, pupates in an excavated chamber and then subsequently emerges through the seed test. The damage after 3 months was 100% in *V. radiata* by *C. maculatus* (Osman *et al.*, 1991). *C. maculatus* is the most important pest of cowpea (*V. unguiculata*) stored in West African countries. The high fecundity of females of *C. maculatus* contributes to increase the larval population in cowpea seeds. This leads to important damage, which can affect 100% of the seeds of *V. unguiculata* between six to seven months (Thomas and Gaspar, 1994).

1.2 Developmental Biology of *Callosobrochus* spp.

Adults of *C. chinensis* are very short lived, usually no more than 12 days under optimum conditions. During this time the females lay about 70 eggs, although oviposition may be depressed in the presence of previously infested seeds. The optimum temperature for oviposition is about 23°C. Eggs are firmly glued to the surface of the host seeds, smooth seed varieties being more suitable for oviposition than rough coat. The eggs are domed structures with Oval flat bases. When newly laid they are looking very small, translucent Gary and inconspicuous. Upon hatching the larva bites through the base of the egg, through the test of the seed and into the cotyledons. Detritus produced during this period is packed into the egg as the insect hatches, turning the egg white and making it clearly visible to the naked eyes. The developing larva feeds entirely within a single seed, excavating a chamber within the cotyledons as it grows. The optimum condition for development is around 32^{0}C and 90% RH and the minimum development period are very short (22-23 days). Eggs hatch within 5-6 days after oviposition. Infestation commonly begins in the field, where eggs are laid on maturating pods. As the pods dry, the pest's ability to infest them decreases. Thus dry seeds stored in their pods are quite resistant to attack, whereas the threshed seeds are susceptible to attack throughout storage. (Haines, 1991)

The biological activities of *C. maculatus* are very similar to those of *C. chinensis* expect that the females of *C. maculatus* lay as many as 115 eggs. The optimum temperature for oviposition is 30-35^{0}C; development period of *C. maculatus breeding on seeds of V. unguiculata* is about 36 days (Haines, 1991).

C. chinensis are rather square in shape, but *C. maculatus* are more elongated. Antennae are pectinate in the males and slightly serrate are in the females; the hind femora have a pair of parallel ridges on the ventral edge, each with an apical spine, the marking on the elytra vary some what, but the dark patches can be quite conspicuous. The eyes are characteristically emarginated (Hill, 1990).

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1.3 Control of *Callosobruchus* spp.

As mentioned earlier, both species are the serious threat to the stored pulses. Control to such stored product pests through insecticides causes emergence of insecticide resistance. Besides it is neither feasible nor appropriate owing to the food poisoning (Oudejans, 1982), health hazard and envirunmental pollution (Bhaduri *et al.*, 1985) and higher costs (Khan and Mannan, 1991). Though attempts have been made, successful biological control programs with these pests have yet to be developed (Islam, 1994a, b).

Sterile male technique is one of the effective means of combating pest species (Knipling, 1967, Waterhouse *et al.*, 1973), where sterile but competitive males are released in the field to compete with normal males, bringing about a suppression of the species. Such agents as radiation (Makee, 1989, Nation and Burditt Jr., 1994), chemosterilants (McDonald, 1974) and endosymbiotic microorganisms (Rousset and Raymond, 1991) gained much popularity in sterilization or complete breakdown in gamete formation. Such promising approach for acquiring sterile male of the pulse beetle has led to design the present investigation.

Several insects species have been controlled successfully by using nuclear techniques which appears to be potential alternatives to chemicals for stored product pests (Gill and Pajni, 1989, Hussain and Imura, 1989, Olaifa *et al.*, 1990). Recent studies on different groups of insects suggests that irradiation is useful for inducing effective sterility in various pest insects ((Hasan and Khan, 1998, Islam *et al.*, 2000, Nation and Burditt

Jr., 1994). Comparative studies of the radiation sensitivity among stored product insect pests are important for nuclear control strategy because many species may be presented in infested pulses and grains and radiation dosage must be high enough to control the most resistant species present. This is crucial because, sensitivity to radiation varies depending on stages and strains of the pest species (Brower, 1972, Cornwell, 1966, Gonen and Fishban, 1974).

1.4 Effect of Cytoplasmic Incompatibility on Insects

Cytoplasmic incompatibility (CI) refers sterile (incompatible) crosses between populations from different geographical locations due to a failure of syngamy. It results therefore in the occurrence of high rate of embryonic mortality in interspecific crosses between individuals possessing different cytoplasmic genomes (Rousset and Raymond, 1991). It is a widespread phenomenon among plants and animals; involving self-replicating but extrachromosomal factors of a variety of types without any involvement of choromosomal genes and resulting in reduced rate of non-hatching but mostly embryonated eggs .by inseminated females (Clements, 1992, Yen and Barr, 1973). One of the most investigated instances is that of *Culex pipiens,* in which crosses between populations from different geographical locations produce sterile insects. The sterility occurs when inseminated females of incompatible strains of an insect mate produce non-hatching eggs. Most of the authors have recognized three categories of crosses, although their definitions have varied.

However the following definitions are consistent with current usage (Clements, 1992).

1. Compatible crosses: Larvae hatch from ≥50% eggs laid and the sex' ratios are normal.
2. Partially compatible crosses: Embryonic development proceeds in a substantial percentage of eggs but these have an intermediate hatch rate (≤50%) and adults that develop include males and females.
3. Incompatible crosses: Embryonic development proceeds in a good number of eggs but larvae hatch from only 0 to ≤ 1% of eggs and adults, if any, that develop (of course, parthenogenetically) are always females.

It was found that crosses of *C. pipiens* from different geographical areas sometimes produced eggs that did not hatch (Marshall and Staley, 1937). Ghelelovitch (1952) and Laven (1951) described this unusual reproductive incompatibility later in *C. pipiens* mosquitoes. He recognized the phenomena as a possible means of controlling mosquitoes and the phenomena was soon studied extensively. Laven (1959) found that the incompatibilities have a cytoplasmic inheritance pattern and he called it "Cytoplasmic Incompatibility". Laven (1967) showed that the factor(s) causing incompatibility maternally transmitted through the cytoplasm, often as many as half of the eggs laid were dead with haploid embryos in them and mating insemination of the females appeared to be normal. After that a reckettsia-like microorganism *Wolbachia pipiens* were first described from the gonadal tissues of the mosquitoes *C. pipiens* L. in 1924 by Hertig and Wolbach. Yen & Barr (1971) first correlated cytoplasmic incompatibility with the presence of *Wolbachia* symbionts. The symbiont *Wolbachia* are intracellular bacteria (alpha subdivision of the protobacteria or purple bacteria) transovarially-transmitted and manipulate the reproduction of hosts. The nature of manipulation varies with *Wolbachla* strain, arthropod taxa and arthropod genetic system. Though Giordano *et al.,* (1995) demonstrated that some strains of *Wolbachia* have caused no reproductive alternation or CI, many investigation revealed that reproductive incompatibilities, Sex-ratio biases, and introduction of thelytoky (i.e. virgin females produce diploid daughters parthenogenetically, rarely producing males) are some of the results of *Wolbachia* symbiosis. Johanowicz and Hoy (1998) reported that when *Wolbachia* infected males of *M. occidentalis* were crossed with cured females, the Incompatibility phenotype includes a unique combination of reduced progeny production and skewed sex ratio of few resulting progeny. However, *Wolbachia,* the reason of cytoplasmic incompatibility, producing two types of cytoplasmic incompatibility, unidirectional, resulting from single *Wolbachia* infection type and Bidirectional, resulting from two different *Wolbachia* infection type.

Whatever may be the types, Cytoplasmic incompatibility (CI) was found not only in insects like mosquito, *Nasonia,* plant hoppers, raisin moths, beetles and *Drosophila,* but also in an isopod (Crustacea), showing that the phenomenon is not restricted to insects. Soon after incompatibility was described in *Drosophila,* major advances were made in understanding both the population dynamics of incompatibility systems and the molecular evolution of incompatibility factors. Unidirectional incompatibility in the flour beetle *Tribolium confusum* was initially detected in crosses between two strains by Stanley in (1961) and this was confirmed in other strains by Wade & Stevens In (1985). Females from these strains produced unproductive eggs when mated with males from other strains. O'Neill (1989) detected *Wolbachia* like microorganisms in the ovaries of *T. confusum.* Further work on *T. confusum* accompanied by the presence of *Wolbachia* and its curing with an antibiotic was reported by Stevens (1989). Using polymerase chain reaction (PCR) assays, O'Neill *et al.* (1992) studied a range of insect species including *T. confusum* and concluded that CI may be a more general phenomenon in insects than it was initially assumed. Based on a multi strain single-pair mating assay designed to look for variation in incompatibility type, Fialho & Stevens (1996) demonstrated that *Wolbachia* infections caused a common incompatibility type across strains of *T. confusum.* Though the presence of any rickettsia like microorganism in *T. confusum* is not yet confirmed, Islam *et al.* (1997) have reported an incompatible cross between a local and an exotic strain of the beetle. Evidence of CI has been obtained in another coleopteran, the alfalfa weevil, *Hypera postica* (Hsiao and Hsiao, 1985a, b). Crossing data from several other insects and recent studies with the PCR determined that 16% of all insects species examined are infected with *Wolbachia* (Werren *et al.*, 1995). The infected species include representatives from a wide variety of orders and families (Giordano *et al.*, 1997) and number of additional cases of CI are expected to be detected in future (Hoffmann and Turelli, 1997).

Recently the infection due to *Wolbachia* in *Callosobruchus* spp. was found. Kondo *et al.* (2002, 1999) identified triple infection with three distinct *Wolbachia* strains in Japanese populations of the adzuki bean beetle, *C. chinensis*. Ijichi *et al.* (2002) attempted to understand the mechanisms underlying the infection with these three organisms, the spatiotemporal infection dynamics of the three *Wolbachia* strains in detail by using a quantitative PCR technique. Despite these publications on *Wolbachia* infection in a species of *Callosobruchus* spp. there is a huge knowledge gap of the infection regarding the other species like *C. maculatus* or *C. analis* which were demonstrated as the most important adzuki bean beetles.

If such a thing happened for the whole *Callosobruchus* spp., CI could be a genetic method for controlling an insect pest species. It could provide 'ready made' sterile-like males for a control operation (Shahid and Curtis, 1987). Since CI produces unviable progeny, it has been used in the control of main vector of bancroftian filariasis, *Culex pipiens* in which crosses between many strains of different geographical origins are sterile in both directions (Krishnamurthy and Laven, 1976). It also helpful for studying chromosome imprinting and chromosomal condensation, as well as elucidating modes of specification (Breeuwer and Warren, 1990). CI, however, *Wolbachia* infections may be responsible for protecting the western biotype of the alfalfa weevil from a parasitoid as indicated by Hsiao (1996). Successful use of CI is dependent in the degree of linkage between *Wolbachia* and the genetically altered symbiont. CI has the advantage over other gene spreading mechanisms, such as P-elements mediated hybrid dysgenesis, in that it may be possible to use it to spread genes into same population more than once (Beard *et al.*, 1993). Recent studies have shown that CI could be a means to drive genetically modified symbionts into a population. Braig *et al.* (1994) were able to replace the natural infection of *D. simulans* by means of micro injecting purified *Wolbachia* from the Asian tiger mosquito, *Aedes albopricti!s.* Turell & Hoffmann (1999) generated models involving microbe-induced CI as mechanism for introducing transgenes into arthropod populations. The successful transfer of *Wolbachia* between distantly related hosts suggest that it may be possible to introduce this symbiont experimentally into arthropod species of medical and agricultural importance in order to manipulate populations genetically.

1.5 *Wolbachia*, Historical Overview

Wolbachia bacteria were first described from the gonadal tissues of the mosquito *Culex pipiens* L. in 1924 by Hertig and Wolbach. Unusual reproductive incompatibilities were described later in *Culex pipiens* mosquitoes by Laven (1951). One type of these incompatibilities was nonreciprocal, meaning that crosses of males from population A with females of population B resulted in normal hybrid progeny, but crosses of males from population B with females from population A (the reciprocal cross) resulted in few viable hybrid progeny. Because the nuclear genetic makeup of the two hybrid crosses is essentially the same and the main difference is which mother's cytoplasm is interacting with the nuclear genes, the incompatibilities have a cytoplasmic inheritance pattern, also called "cytoplasmic incompatibility" (Laven, 1959). In the 1970s Yen & Barr (1973) first correlated these nonreciprocal, cytoplasmic incompatibilities with the presence of *Wolbachia* endosymbionts. They found that when *Wolbachia*-infected males were treated with tetracycline (which is toxic to rickettsia-like microorganisms), they could reproduce successfully with uninfected females. Because *Wolbachia*'s morphological characters are of limited value and *Wolbachia* are difficult to culture outside the host (O'Neill *et al.*, 1992, Weiss and Moulder, 1984), their presence in other arthropods was merely speculative. However, in 1992, *Wolbachia*-specific polymerase chain reaction (PCR) primers were developed (O'Neill *et al.*, 1992). After that *Wolbachia* infection was confirmed in some California populations of *Drosophila simulans* Sturtevant (O'Neill *et al.*, 1992). This symbiont was previously suspected as the causative agent of nonreciprocal reproductive incompatibilities between geographical populations of *D. simulans* (Hoffmann *et al.*, 1986).

1.6 Nature of *Wolbachia*

Wolbachia are intracellular, transovarially-transmitted, rickettsia-like endosymbionts in the alpha-subdivision of the proteobacteria (purple bacteria). *Wolbachia* are transmitted through the egg cytoplasm, and therefore solely by females, except for one reported case of male transmission in laboratory populations of *D. simulans* (Hoffmann and Turelli, 1988). *Wolbachia* are sensitive to high temperatures (Girin and

Boulétreau, 1995, Louis *et al.*, 1993, Stevens, 1989, Stouthamer *et al.*, 1990) and the antibiotics rifampin and tetracycline (Stouthamer *et al.*, 1990). The only success to date in culturing them outside the host has been in an *Aedes albopictus* (Skuse) cell line (O'Neill *et al.*, 1995).

The effects of *Wolbachia* can be influenced by several factors. The strain of *Wolbachia* is important; some strains have been demonstrated to cause no reproductive alterations (Giordano *et al.*, 1995). The phenotype of *Wolbachia*-mediated reproductive alterations also depends on the taxonomic status of the affected arthropod (Insecta, Arachnida, Isopoda) (Werren, 1997), as well as the genetic system of the arthropod. *Wolbachia* symbiosis may have other important effects. *Wolbachia*-mediated reproductive isolation may be one mechanism that could allow sympatric speciation to occur (Giordano *et al.*, 1997, Laven, 1959, Werren, 1997). *Wolbachia* alters sex ratios and progeny survival, as a consequence, may affect laboratory experiments and insect management in field programs. *Wolbachia* infection may have implications for mass rearing projects, especially if the bacteria have an influence on the quality of the natural enemies (Steiner, 1993) or affect the rate of population increase of the individuals being reared.

Wolbachia manipulate arthropod reproduction by causing nonreciprocal incompatibility, bidirectional incompatibility, skewed sex ratios, and thelytoky, depending on the *Wolbachia* strain, arthropod taxa, and genetic system. For example, *Wolbachia* is associated with both reduced egg production and a male-biased sex ratio of the few remaining progeny in *M. occidentalis*, a predatory mite with a parahaploid genetic system (Denise *et al.*, 1998).

1.7 Effect of Gamma Radiation on Insects.

Radiation is neither feasible not appropriate owing to the food poisoning (Oudejans, 1982), health hazards and environmental pollution (Bhaduri *et al.*, 1985) and higher const (Khan and Mannan, 1991). Despite the facts, the use of radiation has proved to be very effective in development of environment friendly integrated pest management technology for the protection and preservation of stored and field crops. Ionising radiation has been used to manage storage pests as an alternative to fumigants and other control methods for several decades. Irradiation of insects has attracted wide attention in various fields from development to genetics and through its possible application to insect pest eradication programms (Knipling, 1955). There have also been two kinds of practical application of radiation to the problem of insect control. One is the direct killing and the other is sterilization by radiation of the whole pest population (O'Brien and Wolfe, 1963).

According to (Bahari, 1994), the selection of insect control by irradiation is either to induce sterility or mortality. A high dose of radiation affects an insect total competitiveness by reducing its ability to seek a native mate and its sexual aggressiveness and vigour by affecting its longevity or disrupting the synchrony of its biological rhythms (Hasan, 1995, LaChance, 1985, Tsubaki and Bumroongsoo, 1990). For the adult cowpea weevil *Callosobruchus maculatus* (F.) the sub-lethal doses of gamma-radiation are 8 GY (El-Sayed *et al.*, 1988). When male *Callosobruchus maculatus* irradiated with a sterilizing dose of gamma radiation and confined with untreated virgin females caused the production of infertile eggs whether mating took place on the day of treatment or three days later (Ahmed *et al.*, 1980). By using dose of gamma radiation on *C. analis* fecundity of parents and their progeny remained unchanged, fertility showed dose-dependent variation whereas sex ratio and longevity of the experimental population remain undistorted (Begum *et al.*, 1981a). When male of *C. analis* irradiated with sub-sterilizing doses of gamma radiation allowed mating with unirradiated virgin female, parental generation respond by the decline in its progeny according to the dose (Begum *et al.*, 1981b). Female of *C. chinensis* exhibited significant affected egg production when treated with gamma radiation doses ranging from 5 to 60Gy (Begum, 1984). Adults of *C. maculatus* resulting from irradiated pupae had lower fecundity and fertility as the dose increased (Ghogomu, 1989). The fecundity and fertility of adults irradiated with gamma radiation either as mature pupae or 1-day old adults decreased with increasing dose. A dose of 640 Gy caused instant mortality of mature and 1-day old adults. (Hussain and Imura, 1989). Adult emergence was prevented when young larvae (up to 10 days old) and older larvae (14 and 16 days old) of *C. maculatus* were treated with 6 and 30 Krad respectively (Ghogomu, 1990). When female of *C. maculatus* were exposed to sub-sterilizing doses the egg production on the first day of oviposition was reduced and both males and females become sterile at a dose of 1 Kard. (Ghogomu, 1991). The combination of sterile males, sterile females, normal males and normal females resulted in suppression of the population of *C. chinensis* at ratio of 7:7:1:1 and 5:5:1:1 following treatment with 3 and 3.5 Kr. respectively when sterile males and females were more competitive than normal insect (Gill and Pajni, 1989). Arrested development was observed at doses of 1.0 4.0, 10.0 and 10.0 Gy in the egg, 1st, 3rd, and 4th larval instars respectively, while 4.0Gy induced sterility of *C. maculatus* (Olaifa *et al.*, 1990).

Remarkable works have also been done on effect of gamma radiation on the different species of insects under Coleoptera. Sharp (1995) , Ito *et al.* (1991) worked on sweet potato weevil Sharp (1995) used the gamma irradiation doses ranging from 100 to 1,000Gy to prevent adults emerging from treated roots infested with different stages of the weevil. He achieved 99.99% mortality stages of sweet potato weevil in 37 Gy (35-42 Gy) for eggs 73 Gy (53-132 Gy) for first instars, 38Gy and 1,497 Gy (963-2.229 Gy) for pupae. Pupae irradiated with greater than or equal to 800 Gy produced no adults. Irradiating adults in roots with 1,000 Gy did not prevent adult emergence. However, males exposed to 150 Gy were sterile, and females exposed to 300 Gy were sterile. Ito *et al.* (1991) irradiated the young females with a dose of 300 Gy had no spermatozoa in their spermatheca after being given the chance to mate with irradiated and non-irradiated males. Changes in the reproductive organs were only visible after 15 days. Complete sterilization occurred when 2-day old adults were exposed to any of the above doses. Several works also found on the granary weevil regarding the effectiveness of the gamma radiation of which Ismail *et al.*(1987b), Ismail *et al.*(1987a) are worth mentionable. Their study was cantered to find out the optimal dose to achieve sterility and mortality of granary weevil. Finally they became able to identify doses of sterility and mortality. Numerous studies happened on the effect of gamma radiation on rice weevil (Rosada *et al.*, 1992) and bowl weevil

(Haynes, 1983, Haynes and Smith, 1989a, b, Villavaso *et al.*, 1986, Winstead *et al.*, 1989).

1.8 Effect of Gamma Radiation on Reproductive Potentials of *Callosobruchus* spp.

1.8.1. Fecundity

According to Begum & Rahman (1974) the females of *C. chinensis* (L.) treated with gamma radiation doses from 0.5 to 0.6 Kr. and mated with untreated males does not affect the mating behaviour, egg production and longevity of *C. chinensis*. A female of *C. chinensis* lays 60 eggs at the rate of 16 per day (Begum *et al.*, 1979). The adults mated within 12 hours after emergence. Pre-oviposition period was very short, 75 ± 15 minutes after mating which lasted for 25 ± 5 seconds. The oviposition period lasted for 3.5 ± 2.5 days. Begum *et al.* (1981b) reported that no change was observed in fecundity when an irradiated male of *C. analis* mated with control virgin female. Parental generation responded by the decline in its progeny according to the does. Fertility showed gradual decreases with the increase of doses. Both F_1 male and female inherited some indication of sterility from their parent. Mating tendency, egg laying and longevity remained unchanged. Begum (1984) treated *C. chinensis* with gamma radiation doses ranging between 5-60Gy and found that the egg production ability of the irradiated females affected significantly. She also found that the mating of irradiated males with normal females did not affect oviposition period. Oviposition period decreased significantly when female was irradiated. According to (Credland and Wright, 1989) females prevented from mating 6 days after emergence but when allowed access to seeds laid fewer eggs than females allowed mating and ovipositing immediately. Females mated at emergence but then prevented from lying for 6 days laid even fewer eggs. Although most female readily re-mated 3 days after their emergence and initial mating, re-mating had no significant effect on the number of eggs laid or the percentage hatching. Similarly, enclosing individual females with different number of males did not markedly affect the number of eggs. There were, however, limited changes in the percentage of egg, which hatched, and the distribution of eggs on the available hosts. Tanaka (1990) reported that age-specific effects of inbreeding on fecundity were assayed in *C. chinensis* by comparing inbred lines and their cross. Four consecutive full-sib-mating reduced total fecundity by only 10.30%, and did not decreased early fecundity at all until the 3rd day from their onset of reproduction. It is suggested that recessive detrimental genes have been estimated from the early period of the adult life span when reproductive value is high. There was a slight tendency that inbreeding depression increased as age proceeded. This was not statistically significant. Thanthianga & Mitchell (1990) found that the fecundity of the South India strain of *C. maculatus* was 73 eggs for females developing in 31 days and increased to 94 eggs for females emerging after 41 days. Fed female bruchids laid 45-55 more eggs than unfed females; this is considered to be the egg-equivalent of the reserves unfed females had to sacrifice for activity. Larval competition did not reduce female fecundity. Oviposition was inhibited when females were given 25 or fewer beans *(V. radiata)* for oviposition and this was reversed when females found fresh beans. Eggs were preferentially laid on the largest egg-free beans. The cues regulating host preferences *(V. unguiculata> V. radiata>> Cajanus cajan >>>Cicer arietinum*) were secondary to the cues hyper-dispersion and the selection of larger beans. Hussain (1994) reported that the fecundity was constant for the first three days and there after it decreased significantly with the maternal age.

1.8.2. Percentages of Adult Emergence

Haque *et al.* (1992) observed that a total of 150 genotypes of *V. mungo* were screened for susceptibility to *C. maculatus* at 30°c and 70% RH. The genotypes SBG No.3 and UH 83-2 were the least susceptible (3.0-3.5% weight loss), while pu-26 and UHB2-46 were the most susceptible. Smoothness and thickness of seed coat and size and mineral content of seed were positively correlated with susceptibility. Protein, crude fibre and phenolic contents appeared to impart resistance. Hatchability of eggs, survival number of F_1 adults and the growth index were significantly greater in highly susceptible genotypes. The reproductive rates and population increase per week on the genotypes UH 82-46, pu-26, SBG No. 3 and UH 83-2 were 60.6, 46.3, 22.14 and 40.3 and 101, 87, 60 and 73% respectively. Males of *C. maculatus* were heavier when reared on UH 82-46 and pu-26 than on SBG No.3 and UH8 3-2.

Javaid *et al.* (1993) proposed that seeds of 2 varieties (ER-7 and Black eye) and 10 landraces of cowpeas were evaluated for susceptibility to *C. maculatus* in the laboratory at 25°C. Differences between the treatments with respect to adult emergence and viability of eggs were significant. Adult emergence varied from 32.89% for ER-7 to 74.01% for BO67-G. The viability of eggs was significantly lower in landraces BO28-B (53.54%) and BOI 3- J (53.87%) than in commercial varieties. Pessoa *et al.* (1993) reported the resistance of 10 cowpea (*V. unguiculata)* cultivars to *C. maculatus* evaluated in the laboratory at 24.25 ± 0.08°c and 74.87 ± 0.87% RH. Cultivar TVx 2907-02D and Praia de Guadaloupe were the least preferred cultivars for oviposition and a variation was observed with regard to non-preference for oviposition. A reduction in the percentage of viable eggs was observed due to an antibiosis resistance mechanism. Hussain (1994) found that the viability of the eggs of *C. chinensis* laid during the first three days of female life was constant which there after declined. The relative fitness of the progeny also decreased with the increased parental age and females oviposited at day 6 had 60.68% less fitness than females oviposited on the first day. The pattern of adult emergence was described in Howe & Currie (1964). They observed that the first male emerged at least 1 day earlier than the females in *C. chinensis* and *C. maculatus*. Islam (1991) postulated that the peak of male emergence was earlier than that of females in the first four days of emergence.

1.8.3. Duration Between Egg to Adult

Begum & Rahman (1974) reported that the development period of *C. analis* from egg to imago took 32.50, 39.00 and 48.50 days in mung bean, gram and peas respectively. The maximum growth of the larvae was observed in mung and peas. Both male and female attained maximum size in mung bean followed by gram and peas. Begum *et al.* (1979) reported that the life cycle of *C. chinensis* was recorded to complete in 28.5, 32.5 and 44.5 days in mung bean, gram and pea respectively. Mbata (1993) found that development was faster and heavier offspring of *C. subinnotatus* were produced when larvae were reared on uninfested seeds. High larval density of up to 5 larvae per seed prolonged the developmental period and caused the production of miniature individuals that laid very few eggs. Sison *et al.* (1996) proposed that several mung bean entries were screened for resistance to *C. chinensis* (L.). The life history of *C. chinensis* was also studied on the susceptible variety of pag-asa 7. It took about 21-30 days for the bruchid to complete development (egg to adult emergence). The eggs were laid on the seed coat white the larvae developed inside the seed completing four instars there. Population takes place in a cell inside the seed and the adult emerges though the entrance hole made by the larva. Pessoa *et al.* (1993) found that the length of development of *C. maculatus* form egg to adult differed among the 10-cowpea cultivars and the study indicated an antibiosis resistance mechanism in this species.

1.8.4. Sex Ratio

According to Begum & Rahman (1974) the sex ratio of *C. analis* was about 1:1. Begum *et al.* (1979) reported that the sex ratio of *C. chinensis* was about 1:1 in mung bean, gram and pea. Begum (1984) said that progeny production decreased in either case of irradiated male or female present in a pair of *C. chinensis.* Irradiated male mated with normal female produced more male progeny. No differences in sex-ratio were observed between beetles on the various pulses (Srivastava and Pant, 1989b).

1.8.5. Adult Longevity

Ryoo & Chan (1993) found that as the number of eggs of *C. chinensis* per adzuki bean seed (*Vigna unguilaris)* increased, larval development time and survival rate decreased. Differences in mortality between treatments were caused largely by competition in the late instars. Begum & Rahman (1974) observed that unmated males of *C. analis* lived longer than the mated one and the case was opposite in case of female. Sugar solution increased the longevity of both sexes considerably. Water-fed adults had shortest life span when compared with those receiving no food. Begum *et al.* (1979) also found that unmated males and females of *C. chinensis* lived longer than the mated ones and sugar solution increased the longevity of both sexes considerably. The water-fed adult and adults receiving no food had shorter life span when compared with the adults fed on sugar solution. Begum (1984) reported that the longevity of male of *C. chinensis* was always greater than that of female irrespective of radiation dose. Irradiated insects showed increased longevity except when irradiated male mated with normal female.

1.9 Oviposition Preference

1.9.1. Oviposition Preference

Selection of seeds of preference is an important characteristic of an egg laying pulse beetles because the reproductive potentials of insects might vary in different types of seeds of pulses. Knowledge about the variation needed for the control of the experimental insects. For this researchers set experimental design for find out the seeds of preference and the reproductive potentials on their preferred seeds of the pulse beetle *Callosobruchus* spp. under study.

Srivastava & Pant (1989a) studied the growth and development of *C. chinensis* on seeds of 11 legumes and found that, *C. chinensis preferred* lentil (*Lens esculenta*) most but not cowpea (*V. unguiculata*). They also found that blackgram was not suitable for development of the pest. In the same year Srivastava & Pant also studied the growth and development of *C. maculatus* and found that growth and development of *C. maculatus* were less favourable in lentil (*Lens esculenta*). Green gram (*V. radiata*) was proved to be most susceptible from the pest species (Srivastava and Pant, 1989b). Begum *et al.,* (1993) found that one generation of five gravid females of *C. chinensis* infested 47.36, 40.12 and 36.36 percent of seeds, and caused 31.28, 23.81, 3.73 percent weight loss In *L. esculenta, V. radiata* and *L. sativus* respectively, during 18- 20 days of storage. The percent oviposition and emergence of adult insects were markedly different in developmental periods of the insects in three types of pulses. The result suggests that *L. esculenta* is more susceptible to *C. chinensis* than *V. radiata* and *L. sativus.* This contradicts with a previous report where Sultana (1974) found that *C. chinensis* caused more damage to mung bean than lentil and pea. Begum *et al.* (1990) observed that black gram did not support *C. chinensis* and the species caused the highest damage in lentil seeds.

Roy (1994) found Bengal gram as the most preferred seed of *C. maculatus* for oviposition both under choice and no choice condition. His study also revealed that *C. maculatus* developed in green gram, cowpea, lentil and red gram but not in Bengal gram. Islam *et al.,* (1990) identified black eyed cowpea and lentils as the most preferable seed and infest resistant seed for oviposition respectively. Thanthianga and Mitchell (1990) reported the cue of host preference of *C. maculatus* as *V. unguiculata> V. radiata >Cajanus cajan > Cicer arietinum.* Ramzan *et al.* (1990) found the highest number of eggs of *C. maculatus* were in green gram (*V. radiata*) and then black gram (*V. unguiculata*). The greatest damage in terms of exit hole and weight loss was found in black gram. They also found that though 90% seeds of *V. umbellata* (rice bean) had eggs but no trace of damage.

The observation on Mannan and Bnuiyan (1996) oviposition, hatchability and emergence of *C. maculatus* indicated that *C. maculatus* preferred to oviposit maximum number of eggs on red kidney bean under no choice test but under free choice test, the insect prefers to oviposit the highest number of eggs on haricot bean and lowest on chickpea while the highest hatchability was observer on cow pea and was found in red kidney bean, haricot bean and soybean. Ofuya & Bambigbola (1991) observed that the development period of *C. maculatus* was longer in soybean and the lightest female were rared from soybean and the insect did not grow and develop on the seeds of winged bean (*Psophocurpus tetrangonolobus*) or mucuna bean (*Mucuna pruriens*). They also found that only a few adults could be rared from lima bean (*Phaseolus lunatus*).

Parr *et al.,* (1996) analysed the activity of female of *C. maculatus* between making contact with a potential host seed and egg deposition on cowpeas, mung bean and mung beans bearing conspecific eggs. They found their simplest behavioural sequence for oviposition of *C. maculatus* females. They observed that *C. maculatus* females deposit eggs on mung bean rather than the other two host like cowpeas and mung bean bearing conspecific eggs i.e. mung bean was shown to be the most acceptable of the three hosts. Nakhla (1988) reported that the highest number of adults of *C. maculatus* emerged from *V. unguiculata* than *Vicia faba, Astragalus cicer* peas and lentils (*Lens esculenta*). Seck *et al.* (1992) screened seeds of 20 varieties of cowpeas in controlled conditions for their resistance to *C. maculatus.* Significant differences among the varieties were found in *C. maculatus* oviposition, percentage survival and damage. On the basis of these three parameters, resistant genotypes were identified among introduced materials while Senegalese varieties appeared highly susceptible. Seck (1993) showed significant differences among the 80 varieties of several cowpea in oviposition, progeny and adult emergence. *C. maculatus* sometimes lay or avoided lying eggs on hilum. The oviposition on plastic beads revealed that the highest number of eggs was laid on white-coloured bead in dark and the lowest on black-coloured bead. The adult female showed its clear preference to oviposit the maximum number of eggs on red kidney bean under no choice test. In the free choice test, the insect prefers to oviposit the highest number of eggs on haricot bean and the lowest on chickpea (Mannan and Bnuiyan, 1996).

Messina (1989) observed those females of *C. maculatus* tend to distribute their eggs uniformly among host seeds and thereby, reduce competition among larvae within seeds. Thanthianga and Mitchell (1990) oviposition rates were found to drop when the beans begin to carry 2 or more eggs. Two choice experiments that were demonstrated by Mbata (1992) found that ovipositing female bruchid *C. maculatus* distributed their eggs evenly on seeds. The species showed less preference for oviposition on the small-sized bean seeds in almost half of the genotypes. The recorded oviposition was 5-8 eggs per seed. The rate of oviposition increased in the remaining medium to large seeded genotypes. The female beetle laid 18 eggs per seed, which was the highest number, found in large seeded beans. These genotypes were highly preferred for oviposition (Sardar and Akhtari, 1992).

Begum *et al.* (1993) found that one generation of five gravid females of *C. chinensis* infested 47.26, 40.12 and 36.36 percent of seeds, and caused 31.28, 23.81 and 3.73 percent weight loss in *Lens esculenta, Vigna radiata* and *Lathyrus sativus* respectively, during 18-20 days of storage. The percent oviposition and emergence of adult insects were markedly different in developmental periods of the insects in three types of pulse. The results suggest that *L. esculenta* is more susceptible to *C. chinensis* than *V. radiata* and *L. sativus*. Ryoo and Chon (1993) studied the relationships among the number of eggs, developmental time and survival rate of *C. chinensis*. In their study life table statistics showed an inverse linear relationship to the number of egg per seed. Depositing 1 egg/seed increased progeny production by 56% compared with 6 eggs/seed. The functional response of larval ectoparasitoid *Anisopteromalus calandrae* showed a liner relationship with host density in the range of 1-64/100 seeds. The parasitoid searched for its host more efficiently when the number of eggs per seed increased 1 to 4; search rate of the parasitoid based on random search increased from 0.045 n 0.013 (mean n SEM) to 0.154 n 0.025. The results indicated that ovipositing fewer eggs per seed is also a good defence tactic for the weevil against the parasitoid. Khan *et al.* (1997) studied on the influences of sensory organs of *C. chinensis* in egg distribution activities. Their experiment revealed that the maxillary and labial palpi of *C. chinensis* play an important role to mediate the even distribution of eggs on seeds. It was also noted that antennae were not required for uniform distribution of eggs. The ablation of pulpy and both pulpy and antennae impair the ability of the beetle to identify seeds without eggs and caused considerable clustering of the egg on a few seeds. Blocking on vision and treatment of tarsi mild acid did not cause any significant change in egg laying behaviour in this bruchid.

1.9.2. Development Under Compulsion

Srivastava & Pant (1989b) observed that black-seeded soybean, soybean, blackgram and French bean were unsuitable for growth and development of *C. chinensis*. Srivastava & Pant (1989b) also observed the growth and development of *C. maculatus* and found that lentils, Bengal gram, peas and yellow and black seeded soybeans were less preferred, while French bean were unsuitable for development of the pest. Ofuya & Bambigbola (1991) observed that *C. maculatus* did not grow and develop on the seeds of winged bean (*Psophocurpus tetragonolobus*) or mucuna bean (*Mucuna pruriens*) and only a few adults could be reared from lima bean (*Phaseolus lunatus*). Developmental period was longer in soybean and the lightest females were reared from soybean. Giga & Moyo (1992) reported that *C. rhodesianus* was unable to develop successfully on all *Vigna* taxa. Mannan & Bhuiyan (1996) reported that no adult (*C. maculatus*) emergence was found in kidney bean, haricot bean and soybean. Population development and interactions of two species of the pulse beetles. *C. analis* (Fab.) and *C. chinensis* (L.) were studied in the laboratory by Begum & Kabir (1990) under controlled conditions of food and space limitations using six types of pulses at 28.31 ± 1^0C and 75.85% RH. Single species cultures of *C. analis* developed highest population level in mung bean on the 80th day. Gram did not support *C. analis* while kheshari (*Lathyrus sativus)*; mushur *(Lens esculenta*) and pea (*Pisum sativum)* showed low level of populations. *C. chinensis* produced almost comparable populations in gram, mung and mushur on the 80th day. Mashkalai (*Vigna* mungo*)* failed to support *C. chinensis* and the insect died out soon.

1.10 Mating Competitiveness

Mating competitiveness refers to the mating success of a treated or sterilized male when the latter is allowed to compete with untreated females of the same species (Islam and Port, 1992). Sexual or total competitiveness of a particular males refers to its overall performance which is an interaction of a number of factors such as mating ability, number of mating, sperm transfer, sperm activity, olfactory responses, vigour and longevity (Fried, 1971, Hooper and Katiyar, 1971). Therefore, competitiveness is a most important propensity of chemo- or radio-sterilized males and should be maintained as naturally as possible. Early studies have shown that chemosterilized males are as successful as untreated males in competition for untreated females (Fye and La Brecque, 1966, La Brecque *et al.*, 1966). More striking findings show that the treated males are actually more successful than untreated males in mating with the wild females (Crystal, 1965, La Brecque *et al.*, 1962, Millar, 1995). Grover & Pillai (1970) and Hafez *et al.,* (1970) reported that sterilized males of *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) and *Culex pipiens* (Unnaeus) (Diptera: Culicidae) respectively were fully competitive. By using a modified Fried's (1971) equation, El-Gazzar & Dame (1983) estimated the competitiveness (C) values of chemosterilized males of *C. quinquefasciatus* Say and showed that the sterilized males were fully competitive under laboratory condition. Sterilized males of *Tribolium* are also fully competitive (Hasan, 1998). Mehta & Bhalla (1983) reported that treated males of *Epilancha vigintioctopunctata* (F.) were fully competitive with untreated ones. Similar results are also reported for *Ephestia cautella* (walker) (Lepidoptera : pyralidae), where 0.2 Gy a substerilizing doses of this species did not lower the mating competitiveness of F1 adults whose fathers were exposed to 0.2 K Gy gamma radiation (Al Taweel *et al.*, 1993). Mansour & Krafsur (1991) applied the sterile insect technique (SIT) to *Musca autumnalis* and reported that sterile males showed reduced mating competitiveness. However in 1989, Hussain & Imura found that the sterilized males of *C. chinensis* were capable of competing sexually with untreated ones but no substantial attempts has been made to determine mating and/or total competitiveness of radiated males of pulse beetles *Callosobruchus* spp. (Coleoptera: Bruchidae) and this led to the present investigation.

Determination of the competitiveness of sterilized males may be made either by direct observations or by the ratio test method. In direct observations, mating competition is estimated by assessing performance of the treated males for untreated females under caged condition (Islam and Port, 1992). In the ratio test method, where a known proportion of treated to untreated males are allowed to mate with a set number of untreated females has been widely used in estimating total competitiveness of sterile males (Brower, 1982, Hooper, 1975, Hooper and Horton, 1981, Hooper and Katiyar, 1971). Perhaps the later method gives the best laboratory based measure of the total competitiveness of sterilized males. This method includes percentage of egg-hatch or percentage pupation data o percentage of adult emergence, which reflects the interaction of a number of factors, e.g., mating ability, sperm complement and competition and the impact of multiple mating. Thus egg-hatch data could be a realistic assessment of incompatible insects competitiveness under laboratory conditions (Hoque and Islam, 1999).

1.11 Aims of the Study

- To study the reproductive potentials such as fecundity, oviposition preference, percentage of adult emergence, adult development time, sex-ratio and adult longevity of the regional strains
- To study the gamma radiation induced inherit sterility of *C. chinensis* and *C. maculatus*.
- To study the cytoplasmic incompatibility of *C. chinensis* and *C. maculatus*
- To study the impact of irradiation on reciprocal crosses and identified incompatible crosses and finally
- To develop an effective sterile male technique for two major pest species of pulses, *C. chinensis* and *C. maculatus*.

1.12 Layout of the Thesis

The thesis furnished according as the following manner. The first chapter introduced the problems and backgrounds of different aspects of the study topic. Second chapter described the methods and materials followed and used in the study. This chapter also included the short descriptions of the statistical methods and introduced software used for the study. The third chapter described the complete output of the experiment of oviposition preference of *C. maculatus* and *C. chinensis.* Fourth chapter was decorated by the results of the experiment of gamma radiation induced sterility up to fifth generation of both species. Fifth chapter included the results of the study of mating competitiveness of irradiated males of *C. chinensis* and *C. maculatus.* The sixth chapter is the core chapter of the thesis, which included the result of the test of presence of cytoplasmic incompatibility within different reciprocal crosses of the study insects. The output of the study of integration of irradiation with cytoplasmic incompatibility was also given in the chapter. The next seventh chapter summarized all the results of the total experiment along with drawbacks and future prospects of research course. At the end reference and three appendices included. The references are cited following the style of Biological Bulletin; an international journal of biology. The appendix chapters mainly contain the monotonous tables whose gist findings are given in the result chapters. Three appendixes correspond to three different chapters.

Chapter 2

Materials and Methods

The study was conducted in the Genetics Research Laboratory, Department of Zoology, University of Rajshahi. The insects used were the common pulse beetle, *C. chinensis* (L.) commonly known as the adzuki bean weevil and *C. maculatus* (Fab.) commonly called the cowpea weevil. They belong to the Order Coleoptera under the Family Bruchidae and their morphological features were studied with the help of the book titled *"Insect and Mite Pests in Food"* by J, Richard Gorham (1991), The following procedures were maintained to carryout the experiment.

2.1. Collection of Pulse Beetle

Seeds of lentil, black gram, Bengal gram, pea and mung bean infested with *Callosobruchus* spp. were collected from stored grain shop from four widely separated regions of Bangladesh *viz.* Rajshahi (RAJ), Chittagong (CHI), Khulna (KHU) and Narayanganj (NAR). The samples were transported to the laboratory in plastic pots. Morphological feature of *C. chinensis* and *C. maculatus* were studied with the help of Gorhan (1987) and Hill (1990). The beetles were inbred in the laboratory for two successive generations in order to eliminate natural and/or deleterious mutations, if any. Each of the regional samples was maintained separately in 500 ml beakers; mouths of which were covered with coarse cloth and tied with rubber bands to prevent the insect from escaping and also to allow the air and moisture in and out of the containers. It should be mentioned that *C. chinensis* were reared in uninfested and sterilized lentil seeds *(Lens esculenta)* and C. *maculatus* in similar black gram seeds *(Vigna mungo)*. For sterilization, the fresh seeds were kept in an oven for overnight at 60° C to destroy the previously laid eggs or immature stage, if any. Whenever required, the insects were cultured in sterilized seeds. The mass cultures of the beetles were maintained in rearing cages in the laboratory at ambient temperature (28 ± 2°C) and at 70 ± 5% RH.

2.2. Experimental Design and Mating Schedules

Experiments were designed to estimate incompatibility relationships between the regional samples of two species of the beetles, collected from main divisional cities or port areas of the country, The regional infested seeds were kept in different beakers for mass culture. Then infested seeds of different region were kept in separate vials for collecting virgin female and unmated males. Single pair mating between the virgin females and unmated males were given in 9cm diameter Petri dishes. Parental through F2 generation was maintained for control line. After this the experiment was conducted with 10 pair of replicates and the reproductive potentials estimated of the mated pair were studied.

2.3. Tests for Cytoplasmic Incompatibility in *Callosobruchus* spp.

Tests for cytoplasmic Incompatibility (CI) in the experimental insects were carried out through evaluating percentage of adult emergence of the purebred and the crossbred samples. Apart from regional controls *viz.* R X R, Ch X Ch, K X K, and N X N, all possible reciprocal crosses between samples were made. A *4 X 4* factorial design for *C. chinensis* and *4 X 4* for *C. maculatus* was considered; because attempts to collect the latter species from Khulna were not successful rather we introduced strains collected from Japan. The strain from England of *C. chinensis* was also tried for raring but it failed to continue the stock. 10 replications per cross were maintained in the incubator at temperature 28 ± 2°C.

2.4. Integration of Irradiation with CI

Suspected and identified cytoplasmic incompatible crosses are selected for further treatment with 8Gy dose of gamma radiation. Mainly the reciprocal crosses were selected for the radiation dose.

2.5. Gamma Radiation Treatments

The experiment was conducted to evaluate the effect of gamma radiation on the reproductive potential of *C. maculatus* (Rajshahi strain). So adult *C. maculatus* were irradiated with gamma radiation at dose 0 (Control), 2, 4, 8 and 12 Gy (1 Gy = 1 Joul/Kg = 0.1 Kr) installed at the "Institute of food and Radiation Biology, Atomic Energy Research Establishment, Dhaka. Each dose had 3 replications where adults or larvae varying from 20 to 50 in number were used. The irradiated insects were kept in separate vials to be used later as per mating schedule. The experiment was continued from parental through F5 generation and each treatment had control (C x C) and treated (T x T) lines.

2.6. Estimation of Reproductive Potentials

Such reproductive potentials as fecundity, percentage of adult emergence, development period, sex -ratio and adult longevity of purebred and cross bread of both the species were recorded, of which percentage of adult emergence was used as indices for evaluating compatibility relationships. Such reproductive potentials were also studied for control (C X C), treated (T X T) and reciprocal (T X C) lines of the gamma irradiated adults of *C. maculatus.*

Among the reproductive potentials the total number of eggs laid by a female within a 24h oviposition period constituted as fecundity of the beetles under study for this experiment. Seeds containing eggs were separated in small vials for observation till the day of emergence. The infected seeds were so separated for collecting virgin females and unmated males. Cotton balls were used to cover the mouth of the small vials. Each vial contained the replication no. and the date of egg collection in a label. The date of emergence and the sex of the emerged insect were recorded regularly. The unmated males were paired with virgin females of the same species in a Petri dish on the same day. About 70-80 lentil seeds for *C. chinensis* and black gram seeds for *C. maculatus* were placed side by side without overlapping in the petridishes containing a mating pair. During the collection of eggs, the insects were separated from the seeds using an aspirator. The duration of developmental period from the day of egg laying to emergence was recorded in days. Number of adults emerged and the percentage of their emergence was worked out. Male to female sex ratio in each replication was calculated for both species. Finally, longevity of males and females were recorded. Adults emerging from the parental generation, if required, were period in the same way as mentioned earlier and raised for subsequent generations.

2.7. Oviposition preference

Experiments for evaluation of seed of preference of the beetles *C. chinensis* and *C. maculatus* were spilt into free choice and no-choice tests described bellow.

2.7.1. 'Free choice' test for oviposition preference

To conduct 'free-choice' test for oviposition preference 300 seed of each of the five different pluses namely, black gram *(Vigna mungo)*, lentil *(Lens esculenta)*, Bengal gram *(Cicer arietinum)* green gram *(Vigna radiata)* and green pea *(Pisum sativum)* were placed in a single Petridish and 10 pairs released in to the petridish. 24 hours observation considered for analysis.

2.7.2. 'No choice' test for oviposition

Seventy seeds of each of the five different pulses *viz.* black gram, lentil, green gram, Bengal gram and green pea were placed separately in the five different Petri dishes for 'no choice' test. A pair of newly emerged male and female was released in each Petri dish. Each species had three replications for every type of seed. After 24 hrs the beetles were removed from the Petri dishes and the eggs laid on the seeds were counted. The reproductive parameters were observed and recorded.

2.8. Estimation of Mating Competitiveness Values (CVs)

To determine the effect of radiation on the competitiveness of male of *C. maculatus*, 6Gy abd 8 Gy treated males, untreated males and untreated females of same age in the ratio of, 1:1:1, 2:1:1 and 3:1:1 were placed in different Petri dishes. Sterilized black gram seeds were used as the oviposition medium. Each assessment was made three times. After 24 hours, egg- carrying seeds were counted and collected. The corresponding percentage of adult emergence was recorded. The ratios of untreated male: Untreated female (1:1) and treated male: untreated female (1:1) was used as control. Same experiment was also conducted on *C. chinensis* in food medium lentil for the males treated with 8Gy gamma radiation. The expected percentage of adult emergence was calculated from the following formula (Fried, 1971)

Expected % of adult emergence = $(C \times H_c + T \times H_t)/(C + T)$

The total competitiveness value (CV) of the treated males was calculated from Fried's (1971) equation was modified by replacing the egg hatch percentages by the corresponding percentage of adult emergence. Hence, equation for total competitiveness stands,

$$CVs = C/T \times (H_c - H_o)/(H_o - H_t)$$

Where,

C = number of untreated males

T = number of treated males

H_c = Percentage of adult emergence from the cross UM:UF

H_t = Percentage of adult emergence from the cross TM:UF

H_0 = Percentage of adult emergence at different ratios.

2.9. Statistical Analysis

2.9.1. Dot Plot

A **dot chart** or **dot plot** is a statistical chart consisting of a group of data points plotted on a simple scale. Dot plots are used for continuous, quantitative and univatiate data. Data points may be labelled if there are few of them. Dot plots are one of the simplest plots available, and are suitable for small to moderate sized data sets. They are useful for highlighting clusters and gaps, as well as outliers. Their other advantage is the conservation of numerical information. When dealing with larger data sets (around 20-30 or more data points). The comparison of sequences can be done in many different ways. The most direct method is to make this comparison via a visual means and this is what "dot plots" attempt was done. Dot plots are a group of methods that visually compare two sequences and look for regions of close similarity between them. The solid line on the main diagonal is a reflection that every base of the sequence is trivially identical to itself. Instead of histograms, it is more useful to think of dot plots as horizontal, one-dimensional scatterplots where tied values are perturbed or displaced vertically (Cleveland, 1985). When points overlap, we may displace them by adding a small amount of uniform random error (Chambers *et al.*, 1984), we may displace them systematically in a textured pattern (Tukey and Tukey, 1990), or we may displace them in increments of one dot width (the method used in the cited hand-drawn examples). These three methods are usually called respectively *jittered plots*, *textured dot strips*, and *dot plots*. Unlike histodot plots, all three of these methods position an outlier (or any case separated from the rest of the data) exactly where it should be on the scale rather than at a lattice point defined by binning.

Reading dot plot is simple. Start with bottom left corner box and go up within the box to observe the pattern. Right direction indicates the increase of scale. The title bar of each box represents the conditions. The advantage of dot plot is that a single trellis dot plot can present the complete scenario of an experiment sometimes, which is very helpful for multiple comparison.

2.9.2. Cluster Analysis

Cluster analysis is the searching for groups in the data, in such a way that objects belonging to the same cluster resemble each other, whereas objects in different clusters are dissimilar. In two or three dimensions, clusters can be visualized. With more dissimilarity than three dimensions, we need some kind of analytical assistance. There are two types of clustering algorithm. 1. Partioning algorithm and 2. Hierarchical algorithm. In the thesis we used the second one. A hierarchical algorithm describes a method yielding an entire hierarchy of clusterings for the Agglomerative

given data set. Methods start with the situation where each object in the data set forms its own little cluster, and then successively merges clusters until only one large cluster remains which is the whole data set (Kaufman and Rousseeuw, 1990).

2.9.3. Dendogram

A dendogram is a tree for visual classification of similarity, commonly used in Biology for grouping species. An object is a case for which we observed different characteristics. Elements of a dendogram are clusters (middle tree elements) and objects (the leaves). An association of two objects or clusters forms a new cluster. In a dendogram, two elements are grouped in one cluster when they have the closest values of all elements available. The arithmetic mean of the two elements is associated with the cluster. It is frequently used to illustrate the arrangement of the clusters produced by a clustering algorithm. Dendrograms are often used in computational biology to illustrate the clustering of genes. Different clustering sometimes used the distance based algorithm. In our study we used Eucleadian distance between a pair of objects and the dendograms are produced based on the Eucleadian distance (Kaufman and Rousseeuw, 1990).

2.9.4. Run Test

The term *run* may in general be explained as a succession of items of the same class. Many concepts to analyze runs in a series of data have been studied. The main concepts are based on (i) the analysis of the total number of runs of a given class (Guibas and Odlyzko, 1980) and (ii) examinations about the appearance of long runs (Feller, 1968). In our study we used the run test to observe the sequential pattern change in the reproductive potentials for the two species of *C. chinensis* and *C. maculatus*.

2.9.5. Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) is a collection of statistical models, and their associated procedures, in which the observed variance is partitioned into components due to different explanatory variables. The initial techniques of the analysis of variance were developed by the statistician and geneticist Prof. R. A. Fisher in the 1920s and 1930s, and is sometimes known as Fisher's ANOVA or Fisher's analysis of variance, due to the use of F - Distribution as part of the test of statistical significance. In practice, there are several types of ANOVA depending on the number of treatments and the way they are applied to the subjects in the experiment: One-way ANOVA is used to test for differences among two or more independent groups. Typically, however, the One-way ANOVA is used to test for differences among three or more groups, with the two-group case relegated to the *t*-test, which is a special case of the ANOVA. The fundamental technique is a partitioning of the total sum of squares into components related to the effects used in the model. For example, we show the model for a simplified ANOVA with one type of treatment at different levels (Ferguson and Takane, 2005).

2.10. Software used

Microsoft word has been used for ward processing. For references and citation management we use EndNote 9x. Data was analyzed by Excel, Splus 2000, Corpstat 11; a free software from International Rice Research Institute.

Chapter 3

Oviposition Preference

Abstract

C. chinensis collected either from lentil or Bengal gram preferred lentil then Bengal gram and then green gram for oviposition. The percentage of emergence of *C. chinensis* collected from Bengal gram and from lentil seed was 53% and 46% respectively on lentil seed. The number of adult emergence of *C. chinensis* in Bengal gram collected from lentil and from Bengal gram was 55% and 70% respectively. The emergence rate is highest in green gram and it was 88% and 65% for *C. chinensis* collected from Bengal gram and from lentil respectively. *C. maculatus* collected from black gram showed the highest propensity to black gram for oviposition. Their second preference was green gram. But *C. maculatus* collected from green gram showed the highest preference to green gram and then the black gram. In both cases the emergences rate is higher in green gram . The emergence rate was 87% and 53% for the *C. maculatus* collected from Black gram and green gram respectively in green gram. *C. maculatus* either collected from green gram or black gram also preferred pea for oviposit but the emergence rate is very poor which is nearer to zero which implies that *C. maculatus* preferred smooth seed coats for oviposition.

3.1. Inrtoduction

Callosobruchus spp. is the most important pest attacking cowpea in storage (Jackai and Daoust, 1986). Females cement their eggs onto the seed surface. Eggs hatch after 3-5 days and larvae bore through the seed coat into the underlying cotyledons. Larval development and pupation takes place in the seed and adults enclose and emerge from the seed. On emergence, adults are already sexually mature and mate immediately, eggs being laid soon after mating. Selection of seeds is an important characteristic of an egg laying pulse beetles because the reproductive potentials of the insects might vary in different types of seeds of pulses.

There are many varieties of cowpeas and they show a wide range of physical characteristics such as seed colour, texture, size, hardness and biochemistry. Smooth seeded varieties are more suitable for oviposition than rough seeded varieties (Haines, 1991). Pessoa *et al.* (1993) reported that Cultivar TVx 2907-02D and Praia de Guadaloupe were the least preferred cultivars for oviposition and a variation was observed with regard to non-preference for oviposition under the laboratory condition.

However, the physical characteristic might control the attack level (Akingbohungbe, 1976, Caswell, 1984, Fatunla and Badaru, 1983). Dick and Credland (1986) have suggested the use of such physical factors to complement the biochemical factors found in TVU 2027.

Eddea and Amatobi (2003) did experiment on 22 cowpea for oviposition of *C. maculatus* and showed that the seed coat may not be a useful aspect to consider for breeding of bruchid resistance into cowpea varieties. In 1989 Srivastava & Pant studied the growth and development of *C. chinensis* on seeds of 11 legumes in the laboratory and found that, 1 preferred seeds of the females of *C. chinensis* were lentil (*Lens esculenta*), green gram (*Phaseolus aureus*), red gram (*Cajanus cajan*), Bengal gram *(C. arietnum)* and cow pea *(V. unguiculata)*. Pea and khesari (*Lathyrus sativus*), were less preferred. Bhut (Black seeded soy 'bean), soybean, black gram *(V. mungo)* and French bean *(P. Vulgaris)* were unsuitable for growth and development of the pest.

Whereas, the growth and development of *C. maculatus* were less preferred on lentils, Bengal gram, peas and yellow-and black seeded soybeans, while French beans were unsuitable for development of the pest. Green gram, black gram, red gram and cowpeas were the most susceptible to *C. maculatus* (Srivastava and Pant, 1989). Roy (1994) studied oviposition and development of *C. maculatus* on 9 legume seeds and found that though Bengal gram was most preferred both under choice and no choice conditions, bruchids developed in green gram, cowpea, lentil and red gram only. Ismail *et al.*, (1990) reported that blackeyed cowpea, chickpea, broad beans and cow pea were the most preferred grain legumes for oviposition by *C. maculatus* whereas kidney beans (*Phascolus vulgaris*), garden pea, fenugreek (*Trigonella corniculata*), dehulled broad beans, soy beans and dehulled lentils were resistant to infestation, The cue of host preference of *C. maculatus* was *V. unguiculata* > *V. radiata* >*Cajanus cajan* > *Cicer arietinum* (Thanthianga & Mitchell, 1990). Females of *C. maculatus* preferred *V. radiata*, *V. unguiculata*, *V. aconitifolia* (moth bean) for oviposition while *V. unguiculata* had the greatest damage in terms of exit holes (69.2%) and weight loss (34.5%), followed by *V. aconitifolia* (53.7% and 21.9%) and *V. radiata* (53.3% and 19.4%) but no damage to *V. umbellata* (rice bean) was recorded, although 40% of seeds had eggs (Ramzan *et al.*, 1990). The observation on oviposition, hatchability and emergence indicated that *C. maculatus* preferred to oviposit maximum number of eggs on red kidney bean under no choice test but under free choice test, the insect prefers to oviposit the highest number of eggs on haricot bean and lowest on chickpea while the highest hatchability was observer on cowpea and was found in red kidney bean, haricot bean and soybean (Mannan & Bhuiyah, 1996). Similarly Ofuya & Bambigbola (1991) observed that the development period of *C. maculatus* was longer in soybean and the lightest female were rared from soybean and the insect did not grow and develop on the seeds of winged bean (*Psophocurpus tetrangonolobus*) or mucuna bean (*Mucuna pruriens*) and only a few adults could be rared from lima bean (*Phaseolus lunatus*). Parr et al., (1996) analysed the activity of female of *C. maculatus* between making contact with a potential host seed and egg deposition on cowpeas, mung bean and mung beans bearing conspecific eggs. The simplest behavioral sequence for oviposition of *C. maculatus* female was observed on mung bean rather than the other two host like cowpeas and mung bean bearing conspecific eggs i.e. mung bean was shown to be the most acceptable of the three hosts.

Knowledge about the variation needed for the control of the experimental insects. Different researcher set experimental designs for find out the seeds of preference the reproductive potentials on their preferred seeds of the pulse beetle *Callosobruchus* spp. Despite the diversified research on the seed preference there is a lack of information about selective food media for the experiment of cytoplasmic incompatibility in the laboratory condition. Since the study insects are *C. chinensis* and *C. maculatus,* we designed an experiment to study the seed preference freely and under compulsion.

3.2. Methods and Materials

Experiments for evaluation of seed of preference of the beetles *C. chinensis* and *C. maculatus* were spilt into free choice and no-choice tests which are described bellow.

3.2.1. 'Free Choice' Test for Oviposition Preference

To conduct 'free-choice' test for oviposition preference in population level seeds of each of the five different pluses namely, black gram *(Vigna mungo),* lentil *(Lens esculenta),* Bengal gram *(Cicer arietinum)* green gram *(Vigna radiata)* and green pea *(Pisum sativum)* were placed in a large size single Petridish. The seeds were arranged in a single layer without overlapping 10 pairs of newly emerged males and females were released in each Petridish. The Petridishes were kept out at fixed temperature in an incubator. After 24 hour, the beetles were removed from the Petridishes and the eggs laid on different seeds were counted, and each of the egg carrying seeds was collected in individual vials. Sterile cotton balls protected the vials and the vials were labelled by unique numbers and date of egg collection. The vials were stored in an incubator for observation till the days of adult emergence. This experiment had three replications for each species.

3.2.2. 'No Choice' Test for Oviposition

Seventy seeds of each of the five different pulses *viz.* black gram, lentil, green gram, Bengal gram and green pea were placed separately in the five different Petri dishes for 'no choice' test. The seeds were arranged in a single layer without overlapping. A pair of newly emerged male and female was released in each Petri dish. Each species had three replications for this type of seed arrangement. After 24 hrs the beetles were removed from the Petri dishes and the eggs laid on the seeds were counted and kept in separate vials labelled with egg collection date and replication number. The reproductive parameters were observed and recorded.

3.3. Results

3.3.1. 'Free Choice' Test for Oviposition

The result of free choice test for oviposition of *C. chinensis* and *C. maculatus* are presented in figure 3.1 and 3.2 respectively. The numerical results are also given in table A.1 in appendix A.

Figure 3.1 showed that *C. chinensis* has strong intensity to oviposit on the lentil seed. Also they like Bengal gram for oviposition when they are free to select the seeds for oviposition. Regarding *C. maculaus*, they are very much fond of Blackgram and then Pea and then green gram for oviposition.

Figure 3.2 showed that for *C. chinensis* preferred seeds are as the order Bengal gram > Green gram > Lentil considering the percentage of emergence. For *C. maculatus* the order is Green gram > Bengal gram > Black gram. The result implies that the study insects prefer seeds base on external physiology of the seeds rather the favourable seed for emergence. *C. maculatus* always preferred the smooth coated seeds for oviposition. Although the emergence rate in pea was zero, we observed that a significant number of eggs laid on the pea seeds.

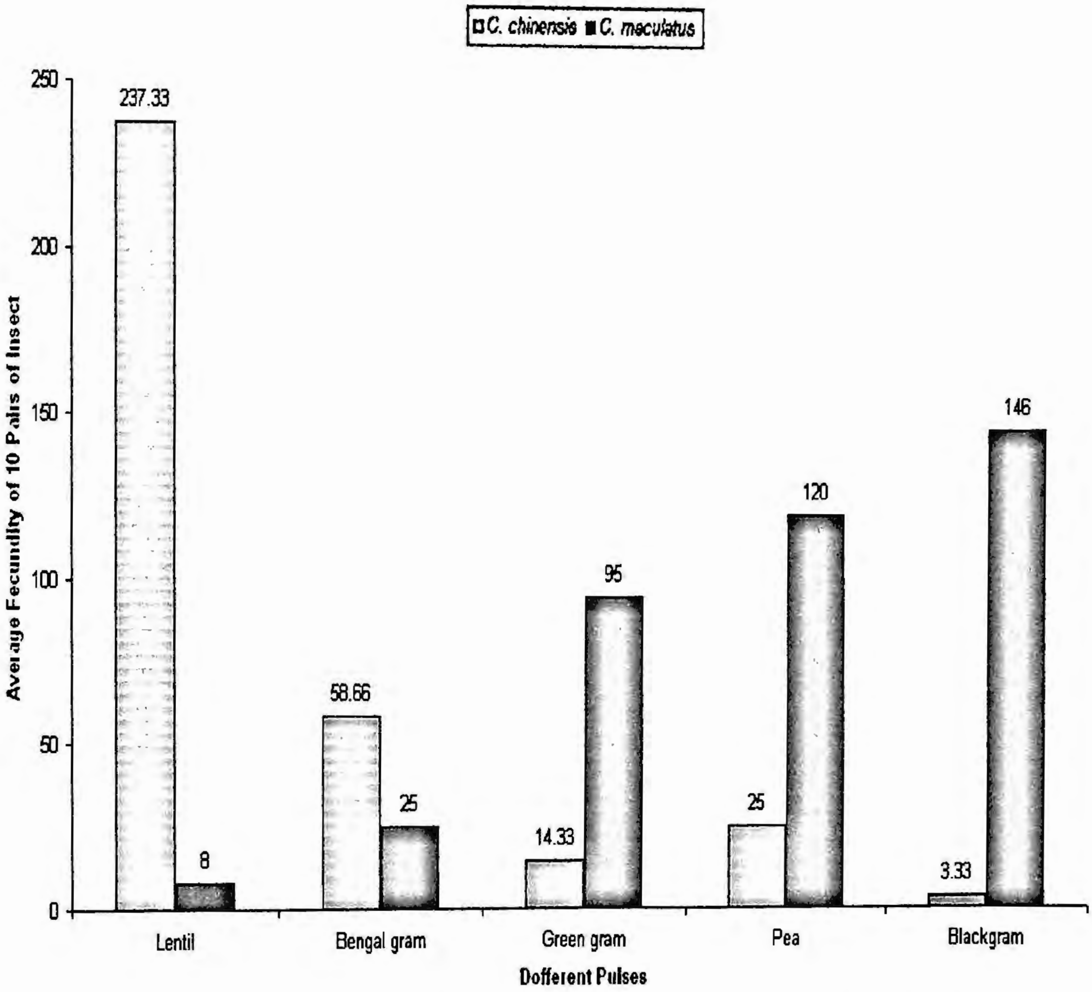

Figure 3.1. Bar chart of the free seed preference for *C. chinensis* and *C. maculatus* for oviposition.

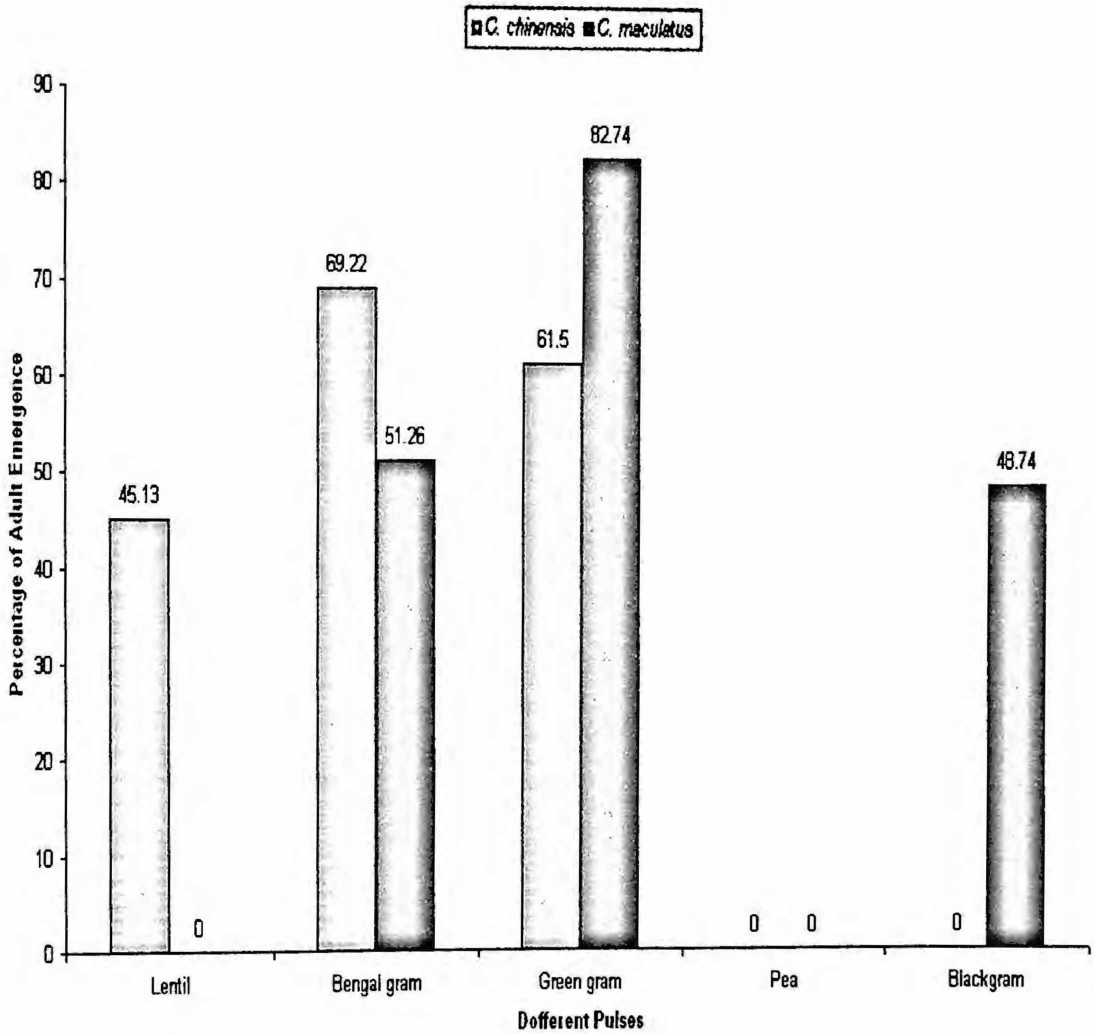

Figure 3.2. Free choice test for oviposition regarding the percentage of adult emergence of *C. chinensis* and *C. maculatus*

3.3.2. 'No Choice' Test for Oviposition

Figure 3.3 showed the fecundity and hatchability under compulsion with all three replicates.

Figure 3.3 showed that if *C. chinensis* were compelled to oviposit on black gram or on lentil or on Bengal gram, they shows the usual fecundity in terms of lying egg, but the hatchability becomes very poor even sometimes no hatch at all. Though *C. chinensis* laid eggs on black gram, the hatch rate was zero. But in Bengal gram, the ratio of total egg and total hatch is nearer to one i.e. almost all the egg hatched. In green gram, the number of total egg was the highest but the number of hatch is relative poor regarding the total egg and total number of hatch ratio of Bengal gram. The third replication of lentil output is little bit confusing which showed that *C. chinensis* might completely emerge from the lentil also. But it was not more preferable than Bengal gram in the free choice test result in figure 3.1. For *C. maculatus*, green gram is the most preferable seed for oviposition and the ratio of total egg and total number of hatch is also high which is nearer to 1. In the seed black gram, they prefer to lay moderate number of egg and the hatch is relatively lower than green gram.

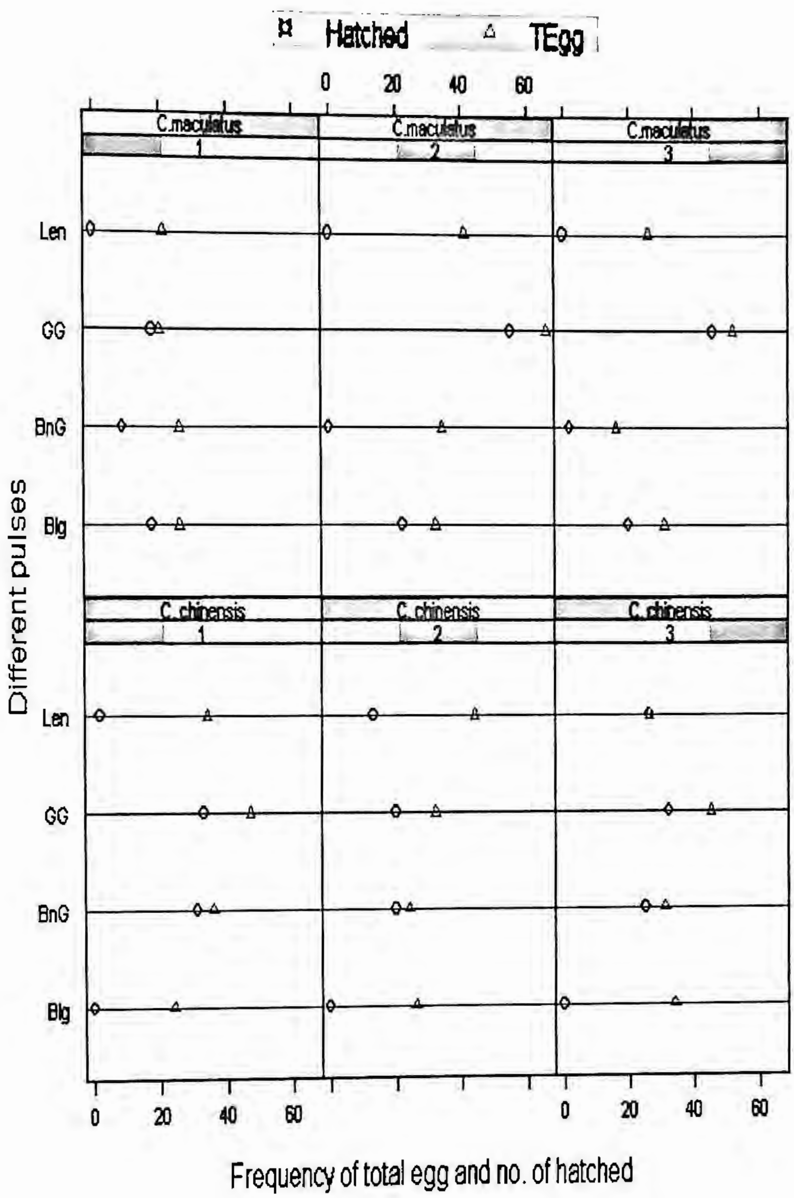

Figure 3.3. Fecundity and Hatchability of *C. chinensis* and *C. maculatus* on different pulses compelled to oviposit. Green title bar consists the name of the species and the orange title bar indicates the replication of the experiment.

Fecundity under compulsion was also studied based on the source pulse i.e. beetles emerged from different seeds were compelled to oviposit on some target pulse. The results summarized in table A.2 for *C. chinensis* and table A.3 for *C. maculatus* in appendix A. Results also illustrated in figure 3.4 for *C. chinensis* and Figure 3.5 for *C. maculatus.*

Figure 3.4 showed that *C. chinensis* collected either from lentil or Bengal gram preferred lentil most for oviposition and the emergence was also moderately high on lentil seeds. Table A.2 showed that the percentage of emergence of *C. chinensis* collected from Bengal gram and lentil is 53% and 46% respectively from Lentil seeds. The next preferable seed of *C. chinensis* from either of the source was the Bengal gram where the number of egg was relatively high followed by lentil. The number of adult emergence of *C. chinensis* in Bengal gram collected from lentil and Bengal gram was 55% and 70% respectively which implies that the emergence in Bengal gram seed is remarkably high. *C. chinensis* collected from Bengal gram also showed a propensity to use green gram for oviposition. The emergence rates were highest in green gram and it was 88% and 65% for *C. chinensis* collected from black gram and lentil respectively. *C. chinensis* never preferred Black gram for oviposition.

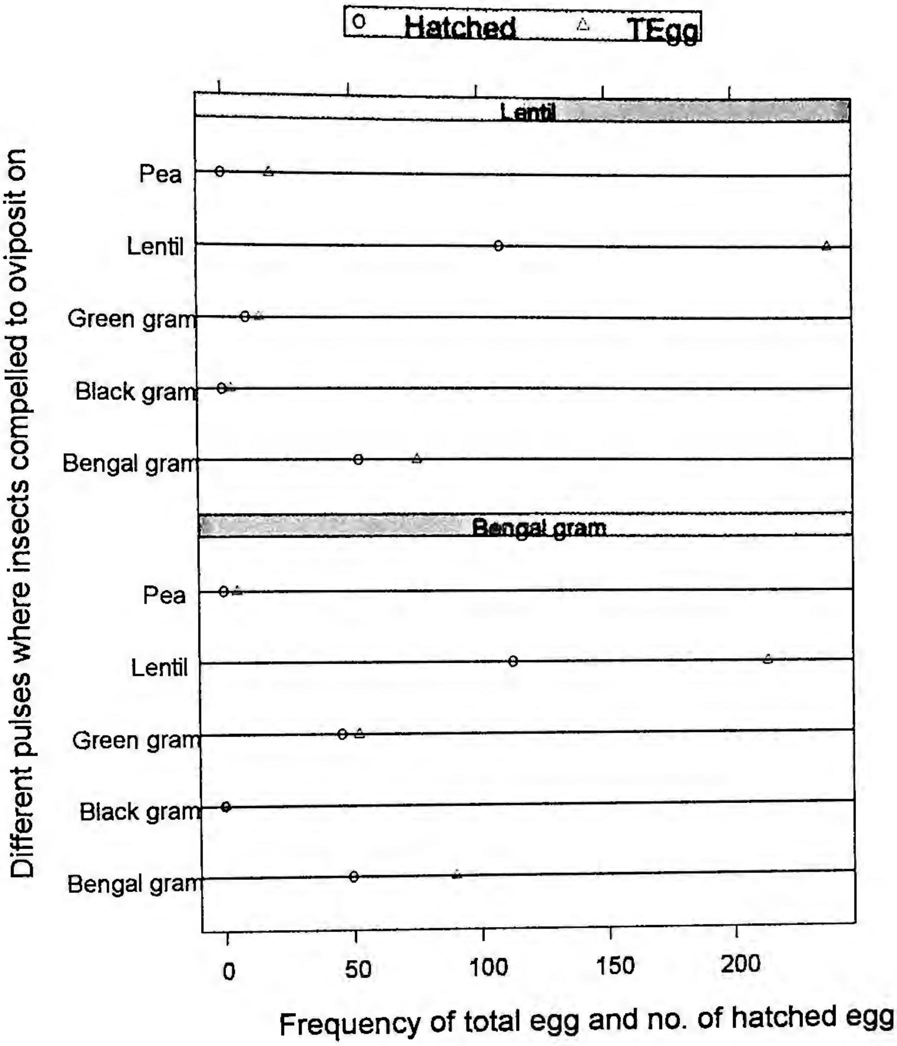

Figure 3.4. Dot plot of seed preference or *C. chinensis* and *C. maculatus* collected from different sources. The bar of each box showed the source pulse they collected from and the left side of each box lists the target pulse they compelled to oviposit.

C. maculatus collected from black gram showed the highest propensity to black gram for oviposition. The second preference is green gram. But *C. maculatus* collected from green gram showed the highest preference to green gram and then the black gram. In both of the cases the emergences rate is higher in green gram. The emergence rate was 87% and 53% for the *C. maculatus* collected from Black gram and green gram respectively. *C. maculatus* either collected from green gram or black gram also preferred pea for oviposit but the emergence was very poor.

3.4. Discussion

In the study it was observed that *C. chinensis* liked lentil for oviposition whatever may be their sources. The next preferable seed is Bengal gram, which is a wrinkled seed. The emergence rate in Bengal gram was 70%. *C. chinensis* prefers green gram less for oviposition, but the emergence rate is very high in green gram and it were 88% for the insect collected form Bengal gram and 63% for the insect collected from lentil.

Begum *et al.,* (1993) found that one generation of five gravid females of *C. chinensis* infested 47.36, 40.12 and 36.36 percent of seeds, and caused 31.28, 23.81, 3.73 percent weight loss In *Lens, esculenta, Vigna radiata* and *Lathyrus satlvus* respectively, during 18-20 days of storage which implies the seed preference for the reproductive potential of *C. chinensis*. The percent oviposition and emergence of adult insects were markedly different in developmental periods of the insects in three types of pulses.

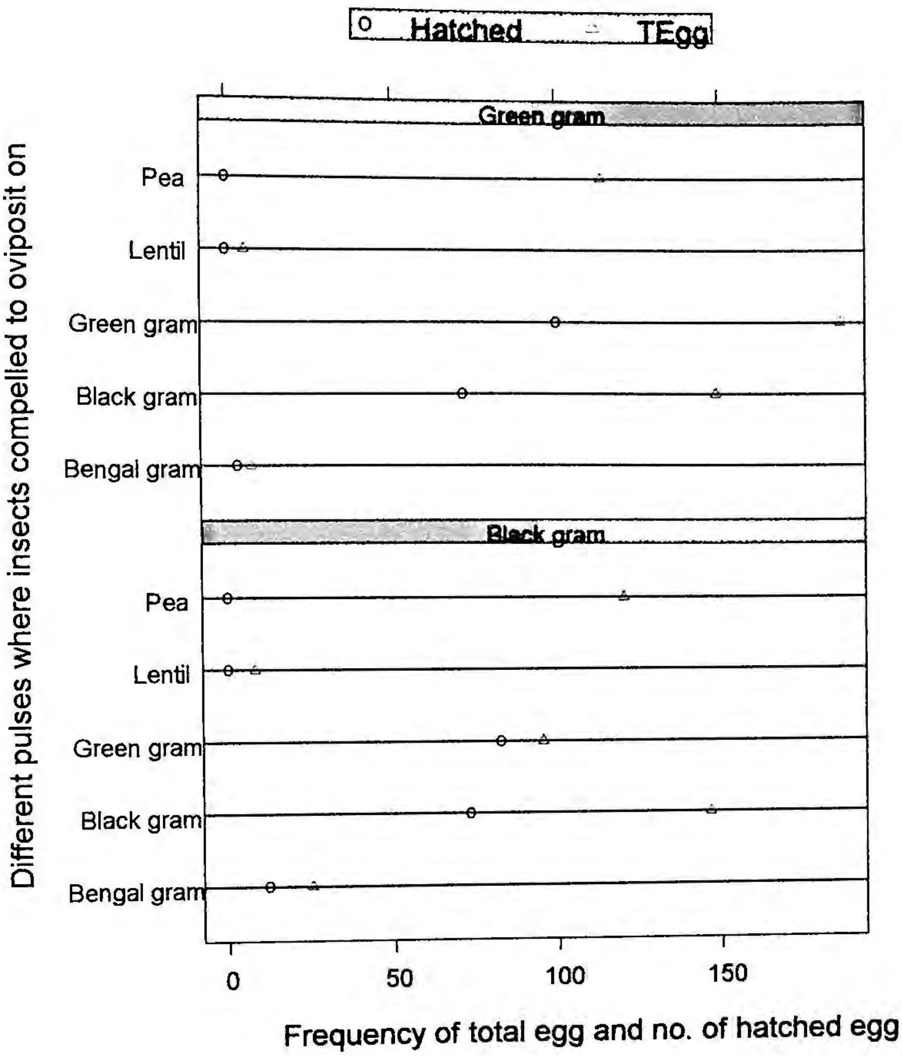

Figure 3.5. Dot plot of seed preference for *C. maculatus* collected from different sources. The bar of each box showed the source pulse they collected from and the left side of each box lists the target pulse they compelled to oviposit.

The result suggests that *L. esculenta* is more susceptible to *C. chinensis* than *V. radiata* and *L. sativus.* This contradicts with a previous report where Sultana (1974) found that C. *chinensis* caused more damage to mung bean than lentil and pea. Begum *et al.,* (1990) observed that black gram did not support *C. chinensis* and the species caused the highest damage in lentil seeds.

C. maculatus has seed specificity. It like to oviposite on the pulse from which it emerged. Considering the physical structure, the insect likes the smooth seed for oviposition. Though *C. maculatus* preferred both black gram and green gram for oviposition, the emergence rate is higher in green gram for either of the cases.

Nwanze and Horber (1976) observed that *C. maculatus* prefer smooth-coated seeds to wrinkled seeds for oviposition; Iloba (1985) and Lale and Efeovbokhan (Lale and Efeovbokhan, 1991), however, demonstrated a selective preference by the bruchid for wrinkled over smooth seeds for oviposition. Hatching and eventual penetration by the first instars larvae were observed by Nwanze and Horber (1976) to be hindered on wrinkled seeds whereas the reverse was reported (Iloba, 1985) on seeds of other cowpea varieties with smooth coats. Lale and Kolo (1998) have suggested that the presence of biochemical factors in the seed coat, irrespective of coat texture, may cause reduced oviposition and poor survival of bruchid eggs on some resistant cowpea varieties.

Chapter 4

Gamma Radiation Induced Inherit Sterility

Summary

Experiment was conducted to study the impact of radiation on five successive generations where only the parent generations were exposed to gamma radiation of different doses. Irradiation is able to increase the time of egg to adult in successive generations of *C. maculatus*. The dose ≥8Gy has dramatic impact on increasing the duration between egg to adult. For the dose 12Gy the maturity time in F5 generation has gone more than 100 days. Dose ≤6Gy has no significant effect on the immature period. Dose 2Gy has no effect on the longevity of males and females the longevity of both sex of the successive generation decreased in dose of 10Gy and 12Gy. In 12Gy radiation dose, males died earlier than females from F1 to F4 generation. The highest longevity increase was found successive generation in 12Gy radiation dose. Inherit sterility induced dose does not affect the sex of the progeny. But considering regarding the independent pair, the average number of independent pair reduces successively in the radiation doses ≥ 6Gy. At 8Gy and above radiation dose, the average number of pair decreases dramatically after F1 generation. Results implied that the radiation doses might have some effect on the sex-ratio of the emerged beetles though the impact is not sequential. In breeding and irradiation do not affect the percentage of adult emergence rather the percentage of adult emergence increased in F2 to F5 generation in 10Gy and 12Gy radiation level.

4.1 Background

Sterilizing with gamma radiation is one of the well-known techniques for pest control. Studies on the effect of gamma radiation on the species *Callosobrochus* spp. are extensive. Males of *C. maculatus* with sterilizing dose of gamma radiation confined with untreated virgin females causes infertile eggs production, whether mating took place on the day of treatment or 3 days latter (Ahmed et al., 1980). The fecundity, sex ratio and adult longevity of gamma irradiated *C. analis* parents and their progeny remain unchanged whether fertility shows a dose dependent variation (Begum *et al.*, 1981b). Ghomomu (1989) found that adults of *C. maculatus* emerged from irradiated mature pupae showed lower fecundity and fertility corresponding with increasing dose of radiation. El-Sayed *et al* (1988) also studied the effect of gamma radiation and found that 8Gy gamma radiation dose on *C. maculatus* is a sublethal dose for the species. Ghogomu (1990) studied the effect of gamma radiation applied to the immature stages of the stored products pest *C. maculatus* and observed the complete mortality before egg hatching occurred when 1- and 3-day-old eggs were treated with 1 and 20 krad, respectively. No adults emerged when 1 and 2 to 3-day old eggs were treated with 1 and 2 krad. Adult emergence was prevented when young larvae (up to 10 days old) and older larvae (14 and 16 days old) were treated with 6 and 30 krad, respectively. Adults emerged when pupae were treated with 30 krad. Adults that developed from early stages treated at <2 krad did not differ from untreated ones in respect of the duration of development or adult lifespan. Ghogomu (1991) found that at 10 Krad dose level both males and females of *C. maculatus* can achieve complete sterility. Hussain and Imura (1989) Studied the effects of gamma-radiation on various stages of the bruchid *C. chinensis* on adzuki beans (*Vigna angularis*). They observed the complete mortality of 1-day-old eggs occurred when they exposed to a radiation dose of 12 Gy. A dose of 640 Gy caused instant mortality of mature pupae and 1-day-old adults. The fecundity and fertility of adults irradiated decreased with increasing dose. Eggs laid by females, which had been exposed, to 20 Gy or more had negligible hatching rates. Complete sterility was obtained by exposing 1-day-old adults to 80Gy; sterilized males were capable of competing sexually with untreated ones. Percentage of egg hatch decreased as the ratio of sterile males to normal males increased, becoming less than 10% at ratios of 9:1 and 15:1.

Olaifa *et al.*(1990) irradiated *C. maculatus* with gamma radiation from a 60Co source. Arrested development was observed at dosages of 1.0, 4.0, 10.0 and 10.0 Gy in the case of egg, 1st, 3rd and 4th instars larvae, repectively. But the lifespan of 2-day-old adults was not affected by 100 Gy dose level. Gill and Pajni (1989) showed that 3 or 3.5 krad gamma irradiated *C. chinensis* failed to develop egg when equal proportions of treated males and normal males and females were used. Saveral works are worth mentionable on the effect of gamma radiation. Khatoon *et al.*(1989), Khattak and Hammed (1986), Howlader *et al.* (1992) are of the others whose works were designed to gain the sterility and mortality of *Callosobruchus* spp.

Remarkable works have also done on effect of gamma radiation on the different species of insects under Colioptera. For example, Sharp (1995) , Ito *et al.* (1991) worked on sweetpotato weevil, Rosada *et al.* (1992) on rice weevil, Haynes (1983), Haynes and Smith (1989a, 1989b), Villavaso *et al.* (1986), Winstead *et al.* (1989) on bowl weevil and Ismail *et al.*(1987a, 1987b) are worth mentionable.

All these studies reviewed are centred on mortality or instant sterility of these insects. The researchers used different radiation doses ranging between 53Gy to 2229Gy on different phase of different weevils. They exposed larva, pupa and different days old adults to radiation with a view to prevent the adult emergence, to change the reproductive organ structures for sterilization and to find out the optimal dose to achieve sterility of those insects. But the long run effect of irradiation of males on their successive progeny has not yet been studied.

In this chapter we studied the effect of different doses of radiation on *C. chinensis* and *C. maculatus* males that was evaluated by means of different reproductive potentials on their offspring till 5th generation.

4.2 Materials and Methods

4.2.1. Collection of Beetles

Seeds of lentil, black gram, Bengal gram, pea and mung bean infested with *Callosobruchus* spp. were collected from stored grain shop from Rajshahi city. The samples were transported to the laboratory in plastic pots. Morphological feature of *C. chinensis* and *C. maculatus* were studied with the help of Gorhan (1987) and Hill (1990). Beetles were inbred in the laboratory for two successive generations in order to eliminate natural and/or deleterious mutations, if any. Each of the regional samples was maintained separately in 500 ml beakers; mouths of which were covered with coarse cloth and tied with rubber bands to prevent the insect from escaping and also to allow the air and moisture in and out of the containers. It should be mentioned that *C. chinensis* were reared in uninfested and sterilized lentil seeds *(Lens esculenta)* and C. *maculatus* in similar black gram seeds *(Vigna mungo)*. For sterilization, the fresh seeds were kept in an oven for overnight at 60° C to destroy' the previously laid eggs or immature stage, if any. Whenever required, the insects were cultured in sterilized seeds. The mass cultures of the beetles were maintained in rearing cages in the laboratory at ambient temperature (28 ± 2°C) and at 70 ± 5% RH.

4.2.2. Experimental Design and Mating Schedule

The experiment was conducted to evaluate the effect of gamma radiation on the reproductive potential of *C. chinensis and C. maculatus* (Rajshahi strain). Adult and 15 days old larvae of *C. chinensis* and *C. maculatus* in Lentil and black gram seeds were irradiated with gamma radiation at dose 0 (Control), 2, 4, 8 and 12 Gy (1 Gy = 1 Joul/Kg = 0.1 Kr) installed at the "Institute of food and Radiation Biology, Atomic Energy Research Establishment, Dhaka. Each dose had 3 replications where adults or larvae varying from 20 to 50 in number were used. The irradiated insects were kept in separate vials to be used later as per mating schedule. The experiment was continued from parental through F5 generation. Different reproductive potentials such as fecundity, hatchability, sex ratio and adult longevity ware observed. The analytical results are presented in the following.

4.3 Results

Gamma radiation induced changes in the reproductive potentials of five successive generations of *C. chinensis* and *C. maculatus* has been studied. The results are presented in the following.

4.3.1. Total Egg

The effects of different level of irradiation dose of *C. maculatus* were presented in figure 4.1. Figure 4.1 showed that for the dose of 2Gy and 4Gy, the total number of egg was increased in F1 generation, was decreased in F2 generation and in F3 generation it remains almost same up to F4 generation and after that it falls permanently. The F5 generation's observation of 4, 6, 8, and 10Gy is absent because of insufficient offspring of F4 generation though the number of egg in F4 generation in 4, 6, and 8Gy were sufficient to carry on the F5 generation. The total number of eggs in successive generations decreased systematically from their parent (F0) generation from near about 32 to 20. The total egg of F1 generation falls dramatically may be due to some external environmental impact. The dose 8Gy also shows almost same pattern as in 6Gy dose. The total number of egg decreased up to F4 generation except the F3 generation. The total egg of F3 generation is extremely large and it was >40. In 10Gy radiation, number of egg pattern is different from the others. Egg number increased up to F3 generation and dramatically fall at F4 generation. The total egg pattern in 12Gy dose group was very much systematic.

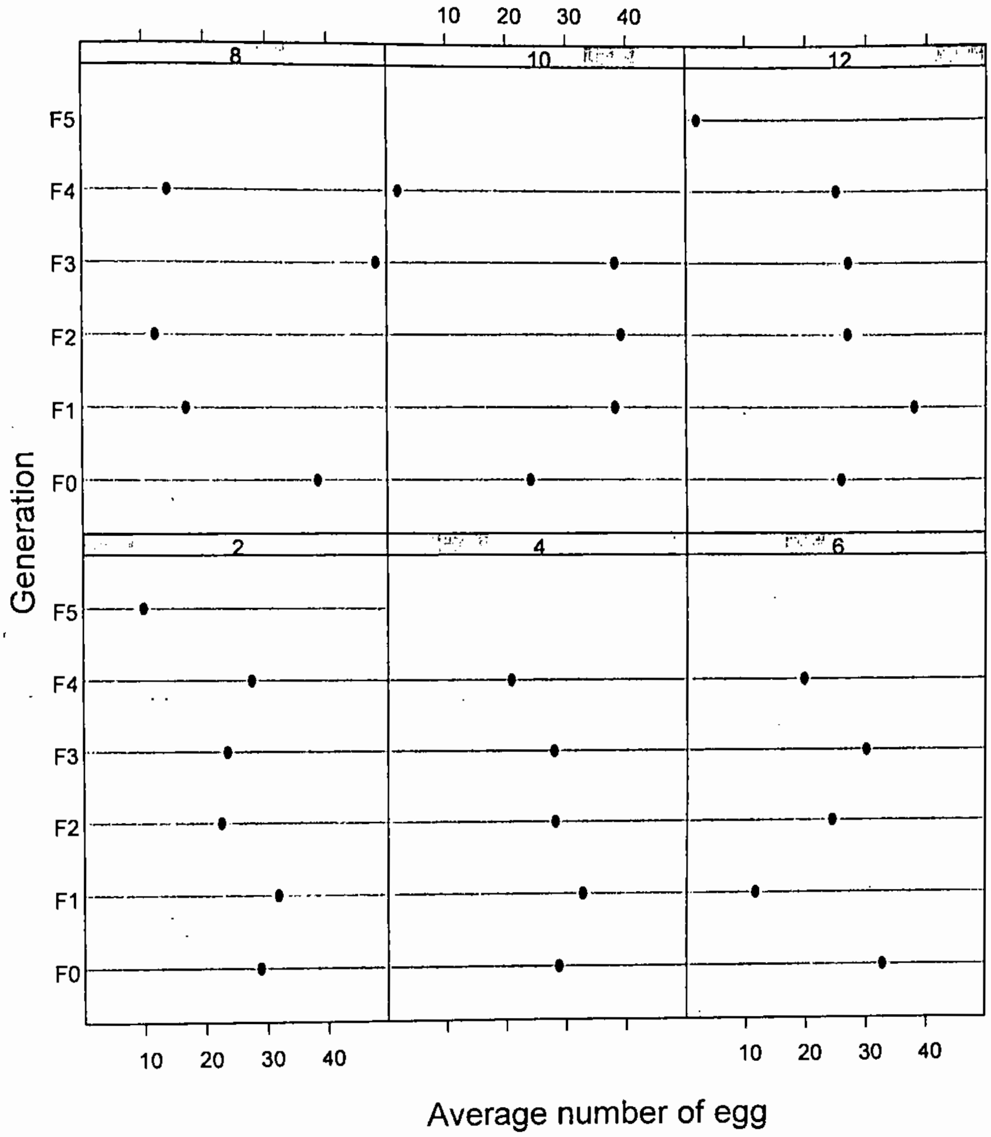

Figure 4.1 Dot plot of average number of egg laid per female in each generation where the parent generation was irradiated with different doses of gamma radiation. Colored bars of each box showed the radiation dose in unit Gy.

In F1 generation the total egg was increased a little and then systematically decreased by successive generation. It is remarkable that the egg lying pattern of 2Gy, 4Gy and 12Gy are almost same in the successive generations, which implies that there is no specific impact on the dose level but systematic, decrease supports the effectiveness of 12Gy.

4.3.2. Duration between Egg to Adult

The durations of egg to adult in successive generation in different doses were presented in figure 4.2. Figure 4.2 showed that all level of doses increased the time to become adult in successive generations. The radiation level ≥ 8Gy is dramatic. Radiation significantly increases the maturity time in each successive generation. Significant impact found in the F4 generations. For the dose 12Gy the maturity time in F5 generation has gone more than 100 days. Early emergence found in F1 generation at the radiation level ≤6Gy. The 2Gy radiation effect was not much significant to increase the emergence time in the successive generations.

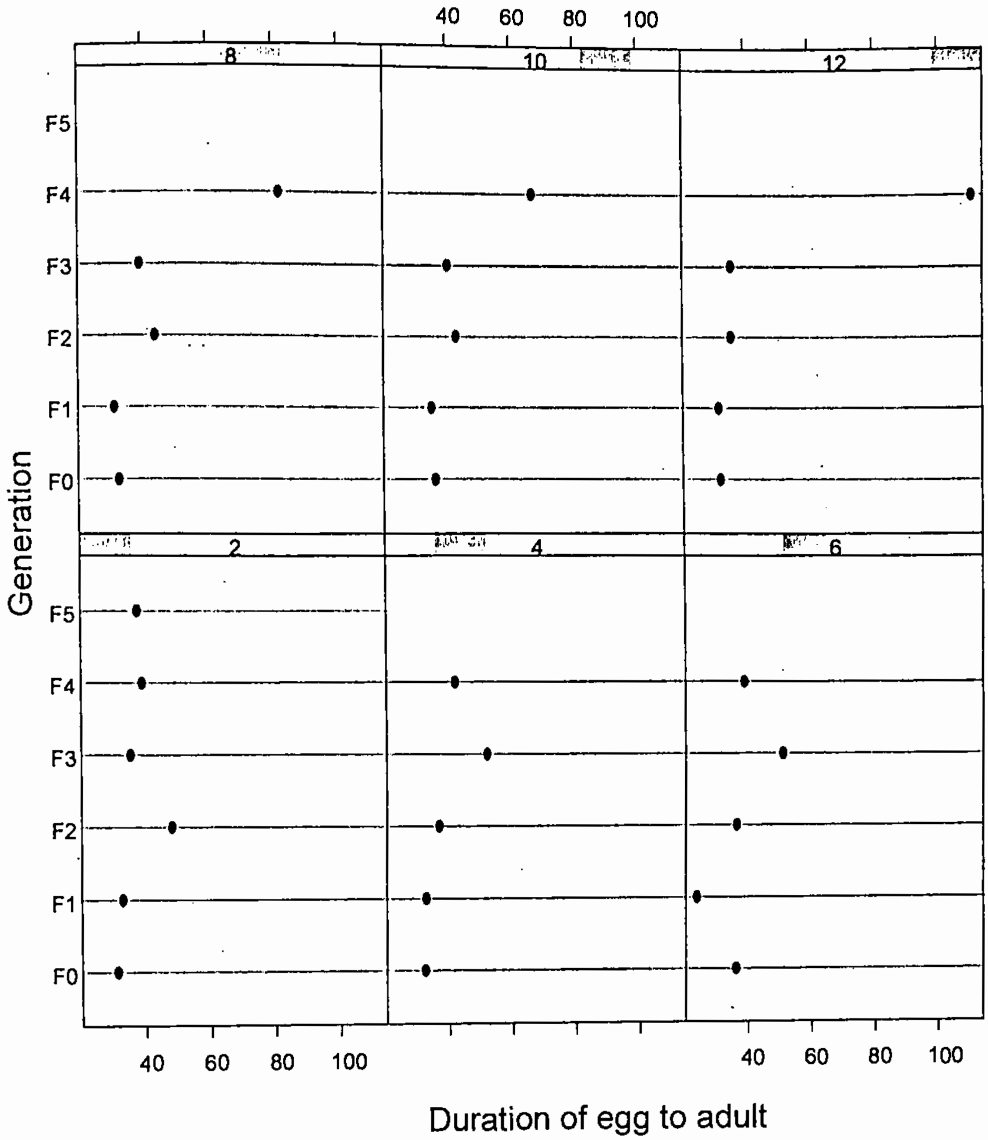

Figure 4.2. Dot plot of average duration to become mature from egg to adult for each generation where the parent generation has been irradiated with different doses. Colored bars of each box showed the radiation dose in unit Gy.

4.3.3. Adults Longevity

Sex-wise adult longevity of males and females of successive generations according to their corresponding doses were illustrated in figure 3. Figure showed that in the radiation level 2Gy, The longevity of males and females in all generation were almost same. The longevity of both sex decreased in the successive generations. Similar result found for the dose 10Gy. In 4Gy and 6Gy radiation dose, the longevity of both sex remained same but the longevity increased in the successive generations. In 8Gy radiation dose longevity of male and female increased up to F2 generation and after that it falls. In 12Gy radiation dose, males died earlier than females from F1 to F4 generation.

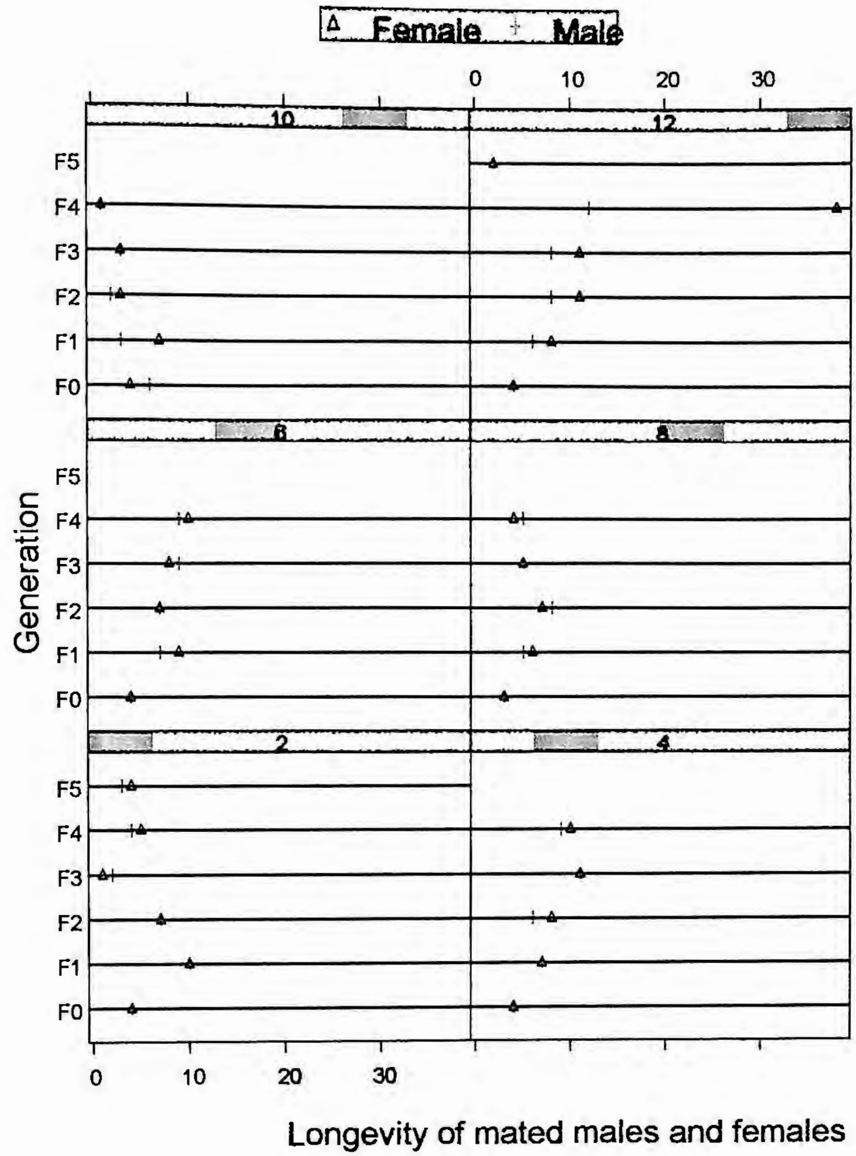

Figure 4.3. Dot plot of longevity of adult males and females for each generation where the parent generation has been irradiated with different doses. Colored bars of each box showed the radiation dose in unit Gy.

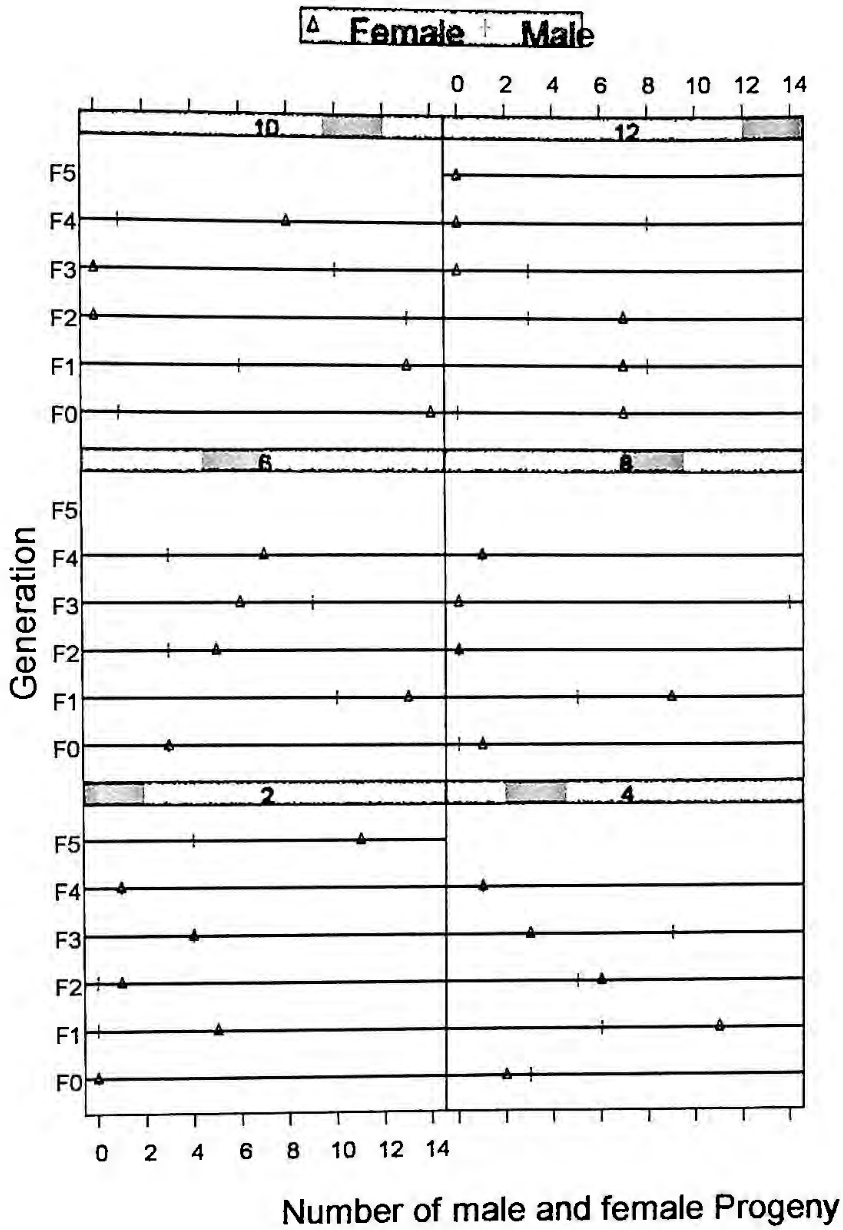

Figure 4.4. Dot plot of the number of males and females emerged from each generation where the parent generation has been irradiated with different doses. Colored bars of each box showed the radiation dose in unit Gy.

4.3.4. Sex-ratio

The number of adult males and females emerged from each successive generation corresponding to the different radiation doses are figured in figure 4. Figure did not show any significant pattern regarding the sex of emerged adult considering the radiation doses or by generation. Enumeration of the numerical value of the sex ratio was sometimes impossible as sometime 0 count found in the male or female.

To avoid the enumeration of numerical value of the sex ratio, we count the number of possible independent pair according to different doses of irradiation and successive generations. The results are given in table 4.1. The calculation has been done averaging the 10 replications, so 0 does not necessarily mean that there was no pair to carry on the experiment for successive generations.

Table 4.1 showed that the average number of individual pair was highest in F2 generation but successively the number of independent pair was decreased. The decrease rate in 4Gy and 6Gy was relatively slow but in 8Gy and above radiation dose the average number of pair was decrease dramatically after F1 generation. Results implied that the radiation doses might have some effect on the sex-ratio of the emerged beetles.

Table 4.1. Table of average count of independent possible pair in each generation

	Generation							
Dose (Gy)	F0	F1	F2	F3	F4	F5	Total	Average
2	0	0	0	4	1	4	9	1
4	2	6	5	3	1	0	18	4
6	3	10	3	6	3	0	25	5
8	0	5	0	0	1	0	6	1
10	1	6	0	0	1	0	9	2
12	0	7	3	0	0	0	10	2
Total	7	34	11	13	7	4	76	13
Average	1	6	2	2	1	1	13	

We also used the non-parametric run test on the difference between the number of males and females on different doses of radiation and on different generation to see whether the difference follows some pattern relevant to the radiation dose or to the generation. Test statistics and the other relevant information of run test are given in table B.1 in appendix B. Run test result shows that the difference of the number of males and the number of females in all dose and all generation appears randomly, which implies that the radiation doses does not have any sequential impact on the sex ratio in different generations.

4.3.5. Percentage of Adult Emergence

The effect of irradiation and inbreeding on percentage of adult emergence was also studied. The results illustrated in figure 4.5. Figure showed that the impact of irradiation and inbreeding does not affect to reduce the percentage of adult emergence rather the percentage of adult emergence increased in F2 to F5 generation in 10Gy and 12Gy radiation level.

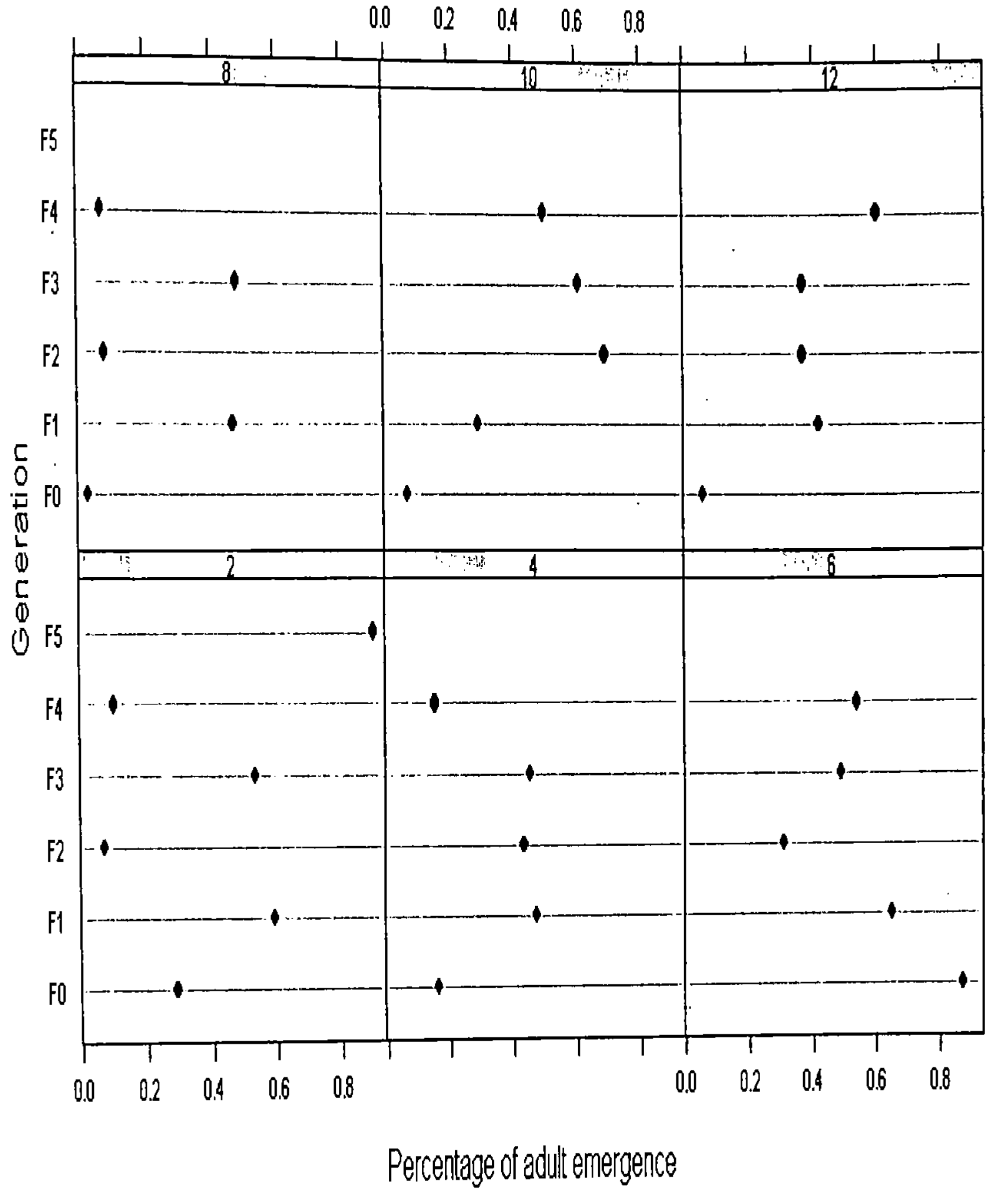

Figure 4.5. Dot plot of the number of males and females emerged from each generation where the parent generation has been irradiated with different doses. Colored bars of each box showed the radiation dose in unit Gy. Percentage will be calculated by multiplying the scale by 100.

4.4 Discussion

Irradiation is able to increase the time of egg to adult in successive generations of *C. maculatus*. The dose ≥8Gy has dramatic impact to increase the duration. For the dose 12Gy the maturity time in F5 generation has gone more than 100 days. Dose ≤6Gy on parent generation has no significant effect on the immature period. Dose 2Gy has no effect on the longevity of males and females. The longevity of both sex of the successive generation decreased in dose of 10Gy and 12Gy. In 12Gy radiation dose, males died earlier than females from F1 to F4 generation. Our result contradicts with the result of (Begum *et al.*, 1981a) where they found that longevity of experimental population of *C. analis* remained undistorted through 6th generation. But regarding the independent possible pair, the average number of independent pair reduces successively in the radiation doses ≥ 6Gy. At 8Gy and above radiation dose, the average number of pair decreases dramatically after F1 generation. The results imply that the radiation doses might have some effect on the sex-ratio of the emerged beetles though the impact is not sequential. In breeding and irradiation do not affect the percentage of adult emergence rather the percentage of adult emergence increased in F2 to F5 generation in 10Gy and 12Gy radiation level. This results is similar to Begum *et al.*(1981a, 1981b) showed that the mating tendency and adult longevity remain unchanged in sterility-inherited progeny. Fertility shows dose dependent variation and male progeny showed higher sterility in the successive generation. Irradiation of female of *C. chinensis* resulted the decrease in egg production as well as progeny number (Begum, 1984). 5-60Gy radiation on *C. analis* resulted that the longevity of the treated females was always greater than that of the treated males (Begum *et al.*, 1985). Our result also supports this result. About the adult as we found that the longevity increase is the highest in each successive generations in 12Gy dose.

Chapter 5

Mating Competitiveness

Abstract

The mating competitiveness of *Callosobruchus* spp. with two radiation doses 6Gy and 8Gy on *C. chinensis* and *C. maculates* is studied. The study showed that for *C. chinensis* the infertility rate increased as the number of treated male increased up to 1(UF):1(UM):2(IM). The degree CVs decreases as the treated males increased. Further increase of the number of males the CVs would be the same. The observed percentage of infertile egg increase was not proportional to the expected percentage of infertile egg increase. The relative percentage of average egg has been decreased as the number of treated male increase in each combination except 1:1:2. For *C. maculatus,* within the treatment combination the maximum average occurred in the ratio 1:1:1 and 1:1:2. In both treatment dose level 6Gy and 8Gy the ratio (UF: UM: IM) 1:1:2 provides the maximum output of average number of egg and the infertility increased compare to the control 1:1:0. The infertility rate was the maximum at 1:1:3 for the 6Gy treated males. But treated with 8Gy shows that as the treated males increased, the infertility is also decreased. The ratio 1:1:1 showed the highest competitiveness values for both radiation doses. The dose with 8Gy showed the highest competitiveness value. The competitiveness values with radiation dose 8Gy are strictly decreasing as the radiated male number increased in each combination. For 6Gy dose radiated males, the competitiveness values are increasing for the ratio 1:1:3 and 1:1:4 and the minimum number of value is attained at the ratio 1:1:2.

5.1 Background

The concept of mass releasing genetically altered insects for pest control is more than 50 yr of age. The use of sterile insect release technique (SIRT) (Knipling, 1955) for the control of insect pests is one of the promising control methods (Brower, 1979, Cornwell, 1966, Tilton and Brower, 1983). SIRT has been used in a variety of insects (Schlilkelmann and Gould, 2000) and its successes rate is encouraging (Klassen *et al.*, 1994). Unlike non-selective insecticide based control, the SIRT and the other genetic pest control methods are proved as environment friendly. Ashraf and Brower (1974) and Tilton and Brower described extensively the advantages of SIRT. Therefore, SIRT is now a growing pest management method of interest. The basic principal of the SIRT is to release the radiated sterilized insects into the wild population and allow them to mate, competing the wild males with females. The radiation dose would be sufficient to induce sexual sterility. Wild females are expected to mate with sterile males, oviposit normally and the embryonic development will be affected to die before hatching (Baumhover, 2002) or to produce sterile progeny. The effectiveness of the method is seriously dependent on the mating competitiveness of the sterilized males. The total mating competitiveness of a particular male refers to its overall performance which is an interaction of a number of factors such as mating ability, number of mating, sperm transfer, sperm activity, olfactory responses, vigour and longevity (Hooper, 1970; Fried, 1971).

Literature on mating competitiveness on other species is much extensive.

Mehta and Bhalla (1983) studied the sterilant effects of penfluron at different doses on the adults of *Epilachna vigintioctopunctata* (F.) (Henosepilachna: vigintioctopunctata) and found that mating competitiveness remains unaffected and the treated males were considered to be fully competitive with untreated males. Baker (1984) reviews the relatively small number of laboratory and field studies carried out on genetic mechanisms for mosquito control and highlighted that field tests using chromosomal rearrangements have had mixed results; failures with some species suggest that colonization, prolonged laboratory maintenance or mass production may have selected an assortative mating behavior that reduces the mating competitiveness of the released mosquitoes. Mansour and Krafsur (1991) applied the sterile insect technique (SIT) to *M. autumnalis*. 6-day-old pupae were exposed to ionizing irradiation at different doses ranging between 100-1600 Krads. Rowland (1991) studied the effects of gamma-HCH or dieldrin resistance genes on flight activity and mating competitiveness in males from backcrossed strains of *A. gambiae* and *A. stephensi*. He showed that the activity pattern of *A. gambiae* males was not affected by resistance genes; in mating competition and predator avoidance experiments.

Mansour and Krafsur (1993) showed that the application of sterile insect technique (SIT) for controlling *Musca autumnalis* exposing six day old pupae to ionizing irradiation at doses in the range of 100-1600 Krads, and found that Eclosion was not affected, while fecundity and fertility were inversely proportional to the radiation dose. They also informed that the sterile males showed a decline in longevity and reduced mating competitiveness and unable to recover fertility.

Success of using irradiation to sterile males of various insect species for control or eradication by the sterile-insect release technique requires a certain dose for sterilizing the insects without affects on their mating behavior. Ratana and Kobayashi (1983) conducted such a study to observe some radiation effect on mating propensity and competitiveness of flies *Ceratitis capitata* (Wiedeman). Flies were irradiated with 145 gray of gamma radiation from a cobalt-60 source in a nitrogen atmosphere 2 days before eclosion and found that the irradiated flies were more competitive than the 4-day-old non-irradiated flies.

Al Taweel *et al.* (1993) tested the mating competitiveness for F1 males and females, whose fathers were exposed as 5-6 day old pupae to 0.2kGy of gamma radiation. The experiment was conducted to for two strains of *Ephestia cautella* by confining them with unirradiated insects. The results indicated that irradiation at 0.2kgy, which was a substerilizing dose for this species, does not lower the mating competitiveness of F1 adults.

Haynes and Smith (1989) sterilized the adults of *Anthonomus grandis* by dipping an aqueous suspension containing 0.4% antibiotics (0.3% kanamycin, 0.015% penicillin and 0.015% chloramphenicol) for 10 min followed by irradiation at 10 krad. The result is that treated males were 77-79% attractive as untreated males to untreated females. When treated males outnumbered untreated males by more than 5:1, the number of matings to untreated females was significantly higher. He suggests that the ratio of 20:1 of treated to untreated males is required to significantly reduce F1 adult emergence.

Brower (1979) studied the mating competitiveness of *Ephestia cautella* (Walker). He treated males with different radiation doses and released them in different ratios. He found that doses of 35 and 50 krad reduced egg hatch to 9.0 and 05% respectively. He also showed that when the ratio was 25 Irradiated males per untreated pair; egg hatch was 1.6 (35 krad) and 2.670 (50 krad) when the ratio was 25 irradiated females per untreated pair. Both males and females were less competitive after treated with 35 krad than treated with 50 krad (based on percentage of egg hatch). Treated females were competitive at all release ratios. The radiated males were competitive with unradiated males except at the lowest release ratio. Thus, irradiated adults were judged sufficiently competitive for field trials.

Hasan (1999) studied the mating competitiveness of *Tribolium* spp. by determining the egg viability resulting from males originating from early and late irradiated pupae concerned in various ratios with unirradiated adults. He showed that infertility increased as the ratios of irradiated males increased to unirradiated males and females. He also showed that within ratios ranging from 5 Irradiated males: 1 Unirradiated males: 1 Unirradiated females to 15 IM: 1 UM: 1 UF, the irradiated males were not fully competitive with normal males since the differences between the observed and expected egg infertility were very high.

Literature regarding the mating competitiveness on *Callosobruchus* spp. seldom found. Hussain and Imura (1989) studied the effects of gamma-radiation on various stages of the bruchid *C. chinensis* on adzuki beans (*Vigna angularis*). He found that the fecundity and fertility of adults irradiated either as mature pupae or 1-day-old adults decreased with increasing dose. Eggs laid by females which had been exposed and/or had mates which had been exposed as mature pupae to 20 Gy or more had negligible hatching rates. Complete sterility was obtained by exposing 1-day-old adults to 80 Gy; sterilized males were capable of competing sexually with untreated ones. Percentage egg hatch decreased as the ratio of sterile males to normal males increased, becoming less than 10% at ratios of 9:1 and 15:1.

Gill and Pajni (1989) showed that for 3 or 3.5 krad gamma irradiated *Callosobruchus chinensis* almost total suppression occurred with a ratio of 8 treated males to one normal male and one normal female. Sterile males were fully competitive with normal males at all ratios. The combination of sterile males, sterile females, normal males and normal females resulted in suppression of the population at ratios of 7:7:1:1 and 5:5:1:1, following treatment with 3 and 3.5 krad, respectively Sterilized males and females were more competitive than normal insects.

Messina *et al.* (2007) conducted mating trials to estimate the degree of precopulatory (behavioral) and postcopulatory (gametic) prezygotic isolation between African and Asian populations of the seed beetle *C. maculatus*. Based on the pairing of males and females of different geographical region they conclude that Asian males obtained a disproportionate share of the matings, and were more likely to disturb a pair already in copulo. Surprisingly, male size did not influence the probability of copulation in the single-male trials, the outcome of male–male competition, or the fecundity of once-mated females. Although the African and Asian populations showed some evidence of mating incompatibility, the absence of symmetrical isolating factors suggests minimal barriers to gene flow.

From the above discussion it can be realized that treatment reduces mating competitiveness as a result, the number of insects to be released must be increased to attain the ratio necessary to produce the decreasing tendency in the population. Thus to evaluate the mating competitiveness of males with desired level of sterile doses is one of the most important area of research for pest control.

Although the effect of different doses of radiation regarding the mating competitiveness is much older in the scientific community to sterilize the males for a viable SIRT planning, the mating competitiveness of *Callosobruchus* is not well studied and there is a few literature found on the *Callosobruchus* spp. about the mating competitiveness of the beetles. Although different reproductive parameters of the *Callosobruchus* have been extendedly studied, the mating competitiveness of radiation treated males of different doses is still in the dark. In this study we tried to find out the possible mating competition behaviour of the *C. chinensis* and *C. maculauts* in different radiation doses.

5.2 Materials and Methods

Seeds of lentil, black gram, Bengal gram, pea and mung bean infested with *Callosobruchus* spp. were collected from stored grain shop of Rajshahi, Bangladesh. The samples were transported to the laboratory in plastic pots. Morphological feature of *C. chinensis* and *C. maculatus* were studied with the help of Gorhan (1991) and Hill (1990). *C. chinensis* were reared in uninfested and sterilized lentil seeds *(Lens esculenta)* and *C. maculatus* in similar black gram seeds *(Vigna mungo)*. The mass cultures of the beetles were maintained in rearing cages in the laboratory at ambient temperature (28 ± 2°C) and at 70 ± 5% RH.

5.3 Experimental Design

To determine the effect of radiation on mating competitiveness, 2-day adults of *Callosobruchus* spp. *C. chinensis* and *C. maculatus* were collected from stock cultures rered in the food media from which they collected. Insects are carried in vials. Adult males of each *Callosobruchus* species were irradiated with a substerilising dose of gamma irradiation of 6gy for *C. maculatus* and 8gy for *C. maculatus* and *C. chinensis* from a 60Co source at a dose rate of approximately 5.955 krad/h at 30 cm distance from the midline of the vials to the ray tube. Immidiately after the day of radiation the irradiated males were placed with unirradiated (U) males and females in the following (Un treated female: Untreated male: Irradiated male) numbers and ratios 0:1:1, 1:0:1, 1:1:1, 1:1:2, 1:1:3, 1:1:4. Three replicates were tested for each combination. The adults of each set were placed in Petri dish containing the food medium for oviposition. Sterilized Black gram is used for *C. maculatus* and Lentil was used for *C. chinensis* oviposition. After 24 hours, egg-carrying seeds were collected and counted. The data recorded until the females death. The corresponding percentage of adult emergence was recorded. To determine the competitiveness value (CV) of irradiated males compared with normal males, the expected percentage egg hatch at each ratio was calculated following the model developed by Fried (1971):

Expected % of adult emergence = $(C \times H_c + T \times H_t)/(C + T)$

The total competitiveness value (CV) of the treated males was calculated from Fried's (1971) replacing the egg hatch percentages by the corresponding percentage of adult emergence modified equation. Hence, equation for total competitiveness stands,

$CVs = C/T \times (H_c - H_o)/(H_o - H_t)$

Where,

C = number of untreated males

T = number of treated males

H_c = Percentage of adult emergence from the cross UM:UF

H_t = Percentage of adult emergence from the cross TM: UF

H_0 = Percentage of adult emergence at different ratios.

5.4 Results

The results of the experiment to study the mating competitivenes of *C. chinensis* and *C. maculatus* are as follows.

5.4.1. Mating Competitiveness Result of *C. chinensis*

The average number of egg laid by each female of *C. chinensis* is presented in figure 5.1. Figure shows that the mean number of eggs varied significantly from each combination. The lowest fecundity was observed in the ratio of 1:1:1 and in 1:1:2. But the maximum fecundity observed in 1:1:3 and 1:1:4. There is no significant difference between the two baseline cases i.e. in the ratio 1:1:0 and 1:0:1. The minimum egg laid by the females where the ratio of female, untreated male and treated male were 1:1:2. Further information on percentage of egg relative to control is presented in the table 5.1. The percentage values show a monotonically increasing. That implies that compared with control, the increased number of treated male increase the females egg lying capacity. It can be argued that treated males are competitive for successful mating.

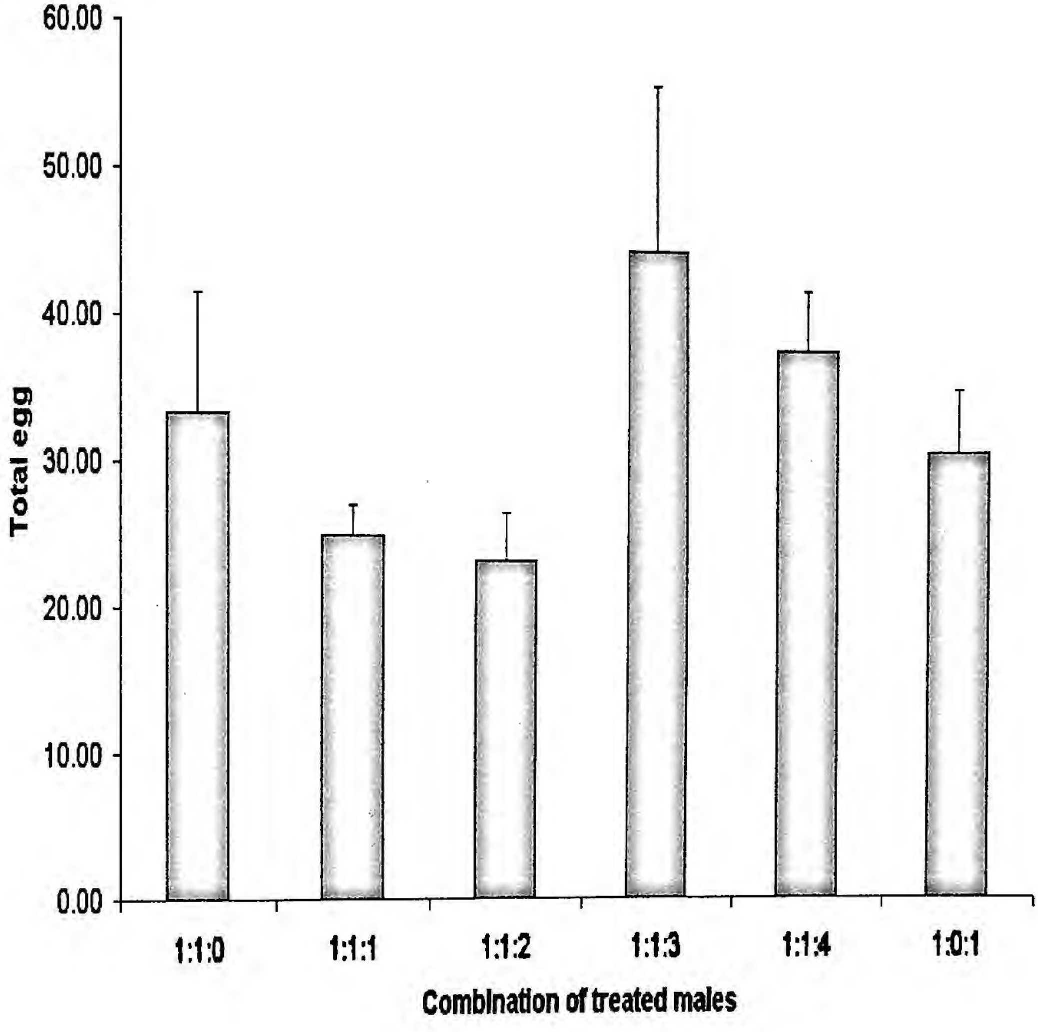

Figure 5.1. Average number of egg laid by the females mated with different combination of treated males of *C. chinensis*. The ratios are presented according as (Untreated female: Untreated male: Irradiated males)

Table 5.1. Number of eggs, percentage egg infertility, and total competitiveness of males irradiated with 8Gy of *C. chinensis*

Dose	Ratio	Total Eggs	% Relative to control	Eggs Infertility	%Eggs Infertility	Expected % of Infertility	CVs
8	1:1:0	33.33 ± 8.22b		2 ±. 0.47a	7.13	-	-
	1:0:1	31.00 ± 2.16b	93.00%	13 ± 10.50bc	44.10	-	-
	1:1:1	25.00 ± 3.27ab	75.00%	16 ± 10.14c	61.61	25.62	2.41
	1:1:2	23.33 ± 11.47a	70.00%	11 ± 7.41b	51.29	31.78	1.61
	1:1:3	44.67 ± 4.03c	134.00%	18 ± 9.46d	41.95	34.86	1.20
	1:1:4	38.00 ± 4.32c	114.00%	15 ± 13.53c	40.66	36.71	1.11

The relative infertility rate according to the increase of the number of the treated males of *C. chinensis* is presented in figure 5.2. Figure shows that the infertility rate increased as the number of treated male increased up to 1:1:2. For the next increase of untreated male in the ratio reduces the infertility rate. It is worth mentionable that the difference of infertility of two base line 1:1:0 and 1:0:1 are very much different. The maximum infertility occurred in the ratio 1:1:2.

The mating competitiveness of the species *C. chinensis* has been enumerated and presented in figure 5.3. Figure 5.3 showed that the degree CVs decreasing as the treated males increased. But the smooth trend of the figure implies that if we would further increase the number of males the CVs would be the same, which might be the future course of research. It is remarkable that all the competitive values are greater than 1. The highest competitiveness value was found in the ratio 1:1:1 and the lowest competitiveness value was found in the ratio 1:1:4. The result implies that as the observed percentage of infertile egg increase is not proportional to the expected percentage of infertile egg increase. The increase of radiated male failed to increase infertile egg as expected. The result implies that increased ratio reduced the competitive values.

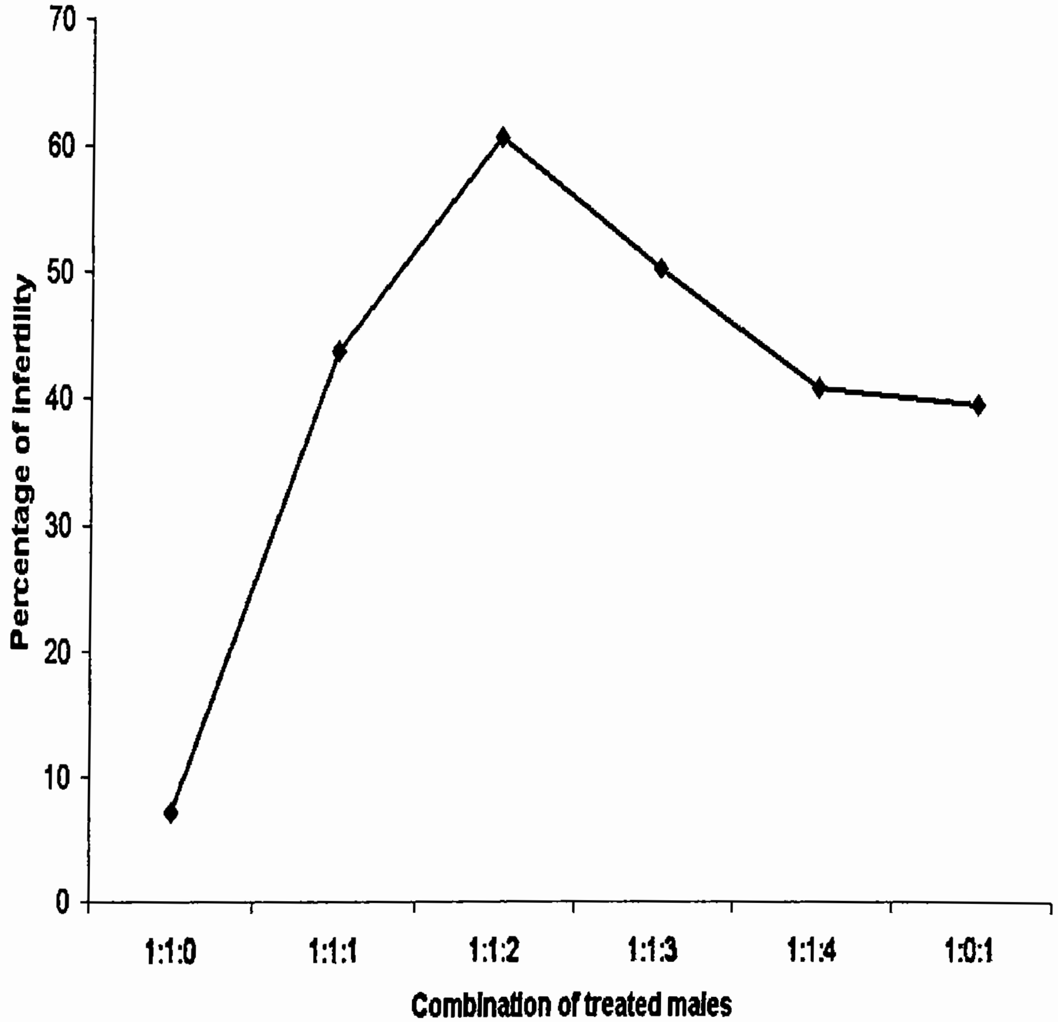

Figure 5.2. Rate of change of infertility in the combination of different ratios of the treated males with untreated males and females of *C. chinensis*. The ratios are presented according as (Utreated female: Utreated male: Irradiated males)

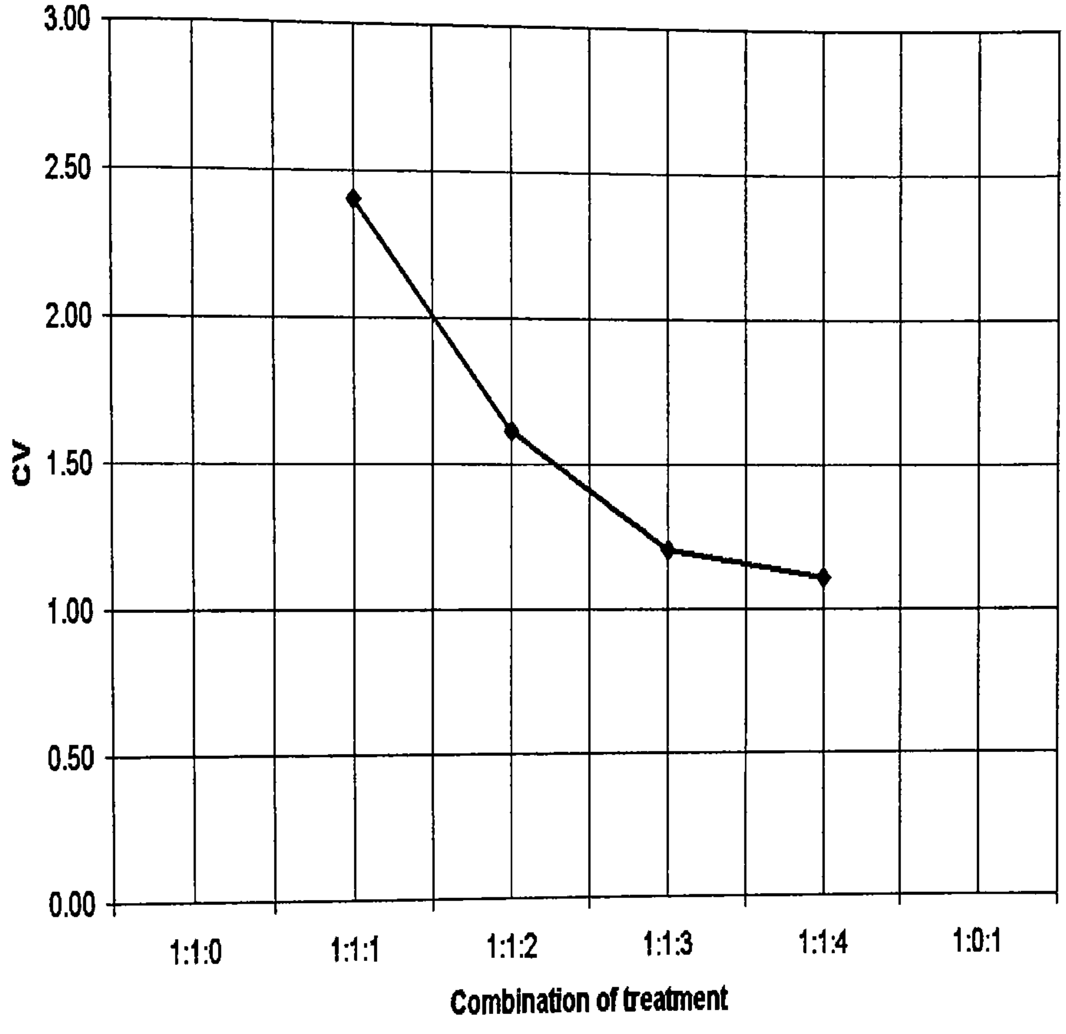

Figure 5.3 Mating competitiveness values of treated males of *C. chinensis*

5.4.2. Mating Competitiveness Result of *C. maculatus*

The average number of egg laid per females of *C. maculatus* in scheduled combination of treated males irradiated with 6Gy and 8Gy with untreated males and untreated females. The outputs regarding the total egg are presented in figure 5.4. Figure shows that the average number of egg laid in each combinations are significantly different from the two control lines 1:1:0 and 1:0:1 for 6gy treated males. The highest average occurred at the ratio 1:1:2, which is significantly different from the other combinations. The combination 1:1:3 and 1:1:4 provides almost the same average. The maximum egg laid in two baseline controls 1:1:0 and 1:0:1. This implies that except the two base lines, as the number of treated females increased, the total egg laid by females was decreased. This phenomenon becomes cleared when we see the percentage of egg relative to control presented in table 5.2 where the relative percentage of average egg has been decreased as the number of treated male increase in each combination except 1:1:2. For 8Gy treated males we see that the average egg laid varies considering the combination. The maximum average occurred at the control line 1:1:0. Within the treatment combination the maximum average occurred in the ratio 1:1:1 and 1:1:2. The others are less and significantly different from 1:1:1 and 1:1:2. This implies that as the number of the treated male increases, the total number of egg laid by the female decreases. The relative percentage of average egg laid that is presented in table 5.2 showed the same result except the ration 1:1:1 and 1:1:2 that produce the different result in both 6gy and 8gy. Compared with the dose 6Gy and 8Gy we see that irradiated male combination with

8Gy remains below regarding the average number of egg except 1:1:1 and 1:1:2. In both treatment dose level 1:1:2 provides the maximum output of average number of egg.

The competitiveness in terms of infertility was illustrated in figure 5.5. Figure 5.5 showed that in all combination of treated males in both 6Gy and 8Gy the infertility increased compare to the control 1:1:0. The percentage of infertility is the maximum for the other baseline control 1:0:1. The combination of irradiated males with 6Gy showed that increasing trend of infertility has some ups and downs in different combination. The infertility rate was the maximum at 1:1:3 for the 6Gy treated males. But treated with 8Gy shows an inverted U shaped trend within the combination, the infertility rate was maximum in the ratio 1:1:1 and the trend shows that as the treated males increased, the infertility is also decreased.

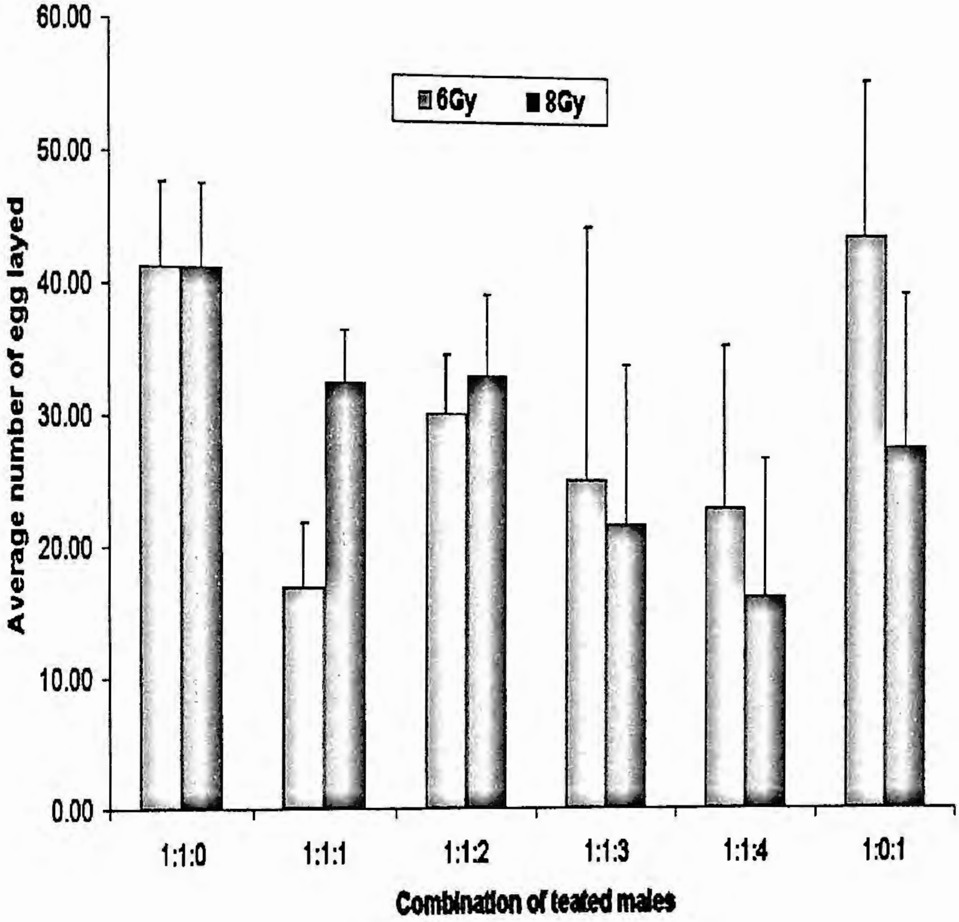

Figure 5.4 Average number of egg laid by the females mated with different combination of treated males of *C. maculatus*. The ratios are presented according as (Untreated female: Untreated male: Irradiated males)

Table 5.2. Number of eggs, percentage egg infertility, and total competitiveness values of males irradiated with 6gy and 8Gy of *C. maculates.* Values with different letters indicates the significant difference between two mean.

Dose	Ratio	Total Eggs	% Relative to control	Eggs Infertility	%Eggs Infertility	Expected % of Infertility	CVs
6	1:1:0	41.33 ± 6.34d		4 ± 2.49a	8.38	-	-
	1:0:1	44.33 ± 4.99d	107.26%	45 ± 3.50d	94.73	-	-
	1:1:1	17.00 ± 4.55a	41.13%	6 ± 3.40a	41.15	51.56	0.80
	1:1:2	30.33 ± 19.48b	73.39%	9 ± 6.34b	20.00	65.95	0.30
	1:1:3	25.33 ± 12.50ab	61.29%	14 ± 7.41c	55.46	73.14	0.76
	1:1:4	20.25 ± 12.02a	49.92%	10 ± 2.5b	30.75	83.26	0.86
8	1:1:0	41.33 ± 6.34d		4 ± 2.49a	8.38	-	-
	1:0:1	28.00 ± 4.00c	67.74%	19 ± 2.00d	68.23	-	-
	1:1:1	32.67 ± 6.13c	79.03%	16 ± 5.19c	52.42	38.30	1.37
	1:1:2	33.33 ± 12.23c	80.65%	13 ± 5.31b	46.47	48.28	0.96
	1:1:3	22.00 ± 10.68b	53.23%	10 ± 7.48b	33.64	53.27	0.63
	1:1:4	16.50 ± 12.02a	39.92%	3 ± 0.00a	24.75	56.26	0.44

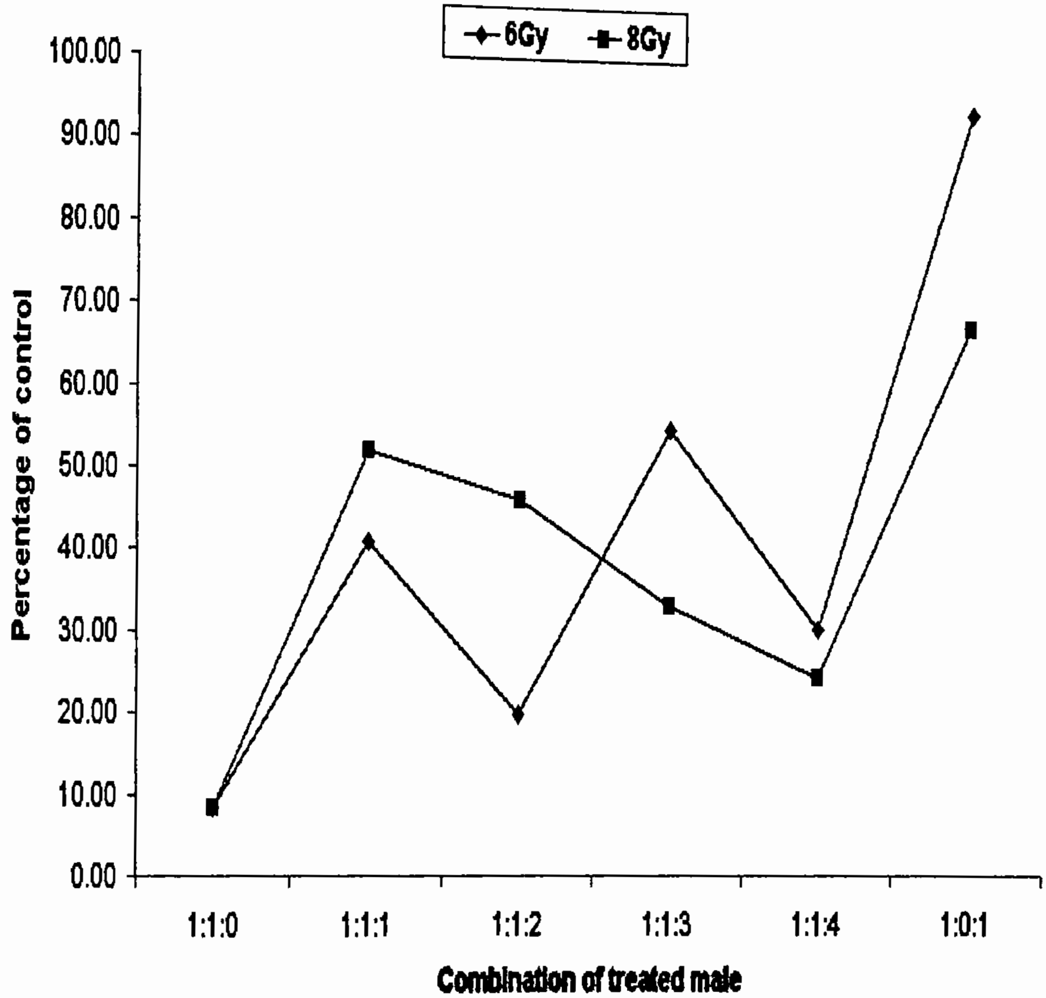

Figure 5.5. Rate of change of infertility in the combination of different ratios of the 6Gy and 8Gy treated males with untreated males and females of *C. maculatus*. The ratios are presented according as (Untreated female: Untreated male: Irradiated males)

The mating competitiveness values of *C. maculatus* treated of both 6Gy and 8 Gy are presented in figure 5.6. The numerical results of mating competitiveness along with the other relevant numerical information and statistic for *C. maculatus* are presented in table 5.2. Figure 5.6 showed that the ratio 1:1:1 showed the highest competitiveness values for both radiation doses. The dose with 8Gy showed the highest competitiveness value. The competitiveness values with radiation dose 8Gy are strictly decreasing as the radiated male number increased in each combination. For 6Gy dose radiated males, the competitiveness values are increasing for the ratio 1:1:3 and 1:1:4 and the minimum number of value is attained at the ratio 1:1:2.

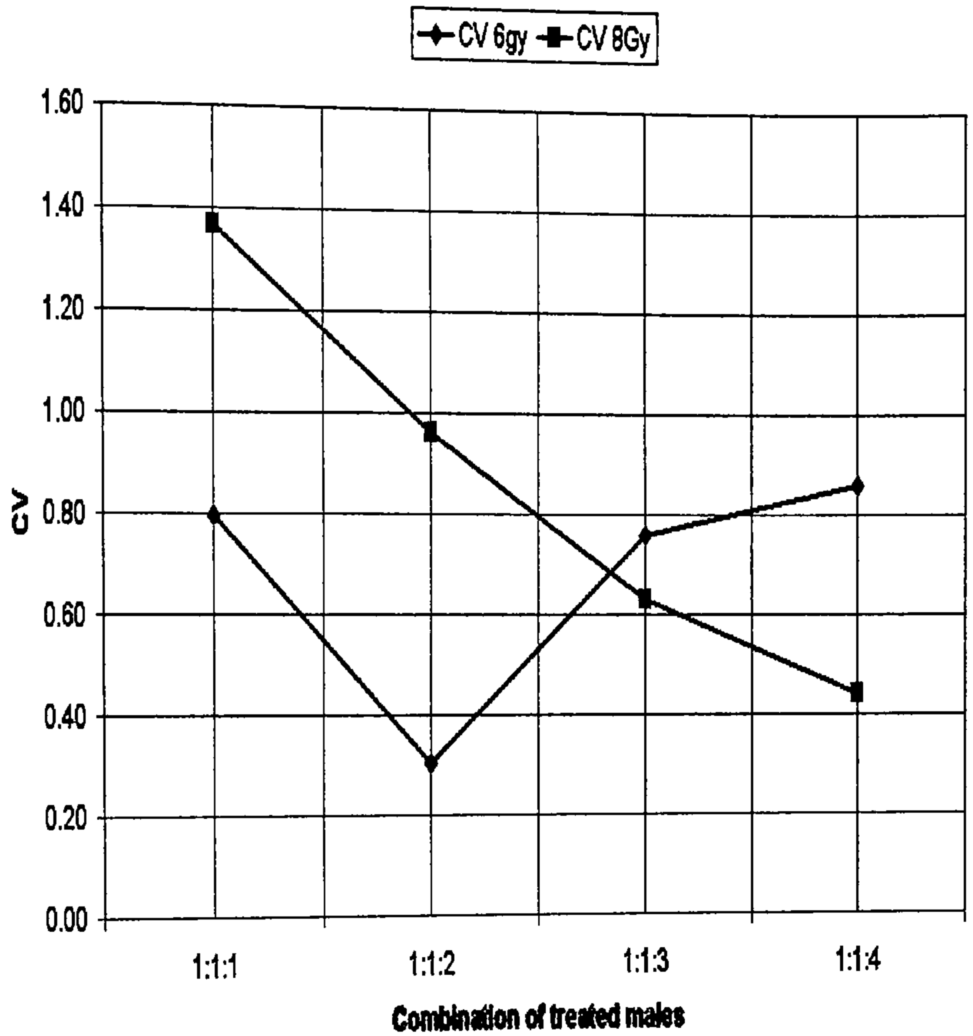

Figure 5.6. Mating competitiveness values of treated males of *C. maculatus*.

5.5 Discussion

Mating competitiveness of the irradiated adult males had not been determined previously and the study provides the needed data. Though the literature on mating competitiveness found with a little extent, there was no literature, which specifically determined the mating competitiveness of *C. chinensis* and *C. maculatus*. In the study the 2-day adult males were irradiated as adults have an adequate level of sexual competitiveness to be effective in a sterile insect release program. Several authors have shown that the program is feasible for different kind of insects that are described in the introductory section of the chapter. For the *Callosobruchus* spp. it was found that *C. chinensis* showed the highest fecundity with the combination 1:1:3 and 1:1:4 and was the lowest in 1:1:1 and 1:1:2. Compared with control, the increased number of treated male increases the femal^'s egg lying capacity. It can be argued that treated males of *C. chinensis* are competitive for successful mating to produce egg. The maximum infertility and the lowest fecundity occurred in the ratio 1:1:2. The degree CVs decreases as the treated males of each combination increased but all the competitive values are greater than 1. The highest competitiveness value found in the ratio 1:1:1 and the lowest found in 1:1:4. The result implies that as the observed percentage of infertile egg increase is not proportional to the expected percentage of infertile egg increase. The increase of males failed to produce infertile eggs in increasing fashion rather they are increasing slowly as the expected increasing faster. The result implies that increased ratio reduced the competitive values 1:1:1 ratio is the best to have the optimum CV in *C. chinensis*.

Regarding the *C. maculatus* highest fecundity occurred at the ratio 1:1:2, which is significantly different from the other combination. The combination 1:1:3 and 1:1:4 provides almost the same average. The maximum egg laid in two baseline controls 1:1:0 and 1:0:1. This implies that except the two base lines, the number of treated females significantly contributed to decrease the total egg by females. Within the treatment combinations the maximum fecundity was occurred in the ratio 1:1:1 and 1:1:2. Compared with the dose 6Gy and 8Gy we see that irradiated male combination with 8Gy remains below regarding the average number of egg except 1:1:1 and 1:1:2. In both treatment dose level 1:1:2 provides the maximum output of average number of egg. The result indicates that 8Gy dose affects more significantly than 6Gy dose on their fecundity. In both 6Gy and 8Gy the infertility increased compare to the control 1:1:0. The infertility rate was maximum at 1:1:3 for the 6Gy treated males. But treated with 8Gy the infertility was maximum in the ratio 1:1:1 and as the treated males increased, the infertility decreased. The ratio 1:1:1 showed the highest competitiveness for both the radiation doses. The dose with 8Gy showed the highest competitiveness value. In both the radiation dose the females of *C. chinensis* and *C. macualtus* are competitive. The competitiveness values with radiation dose 8Gy are strictly decreasing as the radiated male number increased in each combination. For 6Gy dose radiated males, the competitiveness values are increasing for the ratio 1:1:3 and 1:1:4 and the value is minimum at the ratio 1:1:2.

Chapter 6

Cytoplasmic Incompatibility

Abstract

Regional and reciprocal crosses of Rajshahi, Narayangonj, Chittagong and Japan strains of *C. mculatus* and Rrjshahi, Narayangonj, Khulna and Chittagong strains of *C. chinensis* were tested to find the cytoplasmic incompatibility of *Callosobruchus* spp. Result showed that the reciprocal crosses C X K, and N X R showed the highest fecundity in *C. chinensis*. In *C. maculatus* the number of total egg was maximum in reciprocal cross J X C and minimum N X J followed by C X J. In *C. chinensis* C X N showed the lowest emergence rate and it was 51.46% and most of the crosses showed more than 80% of adult emergence except C X N and K X N. In *C. maculatus* the minimum occurred in the reciprocal crosses are N X R and C X N, which was 47% and 43% respectively. Both in *C. chinensis* and *C. maculatus* the regional and reciprocal crosses have significant variation regarding the different components of reproductive potentials. In *C. maculatus* the dissimilarity between J X C and N X J was the maximum followed by J X C to C X N. In *C. chinensis* only the cross C X N showed the instable imcompatibility. In *C. maculatus* there were 4 suspected reciprocal crosses; C X J, C X N, N X J and N X R that are supposed to be infected and showing the incompatible relations. The CI level for these suspected crosses were 0.597, 0.592. , 0.582 and 0.464 respectively and the relative fecundity level estimated and found that C X J, C X N, and N X J fulfil the condition of persistent incompatibility. The critical migration rates for the crosses C X J, C X N and N X J was 0.0040, 0.0032 and 0.0042 respectively. The only about 2% of migration of incompatibility related infection happened in each identified cross. Irradiation with CI seriously affects the fecundity and fertility of *C. maculatus*. Radiation on reciprocal crosses was also able to reduce the fecundity and fertility.

6.1. Background

Cytoplasmic incompatibility in insects is an intra-population sterility phenomenon. Although known for nearly half a century, recently evolutionary biologists having been found that in an increasing number of species belonging to Diptera, Coleoptera, Lepidoptera, Hymenoptera and Homoptera. It has also been reported in an isopod (Crustacea), showing that cytoplasmic incompatibility is not restricted to insects (Berad *et al.*, 1993, O'Neill *et al.*, 1992, Rousset and Raymond, 1991). Many studies have dealt with the mode of inheritance of cytoplasmic incompatibility, its physical cause, its potential in eradication program for pest insects and its possible involvement in specification. Incompatible crosses are characterized by abortive cariogamy, which for most insect species leads to early embryonic death. Although the molecular basis of cytoplasmic incompatibility is unknown yet, it has been demonstrated that the genetic determinants are maternally inherited and correlate with the presence of rickettsia-like bacterial symbionts in the arthropod's gonad tissue (Braig *et al.*, 1994, O'Neill, 1989, Yen and Barr, 1973). Since its first characterization in *Culex pipiens* by Laven (1967), experiments designed to demonstrate incompatibility in a number of such other insects as *Culex* (Barr, 1980, Magnin et al., 1987, Wright and Wang, 1980), *Ades* (Meek, 1984, Meek and McDonald, 1984, Subbarao *et al.*, 1982, Trpis *et al.*, 1981) *Nasonia* (Breeuwer *et al.*, 1992, Breeuwer and Warren, 1990, Breeuwer and Werren, 1993, Williams *et al.*, 1993), *Tribolium* (Fialho and Stevens, 1996, O'Neill, 1989, Wade and Stevens, 1985), *Adalia* (Werren *et al.*, 1994) *Laodelphax* (Noda, 1984), *Henosepilachna* (Mehta and Bhalla, 1983), *Ephestia* (Al Taweel *et al.*, 1993, Ashmed, 1988), *Drosophila* (Bourtzis *et al.*, 1996, Boyle *et al.*, 1993, Callaini *et al.*, 1996, Clancy and Hoffmann, 1998, Giordano *et al.*, 1995, Hoffmann *et al.*, 1994, Hofmann *et al.*, 1998, Jenkins *et al.*, 1996, Mercot *et al.*, 1995, Min and Benzer, 1997, O'Neill, 1991, O'Neill and Karr, 1990, Rousset and

Soligance, 1995, Turelli and Hoffmann, 1991) suggest that it is mediated by mocroorganism in different insect species.

Wolbachia is a genus of obligate, maternally inherited, intracellular bacteria that are widespread in invertebrates. In insects, *Wolbachia* infections are responsible for cytoplasmic incompatibility (CI), which can facilitate the persistence and spread of *Wolbachia* infections. Although the mechanism responsible for the CI phenotype is unknown, a two-component system has been hypothesized (Werren, 1997), consisting of: modification (*mod*) to sperm that occurs in males and rescue (*resc*) that occurs in fertilized embryos. The *mod* component renders sperm incompetent, resulting in karyogamy failure in early embryos. The *resc* component restores sperm competence, allowing successful fertilization and normal embryonic development. Thus, in insect populations that include both infected and uninfected individuals, *Wolbachia*-infected females have a reproductive advantage relative to uninfected females; Infected females are always fertile, suggesting that an infected male produces a sterilizing product against which infected females are protected. This sterility trait is an evolutionary puzzle because it acts in males, but males never transmit the parasites (Frank, 1997). In contrast, uninfected females can only successfully mate with uninfected males. This reproductive advantage can promote the spread of *Wolbachia* infection into a host population (Dobson, 2004, Dobson, 2003, Hoffmann and Turelli, 1997). Olsen (2001) informed the *Wolbachia* effects on fecundity of *Drosophila melanogaster*. He showed that infected flies showed lower fecundity in tropical north Queensland but in temperate southern Victoria, the infected flies were more fecund and the mortality rate of emerged flies increased a little. Super infected females were the longest lived and had the highest oviposition rates, where as in males, uninfected individuals were the longest lived (Dobson *et al.*, 2001). Again *Wolbachia* alters sex ratios and progeny survival. Three different types of cytoplasmic distorters identified in arthropods (Hurst *et al.*, 2001). First, strains of *Wolbachia* are known which induce host pathogenesis through doubling the chromosome complement of haploid males in haplodiploid hosts (Stouthamer, 1997, Stouthamer and Kazmer, 1994). Second, there are strains of both *Wolbachia* and members of the Microspora which alter the sex determination of their host, promoting female development of hosts that would otherwise be male (Rigaud, 1997) and lastly there are a number of bacteria that kill male hosts during embryogenesis (Hurst and Jiggins, 2000). As a result the parasite gains by reducing the fecundity of uninfected females, thereby increasing the relative reproductive rate of infected females. This argument depends on kin selection effects: the parasite in the male does not reproduce, but can aid related parasites in neighbouring females (Frank, 1997).

Kondo *et al.*(1999) and Kondo *et al.* (2002) identified triple infection with three distinct *Wolbachia* strains in Japanese populations of the adzuki bean beetle, *Callosobruchus chinensis*. He used the polymerase chain reaction (PCR) method and molecular phylogenetic analysis of the *wsp* sequences. Both methods unequivocally demonstrated that *C. chinensis* harbours three phylogenetically distinct *Wolbachia*, tentatively designated as wBruCon, wBruOri and wBruAus, respectively. Ijichi *et al.* (2002)

studied the internal spatiotemporal population dynamics of infection with those three *Wolbachia* strains for *C. chinensis.* The aim of their study was to understand the mechanisms underlying the infection with these three organisms, the spatiotemporal infection dynamics of the three *Wolbachia* strains. They showed that during the development of *C. chinensis*, the wBruCon, wBruOri, and wBruAus infection levels consistently increase but the growth patterns are different. Based on the histological data obtained from in situ hybridization and electron microscopy they showed that the peculiar *Wolbachia* composition commonly found in nurse tissues and oocytes, hence they suggested that the *Wolbachia* strains are vertically transmitted to oocytes not directly, but by way of nurse tissue. The study explained interactions among the three coinfecting *Wolbachia* types, reproductive strategies of *Wolbachia*, and factors involved in the different cytoplasmic incompatibility phenotypes.

Though the transmission of *Wolbachia* infection to the females of *C. chinensis* were established by Kondo *et al.* (1999), Kondo *et al.* (2002) and Ijichi *et al.* (2002), the infection in *C. maculatus* is still unknown. On the other hand, those conclusions on *Callosbobruchus* spp. were completely based on the molecular techniques, but in the natural system, the reproductive potentials of *Wolbachia* infected groups of *Callosobruchus* spp. are the special area of interest, which was initiated to know in the chapter. Again the infection type is expected to spread through the species if it shows maternal inheritance and is not deleterious (Caspari and Watson, 1959). So we wanted to see the impact of integration of irradiation with CI, which may be the new concept of pest control.

6.2. Materials and Methods

6.2.1. Experimental Design and Mating Schedule

Beetle collection was described in the earlier chapter. To study the cytoplasmic incompatibility in *Callosobruchus* spp., *C. chinensis* and *C. maculatus* have been collected from different geographic location. For *C. chinensis* we considered the four regions; Chittagong, Khulna and Narayangonj along with Rajshahi. For *C. maculatus* the Khulna region is altered by Japan strain. All possible 4^2 factorial conbination of regional crosses (control) and receiprocal crosses has been studied. Ten replications per crosses are maintained in the incubator at 28 ±1°C temperatures. Eggs laid by females within 24 hours were collected. Total egg cede, total emergence, number of males and females emergence from eggs were observed for the regional and reciprocal crosses.

6.2.2. Estimation of Cytoplasmic Incompatibility

It was assumed that *Wolbachia* is transmitted to be strictly maternally via egg cytoplasm with a certain rate. Not all offspring of infected female host may inherit the infection. However we estimated cytoplasmic incompatibility by the CI level, which is defined as the proportion of zygotes that die fertilized by sperm from an infected male host. So the CI level is estimated as dividing the total emergence of the reciprocal cresses by the regional crosses. Fecundity reduction is also estimated by the same fashion for the total egg. An important insight from this analysis is that a *Wolbachia* infection can stably persist in the population only if $l_{CI} > f$, where l_{CI} is the level of CI and f is the reduction of fecundity relative to control (Flor *et al.*, 2007).

6.2.3. Critical Migration Rate

The critical migration rate was estimated according to (Flor *et al.*, 2007). We first note that the critical migration rate is zero if $l_{CI} \leq f$. Under these circumstances, the infection cannot stably persist in the newly introduce population. However, for $l_{CI} > f$, positive critical migration rates occur. m_{crit} has been calculated using the following formula;

$$m_{crit} = \frac{(f - l_{CI})^2}{4 l_{CI} (1 - f)}$$

Where m_{crit} is the critical migration rate for $l_{CI} > f$, otherwise, m_{crit} is zero

For higher levels of CI, the critical migration rate increases monotonocally as for increasing level of CI, an increasing number of uninfected migrants is needed to suppliment the infection. On the other hand, the critical migration rate decreases with increasing fecundity reduction at fixed CI level. In this case less migration is needed to supersede the infection. The highest possible critical migration rate is m_{crit}=0.25. It is achieved at l_{CI} = *1* and f = *0*.

6.2.4. Integration of Irradiation with CI

During the experiments of the Cytoplasmic incompatibility, the total egg and the total number of hatch have been monitored. Then from the suspected crosses that might showed the cytoplasmic incompatibility were identified and selected. These crosses were selected for irradiation. Selection of reciprocal crosses was completely manual and judgment basis. The selected crosses were irradiated with 8Gy radiation doses and their fecundity, hatchability, adult emergence of males and females and the longevity of the mated pair have been observed.

6.3. Results

6.3.1. Reproductive Potentials of Crosses of *C. chinensis*

First of all the reproductive potentials studied and presented in table 6.1. Table 6.1 showed that almost all the regional crosses produce same number of egg. The average number of egg has no significant difference. The regional cross of Khulna region give up the maximum egg. Within all-possible reciprocal crosses, C X K, and N X R showed the highest fecundity. Regarding the percentage of adult emergence, we see that about 90% egg emerged from the regional cross of Narayangonj. The average number of egg along with DMRT test result is presented in figure 6.1.

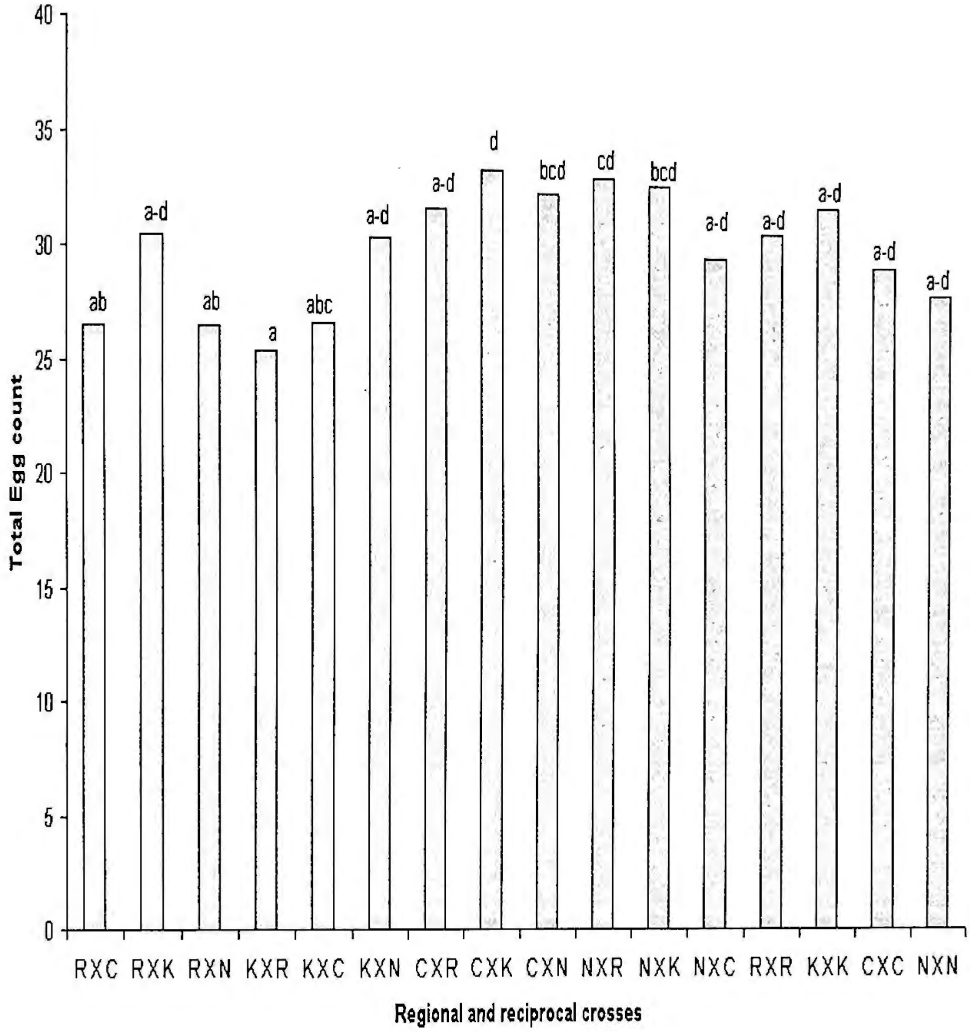

Figure 6.1. Effect of regional and reciprocal crosses on fecundity of *C. chinensis*. Common letters on the bar indicate that there is no significant difference between them. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Narayangonj.

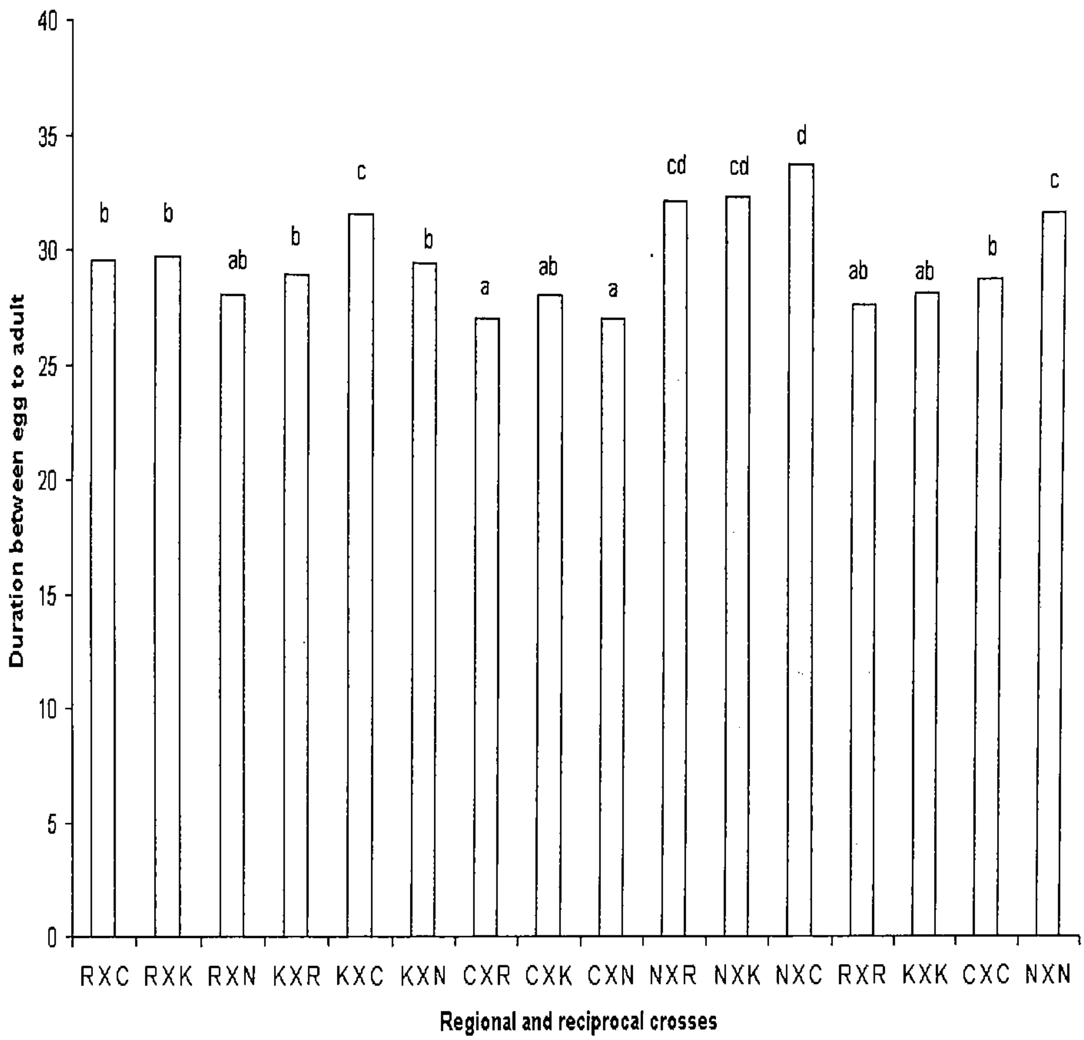

Figure 6.2. Effect of regional and reciprocal crosses on adult development periods of *C. chinensis*. Bars with common letters are not significantly different at 5% level. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Narayangonj.

From the emerged adults from the eggs collected within 24 hours after first mating, the sex ratio was estimated. The detail results of the adult development period and sex ratio of the regional and reciprocal crosses are illustrated in figure 6.2, 6.3 and presented in table 6.1.

Figure 6.2 showed that in the cross N X N, egg took maximum time to emerge and it was about 32 days. The percentage of adult emergence was minimum in the reciprocal crosses C X N followed by K X N. Most of the regional and reciprocal crosses of *C. chinensis* showed more than 80% of adult emergence except C X N and K X N.

Figure 6.3 showed that within the reciprocal crosses, K X R showed the highest adult emergence. On the other hand, C X N showed the lowest emergence rate and it was 51.46%. The other emergence rate was moderately high.

Base on Table 6.1 it was observed that regional cross of Rajshahi maintains the natural sex ratio of M:F = 1:1. The sex ratio of Khulna region is significantly different from the others. The other two regions, the ratio of female is also different from the natural sex ratio, but for large sample the ration may converge to the natural. The female ratio of R X C, R X K, K X R and C X R are different from the natural female ratio. The entire situation, the female progeny was half of the male progeny. Both males and females of the Chittagong region showed the least lifetime compared to the other regions. Maximum Longevity was found in case of males and females in the Rajshahi region. Adult longevity of males and females of the regional crosses K X C was jointly the maximum and it was

11.8 and 10.6 days respectively. Individually the maximum mated male longevity showed in the cross C X K and it was 12.4 days. The mated female longevity of N X K is remarkably the minimum, which was 5.9 days.

For the comparison of different regional and reciprocal crosses about the reproductive potentials we applied analysis of variance to test whether there were any significant difference between the variations of the different factors of the reproductive potential observed and for evaluating the effects of different regional and reciprocal crosses on the general life history. ANOVA tables on total egg, percentage of adult emergence and duration between egg to adult are given in table C.1, C.2 and C.3 respectively in appendix C. ANOVA tables showed that the effect of different regional and reciprocal crosses has significant variation on the reproductive parameters viz. total egg (significant at 5% level), percentage of adult emergence (significant at 1% level) and developmental duration (significant at 1% level).

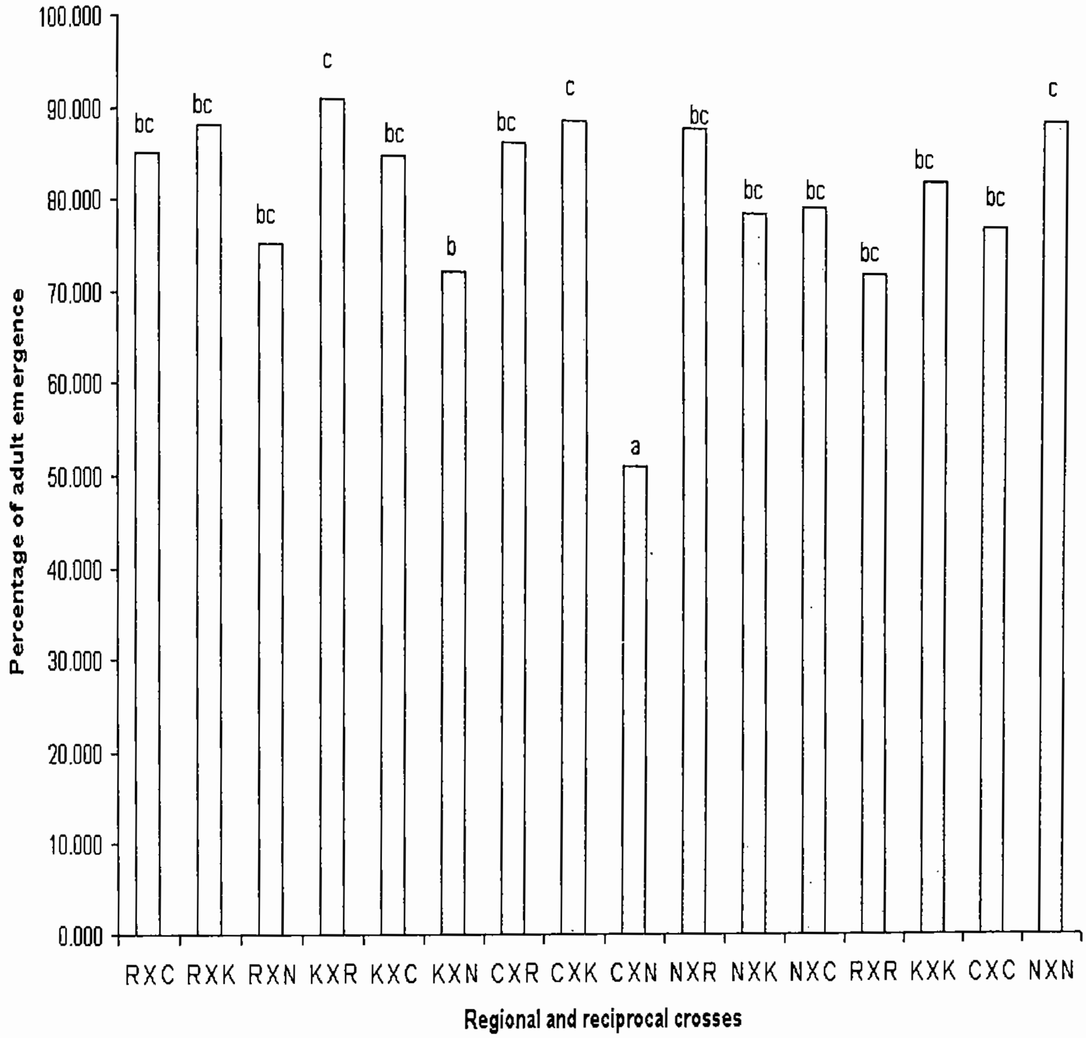

Figure 6.3. Effect of regional and reciprocal crosses percentage of adult emergence of *C. chinensis*. Bars with common letters are not significantly different at 5% level. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Narayangonj.

Table 6.1. Analysis of reproductive potential in the regional and reciprocal crosses of *C. chinensis*

Crosses M x F	Total Egg[1] (Mean ± SD)*	Percentage of adult emergence	Duration between egg to adult stage	Sex ratio	Adult longevity of mated male	Adult longevity of mated female
Regional Crosses						
RAJ X RAJ	30.8 ± 5.16	73.0 ± 15.60	28.10 ± 1.53	1 : 1.01	9.6 ± 1.96	8.9 ± 2.25
KHU X KHU	31.9 ± 7.03	83.25 ± 9.87	28.62 ± 0.92	1 : 0.77	8.8 ± 1.07	7.4 ± 0.80
CHI X CHI	29.3 ± 9.81	78.36 ± 21.22	29.20 ± 1.61	1 : 0.81	7.9 ± 0.94	6.3 ± 0.64
NAR X NAR	28.1 ± 7.07	89.98 ± 6.78	32.18 ± 2.04	1 : 0.81	9.1 ± 1.44	7.6 ± 0.48
Reciprocal Crosses						
RAJ X CHI	26.5 ± 2.22	85.15 ± 16.93	29.56 ± 0.22	1 : 0.55	8.8 ± 2.66	8.3 ± 2.26
RAJ X KHU	30.5 ± 5.48	88.14 ± 9.48	29.70 ± 0.80	1 : 0.43	8.5 ± 2.01	8.5 ± 2.22
RAJ X NAR	26.5 ± 6.07	75.32 ± 15.67	28.09 ± 2.86	1 : 0.79	8.0 ± 1.95	6.9 ± 2.21
KHU X RAJ	25.4 ± 3.86	91.29 ± 7.13	29.01 ± 0.62	1 : 0.51	10.4 ± 1.64	9.8 ± 1.03
KHU X CHI	26.7 ± 5.10	85.36 ± 17.77	31.69 ± 0.82	1 : 0.63	11.8 ± 2.29	10.6 ± 1.79
KHU X NAR	30.5 ± 4.95	72.62 ± 19.93	29.58 ± 3.42	1 : 0.80	10.0 ± 2.57	7.0 ± 1.90
CHI X RAJ	31.8 ± 5.16	86.92 ± 10.57	27.18 ± 0.48	1 : 0.61	10.7 ± 1.95	8.2 ± 0.78
CHI X KHU	33.5 ± 4.52	89.45 ± 9.82	28.28 ± 1.26	1 : 0.72	12.4 ± 1.64	9.9 ± 1.60
CHI X NAR	32.5 ± 4.43	51.46 ± 25.57	27.27 ± 3.44	1 : 0.99	9.3 ± 3.27	7.5 ± 2.55
NAR X RAJ	33.2 ± 6.97	88.91 ± 12.96	32.59 ± 1.67	1 : 0.86	8.1 ± 1.64	6.8 ± 1.72
NAR X KHU	32.9 ± 8.00	79.72 ± 13.56	32.82 ± 2.15	1 : 0.83	7.4 ± 1.34	5.9 ± 1.19
NAR X CHI	29.7 ± 9.12	80.50 ± 17.01	34.24 ± 1.44	1 : 0.69	7.5 ± 1.63	6.5 ± 1.20

[1] 24 hours oviposition. * mean of 10 replicates. RAJ= Rajshahi, CHI = Chittagong, KHU = Khulna, NAR = Narayanganj.

The above results were base on the univariate concept i.e. we analyse each of the reproductive potential individually. But jointly based on the reproductive potentials, the regional and reciprocal crosses may form different clusters in which they are same and between clusters they may be different. Identification for such cluster may provide information about the whole. We performed agglomerative hierarchical clustering method based on the dissimilarity measures of each regional and reciprocal cross. Dissimilarities were calculated using the Euclidian distance. The dissimilarity matrix of each regional and reciprocal cross are presented in table C.4 in Appendix C. The result is illustrated by dendogram in figure 6.4.

Figure 6.4 showed that the regional and reciprocal crosses formed three groups regarding the dissimilarity of the reproductive potentials given in table 5. The first group consists of the reciprocal crosses of R X C, R X K, K X R, K X C, C X R, and C X K. Interestingly no reciprocal crosses from Narayangonj included in the group. The second group consists of all regional crosses and reciprocal crosses of Narayangonj which implied that each regional crosses and the reciprocal crosses of Narayangong with the other regions under study has the higher similarity regarding the reproductive potentials. The third cluster contains only one reciprocal cross C X N. This cross shows the highest dissimilarity. The crosses within the same cluster show the same type of reproductive potential.

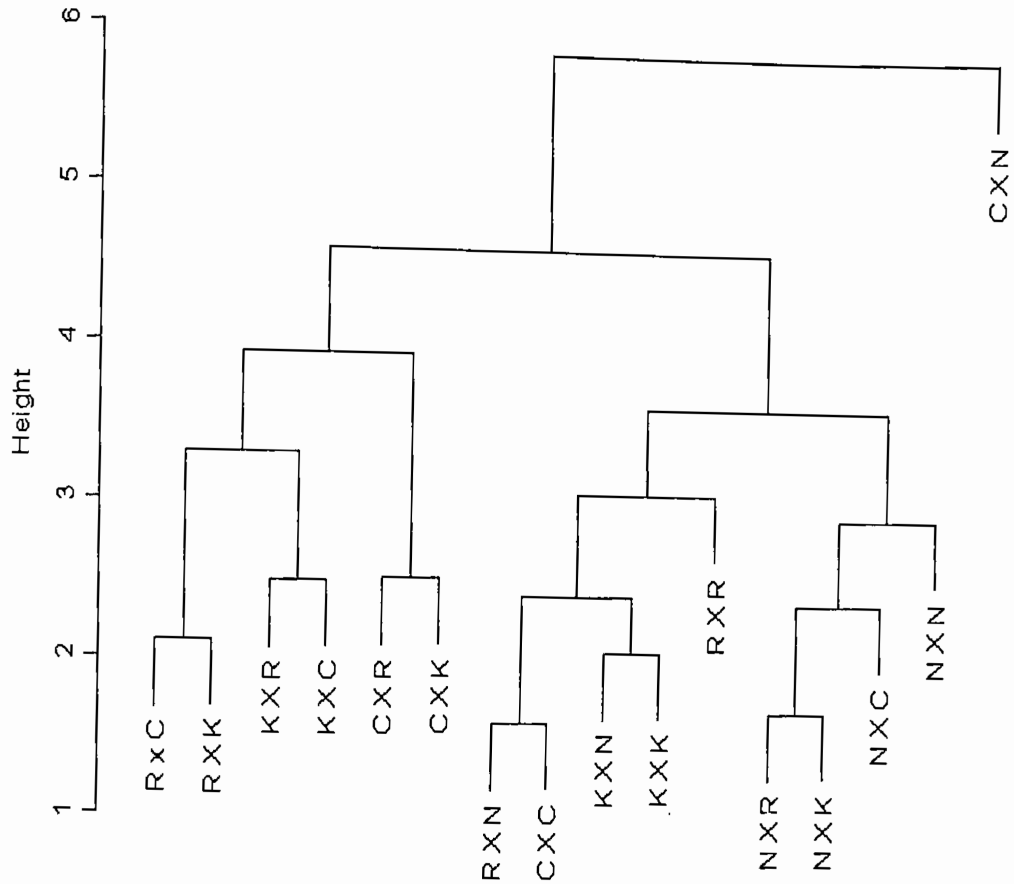

Figure 6.4. Dendogram of the regional and reciprocal crosses of *C. chinensis* based on dissimilarities calculated by Euclidian distance. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Narayangonj.

6.3.2. Cytoplasmic Incompatibility of *C. chinensis*

The CI level, fecundity relative to control and percentage of egg hatched was described along with the number of total egg of different regional and reciprocal crosses were tabulated in table C.5 in appendix C.

Table C.5 showed that no crosses provided the percentage of egg hatched < 50%. Only the cross C X N showed the 51% hatch of total egg. But the fecundity relative to the control cross is more than 110% and the estimated CI level is 0.272. Since the $I_{CI} \leq f$, we can conclude that the incompatibility is not persistent. It is worth mentionable that, N X C formed a different cluster based on the reproductive potentials. We also calculated the CI levels of some other crosses but they are not persistent. Finally we like to conclude that there were no incompatibilities within the reciprocal crosses in the regions.

6.3.3. Reproductive Potentials of Crosses of *C. maculatus*

Different reproductive potentials of *C. maculatus* were informed in table C.6 along with the least significant difference at 5% level. The total number of eggs given up within 24 hours is presented in figure 6.5.

Figure 6.5 showed that the number of total egg within 24 hours was the maximum in reciprocal cross J X C and minimum and N X J, which is significantly different from the others. All the regional crosses C X C, J X J, N X N and R X R differ significantly from each other except, J X J and R X R. these two regional crosses did not differ significantly. From the point of view of the incompatibility relationship, N X J and C X J demands special interest.

The development period of *C. maclatus* was also studied and was presented in figure 6.6. Figure 6.6 showed that the reciprocal cross N X J takes the highest time to become adult from egg. Which was significantly different from the others. The developmental period of the other regional and reciprocal crosses are more or less same.

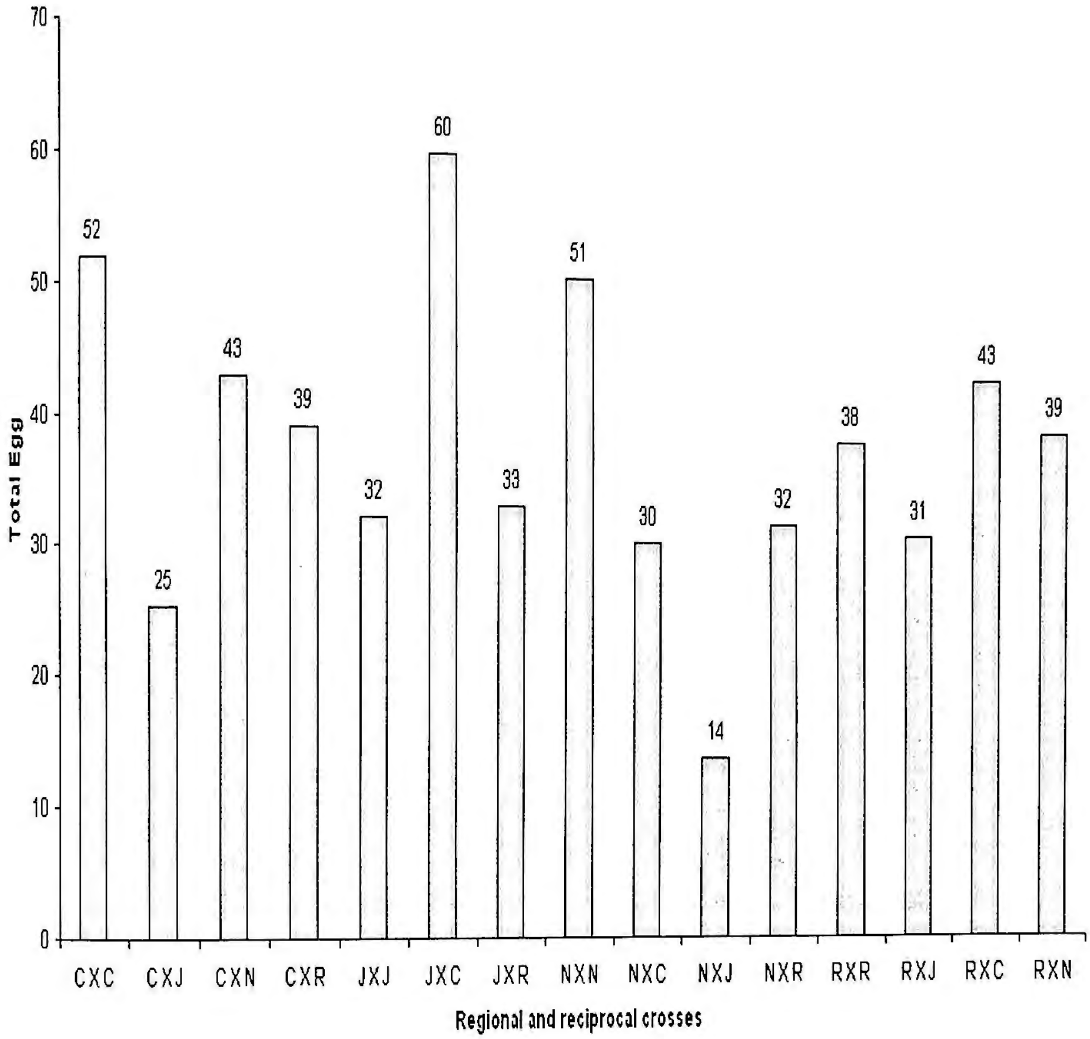

Figure 6.5. Total number of egg laid by the females of different regional and reciprocal crosses of *C. maculatus*. The total egg differed by 8.8 between crosses are significantly different. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Japan.

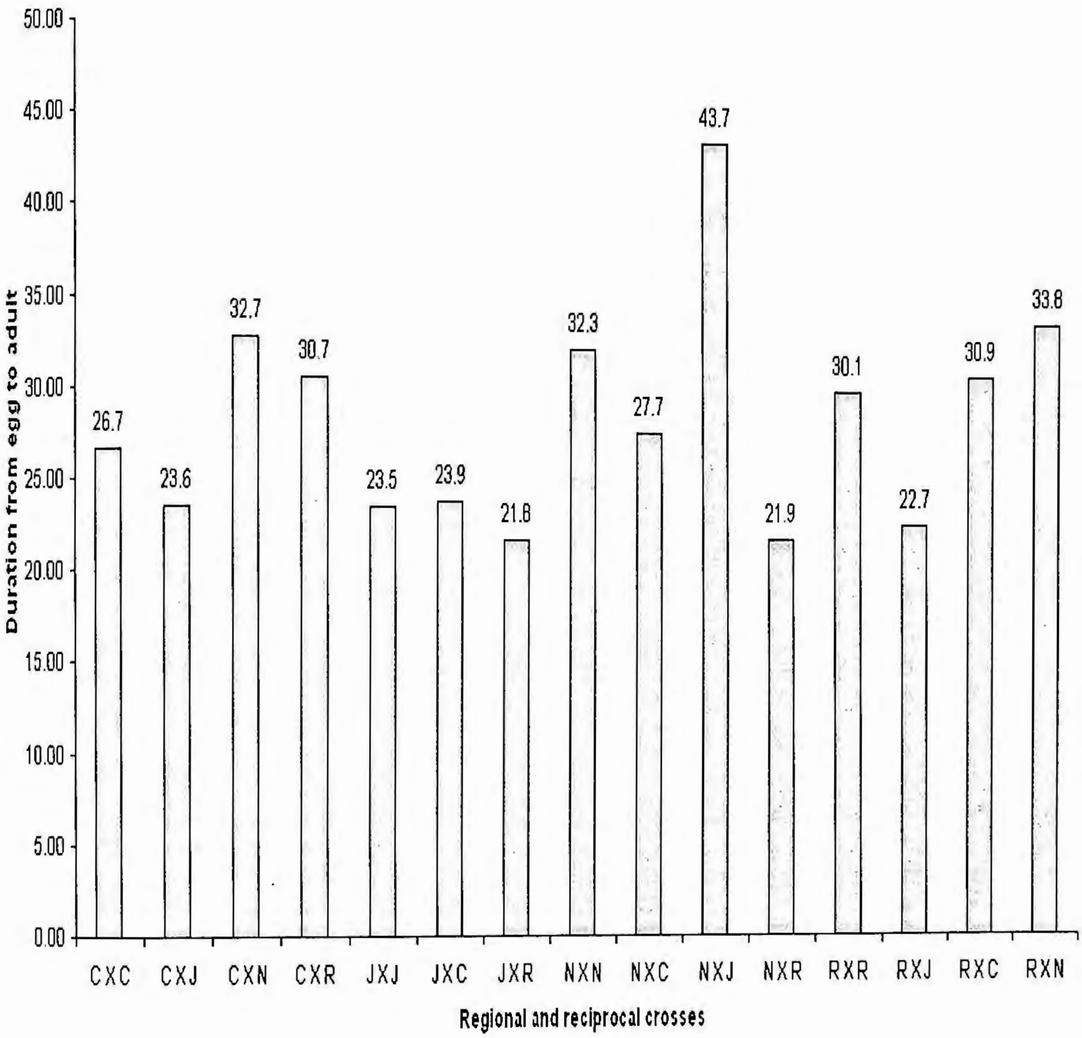

Figure 6.6. Development period of different regional and reciprocal crosses of total egg laid by the females of *C. maculatus*. Total egg differ by 5.2 between crosses significantly different at 5% level. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Japan.

Total number of hatch, number of male emergence and number of female emergence was given in figure 6.7. Figure 6.7 showed that the highest number of egg hatched in the regional cross C X C and reciprocal cross J X C and it was 82% and 95% respectively. The minimum number of egg hatch occurred in the reciprocal crosses are N X R and C X N which was 47% and 43% respectively. These crosses are suspected for having cytoplasmic incompatibility. Regarding the ration of male and female emergence, figure did not show any discrepancy within the regional and reciprocal crosses which indicated that the sex ratio of all regional and reciprocal crosses are the same and it follows the natural sex ratio 1:1.

ANOVA was also performed on the different reproductive potentials of the regional and reciprocal crosses of *C. maculatus*. The results were reported in table C.7 in appendix C. ANOVA of different reproductive potential of regional and reciprocal crosses of *C. maculatus* showed that there was significant variation between the crosses. The result implied that different cross combination able to generate significant variation regarding the total egg, hatchability, developmental period, total number of female and male progeny etc.

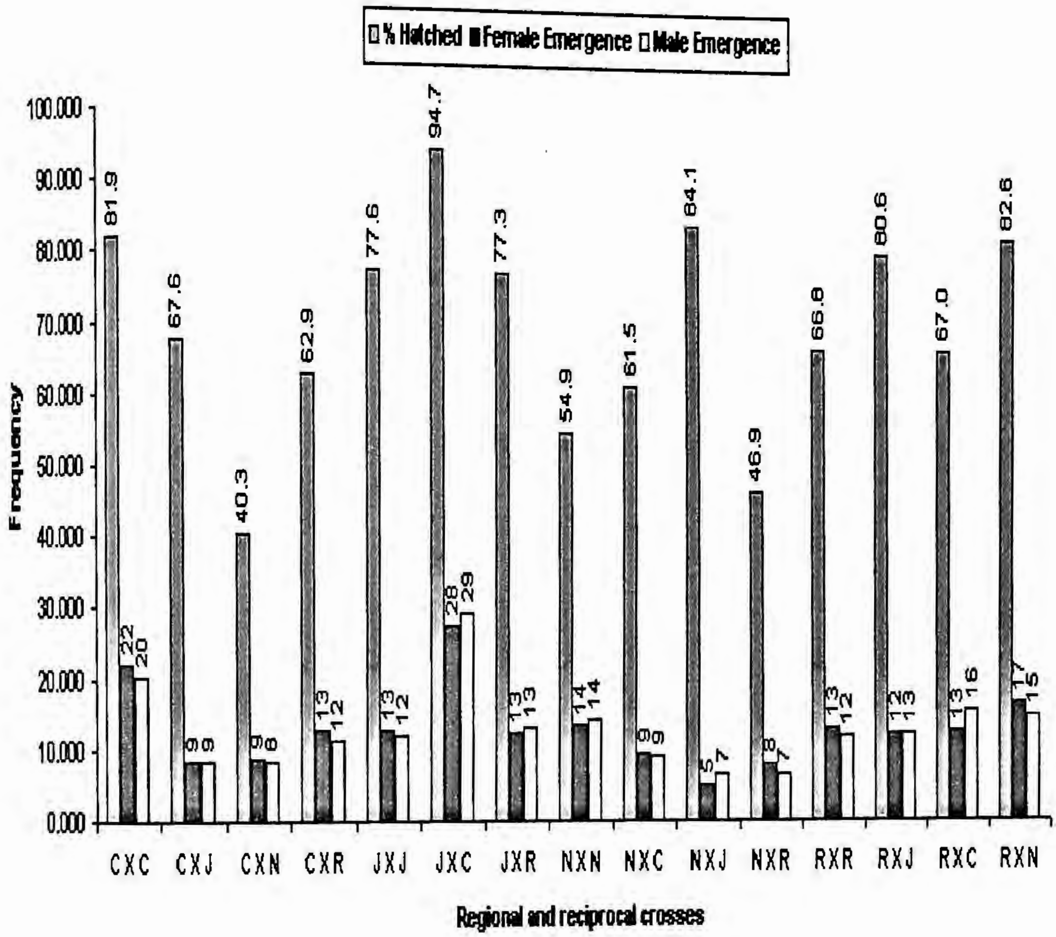

Figure 6.7. Bar chart of the total hatch, number of female emerged and the number of male emerged in different regional and reciprocal crosses of *C. maculatus*. 11.8, 5.93, and 5.94 are the least significant difference or total hatch, number of female emerged and number of male emerged at 5% level of significance. The first letters of the name of the regions represent the respective region of Rajshahi, Khulna, Chittagong, and Japan.

Based on the reproductive potential observed, we applied cluster analysis to identify some groups where within groups they are homogeneous and the similarities are the maximum and between groups they are heterogeneous and the similarity between the group members are the maximum. The dissimilarity matrix along with cluster membership of cluster analysis of regional and reciprocal crosses of *C. maculatus* was given in table C.8 in appendix C. The results of cluster analysis were illustrated in figure 6.8.

Figure 6.8 showed that there exist 4 clusters. The first cluster contains the regional cross C X C and the reciprocal cross J X C. The Third cluster contains only one reciprocal cross C X N and the fourth cluster contains the reciprocal cross N X J. Rest of the regional and reciprocal crosses belongs to second cluster. Crosses of the first, third and fourth cluster has some similarity that the males or females of the crosses are from Chittagong or Narayangonj or from Japan. Neither Rajshahi nor Khulna region's male and females did not provide such types of dissimilarity.

Table C.8 showed that the dissimilarity between J X C and N X J is the maximum followed by J X C to C X N. Sincerely it can be found that strains from Japan and Chittagong contributes to create such level of dissimilarities in the reciprocal crosses of *C. maculatus*.

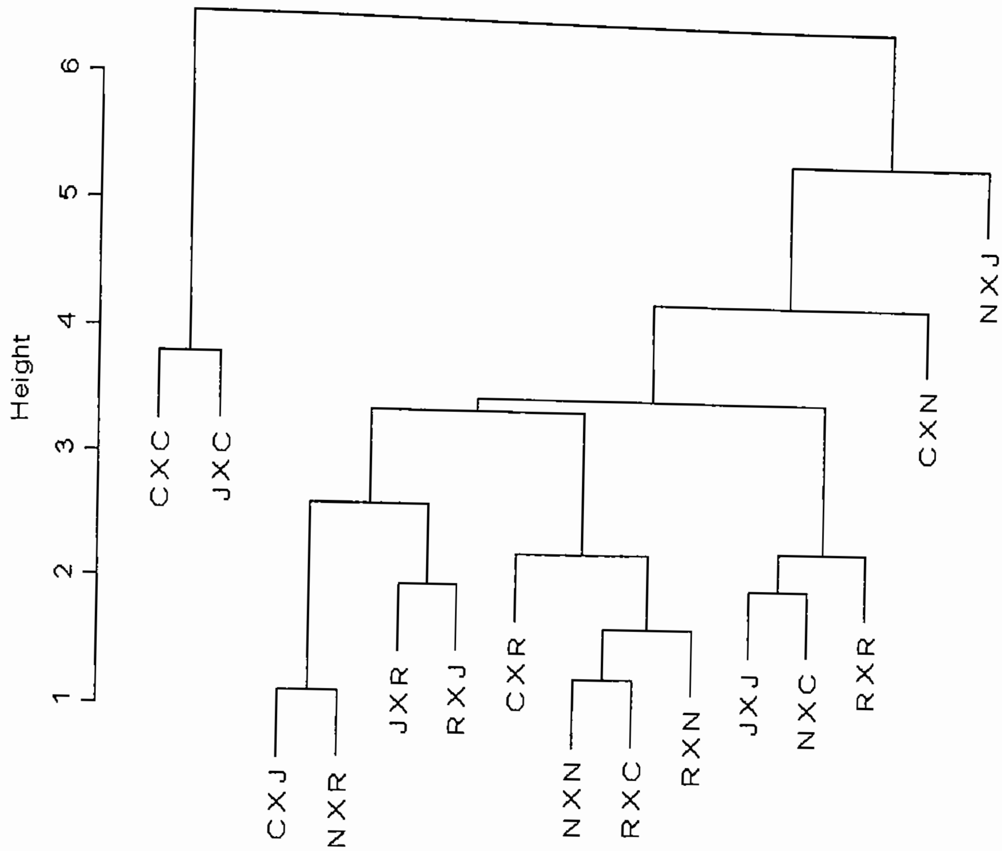

Figure 6.8. Dendogram of the regional and reciprocal crosses of *C. maculatus* based on Euclidian distance. The first letter of the name of the regions represents the respective region of Rajshahi, Khulna, Chittagong and Japan.

6.3.4. Cytoplasmic incompatibility of *C. maculatus*

The estimates related to the estimation of cytoplasmic incompatibility along with the persistence of infection and the critical migration rate of the infection was presented in table C.9 in appendix C. Table C.9 showed that N X R and C X N have less than 50% hatchability. So in general concept these crosses were the most suspected for the presence of cytoplasmic incompatibility. Next we estimate the fecundity of each reciprocal crosses relative to their control crosses about the males. The percentage of fecundity relative to the control is informed for only the suspected crosses and the relative fecundity <50%. We do be able to suspect 4 reciprocal crosses; C X J, C X N, N X J and N X R that are supposed to be infected and showing the incompatible relations. Next the CI level was estimated. The CI level was 0.597, 0.592, 0.582 and 0.464 for the crosses C X J, C X N, N X J and N X R respectively. It is worth mentionable that the maximum CI level can be 1. These incompatibilities are compared to the relative fecundity level and found that C X J, C X N, and N X J fulfil the condition of persistent incompatibility transmission. Finally the critical migration rates have been estimated for the crosses C X J, C X N and N X J, which was 0.0040, 0.0032 and 0.0042 respectively. It should be noted that the maximum critical migration rate is 0.25 when the CI level is 1 and relative fecundity is 0.

6.3.5. Integration of irradiation with CI

From the above analysis we did not found any crosses, which may be suspected for the possible cytoplasmic incompatible relationship in *C. chinensis*. However in *C. maculates* we identified nine reciprocal crosses of which four might show the incompatible relationships and the other five crosses were selected for judgement of the effectiveness. The results of different reproductive of the experiment the irradiated reciprocal crosses are illustrated in figure 6.9. The numerical results of the experiment were presented in table C.10. in appendix C. Figure 6.9 showed that the suspected reciprocal crosses C X J, C X N, N X J showed the reduced fecundity. Their average total egg was not more than 15 and the average emergence of males and females are about to zero. It is remarkable that in the estimation of CI these three crosses showed the symptom of persistent incompatibility. Before irradiation, these crosses yielded 25, 43 and 14 respectively which was reduced to 10, 13 and 11 respectively after irradiation with dose 8Gy. The cross N X R showed the different result though it was suspected to have incompatibility, the fecundity of the cross yield the increased number of total egg though there was no significant difference between before and after radiation. It is worth mentionable that the level of CI was not higher for the cross. The other reciprocal crosses also showed reduced fecundity regarding the total egg. Though these crosses did not show the presence of CI, these crosses were segregated into some individual clusters. Considering the adult emergence, the impact of irradiation with CI was dramatic. The adult emergence of males and females reduced almost to zero. The cross N X R showed the highest emergence and it was not more than 3.

From table C.10 it was observed that the developmental period of the emerged beetles of the experiment were more or less 37 days. The result is informative but not dependable because there are only a few numbers of beetles emerged from egg. The duration of irradiated mated males and females remains almost same as was in the previous results of irradiation.

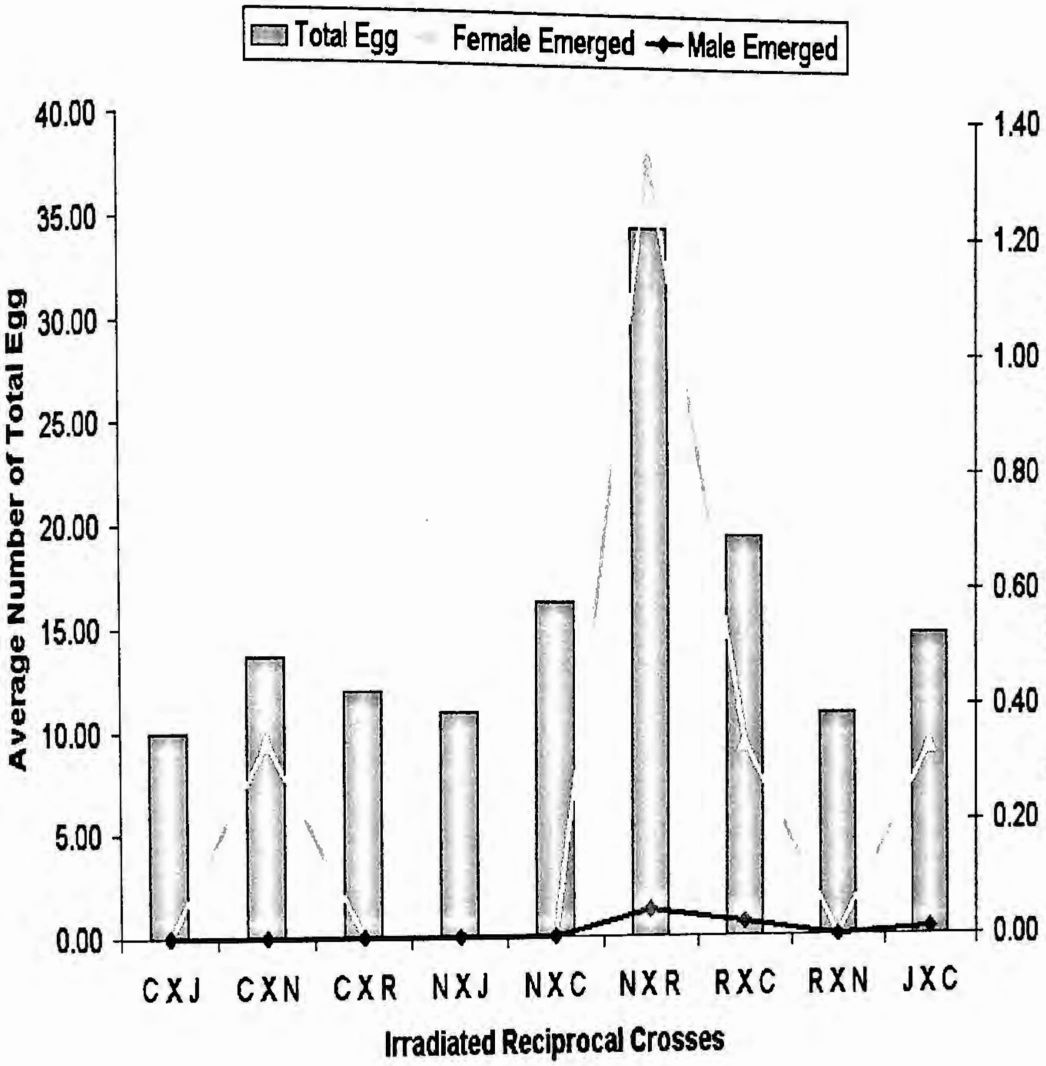

Figure 6.9. Line and bar chart of the reproductive potentials of the reciprocal crosses of *C. maculatus* exposed to 8Gy dose of gamma radiation. The right vertical axis represents the scale of of average number of male and female emerged in each study reciprocal crosses.

6.4. Discussion

In the chapter we tried to identify some incompatible crosses of the species *Callosobruchus* spp. For *C. chinensis* we considered the regional and reciprocal crosses of Rajshahi, Khulna, Chittagong and Narayangonj strains. For *C. maculatus* we considered the strains from Rajshai, Narayangonj, Chittagong and Japan. Different reproductive potential has been observed and analyzed to study whether there exist any incompatible relation.

The outcome and the evolution of host-symbiont associations depend on environmental constraints, but responses are difficult to predict since they arise from a complex interaction between the host, the parasite and the environment. The situation can be even more complex when multiple parasite genotypes, with potentially different responses to environmental changes, coexist within a single host. In this chapter we tried to find some incompatible crosses in two species of *Callosobruchus* spp. In this species, *Wolbachia* infection was proved by Kondo *et al.* (2002) for Japan population. Our result identified 3 reciprocal crosses of which 2 crosses has interaction with Japan females. Again from the cluster analysis we found that in the reciprocal crosses that have Chittagong male and Narayangonj have shown different types of reproductive potentiality. But the presence of imcompatibility relation was not strictly provided. *Wolbacia* induces cytoplasmic incompatibility, a sperm-egg incompatibility that allows it to spread and persist in host populations. It was found that (i) *Wolbachia* density is temperature-specific and highest at 26 °C; (ii) the response of bacterial density to temperature occurs within a single insect generation, during the egg-to-adult developmental period (Mouton *et al.*, 2006).

The nature of the manipulation on reproductive potentials varies with *Wolbachia* strain, arthropod taxa, and arthropod genetic system. Nonreciprocal and reciprocal reproductive incompatibilities, sex ratio biases are some of the results of *Wolbachia* symbiosis. The *Wolbachia* present in the predatory mite *Metaseiulus occidentalis* are genetically similar to those found in insects, and are correlated with nonreciprocal incompatibility in crosses between infected males and uninfected females. The incompatibility phenotype includes reduced numbers of eggs, shrivelled eggs, and a male-biased sex ratio of the few resulting progeny, which may be related to the parahaploid genetic system of this phytoseiid mite (Denise *et al.*, 1998). The identified reciprocal crosses of *C. maculatus* C X J, C X N and N X J which showed the reduced number of eggs and in all the reciprocal crosses the sex ratio was seriously biased to male. These support the presence incompatibility in the crosses. Chittagong and Narayangonj males are the possible host of infection. There is also equally likely possibility to become Japanese strain as the carrier of the infection. In *D. simulans* reduced egg eclosion occurs when females from uninfected strains are crossed with males from infected strains (Moreau *et al.*, 1991). In both C X J and N X J we observed the lowest total egg compared to the other regional and reciprocal crosses which indicates the maternal inheritance as was described in(Hoffmann and Turelli, 1988).

Basic mathematical models of CI show that *Wolbachia* spreads in a single panmictic host population if *Wolbachia* transmission is perfect and the infection does not affect the host fecundity (Caspari and Watson, 1959, Fine, 1978). No such evidence was found in our study rather the relative fecundity has been reduced relative to control. Infected and uninfected host populations can stably coexist if migration is below the critical migration rate and it infection is imperfectly transmitted. Under these circumstances, unidirectional CI acts as a post-zygotic isolation mechanism between the populations. In general, critical migration rates are positive if either *Wolbachia* causes fecundity reductions in infected female hosts. The combination of fecundity rate $f>0$ and transmission rate $t<1$ will generally results in increased critical migration rates and the infection will be imperfect (Flor *et al.*, 2007).

Impact of irradiation on the cytoplasmic incompatible crosses was very significant. The other crosses also showed the same characteristic of reproductive potentials, which were not exposed to show the presence of CI. Irradiated reciprocal crosses from different region's beetles showed the seriously reduced fecundity and fertility.

Finally we like to conclude that strains from Chittagong and Narayangonj males may provide the incompatibility though the stable coexistence between infected and uninfected host population is possible under biologically reasonable conditions (Flor *et al.*, 2007). Integration of irradiation with cytoplasmic incompatibility might be more effective to control the pest.

Chapter 7

Summary

7.1. Summary of the Results

In the thesis, the impact of radiation on successive generations, cytoplasmic incompatibility and integration of irradiation with the presence of cytoplasmic incompatibility of *Callosobruchus* spp. have been studied. *C. chinensis* and *C. maculatus* are considered the study insects. Upholding the objective, some additional experiments such as some reproductive behaviour, mating competitiveness of males were also conducted to know the causal relationships regarding the main objectives. However the experimental component wise results are summarized below.

7.1.1. Oviposition Preference

- *C. chinensis* collected either from lentil or Bengal gram preferred lentil << Bengal gram < green gram for oviposition.
- The percentage of emergence of *C. chinensis* collected from Bengal gram and from lentil seed was 53% and 46% respectively on lentil seed.

- The number of adult emergence of *C. chinensis* in Bengal gram collected from lentil and Bengal gram was 55% and 70% respectively.

- The emergence rate is highest in green gram and it was 88% and 65% for *C. chinensis* collected from Bengal gram and lentil respectively.

- *C. maculatus* collected from black gram preferred black gram >>green gram.

- *C. maculatus* collected from green gram preferred green gram and then the black gram.

- The emergences rate is higher in green gram.

- *C. maculatus* either collected from green gram or black gram also preferred pea for oviposit but the emergence rate is nearer to zero.

7.1.2. Gamma Radiation Induced Inherit Sterility

- Irradiation is able to increase the time of egg to adult in successive generations of *C. maculatus*. The dose ≥8Gy has dramatic impact to increase the duration. For the dose 12Gy the maturity time in F5 generation has gone more than 100 days. Dose ≤6Gy on parent generation has no significant effect on the immature period.

- In 12Gy radiation dose, males died earlier than females from F1 to F4 generation.

- Inherit sterility induced dose does not affect the sex ratio of the progeny. Regarding the independent possible pair, the average number of independent pair reduces successively in the radiation doses ≥ 6Gy. At 8Gy and above radiation dose, the average number of pair decreases dramatically after F1 generation.

- In breeding and irradiation do not affect the percentage of adult emergence rather the percentage of adult emergence increased in F2 to F5 generation in 10Gy and 12Gy radiation level.

7.1.3. Mating Competitiveness

- For *C. chinensis* the infertility rate increased as the number of treated male increased up to 1(UF):1(UM):2(IM). The degree CVs decreases as the treated males increased. Further increase of the number of males the CVs would be the same.

- For *C. maculatus,* the infertility rate was the maximum at 1:1:3 for the 6Gy treated males. Treated with 8Gy the infertility is decreased with increasing number of treated males. The ratio 1:1:1 showed the highest competitiveness values for both radiation doses 6Gy and 8Gy. The dose with 8Gy showed the highest competitiveness value. For 6Gy dose the competitiveness values were the maximum for the ratio 1:1:3 and 1:1:4 and the minimum number of value is attained at the ratio 1:1:2.

7.1.4. Cytoplasmic Incompatibility of *Callosobruchus* spp.

Regional and reciprocal crosses of Rajshahi, Narayangonj, Chittagong and Japan strains of *C. mculatus* and Rrjshahi, Narayangonj, Khulna and Chittagong strains of *C. chinensis* were tested to find the cytoplasmic incompatibility of *Callosobruchus* spp.

- Both in *C. chinensis* and *C. maculatus* the regional and reciprocal crosses have significant variation regarding the different components of reproductive potentials.

- The reciprocal crosses C X K, and N X R showed the highest fecundity in *C. chinensis*. C X N showed the lowest emergence rate and it was 51.46%. Most of the regional and reciprocal crosses showed more than 80% of adult emergence except C X N and K X N. In *C. chinensis* only the cross C X N showed the instable imcompatibility.

- In *C. maculatus* the number of total egg was maximum in reciprocal cross J X C and minimum N X J followed by C X J. The minimum percentage of adult emergence occurred in the reciprocal crosses are N X R and C X N, which was 47% and 43% respectively. The dissimilarity between J X C and N X J was the maximum followed by J X C to C X N.

- In *C. maculatus* there were 4 suspected reciprocal crosses; C X J, C X N, N X J and N X R that are supposed to be infected and showing the incompatible relations. The CI level for these suspected crosses were 0.597, 0.592. , 0.582 and 0.464 respectively and the relative the estimated relative fecundity level estimated and found that C X J, C X N, and N X J fulfil the condition of persistent incompatibility. The critical migration rates for the crosses C X J, C X N and N X J was 0.0040, 0.0032 and 0.0042 respectively. The only 2% of migration of incompatibility related infection happened in the crosses.

- The impact of radiation dose of 8Gy in the incompatible crosses provided a drastic reduction of total egg (<50% from the general) and serious infertility of eggs (nearer to 0%)

7.2. Area of Further Research

There are some drawbacks of the thesis that we proposed for the further area of the research. The study should include the molecular techniques to monitor the *Wolbachia* infection as well as the genetic change due to treatment. This might provide a strong base and explanation of the findings. Experiments based on theoretical mathematical model of incompatibility level, and migration rate of *Wolbachia* infection might provide more meaningful insight of outputs of the research. Impact of irradiation with the presence of cytoplasmic incompatibility should be further studied, as it seems to be prospective.

7.3. Policy Implication

Research on *Wolbachia* infection in *Callosobruchus* spp. is still passing the inception period. So far results found from the study, integration of irradiation with CI may add as a new dimension of pest control. The commercial application of the technique might add a special advantage to safe the stored pulse.

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Appendix A

Table A.1. Average fecundity and percentage of adult emergence of *C. chinensis* and *C. maculatus* in free choice oviposition preference within 300 seeds of each pulses.

	Seeds	*C. chinensis*	*C. maculatus*
Fecundity	Lentil	237.33	8
	Bengal gram	58.66	25
	Green gram	14.33	95
	Pea	25	120
	Blackgram	3.33	146
% Of Adult Emergence	Lentil	45.13	0
	Bengal gram	69.22	51.26
	Green gram	61.5	82.74
	Pea	0	0
	Blackgram	0	48.74

Table A.3. Table of oviposition preference of *C. maculatus* from different pulse sources compelled to oviposit on different pulses

Source pulse	Target pulse	Total egg	Total emerged	Percentage emerged
Black Gram	Bengal Gram	25	12	48%
Black Gram	Black Gram	146	73	50%
Black Gram	Green Gram	95	82	87%
Black Gram	Lentil	8	0	0%
Black Gram	Pea	120	0	0%
Green Gram	Bengal Gram	7	3	45%
Green Gram	Black Gram	149	71	48%
Green Gram	Green Gram	187	100	53%
Green Gram	Lentil	6	0	0%
Green Gram	Pea	114	0	0%

Appendix B

Table B.1. Runs Test of the difference of number of males and number of females

	Difference of male and female emergence
Test Value(a)	3.50
Cases < Test Value	16
Cases >= Test Value	16
Total Cases	32
Number of Runs	21
Z- statistic	1.258
Asymptotic Sig. (2-tailed)	.208

a Median

Table B.2. Numerical summarization of the reproductive potential of the parent and sterility inherited progenies.

Dose	Generation	Total Egg	Male Emerged	Female Emerged	Females Longevity	Males Longevity	Duration Egg to Adult	Male Emerged	Female Emerged	% of Egg Emerged
2	F0	29	1	0.30	3.50	3.5	31.3	0	0	0.29
	F1	32	1	1.20	9.70	9.6	33.0	0	5	0.59
	F2	23	1	0.71	6.78	6.7	48.0	0	1	0.07
	F3	24	1	0.50	1.00	2.0	35.4	4	4	0.53
	F4	28	1	1.00	4.50	3.5	39.0	1	1	0.10
	F5	10	3	1.00	4.00	3.0	37.7	4	11	0.90
4	F0	29	1	0.44	3.80	3.8	32.3	3	2	0.16
	F1	33	1	1.86	6.50	6.5	32.6	6	11	0.47
	F2	28	2	1.33	7.50	6.3	36.7	5	6	0.43
	F3	28	3	0.60	11.40	11.2	51.8	9	3	0.45
	F4	21	2	1.50	9.67	8.7	41.8	1	1	0.15
6	F0	33	2	2.00	3.78	3.9	36.0	3	3	0.87
	F1	12	1	0.33	9.33	7.3	23.8	10	13	0.65
	F2	24	1	1.00	6.67	7.3	36.3	3	5	0.31
	F3	30	3	3.33	8.33	9.3	51.0	9	6	0.49
	F4	20	1	3.00	9.67	8.7	38.9	3	7	0.54
8	F0	38	1	0.80	3.30	3.2	32.8	0	1	0.03
	F1	17	1	2.00	5.50	4.5	31.4	5	9	0.47
	F2	12	0	1.00	7.00	8.0	44.0	0	0	0.08
	F3	48	4	3.00	5.00	5.0	39.3	14	0	0.48
	F4	14	1	0.00	4.00	5.0	82.0	1	1	0.07
10	F0	24	1	0.33	4.00	5.7	36.4	1	14	0.07
	F1	38	1	0.00	7.00	3.0	35.3	6	13	0.29
	F2	39	2	0.00	3.00	2.0	43.1	13	0	0.69
	F3	38	1	0.00	3.00	3.0	40.4	10	0	0.61
	F4	2	1	0.00	1.00	1.0	67.0	1	8	0.50
12	F0	26	1	0.50	3.80	4.3	32.1	0	7	0.06
	F1	38	1	0.00	8.00	6.0	31.6	8	7	0.42
	F2	27	1	2.00	11.00	8.0	35.6	3	7	0.37
	F3	27	1	2.00	11.00	8.0	35.6	3	0	0.37
	F4	25	0	2.00	38.00	12.0	110.8	8	0	0.60

Appendix C

Table C.1. ANOVA for total egg of *C. chinensis*

Source of Variation	Degree of Freedom	Sum of square	Mean sum square	Calculated *F*
Replication	9	363.63	40.40	1.04 ns
Crosses	15	1079.99	71.99	1.85*
Residual	135	5245.06	38.85	
Total	159			

* indicates significance at 5% level

Table C.2. ANOVA for percentage of adult emergence of *C. chinensis*

Source of Variation	**Degree of Freedom**	**Sum of square**	**Mean sum square**	**Calculated *F***
Replication	9	1370.19	152.24	<1
Crosses	15	14374.25	958.28	3.92**
Residual	135	32989.98	244.37	
Total	159			

** indicates significance at 1% level

Table C.3. ANOVA for duration between egg to adult of *C. chinensis*

Source of Variation	Degree of Freedom	Sum of square	Mean sum square	Calculated *F*
Replication	9	35.27	3.919	1.13 ns
Crosses	15	686.63	45.77	13.20**
Residual	135	468.17	3.46	
Total	159			

** indicates significance at 1% level

Table C.4. Dissimilarity matrix and cluster membership of the crosses of *C. chinensis*

	R X C	R X K	R X N	K X R	K X C	K X N	C X R	C X K	C X N	N X R	N X K	N X C	R X R	K X K	C X C	N X N
R X C	0															
R X K	2.1	0														
R X N	2.84	4.16	0													
K X R	2.2	3.17	4.59	0												
K X C	3.56	4.28	5.39	2.48	0											
K X N	3.53	4.05	2.66	4.85	4.67	0										
C X R	3.24	2.83	4.16	3.53	4.16	3.14	0									
C X K	4.92	4.51	5.96	4.39	3.83	4.37	2.5	0								
C X N	6.59	7	4.75	7.81	7.44	3.73	5.92	6.67	0							
N X R	4.51	4.22	4.42	5.92	5.81	3.53	4.49	5.32	6.23	0						
N X K	4.8	4.65	4.13	6.6	6.55	3.39	5.16	6.34	5.62	1.65	0					
N X C	4	3.98	3.96	5.61	5.61	3.72	5.28	6.54	6.49	2.61	2.06	0				
R X R	4.57	5.17	3.44	5.38	5.01	2.52	3.9	4.29	3.38	4.36	4.64	5.24	0			
K X K	3.14	3.04	2.82	4.52	4.95	2.03	2.38	3.99	4.83	2.68	3.09	3.68	2.85	0		
C X C	3.22	3.88	1.58	5.1	5.66	2.15	3.88	5.64	4.67	3.05	2.69	3.05	3.36	1.9	0	
N X N	2.78	3.59	3.37	3.91	3.91	3.17	3.95	4.85	6.51	2.6	3.36	2.71	4.04	2.84	2.85	0
Cluster	**1**	**1**	**2**	**1**	**1**	**2**	**1**	**1**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**2**

Table C.5. Table for calculating the cytoplasmic incompatibility level and relative change in fecundity along with total number egg and total number of hatched egg in *C. chinensis.*

Crosses	Total Egg	Hatched	Percentage Hatched	Fecundity relative to control	CI Level
R X R	31	22	0.7300	1.000	0.000
R X C	27	23	0.8515	0.860	
R X K	31	27	0.8814	0.990	
R X N	27	20	0.7532	0.860	0.112
K X K	32	27	0.8325	1.036	0.000
K X R	25	23	0.9129	0.796	0.127
K X C	27	23	0.8536	0.837	0.142
K X N	31	22	0.7262	0.956	0.166
C X C	29	23	0.7836	1.000	0.000
C X R	32	28	0.8692	1.085	
C X K	34	30	0.8945	1.143	
C X N	**33**	**17**	**0.5146**	**1.109**	**0.272**
N X N	28	25	0.8998	1.000	0.000
N X R	33	30	0.8891	1.181	
N X K	33	26	0.7972	1.171	
N X C	30	24	0.8050	1.057	0.054

Table C.6. Analysis of reproductive potential in the regional and reciprocal crosses of *C. maculatus*

Cross	Total egg	Duration of adult development	Total Hatched	Female emerged	Male emerged	Longevity of mated males	Longevity of mated females
C X C	52	26.66	42	22	20	6.8	7.4
C X J	25	23.58	17	9	9	10.1	7.9
C X N	43	32.74	17	9	8	13.2	11.9
C X R	39	30.65	25	13	12	11.6	7.6
J X J	32	23.55	25	13	12	4.5	6.3
J X C	60	23.88	57	28	29	3.9	4.4
J X R	33	21.83	26	13	13	7.2	10.6
N X N	51	32.28	28	14	14	8.8	8.8
N X C	30	27.66	19	9	9	6.5	7.2
N X J	14	43.73	12	5	7	8.8	10.0
N X R	32	21.94	15	8	7	9.4	7.9
R X R	38	30.10	26	13	12	5.1	4.3
R X J	31	22.68	25	12	13	11.3	10.5
R X C	43	30.92	29	13	16	8.8	9.5
R X N	39	33.83	32	17	15	7.8	8.6
5% LSD	**8.81**	**5.23**	**11.8**	**5.94**	**5.93**	**2.25**	**2.52**

Table C.7. ANOVA for different reproductive potentials of *C. maculatus*

Variate	Treatment MS	df	Residual MS	df	F-ratio	F-prob
Total egg	1332.9	10	98.378	94	13.55	0.000
Total Hatched	277.47	10	34.736	92	7.99	0.000
Male emergence	433.45	10	44.827	94	9.67	0.000
Female emergence	400.42	10	44.596	94	8.98	0.000
Longevity of mated males	86.176	10	6.4021	94	13.46	0.000
Longevity of mated females	60.746	10	8.0872	94	7.51	0.000

Table C.8. Dissimilarity matrix and cluster membership of the crosses of *C. maculatus*. Bold number indicates the remarkable dissimilarities.

Crosses	C X C	C X J	C X N	C X R	J X J	J X C	J X R	N X N	N X C	N X J	N X R	R X R	R X J	R X C	R X N
C X C	0														
C X J	**5.73**	0													
C X N	**6.39**	3.99	0.00												
C X R	4.26	2.67	3.13	0											
J X J	4.17	3.2	5.96	3.82	0										
J X C	3.79	**9.05**	**10.11**	**7.77**	**6.89**	0									
J X R	4.31	2.77	4.25	3.39	2.9	**7.71**	0								
N X N	3.16	4.06	3.58	2.15	3.85	**6.76**	3.32	0							
N X C	**5.17**	2.03	4.61	3.0	1.93	**8.35**	2.85	3.51	0						
N X J	**8.21**	4.81	4.82	**5.09**	**6.14**	**11.52**	**5.83**	**5.79**	4.51	0					
N X R	**5.77**	1.1	3.98	2.94	3.13	**9.11**	2.81	3.98	1.97	5.28	0				
R X R	4	4	**6.16**	3.64	1.98	**6.64**	4.31	3.61	2.51	**6.07**	3.96	0			
R X J	4.99	2.23	3.21	2.62	4.08	8.5	1.97	3.49	3.44	**5.57**	2.62	**5.05**	0		
R X C	3.34	3.53	3.46	2.08	3.59	**6.94**	2.58	1.2	3.2	**5.35**	3.68	3.76	2.71	0	
R X N	2.96	3.95	4.3	2.4	3.38	**6.47**	3.14	1.72	3.29	**5.3**	4.22	3.26	3.53	1.52	0
Cluster	**1**	**2**	**3**	**2**	**2**	**1**	**2**	**2**	**2**	**4**	**2**	**2**	**2**	**2**	**2**

Table C.9. Estimates of relative fecundity increase, cytoplasmic incompatibility level, persistence of possible infection transmission and the critical migration rate of *Wolbachia* infection in *C. maculatus*.

Cross	Total Egg	Total Hatch	% Hatched	% Of Fecundity relative to control (*f*)	CI Level l_{CI}	Persistence of Infection	Critical Migration rate
C X C	52	42	0.819	1.000	0.000	0	
C X J	25	17	0.676	0.387	0.597	1	0.0040
C X N	43	17	0.403	0.401	0.592	1	0.0032
C X R	39	25	0.629				
J X J	32	25	0.776				
J X C	60	57	0.947				
J X R	33	26	0.773				
N X N	51	28	0.549	1.000	0.000	0	
N X C	30	19	0.615				
N X J	14	12	0.841	0.370	0.582	1	0.0042
N X R	32	15	0.469	0.628	0.464	0	
R X R	38	26	0.668				
R X J	31	25	0.806				
R X C	43	29	0.67				
R X N	39	32	0.8256				

Table C.10. Reproductive potentials of suspected incompatible reciprocal crosses exposed to 8Gy radiation dose of *C. maculatus*.

Crosses	Total Egg	Male Emerged	Female Emerged	Duration of Development	Mated Male Longevity	Mated Female Longevity
C X J	10.00	0.00	0.00		3.00	4.00
C X N	13.67	0.00	0.33	37.00	3.67	4.00
C X R	12.00	0.00	0.00		3.00	3.67
N X J	11.00	0.00	0.00		3.50	4.00
N X C	16.33	0.00	0.00		3.00	4.67
N X R	34.67	1.33	1.33	37.22	3.33	3.00
R X C	19.67	0.67	0.33	37.50	6.33	2.33
R X N	11.00	0.00	0.00		4.67	1.67
J X C	15.00	0.33	0.33	38.00	4.96	3.78