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Toxicity of Plant Seed Oils on Some Microbes and the Lesser Meal Worm, Alphitobius Diaperinus (Panzer) (Coleoptera: Tenebrionidae)

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TOXICITY OF PLANT SEED OILS ON SOME MICROBES AND THE LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* (PANZER) (COLEOPTERA: TENEBRIONIDAE)

THESIS SUBMITTED FOR THE DEGREE
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By

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B Sc (Hons), M Sc

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Dedicated
to
My Beloved Parents

DECLARATION

I do hereby declare that the work submitted as a thesis entitled **Toxicity of plant seed oils on some microbes and the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)** to the Institute of Biological Sciences, Rajshahi University, Rajshahi, for the degree of Master of Philosophy is the result of my own investigation and was carried out under the supervisions of Dr Md Ataur Rahman Khan, Professor, Department of Zoology and Dr Md Wahedul Islam, Professor and Director, Institute of Biological Sciences, Rajshahi University, Rajshahi. The work has not been submitted for any other degree.

05.11.2006

November 2006 **Md Raufun Patoary**

CERTIFICATE

This is to certify that Md Raufun Patoary worked under our supervisions as a Master of philosophy (M. Phil.) Fellow. We are pleased to forward his thesis entitled ***Toxicity of plant seed oils on some microbes and the lesser mealworm, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae)*** *which is the record of a bona fide research carried out at the Integrated Pest Management (IPM) laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of the thesis for the award of the degree of Master of Philosophy.*

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The author

ABSTRACT

Bioactivity and insecticidal properties of seeds of different plants, e.g. natai (*Caesalpinia bonduc* L., family-Fabaceae), alkushi (*Mucuna pruriens* L., family-Papilionaceae), rakta chandan (*Adenanthera pavonina* L., family-Fabaceae), bahara (*Terminalia belerica* Geartn., family-Combretaceae), kalojam (*Syzygium cumini* L., family-Myrtaceae) and jayfal (*Myristica fragrans* Houtt., family-Myristicaceae) on bacteria, fungi, brine shrimp and the insect *Alphitobius diaperinus* were investigated.

Toxic effects the methanolic and chloroform seed extracts on some microbes (bacteria: *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Sarcina lutea*, *Streptococcus-β-haemolyticus*, *Shigella shiga*, *Shigella dysenteriae*, *Shigalla boydii*, *Shigella sonnei*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Proteus* sp.; and fungi: *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans*, *Colletotrichum falcatum*, *Fusarium oxysporum*, *Mucor* sp., *Penicellium* sp., and *Vasin factum*) were determined. The bioactivity of the extracts on the Brine Shrimp, Artemia was also observed. In addition, the toxicity of the extracts on the beetle, *Alphitobius diaperinus* was determined. Methanolic extracts of all seed parts showed more toxicity on the microbes, brine shrimp and the beetle.

The seed extracts of the test plants showed toxic effects on the microbes tested by inhibiting their growth.

The findings of the investigation elucidated that the methanolic extract of all test plants possessed strong residual toxic effects on *A. diaperinus* that may have promising potential in controlling storage pest management programs.

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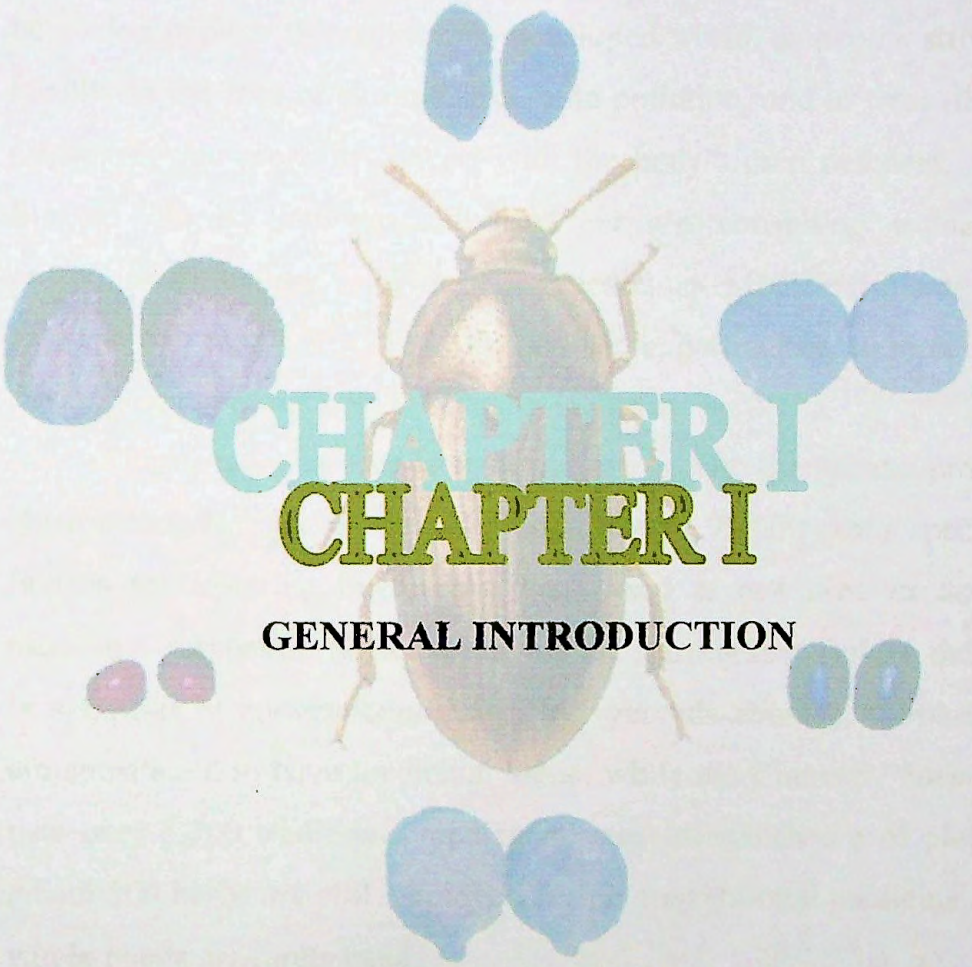


CHAPTER I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1. INTRODUCTION

Medicinal plants are the local heritage with global importance. The world is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in concert with the body's own defenses. People in Europe, North America and Australia are consulting trained herbal professionals and are using the plant medicines. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities.

The variety and sheer number of plants with therapeutic properties is quite astonishing. It is estimated that around 70,000 plant species, from lichens to flowering trees, have been used at one time or another for medicinal purposes. The herbs provide the starting material for the isolation or synthesis of conventional drugs. In Ayurveda about 2,000 plant species are considered to have medicinal value, while the Chinese *Pharmacopoeia* lists over 5,700 traditional medicines, most of which are of plant origin. About 500 herbs are still employed within conventional medicine, although whole plants are rarely used.

Medicinal plants are a living and irreparable resource, which is exhaustible if over used and sustainable if used with care and wisdom. The importance of medicinal plants has been overlooked in the past. However, at present medicinal plants are looked upon not only as a source of affordable health care but also as a source of income. According to WHO report, over

80% of the world population relies on traditional medicine largely plant based for their primary healthcare needs.

The recent past has witnessed an upsurge in the popularity of the herbal medicines. In the developing countries, about 80% of the people depend upon the traditional system of medicine and 95% of the industrial need of this in met through indiscriminate collection from wild. The indiscriminate collection of plants has led to considerable genetic erosion and loss of biodiversity including plants of medicinal value.

Plants are the richest source of bioactive organic chemicals on earth. The total number of plant chemicals may exceed 40,000. Although only about 10,000 secondary plant metabolites, whose major role in the plants against insect pests is defensive (Swain 1977). About 2000 plant species belonging to over 170 families posses insecticidal properties (Feinstein 1952). Recently, Grainge and Ahmed (1988) have listed approximately 2400 plant species as pesticides. Among them 1100 are insecticidal, 2 accaricidal, 230 antifeedant, 225 repellent, 32 growth regulators and 1 chemosterilant.

In Latin *oleum* means oil. They are the most important sources of energy in the diets of mankind. The term oil is a generic expression for a great variety of chemical substances which are composed principally, if not exclusively, of carbon and hydrocarbon (Metcalf and Flint 1962). Chemically fatty oils and fat do not differ, the distribution being a physical one depending on temperature. Fat is a solid at room temperature whereas an oil is a liquid under the same conditions. Fats usually come from animals and oils are generally obtained from plants. Fats and oils are esters of glycerol and fatty acids. They are commonly called triglycerides or simply glycerides.

In tropical countries like Bangladesh, India, Thailand, etc. the climate and storage conditions are much favourable for the growth and development of storage pests (Jacobson 1977, Golob and Muwalo 1984). The presence of the insect in stored foods directly affects both quality and quantity of the commodity (Sinha 1971, Wilbur and Mills 1985, Burkholder and Fanstini 1991, Mondal 1992, 1994). Storage losses from insect attack are often as great as sustained by the growing crops. Estimates of losses to the world's supply of stored grains from insect damage ranges from 5-10% of the world production (Burkholder 1990). In tropical countries 20% or more may occur through the insect attack after harvest (Mondal and Port 1995) because the climate and storage condition in the tropical countries are highly favourable for insect growth and development. In Bangladesh the annual loss amounts to over 100 crores taka (Alam 1971). A large number of insects including many species of beetles and moth attack stored food. In developing countries, the greatest losses during storage to cereals and other durable commodities such as pulses and oilseed are caused by a number of insect pests.

The wide-scale commercial use of plant extracts as insecticides began in the 19850s with the introduction of nicotine from *Nicotiana tabacum,* rotenone from *Lonchocarpus* sp, derris dust from *Derris elliptica* and pyrethrum from the flower heads of *Chrysanthemum cinerariaefolium* (Reed and Arthur 200).

Golob and Webley (1980) produced a bibliography summarising traditional methods used by farmers throughout the world to protect stored products. They also collected published scientific research on the use of extracts of plant materials. Rees *et al.* (1993) produced a bibliography database of 1100 references citing the use of alternative methods to conventional synthetic insecticides, for the control of stored-product insect

pests. These included the use of plant materials, extracts and oils. (Golob *et al.* 1999) reviewed the use of spices and medicinal plants as bioactive grain protectants.

Farmers have been using plant extracts in pest control for centuries. This method of pest control provides an ideal source of low cost, safe and effective pesticides. Extracts of plant material rely on the solubility of the active compounds and it may cause repellency to insects and may show (Sighamony *et al.* 1984), antifeedant or other type of bioactivities against insects (Jayasinghe and Fujimoto 1990, Morallo-Rejesus *et al.* 1990 and 1993, Adalla *et al.*1993, Facknath and Kawol 1993, Kim *et al.* 1994, Naumann *et al.*1994, Niber 1994, Rajuraman and Saxena 1994, Braverman and Chizov-Ginzburg 1997, Ho *et al.* 1997, El-Lakwah and Abdel-Latif 1998, Hermawan *et al.* 1998, Ndungu *et al.* 1999 and Jannet *et al.* 2000).

In Bangladesh, cereals like paddy, wheat, barley, maize, joar, kaon, and bajra oilseeds, pulses, potatoes, spices, etc. are stored both as seeds and foods in a dried condition. Other products, such as oilcakes, tobacco and many other agricultural products are stored in considerable quantities. In such conditions all these products are infested by a large number of insect pests causing severe damage and great economic loss (Alam 1971, Khan and Mannan 1991).

1.2. THE EXPERIMENTAL PLANTS

All living plant cells contain some oil. Large quantities of oils are found usually in the seed of plants and occasionally in the fleshy part of the fruit. Seeds may contain from 1% to over 60% of oil, most species having rather large amounts (Anon. 1967a). Many plant seeds have been used as a source of oil. The plants of the families like Linaceae, Compositae, Leguminosae, Cruciferae, Pedaliaceae, Euphorbiaceae, Oleraceae, Palmae, Zoipotoceae, etc. are important oil producers. There are many types of oilseed. Of these six types of plant seed have been considered as materials for this investigation. These were Natai seed (*Caesalpinia bonduc* L.), Alkushi seed (*Mucuna pruriens* L.), Rakta Chandan seed (*Adenanthera pavonina* L.), Bahera seed (*Terminalia belerica* Geartn.), Kalojam seed (*Syzygium cumini* L.) and Jayfal seed (*Myristica fragrans* Houtt.).

1.2.1. *Caesalpinia bonduc* (L.) Roxb.

Common Name: **Natai, Nickerbean, etc.**

Taxonomic Position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Caesalpinia*
Species	*Caesalpinia bonduc* (L.) Roxb.

Botanical description

An extensive climber; branches finely grey-downy, armed with "hooked and straight" hard yellow prickles. Leaves 30-60 cm. long; petioles prickly; stipules a pair of reduced pinnae at the base of the leaf each furnished with a long mucronate point; pinnae 6-8 pairs, 5-7.5 cm. long, with a pair of book stipulary spines at the base. Leaflets 6-9 pairs, 2-3.8 by 1.3-2.2 cm., membranous, elliptic-oblong, obtuse, strongly mucronate, glabrous above, more or less puberulous beneath; petiolules very short; stipels of short hooked spines. Flowers in dense (usually spicate) long-peduncled terminal and supraaxillary racemes dense at the top, lax downwards, 15-25 cm. long; pedicels very short in bud, elongating to 5 mm. in flower and 8 mm. in fruit, downy; bracts squarrose, linear, acute, reaching 1 cm. long, fulvous-hairy. Calyx 6-8 mm. long, fulvous-hairy; lobes obovate-oblong, obtuse. Petals oblanceolate, yellow. Filaments declinate flattened at the base, clothed with long white silky hairs. Pods shortly stalked, oblong, 5-7.5 by 4.5 cm., densely armed on the faces with wiry prickles. Seeds 1-2, oblong, lead-coloured, 1.3 cm. long.

Distribution

C. bonduc is distributed widely in the tropics and subtropics and is found in moist areas throughout India, Bangladesh, Sri Lanka, Burma, China, Philippines, Malaysia, Tropical Africa, Afghanistan, Madagascar, Pakistan, etc. (Hutton 2001). In Bangladesh it is very common in many parts and often occurs gregariously.

Medicinal Uses

The root-bark is good for tumours and for removing the placenta. The sprouts are useful in the treatment of tumours. The juice of the leaves is

anthelmintic; good in elephantiasis and small pox; destroys the bad odour due to perspiration. The flower is bitter, healing to the body; cures "kapha" and "vata"; the ash is used in ascites. The fruit is acrid, heating to the body; astringent to the bowels, aphrodisiac, anthelmintic; cures urinary discharges, leucorrhwa, piles, wounds. The oil from lie fruit is good for indolent ulcers (*Ayurveda*).

The seed is hot and dry; styptic, antiperiodir, anthelmintic, prevents contagious diseases; cures inflammations; useful in colic, malaria, hydrocele, skin diseases, leprosy (Yununi).

In an official report, the Madras Committee for the propose revision of the Indian Pharmacopoeia, remark that "the seeds an very useful and cheap and antiperiodic, antipyretic, and tonic; valuable in all ordinary cases of simple, continued and intermittent fevers. They have also been found useful in some cases of asthma".

In Madras, an ointment is made from the powdered seeds with castor oil and applied externally in hydrocele and orchitis. In disorders of the liver, the tender leaves are considered very efficacious. In Cochin China they are reckoned as a deobstruent and emmenagogue; and an oil expressed from them is given in convulsions, palsy, and similar complaints.

In Malaya, the young leaves are used in intermittent fevers, and for expelling intestinal worms. In Ceylon, they are applied for toothache, and they are also given for worms in children.

In La Reunion and Madagascar, the roots are considered febrifuge and anthelmintic; they are much used as an astringent in leucorrhoea and blennorrhagia. The seeds are considered tonic, febrifuge, anthelmintic, antiblennorrhagic, and specific in the treatment of hydrocele. The oil from

the seeds is used in convulsions and paralysis. The leaves are a good emmenagogue.

In Guinea, the pounded seeds are considered vesicant; a decoction of the root is prescribed in fever; the boiled leaves are used as a gargle for sore throat.

The powdered seeds were administered mixed with equal part of pepper powder to malarial patients and were found to possess feeble antiperiodic properties. In malignant malaria, they did not do nay good. The leaves and seeds after roasting with castor oil are applied externally to inflammatory swellings especially to inflamed piles, hydrocele, and orchitis with benefit

The seeds contain a fairly good percentage of pale yellow thick oil having a disagreeable odour. It has iodine value of 96.1 and saponification value of 292.8. We could not confirm the presence of a alkaloid as noted by the previous investigators, but they contain a non-glucosidal bitter principle insoluble in water which is pharmacologically inactive. According to some authors the quantity of oil varies between 20 to 25 per cent, whereas in our specimen we did not get more than 14 per cent. As the seeds do not show any marked therapeutic properties and the reinvestigation of their chemical composition does not reveal the presence of any active principles with marked physiological action, further clinical trials were considered unnecessary.

The seeds are ground in water and given internally in snakebite. The seeds are not an antidote to snake-venom. Tumminkatti and Puntambekar have carried out the chemical examination of the seeds and their oil.

Plate 1: Nickerbean, *Caesalpinia bonduc*, with spiny seed pods that mature in fall

1.2.2. *Mucuna pruriens* (L.) DC.

Common Name: **Alkushi, Cowhage, Velvet bean etc**

Taxonomic Position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Papiles
Family	Papilionaceae
Genus	*Mucuna*
Species	*Mucuna pruriens* (L.) DC.

Botanical description

Mucuna is an annual twinning plant. Leaves are trifoliate, gray-silky beneath; petioles are long and silky, 6.3–11.3 cm. Leaflets are membranous, terminal leaflets are smaller, lateral very unequal sided. Dark purple flowers (6 to 30) occur in drooping racemes. Fruits are curved, 4–6 seeded. The longitudinally ribbed pod is densely covered with persistent pale-brown or grey trichomes that cause irritating blisters. Seeds are black ovoid and 12 mm long (Sastry and Kavathekar 1990, Agharkar 1991, Verma *et al.* 1993). The embryo completely fills the seed and is made up of two large fleshy cotyledons. Transverse section of seed shows an outer testa with a palisade epidermis made up of a rod shaped macrosclereids with thickened anticlinal walls.

Distribution

Originated in India, it is now commonly found throughout the tropics.

Medicinal Uses

M. pruriens is one of the popular medicinal plant of India and is constituent of more than 200 indigenous drug formulations. It is widespread over most of the subcontinent and is found in bushes and hedges and dry-deciduous, low forests throughout the plains of India (Sister and Kavathekar 1990, Agharkar 1991, Singh *et al.* 1996). All parts of *Mucuna* posses valuable medicinal properties (Pandey 1998, Pandey 1999, Caius 1989) and there is a heavy demand of *Mucuna* in Indian drug markets. After the discovery that *Mucuna* seeds contain L-dopa, an anti-Parkinson's disease drug, its demand in international market has increased many fold (Farooqi 1999) and demand has motivated Indian farmers to start commercial cultivation.

Roots, according to the *Ayurveda*, are bitter, thermogenic, anthelmintic, diuretic, emollient, stimulant, aphrodisiac, purgative, febrifuge, tonic. It is considered useful to relieve constipation, nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, consumption, ulcers, helminthiasis, fever, and delirum (Lindley 1985, Ramnath 1992, Warrier 1996, Shalini 1997, Upadhyay 2000).

Leaves are popular pot herbs and are used as a fodder crop. Leaves are useful in ulcers, inflammation, cephalagia and general debility.

The trichomes of pods contain mucunain and serotonin and as a result pod causes itching, blisters, and dermatitis. Pods are also used as vegetable. Pod hairs (trichomes) are used as anthelmintic. Hairs mixed with honey have been used as vermifuge. As ointment prepared with hairs act as a local stimulant and mild vesicant (Shastry and Kavathekar 1990, Chandra 1993,

Shastry 1995). Beside medicinal properties, *Mucuna* fixes nitrogen and is as a green manure and cover crop.

Seeds contain L-DOPA (4-3,4-dihydroxy phenylalanine), glutathione, lecithin, gallic acid, glycosides, nicotine, prurenine, prurenidine, dark brown viscous oil. It is a source of minerals (Rastogi and Mehrotra 1991a and b, Singh *et al.* 1995). According to *Ayurveda*, seeds are astringent, laxative, anthelmintic, aphrodisiac, alexipharmic and tonic. Extract of dried seeds taken orally by human adults was active with improvement in erection, duration of coitus, and postcoital satisfaction.

Plate 2: Alkushi, *Mucuna pruriens*, with flowers and seeds

1.2.3. *Adenanthera pavonina* L.

Common Name: Rakta Chandan, Raktakanchan, Red Sandalwood, etc.

Taxonomic position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Adenanthera*
Species	*Adenanthera pavonina* L.

Botanical Description

A small unarmed tree 6-15 m. high; young parts glabrous. Leaves 2-pinnate, 20-30 cm. long; petioles 5-10 cm. long; pinnae 3-6 pairs, opposite, 7.5-15 cm. long, with a stalk 1.3-2 cm. long, Leaflets alternate, 4-8 pairs, 2.5-3.8 by 0.8.2 cm., papery, elliptic-oblong obtuse, glabrous, dark green above, glaucous beneath, base shortly cuneate, unequal sided; petiolules 3 mm. long. Flowers in short-peduncled racemes 5-15 cm. long, axillary or panicled at the ends of the branches; pedicels 2.5-3 mm. long, slender. Calyx minute; lobes short, triangular. Corolla pale yellow, about 3 mm. long; segments united at the base only, linear-lanceolate, acute, valuate, Stamens 10, free, hardly exserted; anthers gland-creasted. Pods 15-23 by 1.6.2 cm., flat, facately curved, pointed, tapering to the base, the values spirally twisted after dehiscence. Seeds 8-15, lenticular-globose, with a blunt keel, smooth, shining, usually brilliant scarlet, 8 mm. diam.

Distribution

Bangladesh, Burma, W. Peninsula, Ceylon- Malay Islands, Timor, China, Philippines.

The Red Sandalwood is believed to be able to fix nitrogen and thus help rejuvenate soils.

Medicinal Uses

The young leaves can be cooked and eaten, but usually only during famine. The leaves were also used to supplement animal fodder, or mulched to fertilise crops. The seeds were eaten in Melanesia and Polynesia and the people there called it the "food tree". The seeds were roasted before eating. Elsewhere they are boiled. In Java, they are roasted, shelled, then eaten with rice. They are said to taste like soybean. The raw seeds are toxic and may cause intoxication. Studies show the cooked seeds to be rich in oil and proteins, and are easily digested by both humans and livestock.

These attractive seeds have been used as beads in jewellery, leis and rosaries. They were also used in ancient India for weighing gold. The seeds are curiously similar in weight. Four seeds make up about one gram. In fact the name "saga" is traced to the Arabic term for "goldsmith". In India, it is believed that a person may have as many wishes as elephants found in a saga seed. The ground seeds can produce an oil which was used as an industrial lubricant. The hard, reddish wood is used to make cabinets, often in place of true sandalwood. With exposure to light, the wood slowly turns purplish-red. It is also valued as firewood as it burns well. The tree resprouts new branches easily and so is not damaged by harvesting for firewood. A red dye is obtained from the wood and used by the Brahmins to make religious markings on their foreheads. In Malaysia and Indonesia, the trees

also provided shade and were planted as "nurse trees" in coffee, clove and rubber plantations.

The powdered seeds make a useful external application hastening suppuration. A decoction is made from the leaves in South India, and given as a remedy for chronic rheumatism and gout. If used for any length of time, it is said to be an aphrodisiac. This decoction is said to be useful in haemorrhage from the bowels and haemanturia. The seeds yield about 14 per cent of oil which contains 25 per cent of lignoceric acid.

A red powder made from the wood is also used as an antiseptic paste. In ancient Indian medicine, the ground seeds are used to treat boils and inflammations. A decoction of the leaves is used to treat gout and rheumatism. The bark was used to wash hair.

Plate 3: Rakta Chandan, *Adenanthera pavonina*, with seeds

1.2.4. *Terminalia bellirica* (Gaertn.) Roxb.

Common Name: Bahera, Boira, Belleric myrobalan, etc.

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Combretaceae
Genus	*Terminalia*
Species	*Terminalia bellirica* (Gaertn.) Roxb.

Botanical Description

A large deciduous tree, 10-20 m. high. Leaves gathered about the extremities of the branches, alternate, coriaceous, 10-20 by 7-15 cm., broadly elliptic or elliptic-obovate, rounded or rarely subacute or shortly acuminate, both surfaces puberulous when young, glabrous and reticulate when old, the margins entire, pellucid, base narrowed; main nerves 6-8 pairs, spreading, prominent, the midrib prominent 'on both surfaces; petioles 2.5-10 cm. long, without glands at apex. Flowers pale greenish yellow, with an offensive odour, in axillary slender spikes longer than the petioles but shorter than the leaves, those in the upper part of the spike male and very shortly pedicelled, those in the lower part hermaphrodite, sessile. Bracts linear, early caducous. Calyx pubescent outside, inside woolly with long brown hairs; teeth broadly triangular, acute. Young ovary always tomenlose/ Drupe 12-25 mm. diam., ovoid, grey, suddenly narrowed into a very short stalk, velvety, obscurely 5-angled when dried.

When mature the leaves are glabrous and usually punctate on the upper side. The punctations are much more permanent than in the other species.

The bark is bluish grey, with many fine vertical cracks. The wood is yellowish grey, hard, no heartwood; annual rings indistinct. Pores very scanty, large, frequently subdivided, joined by irregular wavy, concentric bands of soft loose cellular tissue.

Distribution

It is a characteristic species of the dry forest tracts of the world. It is a common tree occurring throughout deciduous forests of India, and also grown as an avenue tree. The plant is common throughout the greater part of India except arid regions in western Rajasthan, Punjab, Chathisgarh, Uttaranchal, and Haryana and the temperate Himalayan tracts. Naturally it occupies the deciduous forests of the country. It is rarely seen in semi evergreen forests. It is common throughout India in plains and lower hills, chiefly in deciduous forest up to locations of 900 m. Many people grow it in their homesteads. Tree is seen in Burma, Bangladesh, Nepal, Thailand, Indo-China, Malaysis and Sri Lanka.

Medicinal Uses

The fruits contain tannins, α-sitosterol, gallic acid, chebulegic acid etc. It is bitter, asterigent, anthementic, pungent, laxative and are used in piles, enlargement of spleen, diarrhoea, eye troubles, leprosy and headache. The tree is a good source of gum, resembling gum Arabic. The kernel of the nut is said to intoxicate human beings, if eaten in any huge quantity. Mixed with honey it is used in treating ophthalmic problems. The dried fruit has an irregular shape. It is a good astringent. The astringency renders them

valuable in the acts as well as a substitute for falls for lotions, injections and so on. It is also used in dry prolonged coughs, dropsy, diarrhoea, and leprosy. However, overdoses can act as a narcotic poison. Fruits are reported to have antibiotic activity against a wide variety of microorganisms. Wood pulp is suitable for wrapping paper. It is used, to extract a yellow dye from the seed coat. As said earlier, the tree forms important part of *Myrobelans.* Fruits are used for dyeing and tanning, and are also used to increase the potency of spirits. Kernels yield oil, which is reported for preparing soaps.

Plate 4: Test plant-Bahera, *Terminalia bellirica*

1.2.5. *Syzygium cumini* (L.) Skeels

Common names: Jam, Jambolana, Black plum, etc.

Taxonomic position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	*Syzygium*
Species	*Syzygium cumini* (L.) Skeels

Botanical Description

A smooth tree of the Myrtaceae family. Four to fifteen meters in height. Leaves leathery oblong ovate to elliptin or obovate and 6 to 12 cm long, the tip being broad and shortly pointed. The panicles are borne mostly from the branchlets below the leaves often being axillary or terminal, and are four to six cm long. The flowers are numberous, scented, pink or nearly white, without stalks, and borne in crowded fascicles on the ends of the branchlets. The calyx is funnel shaped, about 4 mm long, and 4 toothed. The petals cohere and fall all together as a small disk. The stamens are very numerous and as long as the calyx. Fruit oval to elliptic; 1.5 to 3.5 cm long, dark purple or nearly black, luscious, fleshy and edible; it contains a single large seed.

Distribution

The original home of *S. cumini* is India or the East Indies. It is found in Thailand, Philippines, Madagascar, and some other countries. The plant has been successfully introduced into many other tropical countries such as the West Indies, East and West Africa and some subtropical regions including Florida, California, Algeria and Israel.

Medicinal Uses

Bark paste and curd is taken orally three times a day for two days to cure dysentery. Decoction and fluid extract of dried bark is taken orally for diabetes. Ten grams of dried leaves of *Zanthoxylum armatum* boiled in eight liters of water along with 125 gm of mixture having equal parts of bark of *Acacia nilotica, Mangifera indica,* and *Syzygium cumini* until the quantity of water is reduced to two liters. Fifty milliliters of decoction is taken twice a day after meals. Hot water extract of dried bark is taken orally for dysentery, indigestion, and as a blood purifier. Decoction of dried bark is taken orally for venereal ulcers. *Terminalia arjuna, Pongamia pinnata, Vateria indica, Syzygium cumini, Ficus benghalensis, F. religiosa, F. racemosa, F. talbotii,* and *Azadirachta indica* are used. Fruits are taken orally to cure gastro-intestinal complaints. Hot water extract of dried fruits is used externally as astringent and orally for stomach ulcers and to reduce acidity. Hot water extract of dried fruit and seeds is taken orally for diabetes. Leaves are taken orally for leucorrhea; two young leaves are chewed with cold water for 3-4 days. Decoction of dried seeds is taken orally for diabetes. The fluidextract is taken orally as an anti-inflammatory and the hot water extract is taken orally as an antipyretic, and for diabetes 100-250 mg seed powder is taken orally three times a day with water. Decoction of dried seeds is taken orally for diarrhea; the seeds are taken together with *Cassia auriculata.* Hot water

extract of dried seeds taken orally is prescribed in Ayurvedic medicine for diabetes; it is also used as an astringent in dysentery and diarrhea and to reduce urinary sugars in diabetes. Leaf juice is taken orally to treat diabetes. The juice is taken mixed with milk every morning. Fresh leaf juice is taken orally for stomach pain. Seeds are taken orally for diabetes. Stem bark juice is taken orally for constipation and to stop blood discharge in the feces, mixed with buttermilk and taken every day.

Pharmacological Activities and Clinical Trials

Abortifacient effect: Ethanol/water (1:1) extract of the aerial parts, administered orally to rats at a dose of 200.0 mg/kg was inactive.

Analgesic activity: Ethanol/water (1:1) extract of the aerial parts, administered intraperitoneally to price at a dose of 0.375 mg/kg was inactive vs tail pressure method. Methanol extract of dried seeds, administrated intraperitoneally to mice at a dose of 25.0 mg/kg was active vs acetic acid-induced writhing.

Antiaggression effect: Methanol extract of dried seeds, administered intraperitoneally to mice at a dose of 150.0 mg/kg was active vs foot shock induced agression.

Antibacterial activity: Ethanol (95%) and water extracts of dried fruit at concentrations of 100.0 and 20 mg/disc respectively (expressed as dry weight of the fruit) on agar plate, were inactive on *Bacillus subtilis, Escherichia coli, Salmonella typhosa, Shigella dysenteriae,* and *Staphylococcus aureus.* Ethanol/water (1:1) extract of the aerial parts of a concentration >25.0 mcg/ml on agar plate was inactive on *Bacillus subtilis, Escherichia coli, Salamonella typhosa, Staphylococcus aureus,* and plant pathogen *Agrobacterium tumefaciens.* Saline extract of leaves at a concentration of 1:80 on agar plate was active on *Staphylococcus aureus.*

Antifungal activity: Ethanol/water (1:1) extract of the aerial parts at a concentration > 25.0 mcg/ml on agar plate was inactive on *Microsporum canis, Trichophyton mentagrophytes,* and *Aspergillus niger.*

Antihistamine activity: Ethanol/water (1:1) extract of dried bark at a concentration of 0.01 gm/ml was active on guinea pig ileum. Methanol extract of dried seeds administered intraperitoneally to rats was active vs histamine induced pedal edema.

Antitoxic activity: Methanol extract of dried seeds, administered intraperitoneally to mice at a dose of 50.0 mg/kg was active. Antagonized amphetamine toxicity.

Antiviral activity: Ethanol/water (1:1) extract of dried entire plant at a concentration of 0.1 mg/ml in cell culture was inactive or Ranikhet virus and Vaccinia virus. For Ranikhet virus, infected chorioallantoic membrance viral titre decreased 10% and for Vaccinia virus, 0%. The extract when injected into chick embryo at a dose of 1.0 mg/animal, was inactive on Ranikhet virus and Vaccinia virus. Infected chick embryo viral titre decreased 10% and 0% prospectively. Ethanol/water (1:1) extract of the aerial parts at a concentration of 50.0 mcg/ml in cell culture was inactive on Ranikhet virus and Vaccinia virus. Water extract of the bark was active on Potato X virus.

Antiyeast activity: Ethanol (95%) and water extracts of dried fruit at concentrations of 100.0 and 20.0 mg/disc respectively (expressed as dry weight of the fruit) on agar plate, were inactive on *Candida albicans.* Ethanol/water (1:1) extract of the aerial parts at a concentration >25.0 mcg/ml on agar plate was inactive on *Candida albicans* and *Cryptococcus neoformans.*

Conditioning avoidance response decrease: Methanol extract of dried seeds, administered intraperitoneally to mice at a dose of 150.0 mg/kg was active.

Fish poison: Water extract of fresh bark was active, LD_{50} 0.18%.

Hypotensive activity: Ethanol/water (1:1) extract of dried bark, administered intravenously to dogs at variable dosage levels was inactive.

Hypothemic activity: Ethanol/water (1:1) extract of the aerial parts, administered intraperitoneally to mice at a dose of0.375 mg/kg was inactive. Methanol extract of dried seeds, administered intraperitoneally to mice at a dose of 50.0 mg/kg was active.

Molluscicidal activity: Ethanol (95%) and water extracts at concentrations of 10,000 ppm were inactive on *Biomphalaria glabrata* and *Biomphalaria straminea.* Water saturated with essential oil of fresh leaves at a concentration of 1"10 was inactive on *Biomphalaria glabrata.*

Nematocidal activity: Decoction of a commercial sample of bark at a concentration of 10.0 mg/ml was inactive on *Toxocara canis.* Water and methanol extracts of dried seeds at concentrations of 5.0 and 1.0 mg/ml, respectively, were inactive on *Toxacara canis.*

Protease (HIV) inhibition: Water extract of dried bark at a dose of 200.0 mcg/ml showed weak activity, the methanol extract was active.

Semen coagulation: Ethanol/water (1:1) extract of the aerial parts at a concentration of 2.0% was inactive on rat semen.

Spermicidal effect: Ethanol/water (1:1) extract of the aerial parts was inactive on rat sperm.

Toxicity assessment: Ethanol (95%) extract of dried seeds, when administered intravenously to mice LD_{50} was 0.4 mg/kg and 4.0 mg/kg with

intragastric administration. Ethanol/water (1:1) extract of the aerial parts, administered intrapertoneally to mice showed a LD_{50} of 0.75 mg/kg.

Weight increase: Powdered commercial sample of seeds administered by gastric intubation to rats at a dose of 53.2 mg/kg was active.

Plate 5: Jam, *Syzygium cumini*, with mature and immature seeds

1.2.6. *Myristica fragrans* Houtt.

Common Names: Nutmeg, Jayfal, Mace, etc.

Taxonomic position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Magnoliales
Family	Myristicaceae
Genus	*Myristica* Gronov.
Species	*Myristica fragrans* Houtt.

Botanical Description

A lofty tree, branches slender. Leaves coriaceous, 7.5-8.8 cm., elliptic -oblong or -lanceolate, acuminate, sometimes oblanceolate, and tip caudate, base acute, pale yellow-brown, paler with red-brown nerves beneath, nerves about 8 pairs, slender; petiole 6-13 mm. Male racemes 2.5-5 cm.; flowers bracteolate, 6 mm. long, ellipsoid or urceolate, nodding; males in lax slender supra-axillary racemes; bracteole-a scale under the glabrate perianth; anthers 9-12, connate in a cylindric stipitate column. Fruit ovoid, subglobose or pyriform. 3.8-5 cm. long.

Distribution

Indigenous to the Moluccas and Banda Islands in the South Pacific although it is seldom found truly wild. It is now cultivated in tropical regions, especially Indonesia, Grenada in the West Indies and Sri Lanka (Purseglove 1968, Bown 1995).

Medicinal Uses

The fruit is bitter, hot, pungent; astringent to the bowels, anthelmintic, aphrodisiac; improves appetite, taste, voice; useful in "kapha", "vafa", foul breath, bronchitis, vomiting, thirst, diseases of the heart, urinary discharges. The oil is stimulant, carminative; useful in diarrhoea, convulsions, pains, ulcers; improves the appetite. The mace has a bitter pungent taste; anthelmintic, alexiteric; useful in bronchitis, asthma, thirst; improves the appetite (*Ayurveda*).

Nutmeg oil is also known as oleum myristicae, ol. myrist., myristica oil, essence de muscade, atheririsches muskatol, essencia de moscada and essencia de nuez moscada. It is a volatile oil obtained by steam distillation from the seed. It is a colourless or pale yellow liquid with an odour and taste of nutmeg. It is scarcely distinguishable from the volatile oil of mace and frequently no commercial distinction is made between the two. There are two types of nutmeg oil, mainly East Indian Nutmeg Oil and West Indian Nutmeg Oil. The East Indian Nutmeg Oil has a weight of 0.885 to 0.915 g/mL and is soluble in 90% alcohol at a ratio of 1 part oil to 3 parts alcohol. West Indian Nutmeg Oil has a weight of 0.86-0.88 g/mL and is soluble in 90% alcohol at a ratio of 1 part oil to 4 parts alcohol. Nutmeg oil should be stored in a cool place in well filled airtight containers and protected from light.

The nut and the mace are diuretic, laetagogue, stimulant, hypnotic, digestive, tonic, aphrodisiac; useful in choleraic diarrhoeas, diseases of the liver and spleen, headache, palsy, eye troubles (Yunani).

The seeds are carminative and stomachic; useful in flatulency, a, and vomiting. When given at all largely it is essentially narcotic.

Actually in Europe it is mostly employed as a flavouring for medical or culinary purposes.

The Chinese do not use nutmeg much as a spice, but as a carminative, stomachic, and antispasmodic among medicinal remedies for children and the aged. The nut is a Cambodian remedy for looseness of bowels. The nutmeg is useless in the symptomatic treatment of snake-bite.

Plate 6: Nutmeg, *Myristica fragrans*, with mature seeds

1.3. The Test Insect: *Alphitobius diaperinus* (Panzer)

The darkling beetle or black bug or lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is a notorious and serious pest of a great variety of stored products. It is cosmopolitan in distribution and is associated with wheat, barley, rice, oatmeal, soybeans, cowpeas, peanuts, etc. and it has also been reported from linseed, cotton seed, oilseed products, tobacco, skins, drugs, poultry litter, etc. (Lepesme 1944, Lancaster and Simco 1967, Spilman 1987).It is also noted that this is a serious pest of poultry farms throughout the world (Nemerseri and Gerztessy 1973, Ichinose 1980, Vaughan and Turner 1972, Wildey 1983).

A. diaperinus is a worldwide pest and reaches immense populations in litter in broiler breeder houses and grow outhouses and as well as in the accumulated manure under caged layers and under the slates in breeder houses (Pfeiffer and Axttel 1980, Rueda and Axtell 1997). The beetle life cycle includes eggs, larvae (6-9 stages), pupae and adults, all of which are found in the litter or manure. A complete life cycle from egg to adult requires about 5 weeks, depending on the temperature (Rueda and Axtell 1996). The adults are extremely long-lived (at least a year) and able to survive adverse conditions. Some of the beetle larvae in the presence of dense populations move into the building insulations to pupate and in the process destroy the insulating value (Geden and Axtell 1987). Repair of damage to house is costly and the reduced insulation interferes with bird production and by making temperature more difficult. In addition to this costly structural damage, the beetles are excellent reservoirs of disease organisms affecting both humans and birds and are a significant hazard to bird production (Jones *et al.* 1991a and b, McAllister *et al.* 1994, 1995 and

1996). Young birds can eat large numbers of beetle larvae which interferes with normal feed consumption and growth, and provides an avenue for disease transmission (Despins and Axtell 1994 and 1995, Despins *et al.* 1994). Adult beetles are capable of flying and flight takes place mostly at night. When beetle infested litter is removed from poultry houses and spread on fields, the adults quickly leave the unsuitable habitat and fly to nearby human dwelling causing great annoyance and often low suits.

The lesser mealworms are identified by some of their external characters (Lyon, 1991):

- The lesser mealworm adult beetle is $\frac{1}{4}$ inch long, reddish brown to brown coloured, with distinct longitudinal grooves on the wing covers, body oval shaped and slightly flattened.
- Eggs are about $\frac{1}{16}$ inch long and creamy white to tan coloured. They are slender with slightly rounded ends.
- Larvae are about $\frac{1}{3}$ inch long, tan to light brown coloured with slender, segmented bodies and three pairs of legs.
- Pupae are about $\frac{1}{4}$ inch long, creamy white to tan coloured with legs tucked alongside to the body (segmented appearance).

LESSER MEALWORM FACTS

- Originated in Eastern Africa where associated with the nests and guano of various bird and bat species.
- Occurs in accumulations of immense, protected, moist amounts of manure and litter from broilers, breeders, pullets, and caged layer chickens, turkeys, and other avian commercial operations such as quail and Cornish hens.

- Highly active both through running and, at night, through flight. (Prefer a dark daytime hiding place).
- Congregate in areas where there are warm, moist conditions.
- Sensitive to freezing and high temperatures with dehydration occurring in environments not containing enough moisture.
- Tend to avoid excessively wet areas, especially where anaerobic microbial activity is high (fermentation of wet feed or soupy manure).
- All life stages tend to clump together in close contact in areas where food, water, and environmental conditions are best for survival.
- In native Africa, beetles were nest scavengers, consuming bits of feed, nest litter, portions of manure, dead plant and animal material.
- In USA, poultry operations, beetles eat feed , litter, manure solids, and even portions of dead and dying birds.
- Will move out of the chicken area of these building and in to insulation.
- Have no natural enemies and carry a large variety of avian viruses, bacteria, fungi, and parasites without observable negative effects on the beetles. They are exceptionally sturdy and durable.
- Cannibalistic at all stages, and can actually lower their own populations through predation when conditions are too dry or food is in short supply.
- Conditions favoring beetle cannibalism often stimulate their migration, resulting in them becoming pests in insulation or in other buildings near poultry operations.
- They cannot survive freezing or temperatures above 90°F for any length of time unless they have access to much free moisture.
- They can consume large quantities of feed in a short period of time.

- Through their active consumption of edible organic matter and their burrowing, these beetles do help recycle poultry wastes and remove material that otherwise would be excellent for fly development.
- Low to moderate beetle populations can have a very positive effect on controlling fly populations in large, caged-layer houses where manure and litter are allowed to build-up.
- Both adults and larvae are nocturnal and remain active 24 hours a day, favoring dark interiors of buildings during the day.
- Beetles are actively attracted to artificial light sources at night. Security lights around poultry facilities may help control nocturnal migration to a slight extent by keeping them in an area and preventing them from detecting distant light sources.

Stored products insects present a number of problems associated with insecticidal treatment. In general, insecticides present the following important problems:

- Resistance of pests to chemical insecticides.
- Elimination of beneficial insects.
- Persistence of several insecticides and their accumulation in the food chain by biological concentration.
- Higher cost of production or application.
- Toxicity to human and wildlife.

Control of lesser mealworm larvae by spraying insecticides in the poultry farms is quite difficult due to their climbing and tunneling behaviour (Geden and Axtell 1987). This behaviour greatly affected the insulated walls of piggery in Ireland (O'conor 1987), and that of poultry (Gall 1980, Ichinose *et al.* 1980).

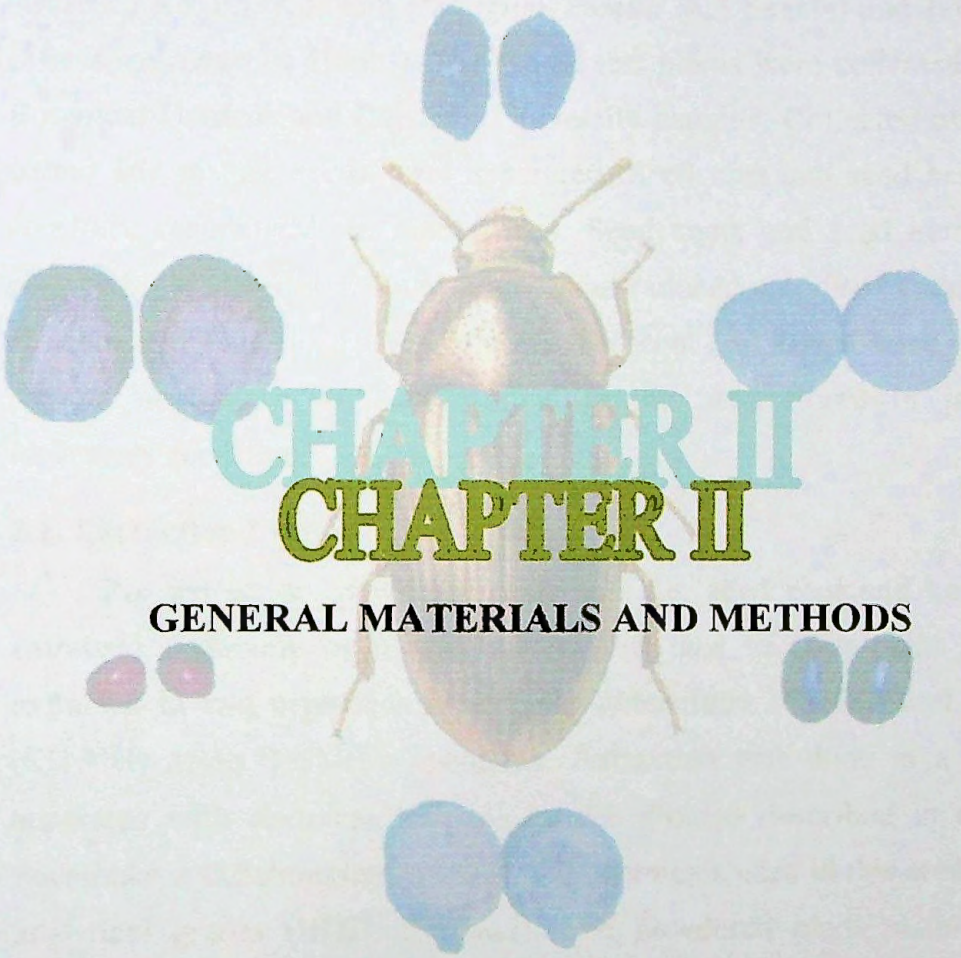
This beetle has been incriminated in the transmission of several diseases and disease agents. These include Newcastle disease, avian influenza, infectious bursal disease, Mark's disease, fowl pox, Salmonellasis, *Aspergillus* spp., Reovirus, Rotavirus, *Eimeria* (coccidiosis), tapeworms and cecal worms (De las Casas *et al.* 1973 and 1976, Despins *et al.* 1994, McAllister *et al.* 1994, 1995 and 1996).

The mouthparts of the lesser mealworm reveal that the adults are general feeders, while the larvae are adapted for feeding on cemented food substances (Leschen and Steelman 1988).

State some words on the effect of oils on bacteria, fungi and the brine shrimp. The potentials for developing antimicrobials into medicines seem to be very much promising from the point of both drug development and phytomedicines.

1.4. OBJECTIVES OF THE RESEARCH WORK

Objectives of the present work was to investigate the effectiveness of some plant seed oils, *viz.* Natai (*Caesalpinia bonduc* L.), Alkushi (*Mucuna pruriens* L.), Rakta Chandan (*Adenanthera pavonina* L.), Bahera (*Terminalia bellerica* Gaertn.), Kalojam (*Syzygium cumini* L.) and Jayfal (*Myristica fragrans* Houtt.) against bacteria, fungus, brine shrimp and the insect, *Alphitobius diaperinus* (Panzer). Selection of the plant seed oils as chemicals in the experiment was based on their effectiveness against other stored product insect pests. The main objective of the project was also to study the effects of these oils on behavioural and biological aspects of *A. diaperinus* as well as their toxicity causing the mortality of adults.



CHAPTER II

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

2.1. Plant Materials

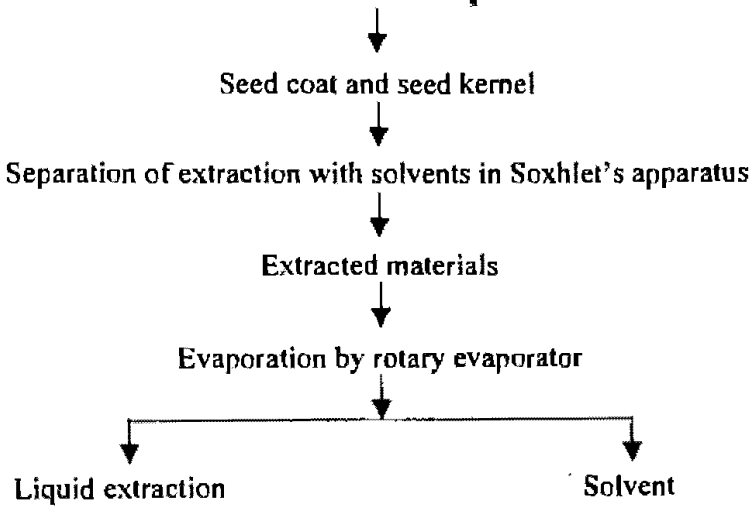
Six plant seeds have been considered for this investigation. These were Natai (*Caesalpinia bonduc* (L.) Roxb.), Alkushi (*Mucuna pruriens* L.), Rakta Chandan (*Adenanthera pavonina* L.), Bahera (*Terminalia bellirica* (Gaertn.) Roxb.), Kalojam (*Syzygium cumini* (L.) Skeels) and Jayfal seeds (*Myristica fragrans* Houtt.). The whole test plants were collected from the Botanical Gardens and Rajshahi University campus. Collected plants were sorted and mature seeds were separated. Seed coat and seed kernel were carefully separated from plant seeds. Seed coats and seed kernels were chopped into small pieces and dried under reduced pressure (Vacuum drier) at room temperature. Different plant seed coat and kernel were grinded to powder separately with an electric grinder and stored in jars under laboratory conditions.

2.2. Extraction Process

For extraction, powdered materials, i.e. seed coat and kernel were extracted separately. Sufficient amounts of dust of each plant part were extracted in two organic solvents, *viz.* chloroform ($CHCl_3$) and methanol (CH_3OH) using Soxhlet's apparatus. Extraction was done in a Soxhlet's apparatus with chemical solvents by the process described in details by Feuerhake and Schmutterer (1982). All chemicals used in this study were of analytical grades (BDH, England). The powdered plant materials were weighed on an electronic balance and were kept in a thimble. The thimble was then placed inside the Soxhlet's apparatus and requisite amounts of the solvents were poured into the apparatus. Extraction procedure was continued till the extract became colourless. When the extraction was completed for one solvent, the materials inside the thimble were dried up and then extraction was started with another solvent. The extracts were

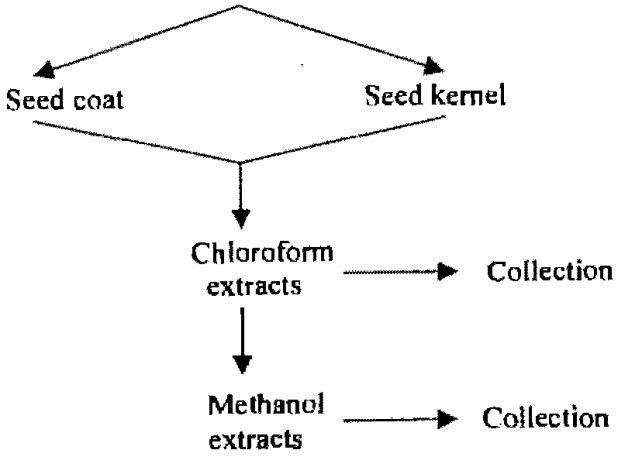
collected separately and the mixed solvent was removed from the extracts with a vacuum rotary evaporator under reduced pressure. The oily extracts of seed coat and kernel were labeled and stored in a refrigerator at 20°C. The scheme for a liquid extraction method to produce standardized and enriched plant seed coat and seed kernel extracts is outlined below:

Outline of extraction procedure



Scheme for a liquid extraction method to produce standardized and enriched plants seed coat and seed kernel

Collection of extracts in chloroform and methanol solvents from the plant materials



2.3. Test Organisms

The test organisms for medicinal tests were bacteria and fungi, and the brine shrimp, (*Artemia salina* Leach.), and the insecticidal tests were done on the lesser mealworm, *Alphitobius diaperinus* (Panzer).

2.3.1. Culture of bacteria

Collection of Bacteria: Fourteen pathogenic bacteria (five gram positive and nine gram negative) selected for the test were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh. The pure culture was previously collected from the Microbiology Department, Dhaka University, Bangladesh.

Table 1: List of test bacteria

Gram-positive	**Gram-negative**
1. *Bacillus megaterium*	1. *Shigella shiga*
2. *Bacillus cereus*	2. *Shigella dysenteriae*
3. *Staphylococcus aureus*	3. *Shigalla boydii*
4. *Sarcina lutea*	4. *Shigella sonnei*
5. *Streptococcus-β-haemolyticus*	5. *Salmonella typhi*
	6. *Escherichia coli*
	7. *Klebsiella* sp.
	8. *Pseudomonas aeruginosa*
	9. *Proteus* sp.

Apparatus and Reagents used

i. Filter paper discs (5mm diameter)

ii. Test tubes

iii. Petridishes (20 cm diameter)

iv. Sterile forceps
v. Sterile cotton wool
vi. Inoculating loop
vii. Bunsen burner
viii. Micropipette (10-100µl)
ix. Laminar Air Flow Unit (Biocraft and Scientific Industries, India).
x. Autoclave (ALP Co. Ltd. KT-301, Tokyo)
xi. Incubator (OSK, 9639 A, Japan).
xii. Punch machine
xiii. Beaker
xiv. Nutrient agar media (DIFCO)
xv. Alcohol (95%)
xvi. Methanol
xvii. Vials.

Sterilization Procedure: Antibacterial screening was done in Laminar hood and all types of other precautions were highly maintained to avoid any contamination by the organisms under test. Ultraviolet (UV) light has switched on before one hour of working in laminar hood to avoid any accidental contamination. Petridishes and other glasswares were sterilized by autoclaving at 121°C and a pressure of 15-lbs./sq. inch for 20 minutes. Blank or control discs were first kept in a covered petridish and then subjected to dry heat sterilization at 180°C for 1 hour. Later they were kept in laminar hood under UV light for 30 minutes.

Culture Media: The following media are usually used to demonstrate the antibacterial activity and to make subculture of the test organisms:

i. Nutrient agar medium
ii. Nutrient broth medium

iii. Muelle- Hinton medium

iv. Tryptic Soy broth (TSB) medium

v. Adams and Roe medium

vi. NIH agar or broth medium.

Preparation of Subculture: The media prepared were then dispensed in 20 ml and 5 ml to prepare plates and slants respectively in a number of clean tests. The slants were used for making fresh culture of microorganisms which in turn was used for sensitivity tests. The tubes were then plugged with cotton and sterilized in an autoclave at 121°C and a pressure of 15 lbs./sq. inch for 15 minutes. With the help of a inoculation loop the test organisms were transferred from the pure culture to the agar slants in an aseptic condition (a laminar airflow unit). The inoculated slants were then incubated at 37.5°C for 18.24 hr. to assure the growth of test organisms. This culture was used within one week (Bauer *et al.* 1966).

Preparation of Test Plates: The test organism was transferred from the subculture to the test tube containing 20ml autoclaved medium with the help of an inoculating loop in an aseptic area. The test tube was shaken by rotation to get a uniform suspension of the organism. The bacterial suspension was immediately transferred to the sterile petridishes in an aseptic area and was rotated several times, firstly clockwise and then anti-clock wise to assure homogeneous distribution of the test organisms. The depth of media into each petridish (20 cm diameter) was approximately 4 mm. After the medium had cooled to room temperature, it was stored in a refrigerator at 4°C (Bauer *et al.* 1966).

Preparation of Discs and Test Samples

Preparation of discs: Three types of discs were prepared for antibacterial screening. These are as follows:

a. Sample discs,

b. Standard discs, and

c. Control/blank discs.

a. Sample discs: Sterilized filter paper discs .5mm in diameter (BBL, Cocksville U.S.A.) were prepared with the help of punch machine and were taken in a blank petridish. Sample solution of desired concentration was applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for a few minutes for complete removal of solvent.

b. Standard discs: These are used to compare the antibacterial activity of test material. In our investigation, Ciprofloxacin (30µg/discs) standard disc was used as a reference.

c. Control /blank discs: These were used as negative control to ensure that the residual solvent and the filter paper were not active themselves. These were prepared in the previous manner applying only solvent to the discs and were used to examine the effect of solvent.

Preparation of Test Samples: Sample discs were prepared by dissolving 6 mg of each oily extract (seed coat and seed kernel) in 200 µl of methanol to get a concentration of 300 µg/10 µl. The solutions were applied on sterile blotting paper disc (5 mm diameter) to obtain the desired concentration.

Placement of the Discs, Diffusion and Incubation

The sample discs impregnated separately with the test material, standard antibiotic discs and control disc were placed gently on the solidified agar plates, freshly needed with the test organisms with the help

of sterile forceps to assure complete contact with the surface of the medium. The spatial arrangement of the discs was such that the discs were not closer than 15mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition.

The plates were then inverted and kept in a refrigerator for about 24 hours at 4°C to obtain maximum diffusion of the test material. Finally, the plates were incubated at 37°C for 12-18 hours.

Determination of Antibacterial Activity (Zone of Inhibition)

After 12 hours of incubation, the antibacterial activity of the test agents was determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale and compared with that of the standard disc.

Precautions

Necessary instrument and surrounding area were cleaned with absolute alcohol. Room heater was placed in order to prevent any pathogen contamination from outside.

2.3.2. Culture of Fungi

Collection of Fungi: Eight pathogenic fungi selected for the test were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh. The fungi were *Aspergillus fumigatus*, *Aspergillus flavus*, *Candida albicans*, *Colletotrichum falcatum*, *Fusarium oxysporum*, *Mucor* sp., *Penicellium* sp., and *Vasin factum*.

Necessary Equipment: The following equipment were used:

i) Glass petridish (20 cm diameter)

ii) Test tube (8-10 inch)

iii) Conical flask (1000 and 500ml)

iv) Needle with hook.

Cleaning of Glassware: Petridishes, tube, and conical flasks were regularly washed with a detergent solution. Distilled water was used for final wash and rinsed carefully. Pencil marks, paraffin and Canada balsam were removed effectively from the glassware with xylene. Sometimes the dirty or spotted glasswares were cleaned and washed with recommended cleaning solution, which was prepared in the laboratory as follow:

Potassium dichromate	100gm
Acid/sulfuric	500gm
Water	1000mg

Glasswares were sterilized in an autoclave for 15 to 20 minutes at 250°C and 15 lbs. pressure. Glasswares were also sterilized by dry heat in a hot air oven. The needle was sterilized in a flame, which was used for inoculation of fungal spores on surface of agar media.

Media For Growing Fungi

Fungi may be cultured on a variety of substrata or media. Such media may be either liquid or may be made solid by the addition of agar. Most fungi will grow in media high in carbohydrates, with a pH ranging between 5 and 6. There is no medium ideally suited for the culture of fungi because the nutritional requirements vary considerably with the species (Alexopo los and Beneke 1962). Fungi were cultured on Potato Dextrose Agar (PDA) media.

Composition: Composition of the Potato Dextrose Agar medium used was as follows:

Peeled and sliced potatoes (Old potatoes were used)	200gm
Sucrose	20 gm
Agar (Powder)	20gm
Distilled water	1000gm

Sliced potatoes were cooked in a beaker containing 500ml of water for 30 minutes. Extracts were filtered through a fine piece of cloth. Agar was added to the potato extract gradually while boiling. Boiling was continued till the agars melt, twenty gram of sucrose was dissolved and restored to 1000ml volume with distilled water.

Sterilization: Since media were prepared in order to grow fungi in pure culture, it was necessary to sterilize the media before using in order to kill any bacterial or fungal spores, if present. Sterilization was accomplished by placing the media in an autoclave and steaming them for 15-20 minutes at 121°C and 15 P.S.I. pressure. Glasswares such as tubes were also sterilized in the autoclave. A flame-sterilized needle was used for inoculation of fungal spores onto the surface of agar media.

Preparation of the test plates, discs, test sample, placement of the discs, diffusion and incubation

Preparation of the test plates, discs, test samples, placement of the discs, diffusion and incubation processes were almost the same as done for the antibacterial screening. Here, only the incubation period was replaced by 48 hours at room temperature.

Precautions: Necessary instrument and surrounding area were cleaned with absolute alcohol. Room heater was placed in order to prevent any pathogen contamination from out side.

2.3.3. Culture of Brine Shrimp

Collection of eggs: *Artemia salina* Leach. eggs were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh.

Necessary equipment

i. Sea salt (NaCl)
ii. Small tank with perforated dividing partition, cover and lamp to hatch the shrimps.
iii. Pipettes (5 ml, 1 ml).
iv. Micropipettes (10µl and 100µl)
v. Glass vials (10ml).
vi. Magnifying glass.

Procedure

Preparation of Simulated Seawater: Since the lethality test involves the culture of brine shrimp nauplii, the nauplli should grow in seawater. Seawater nature contains 3.8% sodium chloride and hence 38.0 gm sea salt was weighed, dissolved in one liter of distilled water and filtered off.

Hatching of Shrimps: Simulated seawater was taken in a small tank. Shrimp eggs were added to the divided tank. A constant oxygen supply and a constant temperature (around 37°C) were maintained. Two days were need for hatching of shrimp eggs, and mature nauplii were collected.

Application of the Sample and Brine Shrimp Nauplii to the Vials

Clean vials were taken for the 10 samples in five concentrations (two vials for each concentration) and 10 vials were also taken for control test. Five ml of seawater containing 10 brine shrimp nauplii was kept in each vial. With the help of a micropipette specific volumes of samples were

transferred from the stock solutions to the vials to get final concentration of 5.0, 10.0, 20.0, 40.0, and 80.0 µg/ml. The concentration of DMSO in these vials was not allowed to exceed 50µl/5ml of brine, because above this concentration cytotoxicity due to DMSO may arise.

In the control vials the same volume of DMSO (as in the sample vials) and 5 ml of seawater were taken.

Counting of Nauplii: After 24 hours, the contents vials were examined, and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

Analysis of Data

The dose-mortality data were analyzed statistically. The effectiveness of the dose mortality relationship (concentration-mortality relationship) of any sample is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

In this investigation, mortality in the control was not adjusted or corrected because control mortality was during the exposure periods.

2.3.4. Culture of *Alphitobius diaperinus*

Necessary equipments

For maintaining the culture of beetles, the following laboratory materials were used:

I. Plastic jars,	II. Beakers,	III. Petri dishes,
IV. Sable hair brush,	V. Pieces of cloth,	VI. Spoons,
VII. Sieves,	VIII. Rubber bands,	IX. Potato slices, etc.

All the materials mentioned above were sterilized by keeping in an oven for about six hours at 120°C (Khan 1981).

Collection of beetles

A. diaperinus adults were collected from the storehouse of a flour mills at Saheb Bazar, Rajshahi.

Culture of beetles

The insect used in the present study was the lesser mealworm, *A. diaperinus*. This is a common pest of stored grains all over the tropical and sub-tropical countries of the world including Bangladesh. It is easily obtainable.

About 1500 beetles were collected for culture. The beetles were sieved through U.S. standard No. 16 and 20 sieves. The healthy and active beetles were taken in plastic jars covered with a cloth, secured with a rubber band at the top. Slices of potato were kept with in jars for humidity control, which were replaced when necessary. Several sub-cultures were maintained, each containing 100 beetles in 250g of food in a jar.

Collection of eggs

Adult beetles were put in Petri dishes (9 cm diameter) containing wheat flour. On the next day, eggs were collected by sieving the medium with sieves of 500 and 250 micrometer apertures (Khan and Selman 1981). The eggs were placed in a Petri dishes (9.5 cm diameter) and incubated at room temperatures.

Transfer of larvae to the food

The larvae hatched out in 3-4 days which were collected by a fine sable hair brush. Newly hatched larvae were transferred to the jars (20 cm × 8 cm) containing wheat flour with the aid of a hair brush. Three hundred larvae were used for each food and the experiment was replicated four times. Slices of potato were kept inside the jars to maintain humidity which were changed weekly. Larvae were checked from time to time for pupation.

Collection of pupae and adults

The young pupae were collected with a fine sable hair brush and transferred to a Petridish for the emergence of adults.

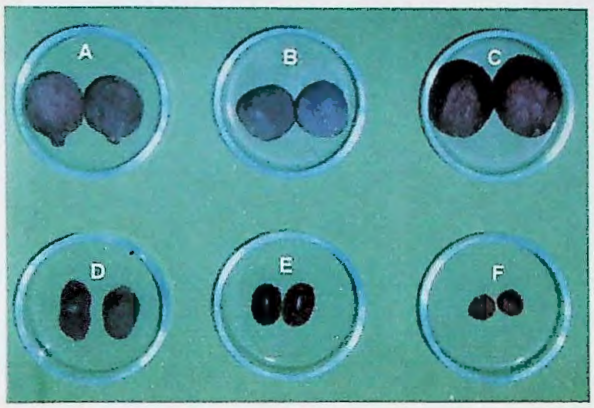


Plate 7a: Mature seeds

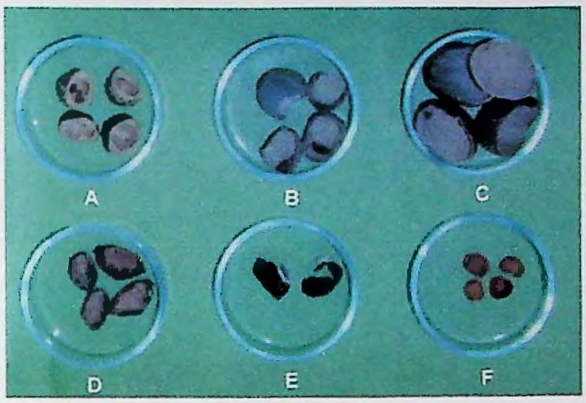


Plate 7b: Seeds coats

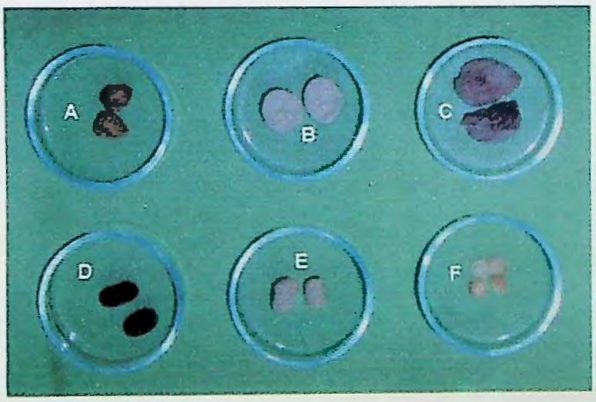


Plate 7c: seeds kernels

Plate 7d: Dust of seed coats

Note: A.= Bahera; B= Natai; C= Jayfal;
D= Kalojam; E= Alkushi and F= Rakta chandan

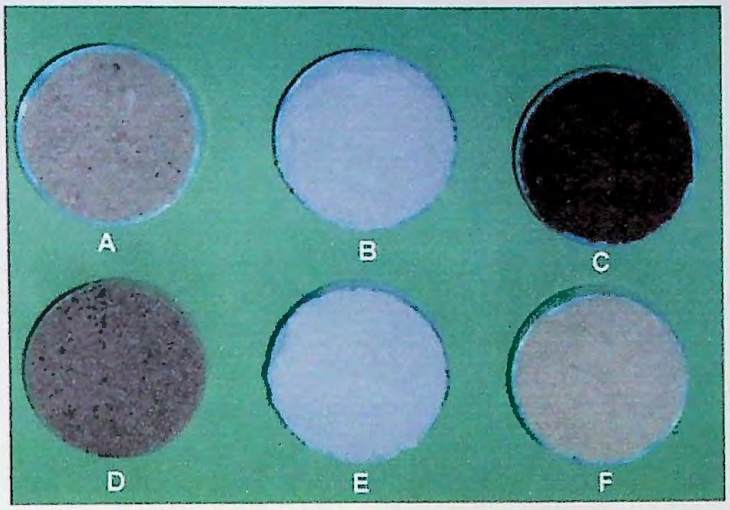


Plate 7e: Dust of seed kernels

Note: A.= Bahera; B= Natai; C= Jayfal; D= Kalojam; E= Alkushi and F= Rakta chandan

Plate 8: Extract preparation

Plate 9: Shaking of extracts on shaker

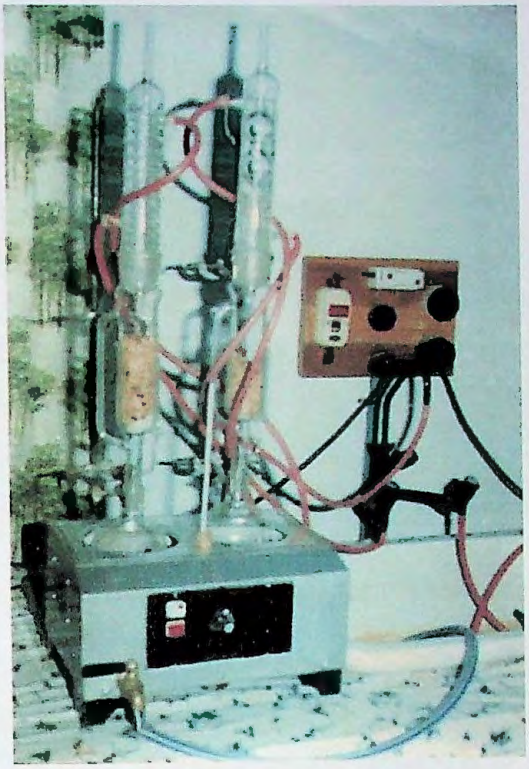


Plate 10: Plant seed extraction in a Soxhlet's apparatus

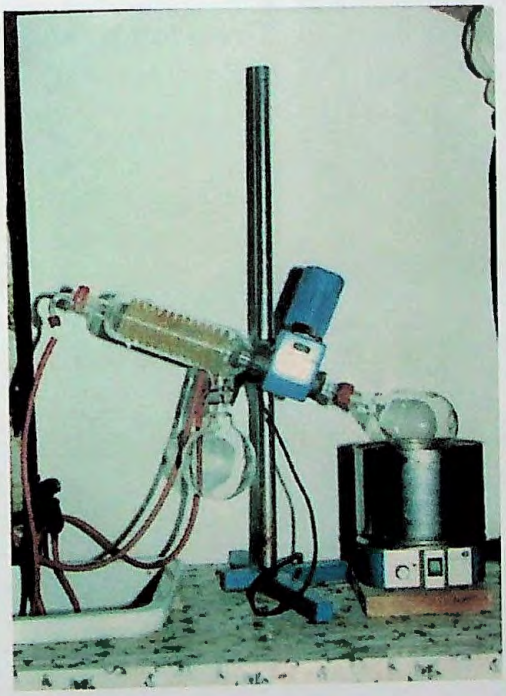


Plate 11: Evaporation of mixed solvent from extraction by a Vacuum Rotary Evaporator

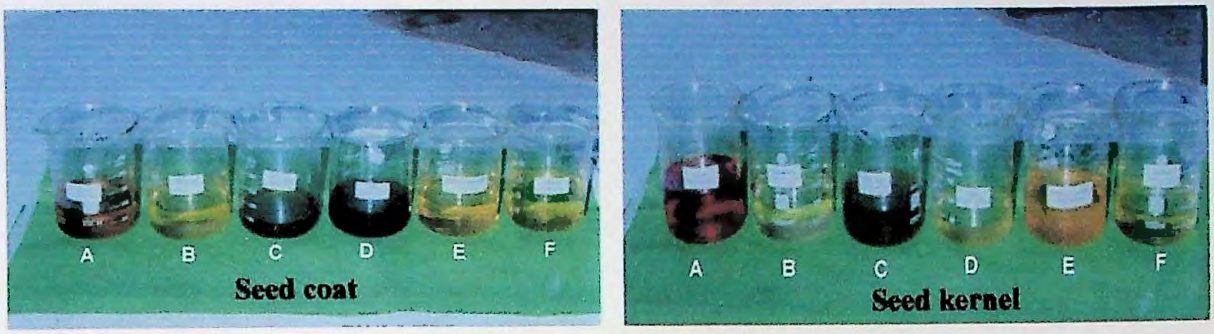


Plate 12: Chloroform extracts of seed coat and seed kernel with solvent

Note: A.= Bahera; B= Natai; C= Jayfal; D= Kalojam; E= Alkushi and F= Rakta chandan

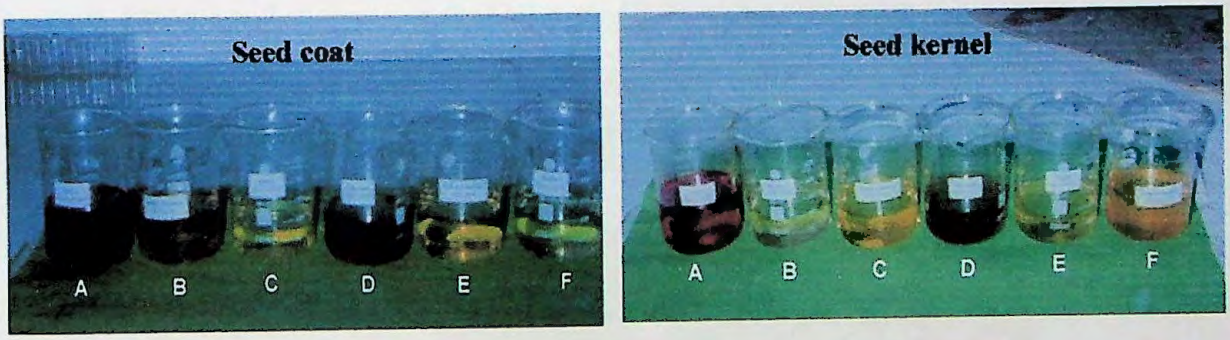


Plate 13: Methanol extracts of seed coat and seed kernel with solvent

Note: A.= Bahera; B= Natai; C= Jayfal; D= Kalojam; E= Alkushi and F= Rakta chandan

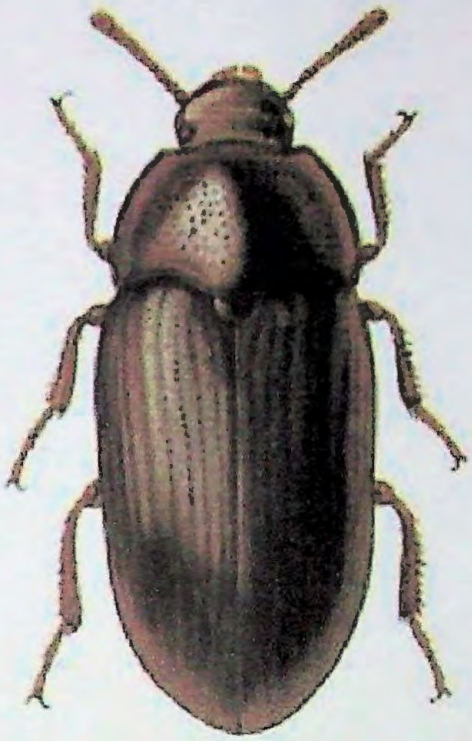
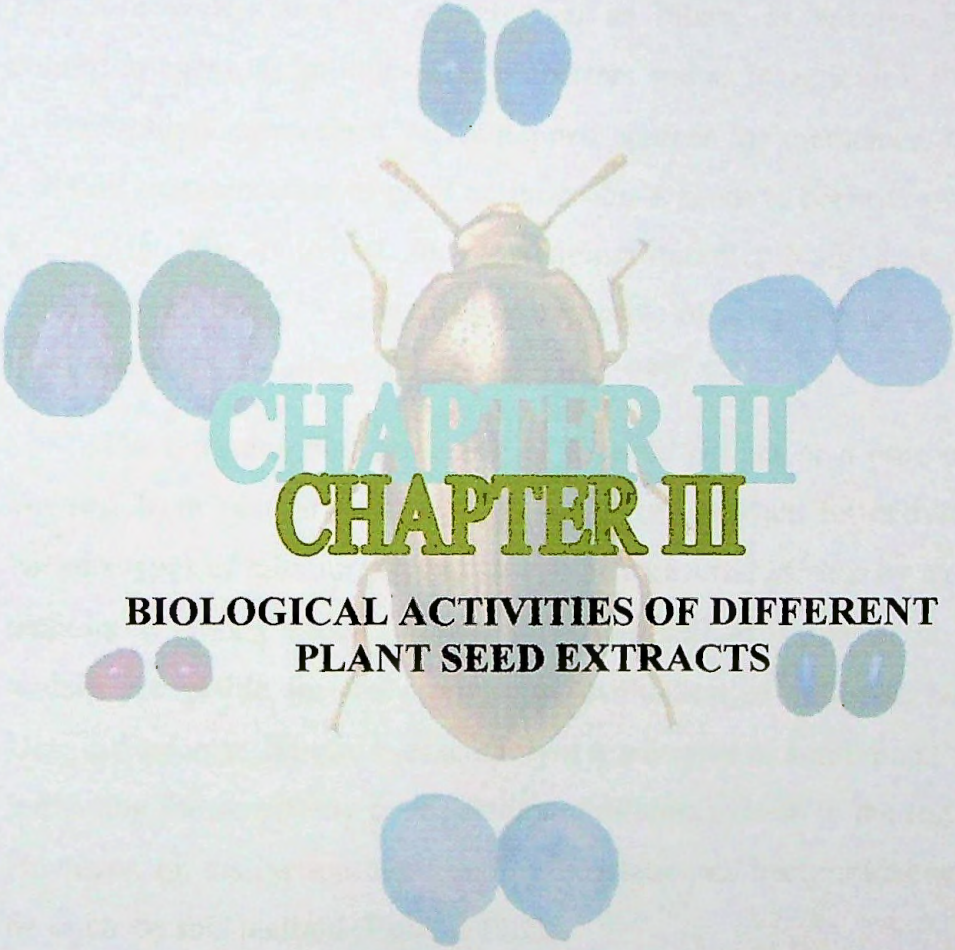


Plate 14: Adult lesser mealworm, *Alphitobius diaperinus* (Panzer)

Plate 15: Mass culture of *Alphitobius diaperinus* (Panzer)



CHAPTER III

BIOLOGICAL ACTIVITIES OF DIFFERENT PLANT SEED EXTRACTS

BIOLOGICAL ACTIVITIES OF DIFFERENT PLANT SEED EXTRACTS

3.1.Introduction

Medicinal plants help in alleviating human suffering. These plants are being integrated to the field of foods as additives, beverages and cosmetics. They are widely used as sweeteners, as biters, as species, as natural colouring agent, as antimicrobial properties and as insecticides. Plant based antimicrobials represent a vast untapped sources for medicines. Continued and further exploration of plant antimicrobials needs to occur (Farnsworth *el al.* 1991). The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines (Iwu *el al.* 1999).

The antimicrobial screening of a crude extract or a pure compound isolated from natural sources is essential to ascertain its activity against various types of microorganisms. It can be measured *in vitro* by a number of techniques among which the disc diffusion method (Bauer *et al.* 1966), is widely acceptable for the preliminary evaluation of antibacterial activity. Disc diffusion technique is essentially a qualitative or semi quantitative test indicating the sensitivity or resistance of microorganism to the test material. However, no distinction between bacteriostatic and bactericidal activity can be made by this method (Roland 1982).

3.2. Antibacterial activity

In the present investigation the antimicrobial activities of the plant seeds was determined.

Materials and Methods

The antibacterial activity of plant seed coats and seed kernels were performed using the disc diffusion assay method of Calabrasi and Chabnep (1996). The Gram-positive bacteria, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Sarcina lutea*, *Streptococcus β-haemolyticus* and the gram-negative bacteria *Shigella shiga*, *S. dysenteriae*, *S. boydii*, *S. sounei*, *Salmonella typhi*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonus aeruginosa*, and *Proteus* sp. were used as the test organisms to determine the medicinal effect of the title plants. The initial stocks were collected from the Microbiology Laboratory, Department of Pharmacy, University of Rajshahi.

Nutrient Agar Media (DIFCO) and Nutrient Broth Media were used in the present investigation. The methods given by Bauer *et al.* (1966) were followed for media preparation, bacterial culture initiation and for the maintenance of culture.

The instant nutrient agar media was accurately weighed and then reconstituted with distilled water in a conical flask according to specification (2.3%). It was then heated in water bath to dissolve the agar until a clear solution of medium was obtained. The medium was then transferred in 20 ml and 5 ml respectively to 20 cm petridishes and 15 ml culture tubes to prepare plates and slants, respectively, in a number of required petridishes and test tubes. These slants were used for making fresh culture of microorganisms, which in turn were used for sensitivity tests. The lest tubes were then plugged with cotton and sterilized in an autoclave at 121°C and a pressure of 15 lbs/sq/inch for 15 minutes. After sterilization, the test tubes were kept in an inclined position for solidification.

With the help of inoculating loop, the test organisms from the pure cultures were transferred to the agar slants in an aseptic condition. The inoculated slants were then incubated at 37°C for 18-24 hrs to assure the growth of test organisms. This culture was used for the initiation of fresh culture for sensitivity test.

Determination of the antibacterial activity: After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones in term of millimeter (mm).

Determination of minimum inhibitory concentration: The minimum inhibitory concentration (MIC) is the lowest concentration of the test sample or drug at which it shows the highest activity against microorganisms. The minimum inhibitory concentration was determined by "Serial dilution technique" using nutrient broth media (Roland *et al.* 1982). The minimum inhibitory concentration of oily extract of seed coats and seed kernels were determined against gram positive (*Bacillus cereus, Bacillus megaterium, Staphylococcus aureus*) and gram negative (*Shigella sonnei* and *Shigella dysenteriae*) bacteria. Nutrient agar and nutrient broth (DIFCO) were used as bacteriological media.

Preparation of the solution: Each of the oily extract of seed coat and seed kernel of 2.048 mg were taken in two different vials. Then, 2 ml of suitable solvent was added to each of the vials and agitated well to make sample solution. Thus, solutions with a concentration of 1024 µg/ml were obtained for each sample.

Preparation of the inoculums: The test organisms were grown overnight at 37.5°C in nutrient broth medium. The agar medium with the organism was

diluted in such a way that the medium contained about 10^7 cells/ml. This suspension was used as inocula.

The following procedure was followed:

1. Twelve autoclaved test tube were taken, of which nine were marked 1, 2, 3, 4, 5, 6, 7, 8, 9 and the rest three were assigned as C_M (medium), C_S (medium + sample) and C_I (medium + inoculums).
2. To each of twelve test tubes, 1 ml of sterile nutrient broth medium was added.
3. Then to the first test tube, 1 ml of the sample solution was added and mixed well.
4. One ml content from the first test tube was transferred to the second test tube, was mixed uniformly and again 1 ml of this mixture was transferred to the third test tube. This process of serial dilution was continued up to the ninth test tube.
5. Then 10 µl of the diluted inoculum of *B. cereus* (10^7 cells/ml) was added to each of the nine test tubes and mixed well.
6. One ml of the sample solution was added to the control test tube, C_S and mixed well and 1 ml of this mixed content was discarded. This was done to check the clarity of the medium in presence of diluted solution of the compound.
7. Ten µl of the inoculum (10^7 cells/ml) was added to the control test tube C_i to observe the growth of the organism in the medium used.
8. The control test tube C_M containing the medium only was used to confirm the sterility of the medium.
9. At last all the test tubes were incubated at 37.5 °C for 12-18 h.

The same procedure was also applied to determine the minimum inhibitory concentration against *Bacillus megaterium*, *Staphylococcus*

aureus. Streptococcus-β-haemolyticus and *Pseudomonus aeruginosa* for each of the sample. The results are given in **Tables** 8 and 9.

3.3. Anti fungal activity

The same procedure as used for the antibacterial activity test was performed for the antifungal activity of the oily extract of seed coat and seed kernel. The only difference was that the period of incubation was 48 h at room temperature.

Three identified fungi collected from the Department of Pharmacy, University of Rajshahi were used for the test of antifungal activity. The fungi are listed below:

1. *Aspergillus fumigatus*
2. *Aspergillus flavus*
3. *Candida albicans*
4. *Colletotrichum falcatum*
5. *Fusarium oxysporum*
6. *Mucor* sp.
7. *Penicellium* sp.
8. *Vasin factum*

Potato Dextrose Agar (PDA) medium was used for fungal culture.

3.4. Brine shrimp lethality bioassay

Toxicity of oily extracts of seed coats and seed kernels were determined by using Brine Shrimp Lethality Bioassay (Persoone 1980, Meyer *et al.* 1982). The eggs of shrimps were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh.

Preparation of stimulated seawater: Thirty-eight gram of sea salt (NaCl, non-iodized) was dissolved in distilled water to make 1000 ml and then filtered off. The pH of seawater was maintained between 8.0 and 9.0 by using $NaHCO_3$.

Hatching of the brine shrimp eggs: Seawater was kept in small tank and shrimp eggs were added (1.5 gm/1) to one side of the divided tank. Constant temperature (35 ± 2°C), sufficient light and air supply were maintained to give the sufficient aeration. After 48 h, matured nauplii were collected and used for the experiment.

Preparation of the sample solution: One milligram of each of the sample of seed extract and standard antibiotic amphicilin trihydrate were dissolved in 200 µl DMSO to get a concentration of 5 µg/ml for each of the sample.

Application of test sample and brine shrimp nauplii to the vials: The experiment was done into five groups for each sample. Each group contained three vials consisting of 10 nauplii in 5 ml of sea water. The concentrations of the sample in each vial of the group was made 5, 10, 20, 40 and 80 µg/ml respectively. For control, group three vials containing 10 brine shrimp nauplii in 5 ml seawater were taken and 20µl DMSO was added to each vial.

Counting of nauplii and data analysis: After 24 h, the vials were examined and the number of surviving nauplii in each vial was counted using magnifying glass and the results were noted. The median lethal concentration (LC_{50}) was calculated using Probit analysis. Regression lines were drawn by plotting log concentration versus probit mortality (Goldstein 1974) for the compounds ampicillin trihydrate, oily extract of seed coat and seed kernel separately.

3.5. Results and Discussion

The biological activities of the different plant seed extracts were assayed against gram positive and gram negative pathogenic bacteria, fungi and brine shrimps. The results of these experiments are discussed below under separate headings.

Antibacterial activity

The antibacterial activities of the oily extracts of seed coat and seed kernel were determined at a concentration of 50 and 200 µg/disc against a total of 5 gram-positive and 9 gram-negative pathogenic bacteria and compared with standard antibiotic Ciprofloxacin (30 µg/disc). Zone of inhibition was prominent for the control Ciprofloxacin at both concentration 50 and 200 µg/disc. Clear inhibition zone was also observed for both seed oil at both the concentration for most of the pathogenic bacteria. However, the degree of bactericidal properties of seed coat and seed kernel was observed to be different. At 200 µg/disc CH_3OH extract exhibited bigger and more prominent clear zone than 50 µg/disc. At 200 µg/disc of oily extracts of Joyfal and Kalojam seed coat and seed kernel were more effective than the other extracts. The oily extracts of the seed coat exhibited largest and more prominent clear zone at 200 µg/disc (Table- 2, 3, 4, 5, 6 and 7) (Plate- 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 and 29).

Table-2. Antibacterial activity of Methanol and Chloroform extracts of seed of *Caesalpinia bonduc* (L.) and standard Ciprofloxacin

	Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	-	-	-	-	-	7	-	-	34
	Bacillus cereus	-	13	-	-	-	-	-	7	33
	Staphylococcus aureus	-	10	-	-	-	-	-	-	35
	Sarcina lutea	-	12	-	-	-	-	-	-	34
	Streptococcus-β-haemolyticus	-	10	-	-	-	7	-	-	33
Gram negative bacteria	*Shigella shiga*	-	9	-	-	-	10	-	-	34
	Shigella dysenteriae	-	10	-	-	-	12	-	-	33
	Shigella boydii	-	13	-	-	-	9	-	-	34
	Shigella sonnei	-		-	-	-	-	-	-	33
	Salmonella typhi	-	8	-	-	-	-	-	-	33
	Escherichia coli	-	9	-	-	-	-	-	-	34
	Klebsiella sp.	7	15	-	-	-	-	-	-	35
	Pseudomonas aeruginosa	-	-	-	-	-	10	-	-	34
	Proteus sp.	-	-	-	-	-	-	-	-	34

Table-3. Antibacterial activity of Methanol and Chloroform extracts of seed of *Mucuna pruriens* (L.) and standard Ciprofloxacin

	Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	-		-	-	-	-	-	-	33
	Bacillus cereus	-	-	-	-	-	-	-	-	34
	Staphylococcus aureus	-	-	-	-	-	-	-	-	34
	Sarcina lutea	-	-	-	-	-	-	-	-	35
	Streptococcus-β-haemolyticus	-	-	-	-	-	-	-	-	33
Gram negative bacteria	*Shigella shiga*	-	-	-	-	-	-	-	-	35
	Shigella dysenteriae	-	8	-	9	-	-	-	-	34
	Shigella boydii	-	-	-	-	-	-	-	-	33
	Shigella sonnei	-	-	-	-	-	-	-	-	34
	Salmonella typhi	-	-	-	-	-	-	-	-	33
	Escherichia coli	-	7	-	-	-	9	-	-	34
	Klebsiella sp.	-	-	-	-	-	-	-	-	34
	Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	35
	Proteus sp.	-	-	-	-	-	-	-	-	34

Table-4. Antibacterial activity of Methanol and Chloroform extracts of seed of *Adenanthera pavonina* (L.) and standard Ciprofloxacin

Test Organisms µg disc $^{-1}$		Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	-	13	-	9	-	8	-	-	35
	Bacillus cereus	-	7	-	-	-	8	-	-	33
	Staphylococcus aureus	-	-	-	-	-	-	-	-	34
	Sarcina lutea	-	11	-	-	-	-	-	-	34
	Streptococcus-β-haemolyticus	7	17	-	-	-	7	-	-	34
Gram negative bacteria	*Shigella shiga*	-	10	-	-	-	9	-	-	35
	Shigella dysenteriae	-	10	-	-	-	8	-	10	34
	Shigella boydii	-	9	-	-	-	10	-	-	34
	Shigella sonnei	-	14	-	-	-	8	-	-	33
	Salmonella typhi	7	15	-	-	-	-	-	-	34
	Escherichia coli	-	14	-	13	-	10	-	-	33
	Klebsiella sp.	-	13	-	-	-	-	-	-	34
	Pseudomonas aeruginosa	-	11	-	8	-	10	-	-	35
	Proteus sp.	-	14	-	-	-	7	-	-	35

Table-5. Antibacterial activity of Methanol and Chloroform extracts of seed of *Terminalia belerica* (Roxb.) and standard Ciprofloxacin

Test Organisms µg disc $^{-1}$		Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	-	-	-	-	-	-	-	-	33
	Bacillus cereus	-	-	-	-	-	-	-	7	34
	Staphylococcus aureus	-	-	-	-	-	-	-	-	34
	Sarcina lutea	-	-	-	-	-	-	-	-	35
	Streptococcus-β-haemolyticus	-	10	-	-	-		-	-	34
Gram negative bacteria	*Shigella shiga*	-	-	-	-	-	-	-	-	35
	Shigella dysenteriae	-	-	-	-	-	-	-	-	33
	Shigella boydii	-	-	-	-	-	-	-	-	34
	Shigella sonnei	-	-	-	-	-	-	-	-	34
	Salmonella typhi	-	-	-	-	-	-	-	8	33
	Escherichia coli	-	-	-	-	-	-	-	-	35
	Klebsiella sp.	-	-	-	-	-	-	-	-	33
	Pseudomonas aeruginosa	-	9	-	-	-	9	-	-	34
	Proteus sp.	-	-	-	-	-	-	-	-	35

Table-6. Antibacterial activity of Methanol and Chloroform extracts of seed of *Syzygium cumini* L. and standard Ciprofloxacin

	Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	-	-	-	-	-	9	-	11	34
	Bacillus cereus	-	8	-	13	-	7	-	10	35
	Staphylococcus aureus	-	10	-	8	-	7	-	12	33
	Sarcina lutea	-	10	-	13	-	8	-	9	35
	Streptococcus-β-haemolyticus	-	14	7	15	-	8	-	9	34
Gram negative bacteria	*Shigella shiga*	-	-	-	8	-	-	-	13	34
	Shigella dysenteriae	-	10	8	17	-	-	7	15	33
	Shigella boydii	-		-	10	-		-	8	35
	Shigella sonnei	-	14	-	10	-	9	-	11	33
	Salmonella typhi	-	9	-	14	-	-	-	13	34
	Escherichia coli	-	11	8	19	-	-	-	12	34
	Klebsiella sp.		12	7	18	-	8	-	10	33
	Pseudomonas aeruginosa	-	-	-	11	-	9	-	14	34
	Proteus sp.	-	10	-	9	-	-	-	8	33

Table-7. Antibacterial activity of Methanol and Chloroform extracts of seed of *Myristica fragrans* (Houtt.) and standard Ciprofloxacin

	Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
		Methanol extract				Chloroform extract				Ciprofloxacin 30 µg/disc
		Seed coat		Seed kernel		Seed coat		Seed kernel		
		50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria	*Bacillus megaterium*	7	16	-	13	-	9	-	11	35
	Bacillus cereus	-	14	-	10	-	8	-	9	34
	Staphylococcus aureus	8	20	7	15	-	8	-	7	34
	Sarcina lutea	7	18	-	11	-	11	-	9	34
	Streptococcus-β-haemolyticus	-	12	-	13	-	10	-	7	35
Gram negative bacteria	*Shigella shiga*	-	14	7	15	-	14	-	8	33
	Shigella dysenteriae	-	13	-	14	-	8	-	11	33
	Shigella boydii	-	14	-	13	-	10	-	11	35
	Shigella sonnei	7	18	-	13	-	8	-	10	34
	Salmonella typhi	-	10	-	13	-	9	-	-	34
	Escherichia coli	-	13	7	15	-	-	-	10	35
	Klebsiella sp.	-	13	-	10	-	13	-	10	35
	Pseudomonas aeruginosa	-	14	-	13	-	11	-	9	35
	Proteus sp.	-	8	-	11	-	9	-	11	34

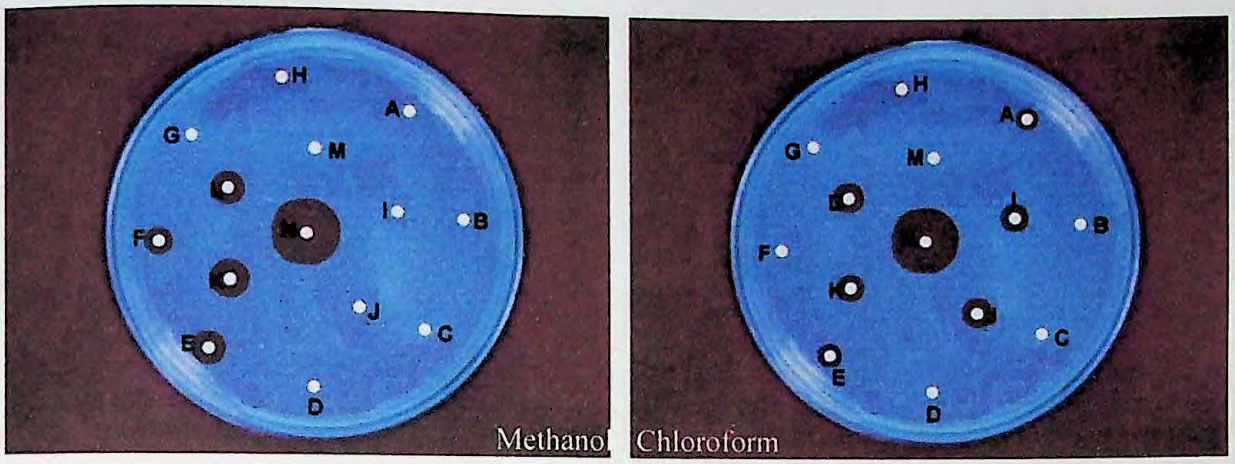


Plate 16: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Bacillus megaterium*

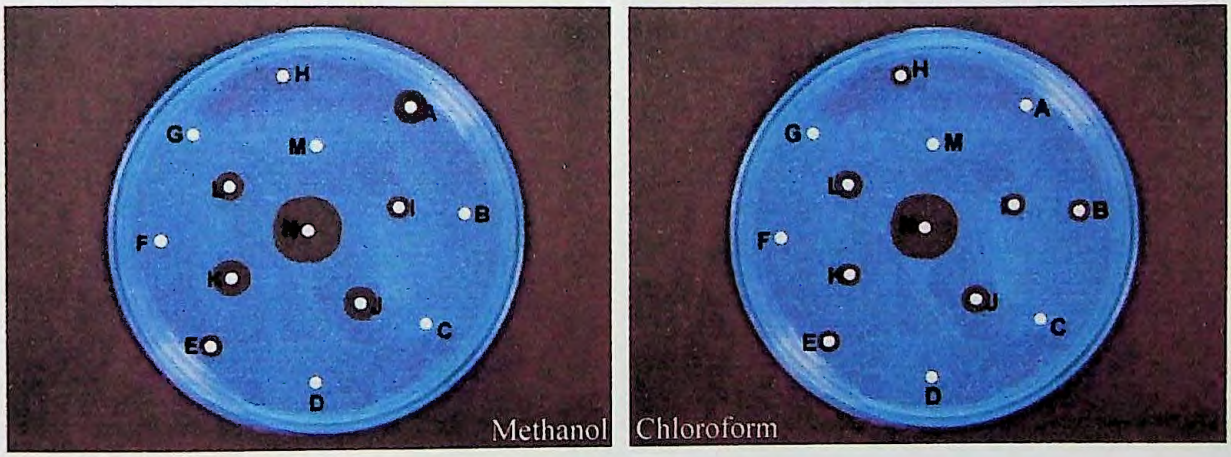


Plate 17: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Bacillus cereus*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

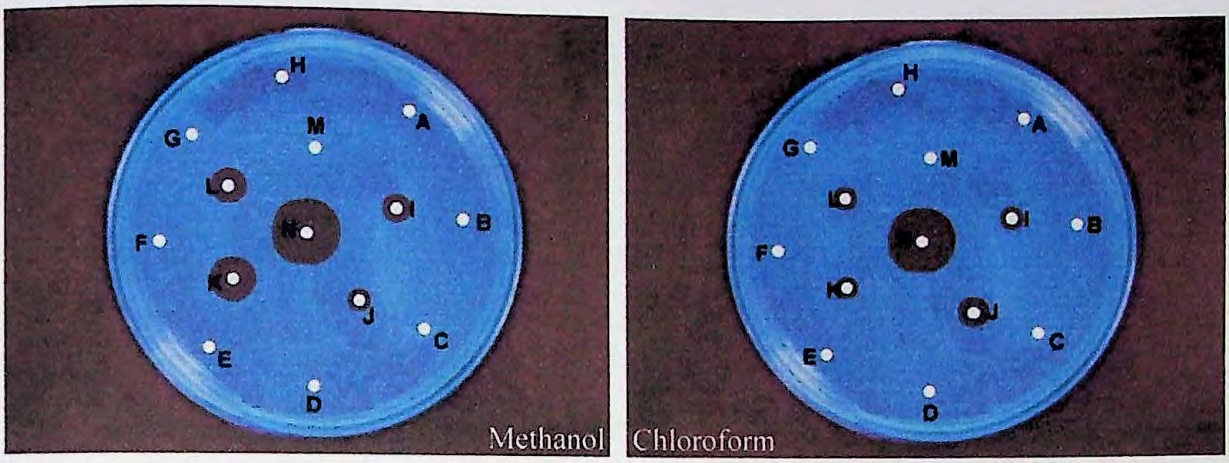


Plate 18: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Staphylococcus aureus*

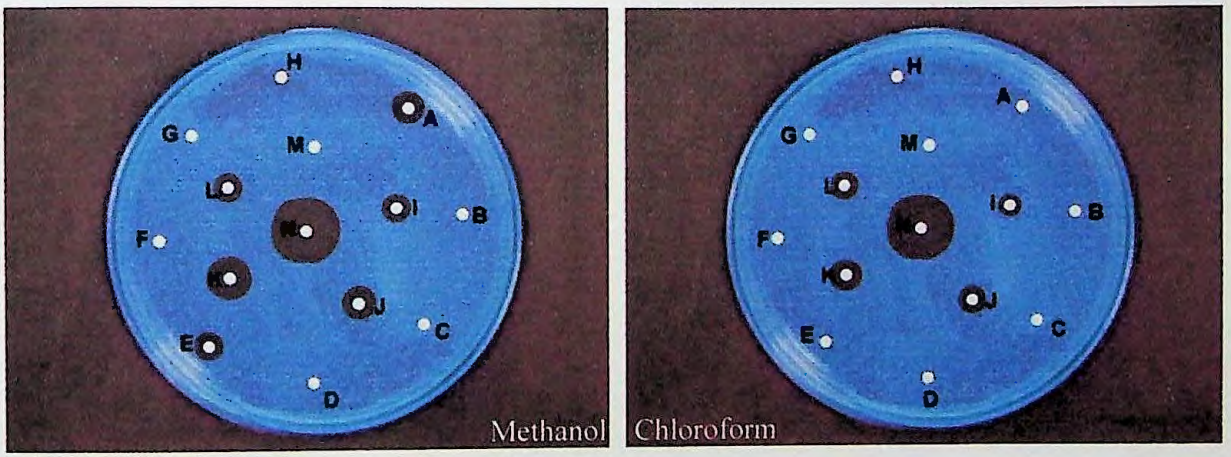


Plate 19: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Sarcina lutea*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

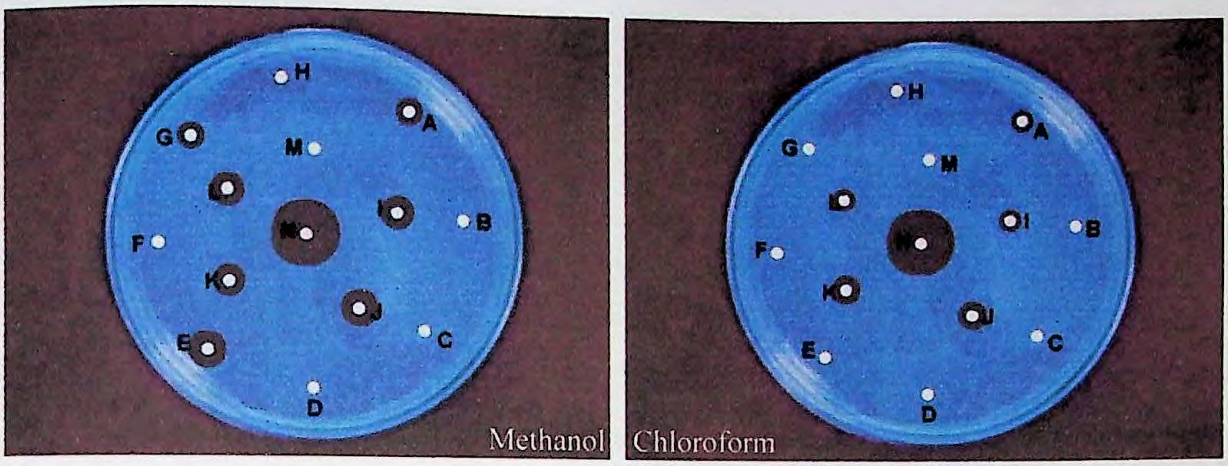


Plate 20: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Streptococcus-β-haemolyticus*

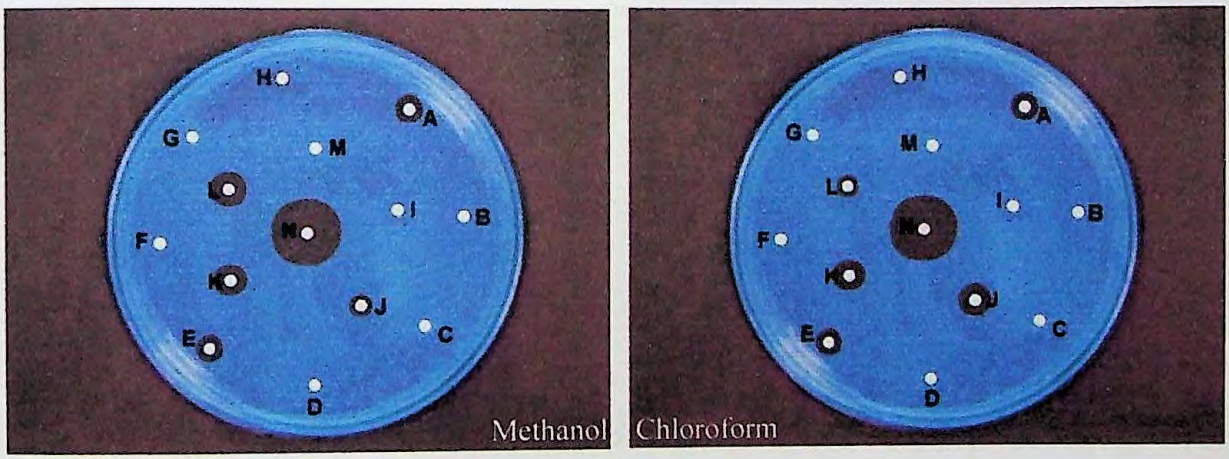


Plate 21: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Shigella shiga*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

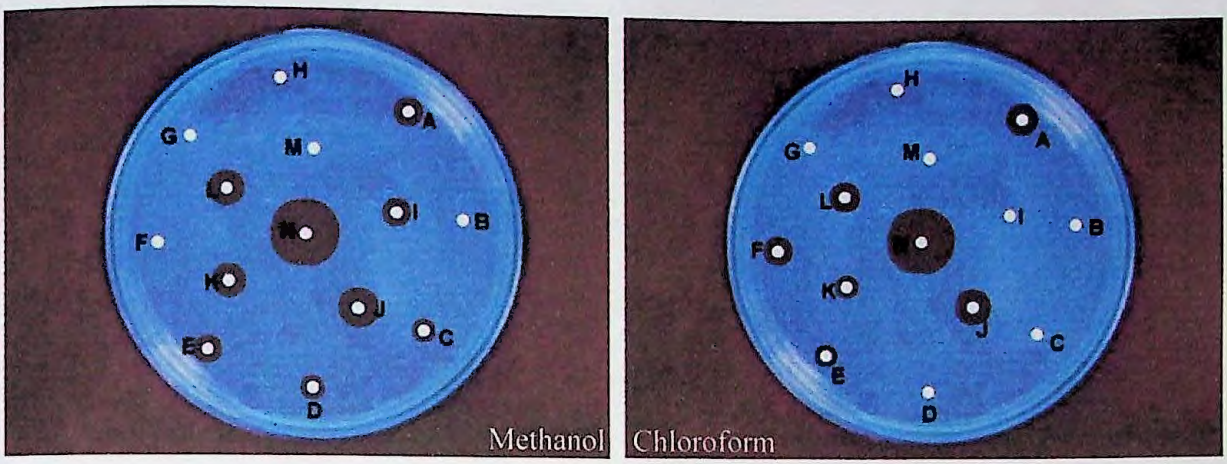


Plate 22: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Shigella dysenteriae*

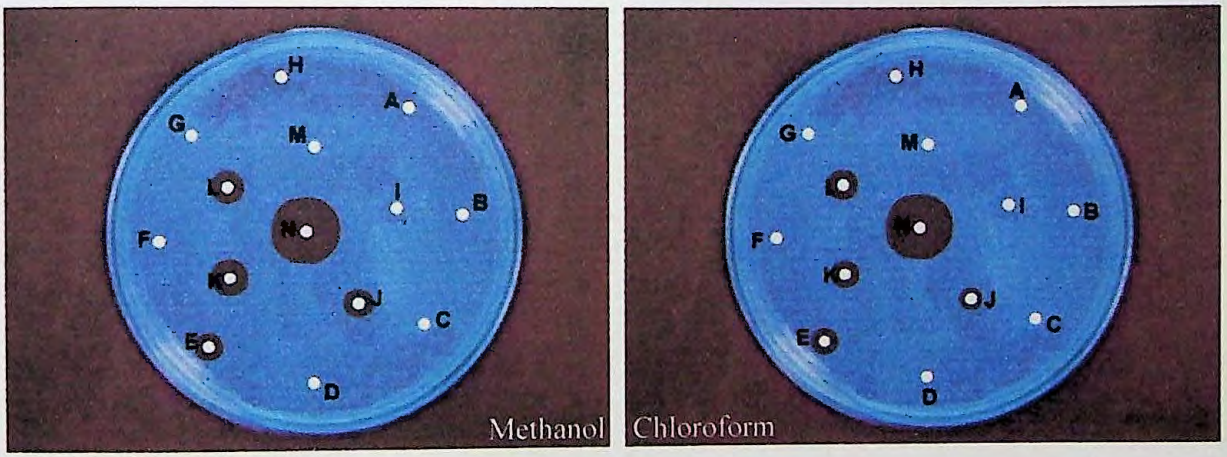


Plate 23: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Shigella boydii*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

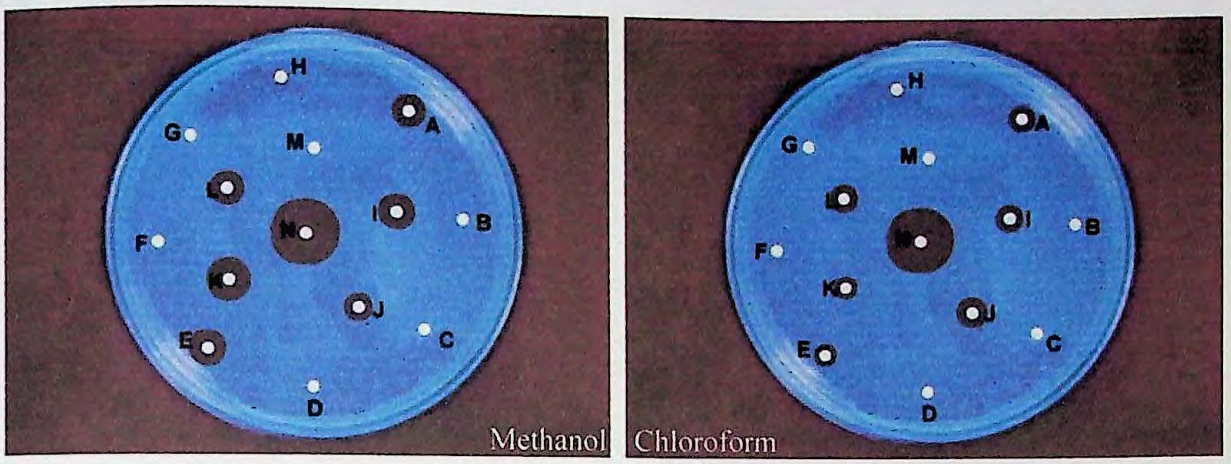


Plate 24: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Shigella sonnei*

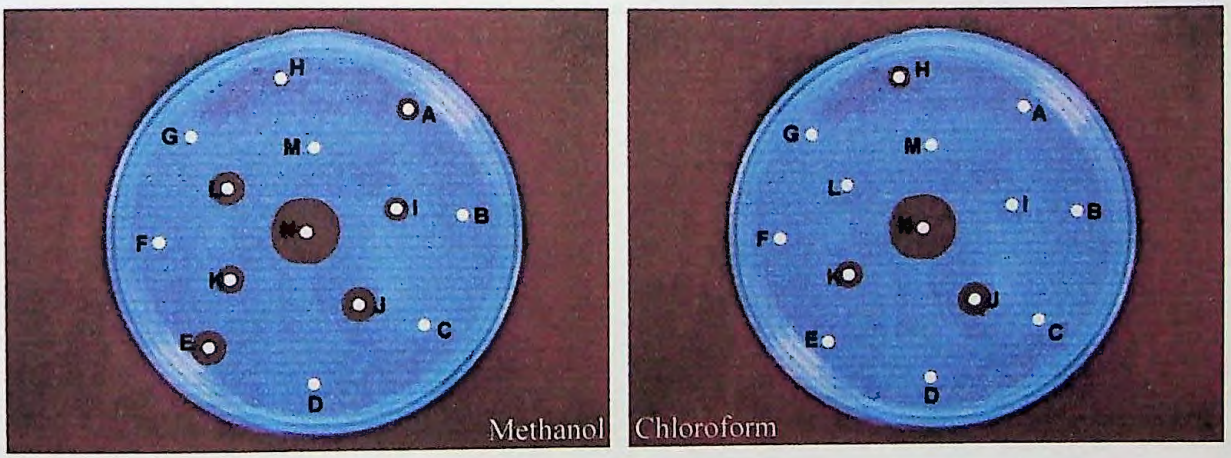


Plate 25: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Salmonella typhi*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

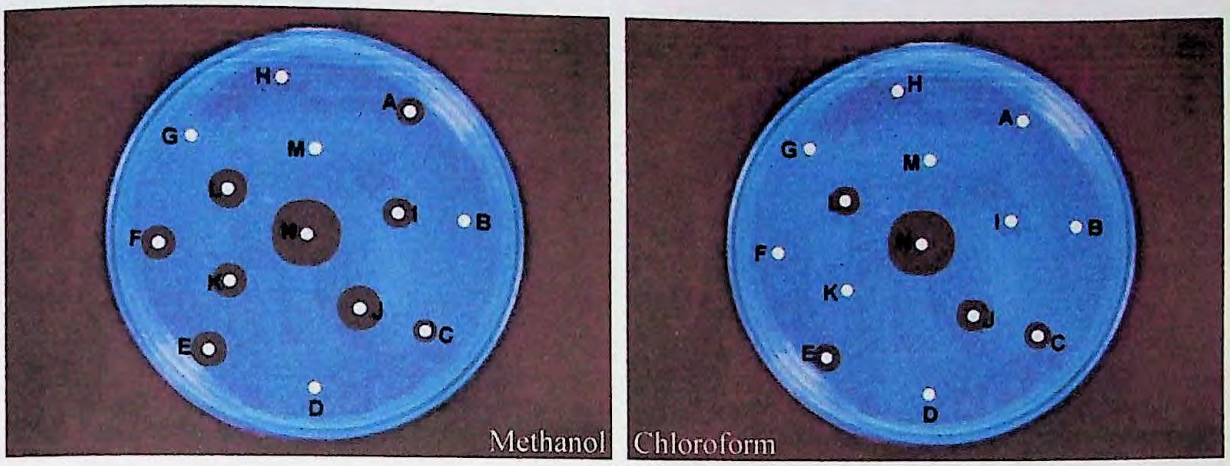


Plate 26: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Escherichia coli*

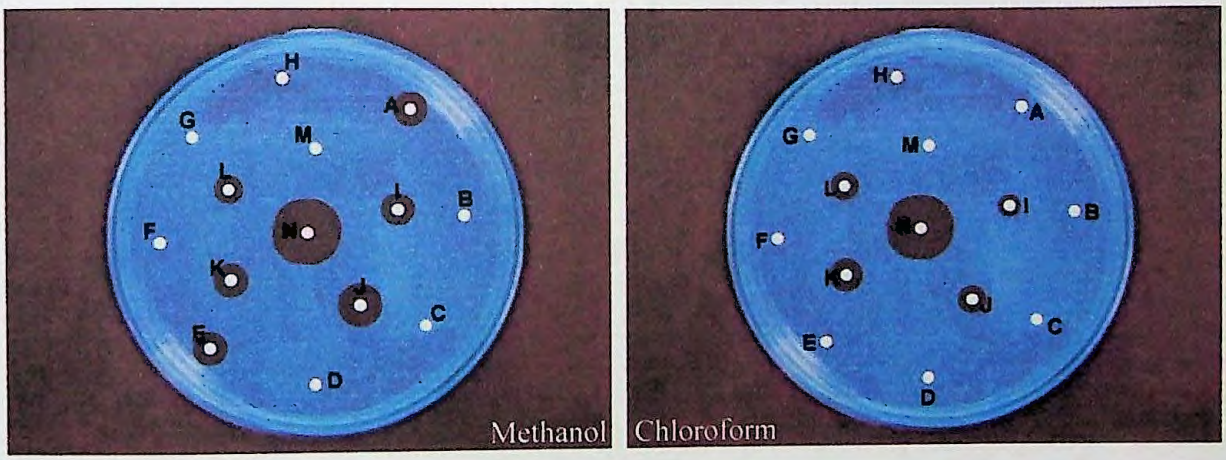


Plate 27: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Klebsiella* sp.

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

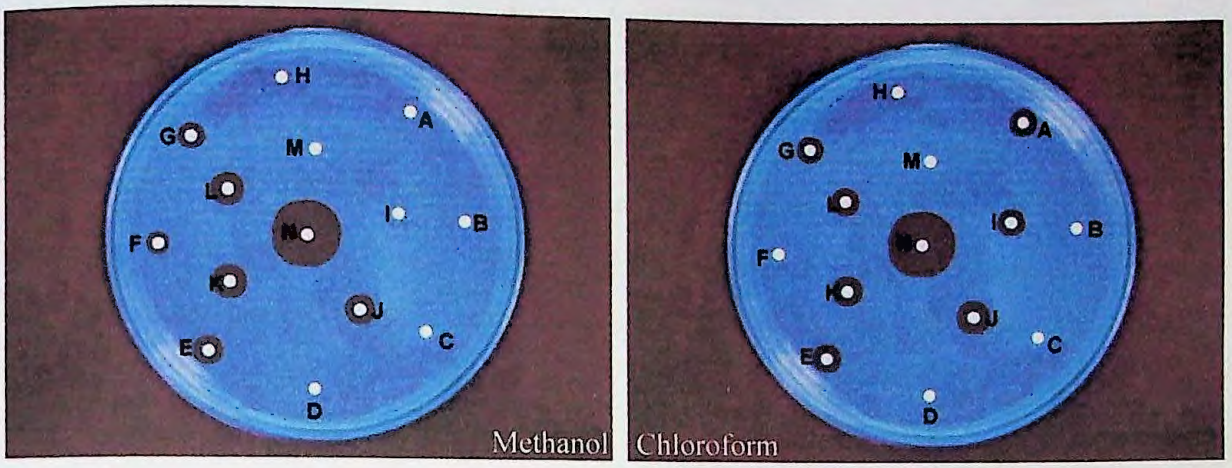


Plate 28: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Pseudomonas aeruginosa*

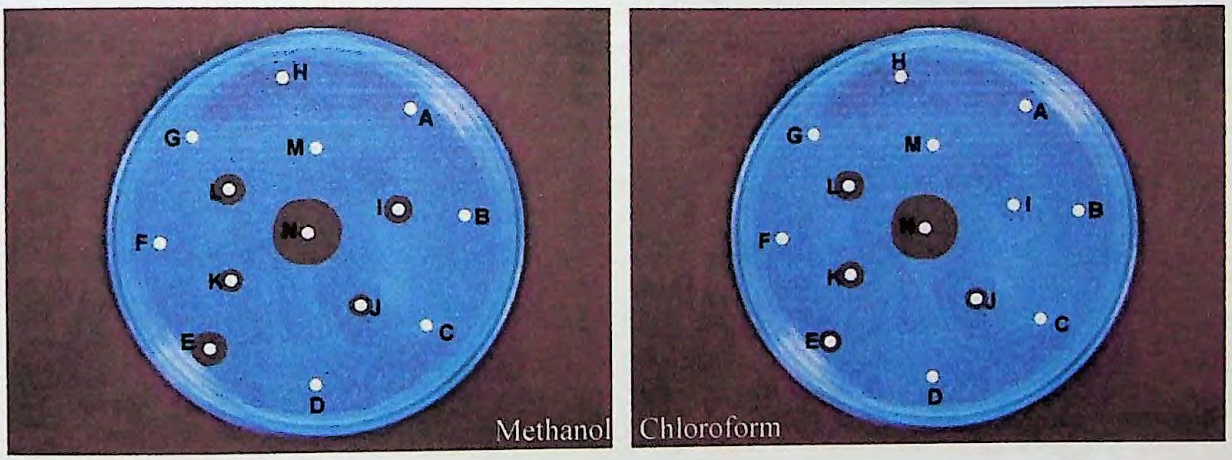


Plate 29: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Ciprofloxacin (30 µg/disc) against *Proteus* sp.

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Ciprofloxacin (Control)

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of the test sample at which it shows the highest activity against microorganisms. The minimum inhibitory concentrations of the oily extracts (CH_3OH and $CHCl_3$) were determined by serial dilution technique against *B. cereus*, *B. megaterium*, *Staphylococcus aureus*, *Shigella sonnei* and *S. dysenteriae* and the results are shown Tables 8 and 9.

In this method a large number of sterilized test tubes were used and each of the test tube contained sterile nutrient broth medium in a serial dilution (2-512 µg/ml) and 10 µl of test organisms (cells/ml) and mixed well. The test samples in various concentrations were applied to the nutrient medium (1 ml) in each test tube and incubated at 37.5°C for 24 h.

No sign of growth of the test organisms *B. cereus*, *B. megaterium*, *Staphylococcus aureus*, *Shigella sonnei* and *S. dysenteriae* was observed in the test tube (No. 3) containing 128 µg/ml of the methanol extract. The minimum inhibitory concentration for *Bacillus cereus* and *Shigella dysenteriae* was 128 µg/ml whereas, for the rest of bacteria the MIC was 64 µg/ml of the chloroform extract.

The MIC for the chloroform extract was observed to be 128 µg/ml for *B. cereus*, *B. megaterium*, *Shigella sonnei* and *S. dysenteriae*. However, the MIC was 64 µg/ml of the chloroform extract for *S. aureus*.

No inhibition was observed in the test tube containing sample lower than the concentrations. Three control tests were performed using nutrient C_M (medium), C_S (medium + sample) and another is C_I (medium + inoculum) where bacterial growth was observed in C_I only but the other two were clear. The results are presented in the Tables 8 and 9

It is evident from these results that both the CH_3OH and $CHCl_3$ extracts have properties to inhibit bacterial growth even at low concentrations (64 µg/ml of CH_3OH or 128 µg/ml of $CHCl_3$).

Table 8: Minimum inhibitory concentrations of methanolic extract of *Syzygium cumini* L. against five pathogenic bacteria

Test tube No.	Nutrient broth or potato dextrose broth medium added (ml)	Diluted solution of methanol extract from kernel (µg/ml)	Inocula added (µl)	*Bacillus cereus*	*Bacillus megaterium*	*Staphylococcus aureus*	*Shigella sonnei*	*Shigella dysenteriae*
1	1	512	10	-	-	-	-	-
2	1	256	10	-	-	-	-	-
3	1	128	10	-	-	-	-	-
4	1	64	10	+	-	-	-	+
5	1	32	10	+	+	+	+	+
6	1	16	10	+	+	+	+	+
7	1	8	10	+	+	+	+	+
8	1	4	10	+	+	+	+	+
9	1	2	10	+	+	+	+	+
10	1	1	10	+	+	+	+	+
Cm	1	0	0	-	-	-	-	-
Cs	1	1024	0	-	-	-	-	-
Ci	1	0	10	+	+	+	+	+
Results of MIC values (µg/ml)				128	64	64	64	128

Notes: + = Indicates growth, - = Indicates no growth

Table 9: Minimum inhibitory concentrations of chloroform extract of *Syzygium cumini* L. against five pathogenic bacteria

Test tube No.	Nutrient broth or potato dextrose broth medium added (ml)	Diluted solution of chloroform extract from kernel (µg/ml)	Inocula added (µl)	*Bacillus cereus*	*Bacillus megaterium*	*Staphylococcus aureus*	*Shigella sonnei*	*Shigella dysenteriae*
1	1	512	10	-	-	-	-	-
2	1	256	10	-	-	-	-	-
3	1	128	10	-	-	-	-	-
4	1	64	10	+	+	-	+	+
5	1	32	10	+	+	+	+	+
6	1	16	10	+	+	+	+	+
7	1	8	10	+	+	+	+	+
8	1	4	10	+	+	+	+	+
9	1	2	10	+	+	+	+	+
10	1	1	10	+	+	+	+	+
Cm	1	0	0	-	-	-	-	-
Cs	1	1024	0	-	-	-	-	-
Ci	1	0	10	+	+	+	+	+
Results of MIC values (µg/ml)				128	128	64	128	128

Notes: + = Indicates growth, - = Indicates no growth

Antifungal activity

All the extracts were tested against the eight pathogenic fungi at the concentration of 50 µg/disc and 200 µg/disc and compared with the standard antibiotic Nystatin (50 µg/disc). No fungal activity was observed for *A. pavonina* seed extracts but prominent zone of inhibition was observed for all seed coat extracts at 200 µg/disc and for seed kernel extract at both 50 and 200 µg/disc. The antifungal activity was determined after 48 h of incubation at room temperature (30°C) and the results are shown in **Tables** 10, 11, 12, 13, 14 and 15.

A clear inhibition zone was observed for seed coat extract at both the concentrations for all the test fungi (Plates 30, 31, 32, 33, 34, 35, 36 and 37). At 200µg/disc *Penicellium* sp. Exhibited a bigger and prominent clear zone.

Table-10. Antifungal activity of Methanol and Chloroform extracts of seed of *Caesalpinia bonduc* (L) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (mm)								
	Methanol extract				Chloroform extract				Nystatin 50 µg/disc
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Aspergillus fumigatus	-	12	-	-	-	-	-	-	27
Aspergillus flavus	-	7	-	-	-	-	-	-	29
Candida albicans	-	9	-	-	-	-	-	-	28
Colletotrichum falcatum	-	8	-	-	-	-	-	-	28
Fusarium oxysporum	-	12	-	-	-	-	-	-	27
Mucor sp.	-	13	-	-	-	-	-	-	29
Penicellium sp.	7	14	-	-	-	-	-	-	29
Vasin factum	7	15	-	-	-	-	-	-	28

Table-11. Antifungal activity of Methanol and Chloroform extracts of seed of *Mucuna pruriens* (L.) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
	Methanol extract				Chloroform extract				Nystatin 50 µg/disc
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Aspergillus fumigatus	-		-	-	-	8	-	-	28
Aspergillus flavus	-	-	-	-	-	9	-	-	29
Candida albicans	-	-	-	-	-	7	-	-	27
Colletotrichum falcatum	-	-	-	-	-	8	-	-	27
Fusarium oxysporum	-	-	-	-	-	7	-	-	28
Mucor sp.	-	-	-	-	-	9	-	-	27
Penicellium sp.	-	-	21	47	-	-	-	-	29
Vasin factum	-	-	-	-	-	10	-	-	28

Table-12. Antifungal activity of Methanol and Chloroform extracts of seed of *Adenanthera pavonina* (L.) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
	Methanol extract				Chloroform extract				Nystatin 50 µg/disc
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Aspergillus fumigatus	-	-	-	-	-	-	-	-	29
Aspergillus flavus	-	-	-	-	-	-	-	-	28
Candida albicans	-	-	-	-	-	-	-	-	28
Colletotrichum falcatum	-	-	-	-	-	-	-	-	27
Fusarium oxysporum	-	-	-	-	-	-	-	-	27
Mucor sp.	-	-	-	-	-	-	-	-	29
Penicellium sp.	-	-	-	-	-	-	-	-	28
Vasin factum	-	7	-	-	-	-	-	-	27

Table-13. Antifungal activity of Methanol and Chloroform extracts of seed of *Terminalia belerica* (Roxb.) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
	Methanol extract				Chloroform extract				Nystatin 50 µg/disc
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Aspergillus fumigatus	-	-	-	-	-	-	-	-	27
Aspergillus flavus	16	39	-	-	8	15	-	-	28
Candida albicans	-		-	-	-	-	-	-	28
Colletotrichum falcatum	-		-	-	-	-	-	-	29
Fusarium oxysporum	7	16	-	-	-	8	-	-	27
Mucor sp.	-	-	-	-	-	8	-	-	29
Penicellium sp.	-	-	-	-	-	-	-	-	28
Vasin factum	-	13	-	-	-	-	-	-	28

Table-14. Antifungal activity of Methanol and Chloroform extracts of seed of *Syzygium cumini* (L.) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
	Methanol extract				Chloroform extract				Nystatin
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc
Aspergillus fumigatus	7	17	8	17	-	10	-	9	29
Aspergillus flavus	10	18	8	19	-	8	-	9	28
Candida albicans	7	15	7	16	-	-	-	-	28
Colletotrichum falcatum	-	13	-	14	-	-	-	-	27
Fusarium oxysporum	9	17	10	19	-	10	-	8	29
Mucor sp.	9	18	8	19	-	9	-	8	27
Penicellium sp.	8	15	-	14	-	8	-	12	29
Vasin factum	-	14	8	15	-	9	-	8	27

Table-15. Antifungal activity of Methanol and Chloroform extracts of seed of *Myristica fragrans* (Houtt.) and standard Nystatin

Test Organisms µg disc $^{-1}$	Diameter of zone of inhibition (in mm)								
	Methanol extract				Chloroform extract				Nystatin
	Seed coat		Seed kernel		Seed coat		Seed kernel		
	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	50 µg/disc
Aspergillus fumigatus	-	13	7	15	-	9	-	10	27
Aspergillus flavus	7	15	7	16	-	8	-	9	28
Candida albicans	-	12	-	14	-	7	-	7	29
Colletotrichum falcatum	-	13	-	12	-	7	-	8	28
Fusarium oxysporum	8	17	7	16	-	9	-	9	29
Mucor sp.	-	12	-	13	-	8	-	8	27
Penicellium sp.	7	16	9	19	-	11	-	9	29
Vasin factum	-	10	8	17	-	9	-	-	27

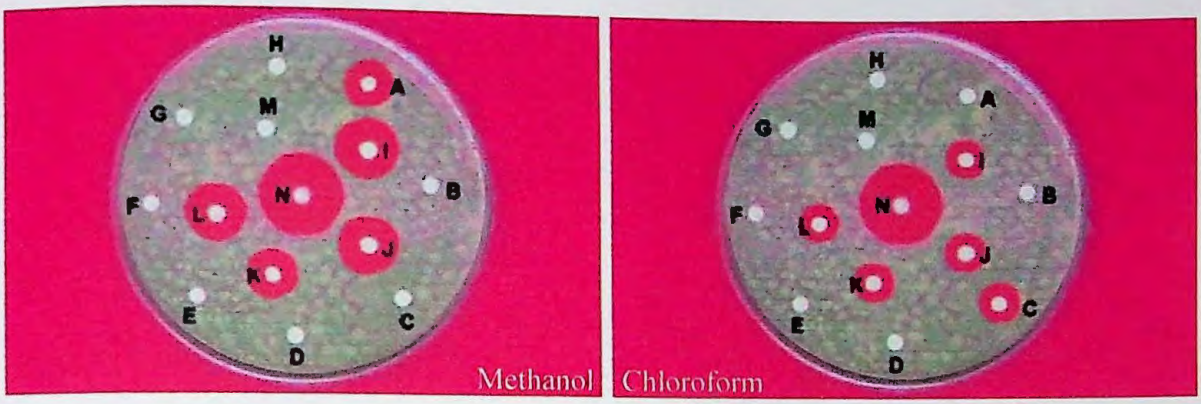


Plate 30: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Aspergillus fumigatus*

Plate 31: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Aspergillus flavus*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Nystatin (Control)

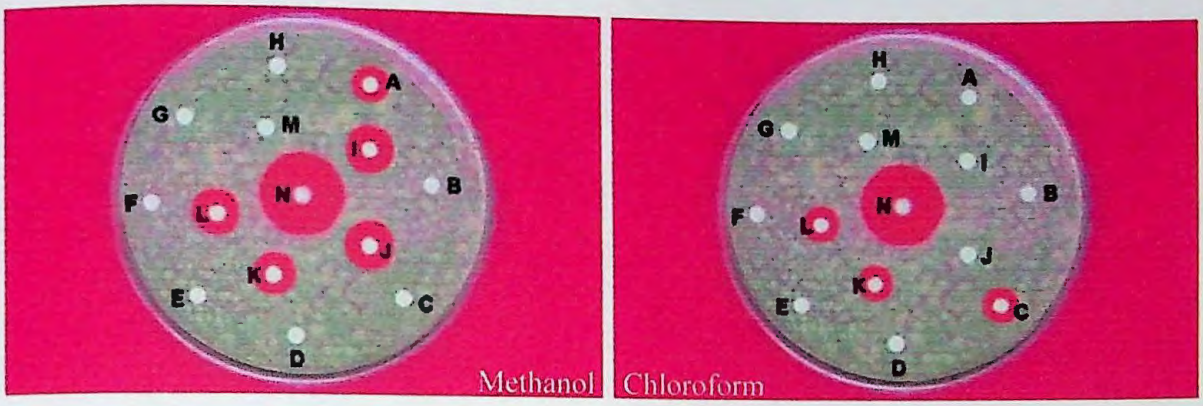


Plate 32: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Candida albicans*

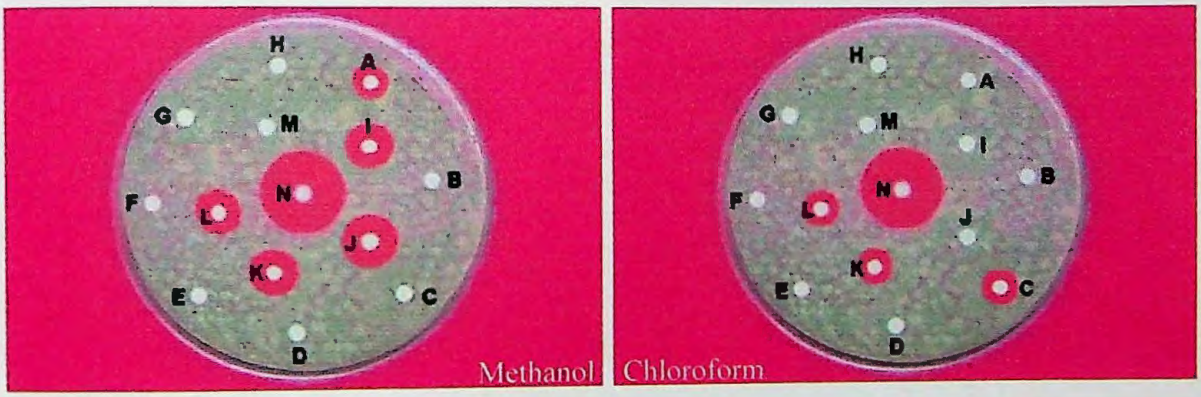


Plate 33: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Colletotrichum falcatum*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Nystatin (Control)

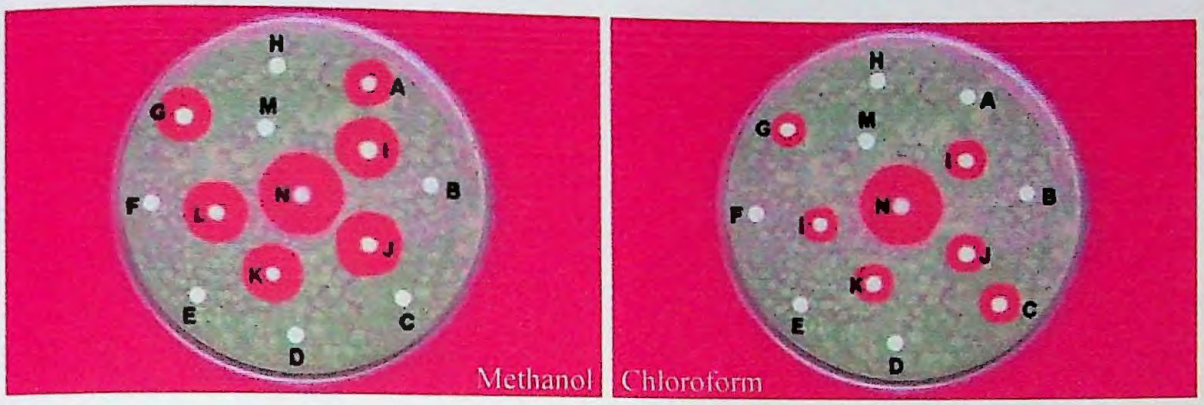


Plate 34: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Fusarium oxysporum*

Plate 35: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Mucor* sp.

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Nystatin (Control)

Plate 36: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Penicellium* sp.

Plate 37: Effect of methanol and chloroform oily extracts from seed coat and seed kernel and standard Nystatin (50 µg/disc) against *Vasin factum*

Note:

A-Natai coat	B- Natai kernel
C- Alkushi coat	D- Alkushi kernel
E- Chandan coat	F- Chandan kernel
G- Bahera coat	H- Bahera kernel
I- Jam coat	J- Jam kernel
K- Jayfal coat	L- Jayfal kernel
M- Solvent	N- Nystatin (Control)

Brine shrimp lethality bioassay

The results of the brine shrimp lethality bioassay are shown in the Table 16. From this experiment, it was revealed that each of the test samples showed different mortality rates at different concentrations. The mortality rates of brine shrimps nauplii were found to increase with the increase of the concentration of the tested extracts. Regression lines drawn by plotting log concentrations of the tested extracts against probit mortality reveal linear correlation between doses and mortality. The LC_{50} values of different plant seed oil and standard ampicilin trihydrate are given in **Table** 16.

It is evident that different plant seed oils were found to be lethal to brine *nauplii* indicating that the extracts are biologically active. The methanol extract was more active with lower LC_{50} values whereas the Chloroform extracts were comparatively less active with higher LC_{50} values.

Brine shrimp lethality bioassay is a recent development in the bioassay for the bioaclive compounds (Chaterjee 1975, Mayer *et al.* 1982, Alkofahi *et al.* 1989, Pelcjar *el al.* 1986, McLaughlin 1988 and 1992, Persoone 1980). The brine shrimp assay has advantages of being rapid (24 h), inexpensive and simple. It easily utilizes a huge number of organisms for statistical validation and requires no special equipment and needs relatively small amounts of sample. Natural product extracts and pure compounds can be tested for their bioactivity by this method. The bioassay indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer. antiviral, insecticidal, pesticidal, etc,) of the compound. Therefore, in addition to antimicrobial properties, brine shrimp lethality bioassay was also included in the present part of investigation to determine the degrce of cytotoxicity and pharmacological activities of the fractionated extracts.

Table 16: Ampicillin trihydrate, seed coat and seed kernel extract lethality bioassay on brine shrimp nauplii.

Extracts		LC_{50} (µg/ml)	95 % Confidence limit		Regression equation	χ^2 values
			Lower	Upper		
Methanol extract	Control (20µg DMSO)	0	0	0	0	0
	Ampicillin	6.721	3.148	14.352	Y = 4.236489 + .9227454 X	0.20158
	Myristica fragrans					
	Seed coat	34.23474	24.08017	48.6715	Y = 2.617475 + 1.552673 X	1.17796
	Seed kernel	30.21676	21.61476	42.24208	Y = 2.671838 + 1.572819 X	0.13132
	Syzygium cumini					
	Seed coat	41.12985	28.25775	59.86555	Y = 2.466711 + 1.569419 X	5.03311
	Seed kernel	28.72247	20.76573	39.72799	Y = 2.634581 + 1.622125 X	2.10562
	Terminalia bellirica					
	Seed coat	34.28775	24.38909	48.20393	Y = 2.508102 + 1.62324 X	0.32606
	Seed kernel	26.51602	19.22125	36.57927	Y = 2.723973 + 1.598886 X	1.45988
	Mucuna pruriens					
	Seed coat	47.93552	32.12584	71.52542	Y = 2.331575 + 1.587727 X	0.92116
	Seed kernel	33.80515	23.37058	48.89861	Y = 2.747884 + 1.472951 X	0.15647
Chloroform extract	***Myristica fragrans***					
	Seed coat	95.58479	42.39436	215.511	Y = 2.750502 + 1.135887 X	1.77241
	Seed kernel	45.00616	29.06815	69.68294	Y = 2.683566 + 1.401121 X	0.32692
	Syzygium cumini					
	Seed coat	88.28874	42.93272	181.5609	Y = 2.604101 + 1.231251 X	1.53685
	Seed kernel	41.92609	27.55824	63.78479	Y = 2.701551 + 1.416623 X	0.59163
	Terminalia bellirica					
	Seed coat	64.79947	35.06378	119.7524	Y = 2.816124 + 1.205515 X	0.39005
	Seed kernel	58.82652	32.51715	106.4225	Y = 2.910908 + 1.180563 X	0.89138
	Mucuna pruriens					
	Seed coat	79.46446	42.78583	147.5863	Y = 2.439498 + 1.34751 X	0.61027
	Seed kernel	71.83673	36.79804	140.2389	Y = 2.831675 + 1.168061 X	0.28685

CHAPTER IV

DOSE MORTALITY RESPONSE OF ADULT *ALPHITOBIUS DIAPERINUS* TO DIFFERENT PLANT SEED EXTRACTS

DOSE MORTALITY RESPONSE OF ADULT *ALPHITOBIUS DIAPERINUS* TO DIFFERENT PLANT SEED EXTRACTS

4.1. Introduction

The use of natural pesticides in agricultural and horticultural industries has increased in recent years. These bio-pesticides offer desirable alternatives to synthetic chemicals in agricultural systems where protection of the environment and preservation of beneficial organisms are important. Botanical compounds are effective, and biodegradable, and rapidly metabolize in the environment (Islam 1999).

The present investigation reports the effect of seed extracts on the mortality of *A. diaperinus* adults.

4.2. Materials and Methods

Extracts collected from different solvents were weighed and dissolved in the respective solvents according to the proportion of dry weight of the dust. Various concentrations of the extracts were poured on petridishes (6 cm) with the help of a pipette, to cover the whole area of the petridish uniformly. Petridishes were kept in the air at room temperature for drying.

To observe the mortality of adults of *A. diaperinus* the surface film method was used. The concentrations used were 0.177, 0.354, 0.708, 1.416 and 2.832 mg/cm^2.

The adult mortality was recorded 24, 48 and 72 h after treatments. Three replications were used for each concentration and a control batch was maintained for each treatment where only the solvent was used.

The mortality was corrected using Abbott's formula (Abbott 1925):

$$P_t \frac{P_o - P_c}{100 - P_c} \times 100$$

Where,

P_t= Corrected mortality,

P_o= Observed mortality and

P_c= Control mortality.

The observed data was then subjected to Probit analysis according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agriculture and Environmental Science, University of Newcastle Upon Tyne, UK.

Different plant seeds viz. seed coats and seed kernels were extracted separately with methanol and chloroform. The extract condensed using rotary evaporator and weighed. The yield of the extracts according solvents and plant seeds are given in **Table** 17. Extract out put was the lowest for seed coat and highest seed kernel.

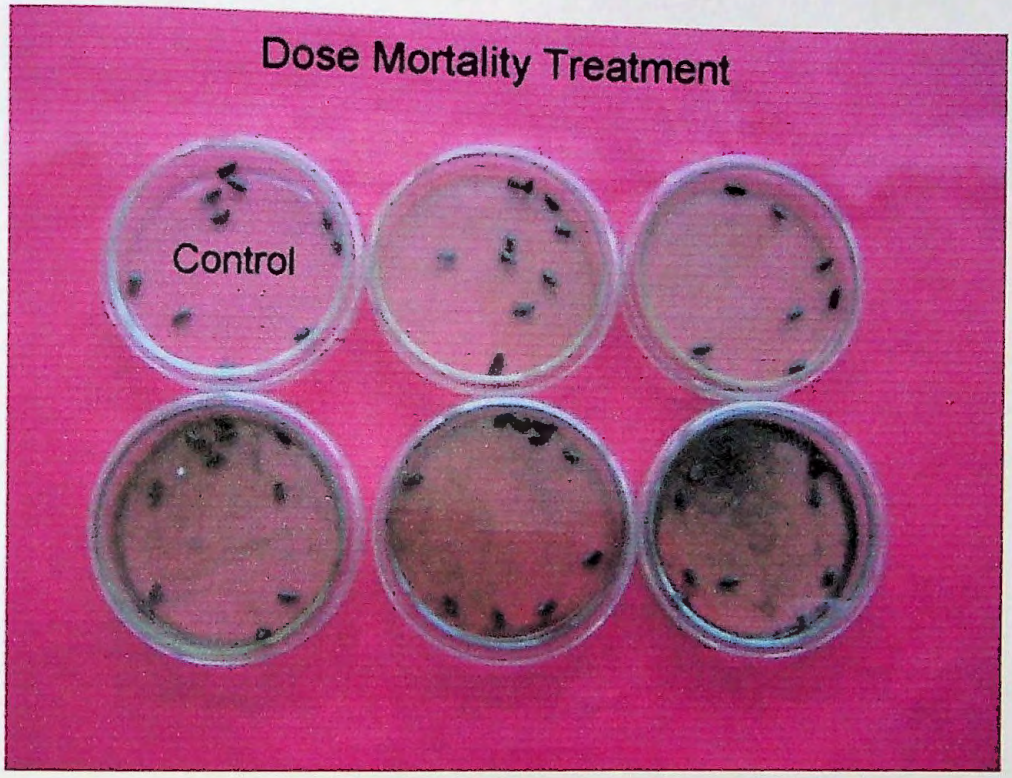


Plate 38: Mortality test of *A. diaperinus* with different extract of test plants

Table 17: Extract from seed coat and seed kernel of test plants with different solvents

Plant parts	Weight of dust (gm)	Weight of extracts (gm) (Methanol)	Weight of extracts (gm) (Chloroform)
Natai coat	100	3.212	0.595
Natai kernel	100	25.772	19.010
Alkushi coat	100	4.176	1.085
Alkushi kernel	100	17.896	14.332
Rakta Chandan coat	100	7.236	5.79
Rakta Chandan kernel	100	25.031	14.704
Bahera coat	100	1.540	1.296
Bahera kernel	100	8.736	3.06
Kalojam coat	100	0.680	0.459
Kalojam kernel	100	18.628	13.08
Jayfal coat	100	5.092	2.952
Jayfal kernel	100	12.736	5.596

4.3. Results and Discussion

The LD_{50} values, 95% confidence limits, regression equations (Y), chi-square (χ^2) values and probit regression lines are shown in **Tables** 18-23 and **Figures** 1-24. The chi square values showed no significant heterogeneity. The methanolic extracts were found to be more toxic to the beetles than the chloroform extract.

Oils as well as other plant materials increase adult mortality in insects. Some earlier workers reported effective use of plant materials, *viz.* akanda (*Calotropic procera*), black pepper (*Piper nigrum*), nishinda (*Vitex negundo*), biskhathali (*Polygonum hydropiper*), alkushi (*Mucuna pruriens*), Bahera (*Termenalia bellirica*), dhutura (*Detura metal*) and *Sapindus mukarasis* against many stored-product insects pests (Khanam *et al.* 1990a and b, 1991, Khalequzzaman and Rahman 1992, Khalequzzaman and Islam 1992a and b, Malek and Wilkins 1993, Talukdur and Howse 1995).

Table 18: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Caesalpinia bonduc* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.478976	1.021764	2.140779	Y = 3.122631 + 1.604643 X	1.63864
		48 h	0.9306264	0.6727485	1.287354	Y = 3.461391 + 1.5882 X	2.06518
		72 h	0.6671214	0.4892816	0.9096008	Y = 3.658389 + 1.627765 X	1.1439
	Seed kernel	24 h	1.224791	0.8450087	1.775262	Y = 3.388039 + 1.481498 X	1.07820
		48 h	0.8292164	0.6023791	1.141474	Y = 3.540655 + 1.588545 X	1.30752
		72 h	0.5797489	0.4407189	0.7626373	Y = 3.525792 + 1.931514 X	0.44886
Chloroform extract	Seed coat	24 h	2.302907	1.318479	4.02235	Y = 3.184026 + 1.333044 X	1.59600
		48 h	1.293737	0.8514092	1.965866	Y = 3.522915 + 1.328498 X	2.01509
		72 h	0.7783415	0.5444667	1.112677	Y = 3.75927 + 1.392248 X	1.59316
	Seed kernel	24 h	1.621713	1.000864	2.627682	Y = 3.454967 + 1.276915 X	1.71158
		48 h	0.9942279	0.655636	1.50768	Y = 3.787228 + 1.215829 X	1.49181
		72 h	0.6583311	0.4582781	0.945714	Y = 3.892942 + 1.352637 X	1.18821

Table 19: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Mucuna pruriens* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.522318	1.038421	2.231709	Y = 3.135489 + 1.576747 X	1.50939
		48 h	0.9537388	0.6638391	1.370238	Y = 3.624462 + 1.404429 X	1.84053
		72 h	0.6156176	0.4471281	0.8475982	Y = 3.742566 + 1.593079 X	2.01823
	Seed kernel	24 h	1.314728	0.8921169	1.937538	Y = 3.364875 + 1.461452 X	0.69317
		48 h	0.8295285	0.5980068	1.150685	Y = 3.574523 + 1.551403 X	1.41006
		72 h	0.6255962	0.4684772	0.8354103	Y = 3.59006 + 1.770627 X	0.89616
Chloroform extract	Seed coat	24 h	2.539317	1.38525	4.65485	Y = 3.176921 + 1.297827 X	1.30932
		48 h	1.269403	0.8267301	1.949106	Y = 3.580759 + 1.286011 X	2.53676
		72 h	0.8156945	0.5697673	1.167771	Y = 3.732092 + 1.390971 X	2.98937
	Seed kernel	24 h	2.082525	1.151147	3.767468	Y = 3.443318 + 1.180566 X	.891273
		48 h	1.056869	0.6990381	1.597871	Y = 3.71662 + 1.253275 X	2.14442
		72 h	0.6304605	0.4551897	0.8732194	Y = 3.753821 + 1.55839 X	2.07671

Table 20: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Adenanthera pavonina* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.440276	0.9236957	2.245754	Y = 3.472919 + 1.318216 X	1.62771
		48 h	1.008387	0.6771971	1.501548	Y = 3.701776 + 1.293533 X	2.14629
		72 h	0.7035133	.494771	1.000323	Y = 3.81209 + 1.402041 X	2.19368
	Seed kernel	24 h	1.236938	0.8228445	1.859422	Y = 3.525444 + 1.349896 X	2.36307
		48 h	0.7953909	0.5539598	1.142044	Y = 3.767296 + 1.368788 X	1.74682
		72 h	0.6335851	0.4639357	0.8652707	Y = 3.69485 + 1.627765 X	1.49371
Chloroform extract	Seed coat	24 h	1.978017	1.207402	3.240471	Y = 3.196601 + 1.391265 X	1.96270
		48 h	1.201118	0.8021061	1.79862	Y = 3.544232 + 1.34845 X	2.20796
		72 h	0.8156945	0.5697673	1.167771	Y = 3.732092 + 1.390971 X	2.98937
	Seed kernel	24 h	1.357389	0.8987511	2.05007	Y = 3.434923 + 1.381718 X	1.26129
		48 h	0.9112725	0.6354845	1.306747	Y = 3.659542 + 1.396822 X	2.06184
		72 h	0.7539458	0.5349434	1.062607	Y = 3.731028 + 1.446386 X	1.41295

Table 21: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Terminalia bellirica* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.357389	0.8987511	2.05007	Y = 3.434923 + 1.381718 X	1.26129
		48 h	0.8463201	0.5979505	1.197855	Y = 3.662792 + 1.44168 X	2.36665
		72 h	0.6192901	0.4441412	0.8635098	Y = 3.802214 + 1.512558 X	2.46234
	Seed kernel	24 h	1.168178	0.8009703	1.703732	Y = 3.475375 + 1.428209 X	1.72105
		48 h	0.6699407	0.4785863	0.9378052	Y = 3.779617 + 1.477396 X	2.00673
		72 h	0.4985383	0.3611395	0.6882115	Y = 3.86098 + 1.63254 X	1.70774
Chloroform extract	Seed coat	24 h	1.638713	1.060978	2.531041	Y = 3.264633 + 1.42887 X	2.77253
		48 h	1.02324	0.7088272	1.477117	Y = 3.577416 + 1.40853 X	3.18307
		72 h	0.7750141	0.5390637	1.114241	Y = 3.788637 + 1.362139 X	4.20612
	Seed kernel	24 h	1.27797	0.8695032	1.878322	Y = 3.394781 + 1.45069 X	0.93775
		48 h	0.8069778	0.5698323	1.142816	Y = 3.700149 + 1.433352 X	2.68056
		72 h	0.5295841	0.3562501	0.787254	Y = 2.791475 + 1.281096 X	0.14204

Table 22: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Syzygium cumini* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.283756	0.8221173	2.004615	Y = 3.621953 + 1.243184 X	1.17337
		48 h	0.8074514	0.5489873	1.187601	Y = 3.847263 + 1.27077 X	2.38401
		72 h	0.5552243	0.3964113	0.7776622	Y = 3.873948 + 1.512558 X	1.60024
	Seed kernel	24 h	1.008189	0.6823361	1.489655	Y = 3.654263 + 1.340988 X	1.88495
		48 h	0.7346876	0.5048813	1.069095	Y = 3.863994 + 1.31163 X	2.91901
		72 h	0.4896422	0.3607544	0.6645781	Y = 3.803037 + 1.735035 X	1.27344
Chloroform extract	Seed coat	24 h	1.600207	0.9739899	2.629044	Y = 3.515515 + 1.232781 X	1.86552
		48 h	1.050938	0.7032235	1.570583	Y = 3.68493 + 1.287294 X	2.23643
		72 h	0.6812436	0.478802	0.9692792	Y = 3.832571 + 1.400967 X	2.82552
	Seed kernel	24 h	1.318253	0.8757347	1.98438	Y = 3.463222 + 1.372125 X	1.74029
		48 h	0.8615255	0.5924073	1.252899	Y = 3.762158 + 1.323516 X	3.49025
		72 h	0.6093798	0.4331106	0.857388	Y = 3.842322 + 1.47496 X	3.29659

Table 23: LD_{50}, 95% confidence limits and regression equations of methanolic and chloroform extracts of *Myristica fragrans* against *Alphitobius diaperinus* adults

Test extracts		Exposure period	LD_{50} values mg/cm^2	95 % confidence limits		Regression equation	χ^2 values
				Lower	Upper		
Methanol extract	Seed coat	24 h	1.218091	0.7942018	1.868223	Y = 3.612241 + 1.27824 X	1.03308
		48 h	0.7877938	0.5345827	1.160941	Y = 3.865469 + 1.265635 X	2.47972
		72 h	0.5469572	0.3867455	0.7735376	Y = 3.915061 + 1.470201 X	1.86727
	Seed kernel	24 h	1.008387	0.6771971	1.501548	Y = 3.701776 + 1.293533 X	2.14629
		48 h	0.6093798	0.4331106	0.857388	Y = 3.842322 + 1.47496 X	3.29659
		72 h	0.4690833	0.3412604	0.6447836	Y = 3.861735 + 1.69574 X	2.14050
Chloroform extract	Seed coat	24 h	1.450682	0.9110546	2.309935	Y = 3.53293 + 1.263004 X	1.97871
		48 h	0.9085649	0.6199273	1.331592	Y = 3.738358 + 1.316465 X	2.73264
		72 h	0.7092794	0.4948258	1.016675	Y = 3.834048 + 1.37039 X	2.34143
	Seed kernel	24 h	1.264572	0.8459302	1.890395	Y = 3.49088 + 1.369508 X	1.89416
		48 h	0.6777083	0.4847582	0.9474592	Y = 3.759146 + 1.493129 X	4.25291
		72 h	0.5540011	0.3992872	0.7686625	Y = 3.827943 + 1.576382 X	3.17786

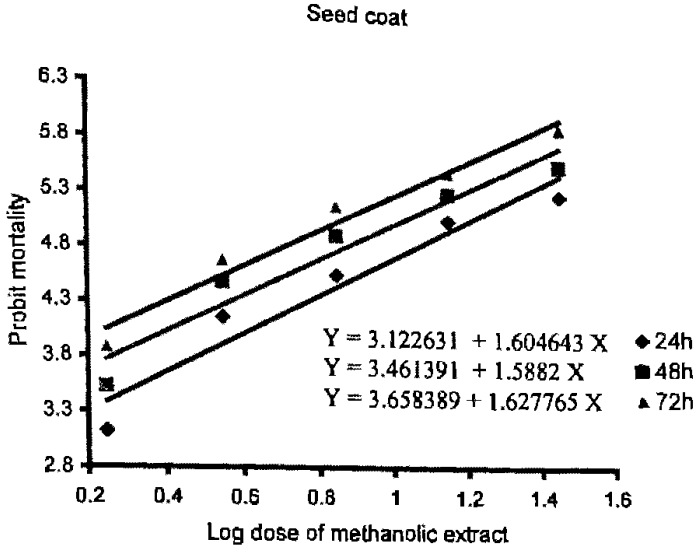


Figure 1: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Caesalpania bonduc* seed coat extract in methanol after 24, 48 and 72 hours of exposure

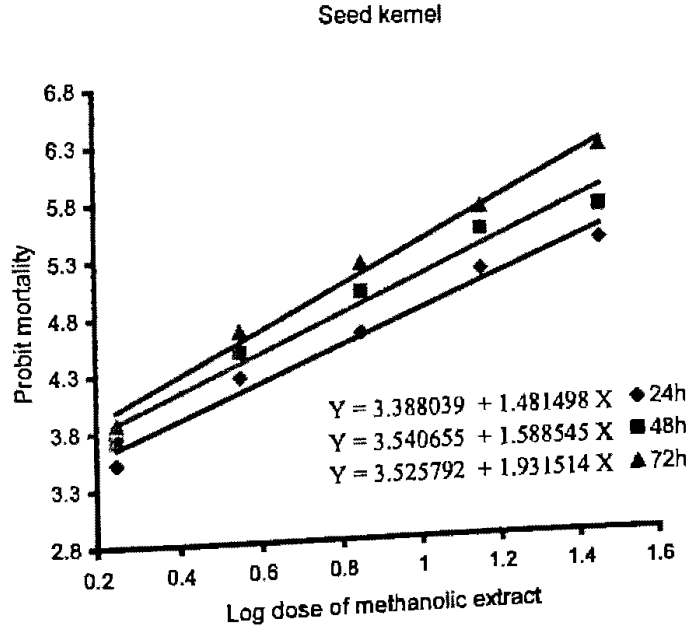


Figure 2: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Caesalpania bonduc* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

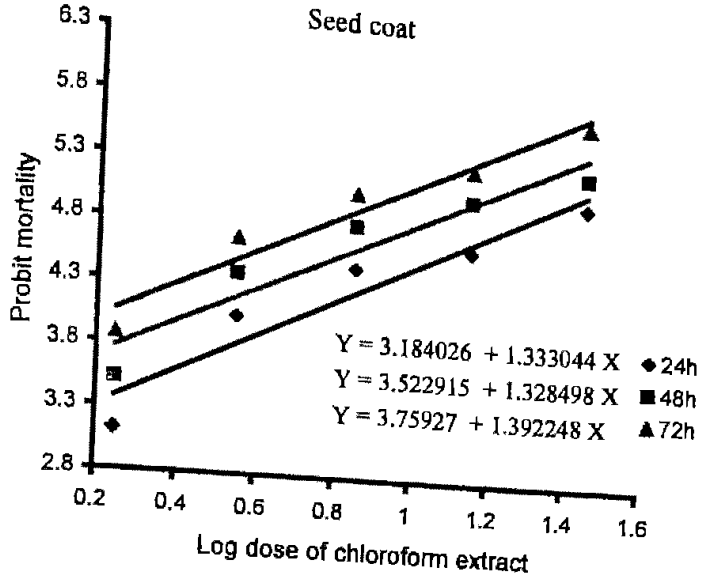


Figure 3: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Caesalpania bonduc* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

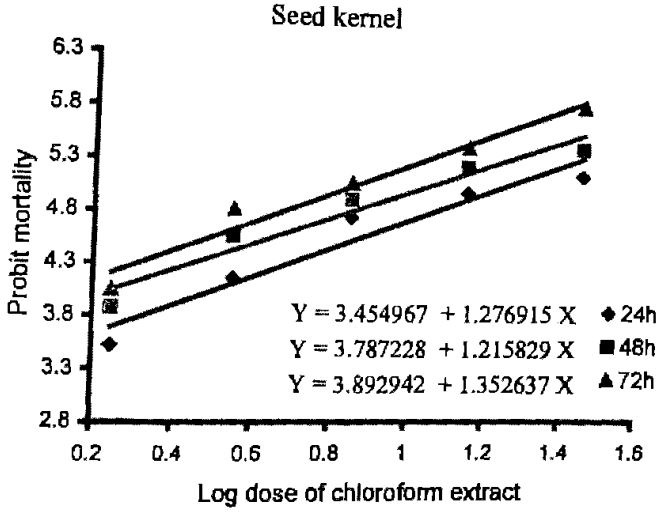


Figure 4: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Caesalpania bonduc* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure

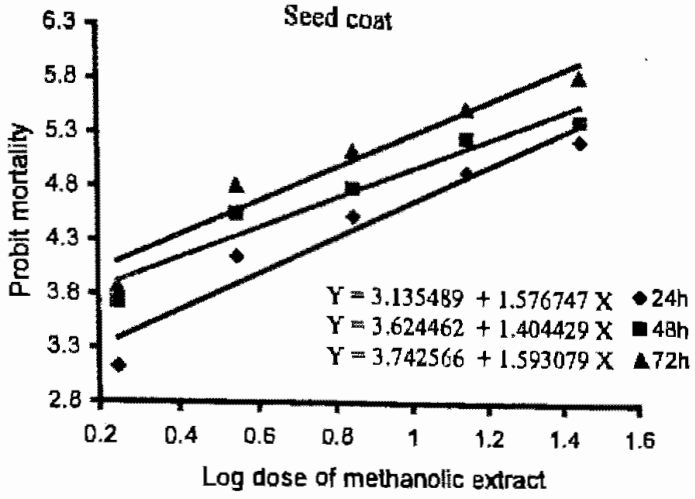


Figure 5: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Mucuna pruriens* seed coat extract in methanol after 24, 48 and 72 hours of exposure

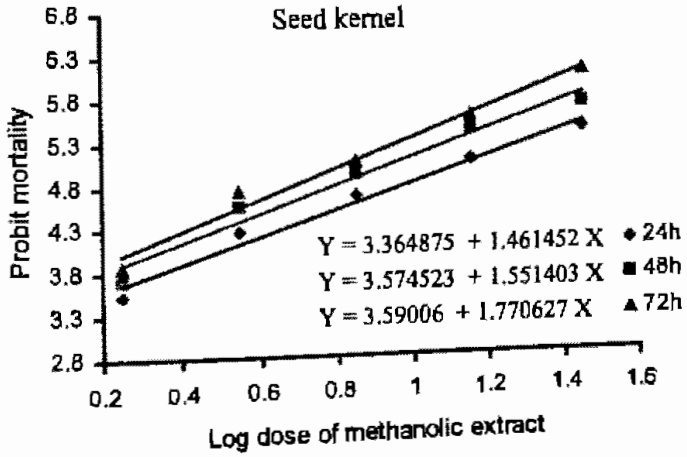


Figure 6: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Mucuna pruriens* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

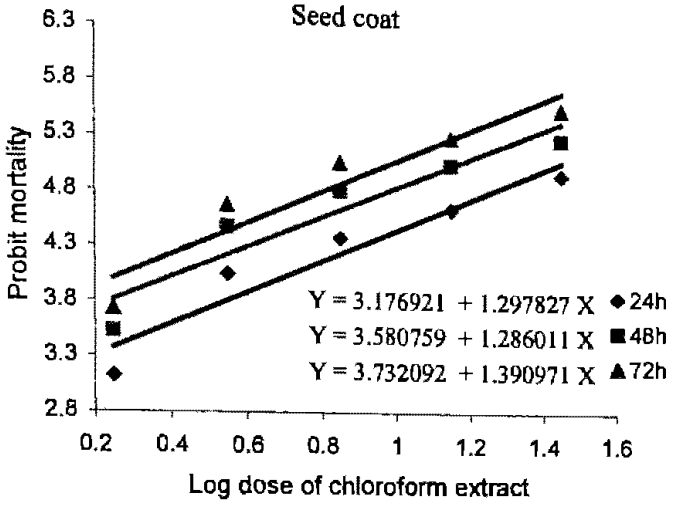


Figure 7: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Mucuna pruriens* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

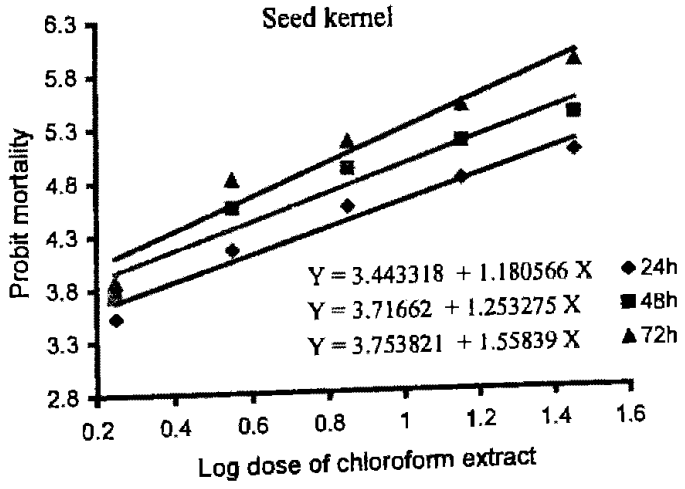


Figure 8: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Mucuna pruriens* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure

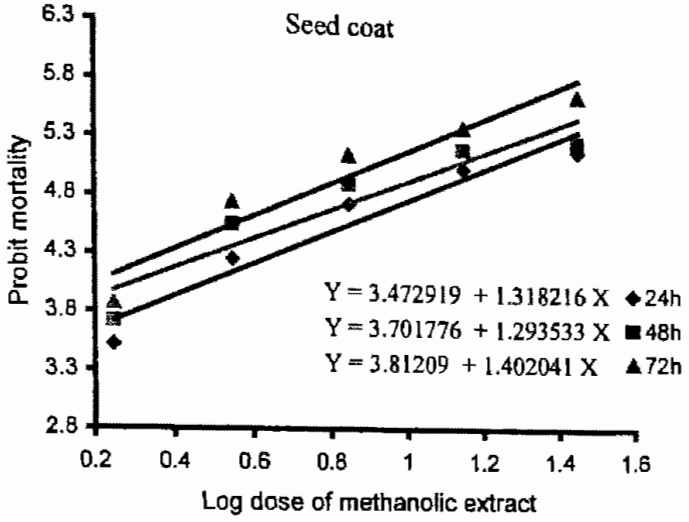


Figure 9: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Adenanthera pavonina* seed coat extract in methanol after 24, 48 and 72 hours of exposure

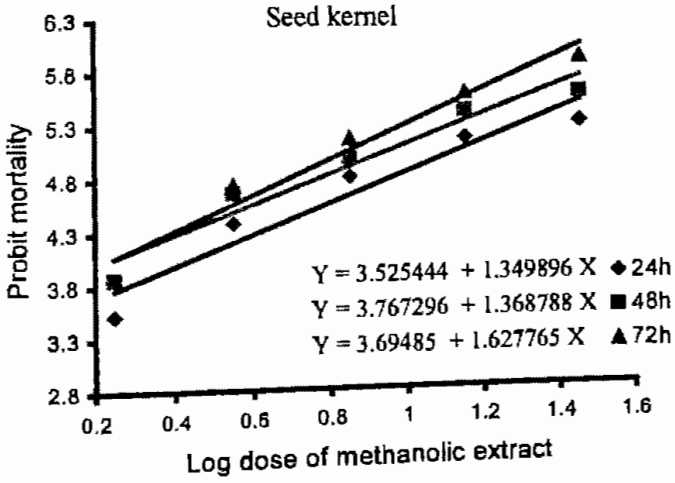


Figure 10: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Adenanthera pavonina* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

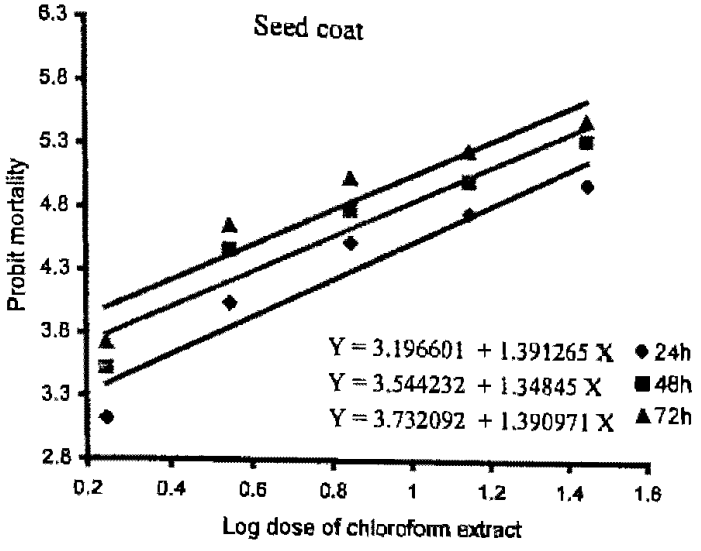


Figure 11: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Adenanthera pavonina* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

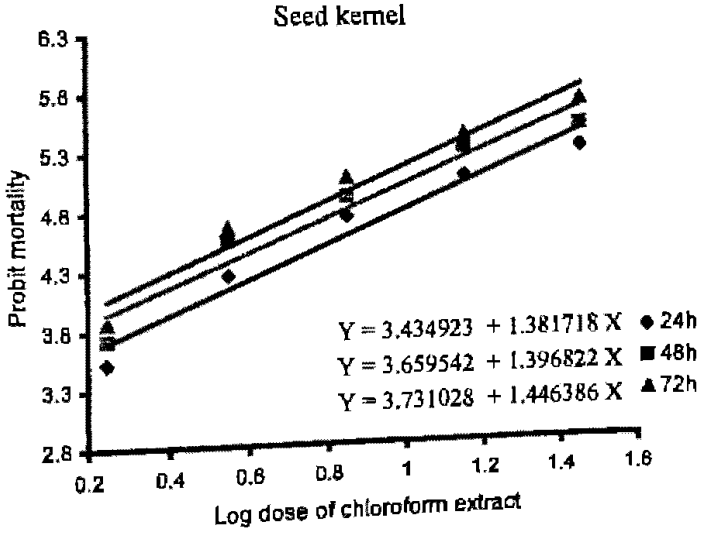


Figure 12: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Adenanthera pavonina* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure

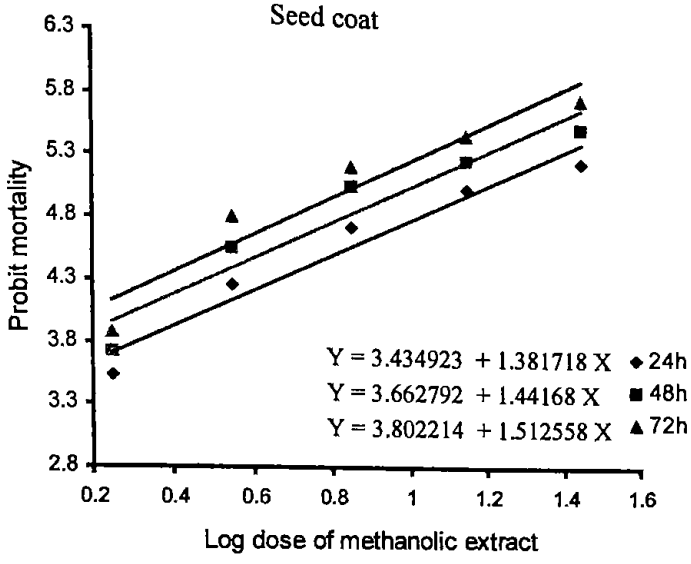


Figure 13: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Termenalia bellirica* seed coat extract in methanol after 24, 48 and 72 hours of exposure

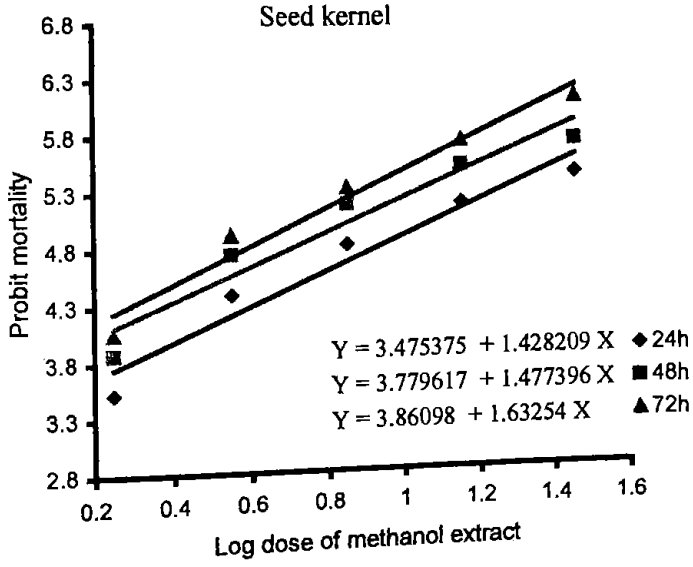


Figure 14: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Termenalia bellirica* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

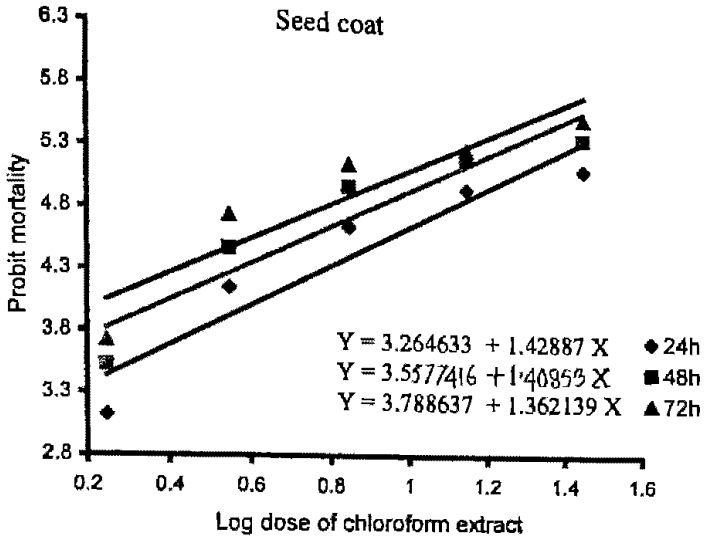


Figure 15: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Termenalia bellirica* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

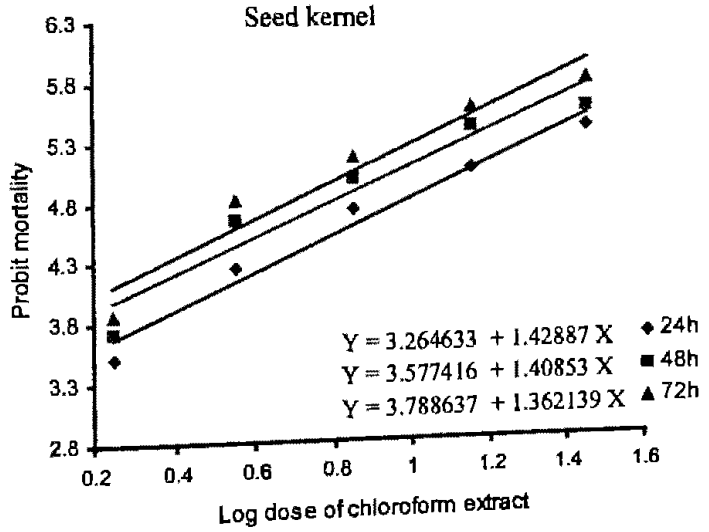


Figure 16: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Termenalia bellirica* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure

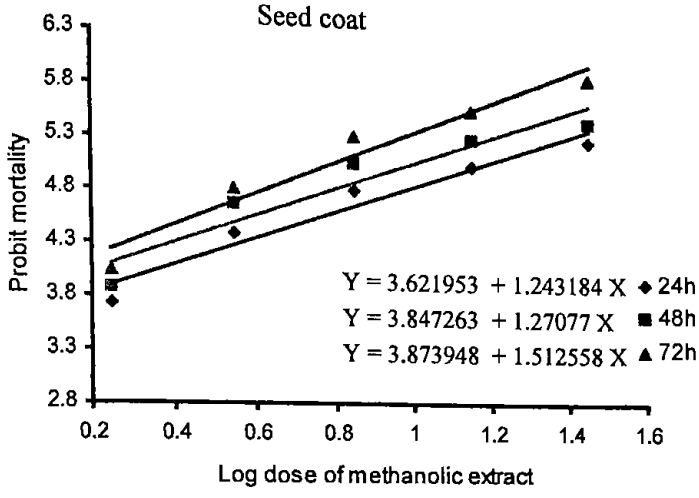


Figure 17: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Syzygium cumini* seed coat extract in methanol after 24, 48 and 72 hours of exposure

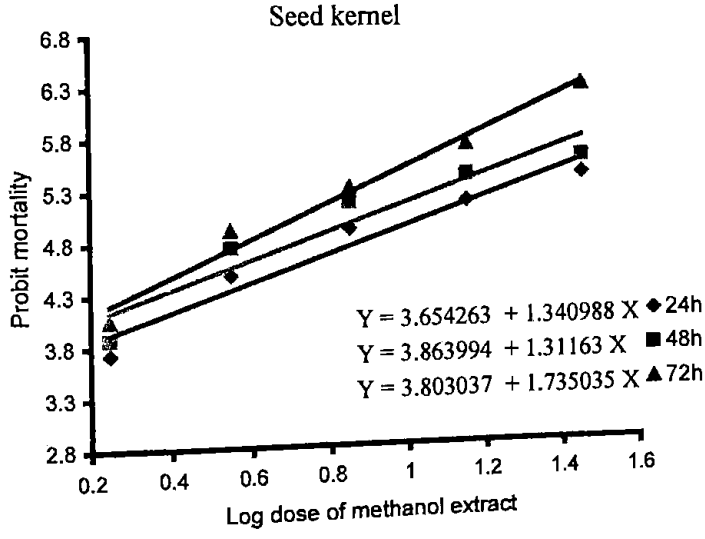


Figure 18: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Syzygium cumini* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

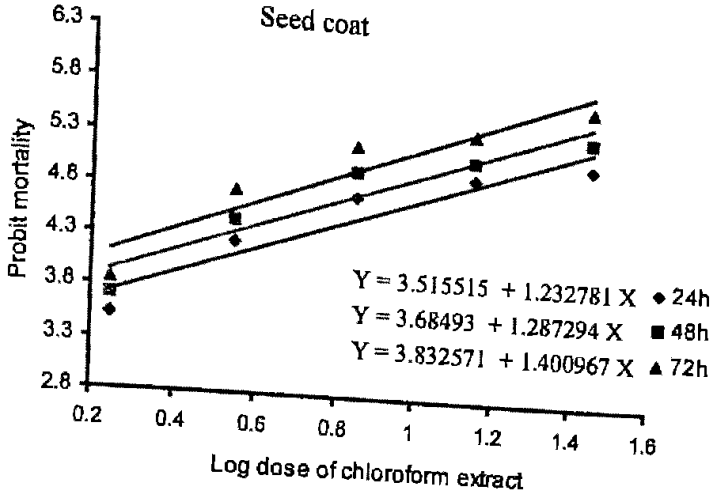


Figure 19: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Syzygium cumini* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

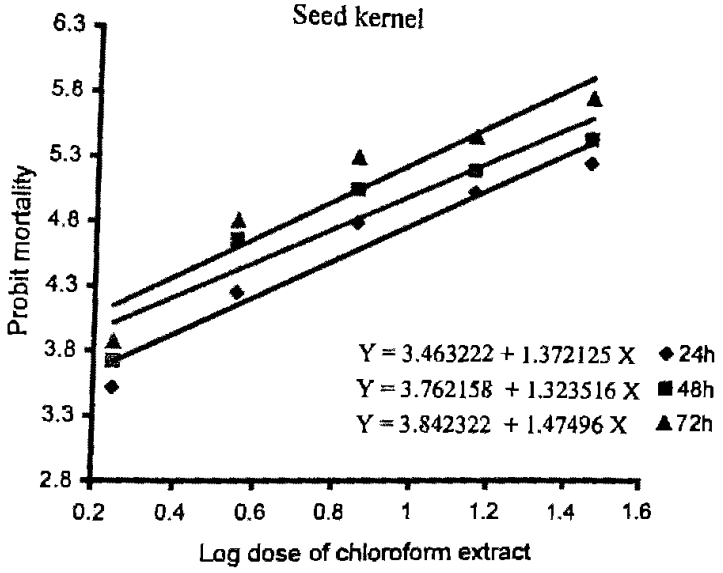


Figure 20: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Syzygium cumini* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure

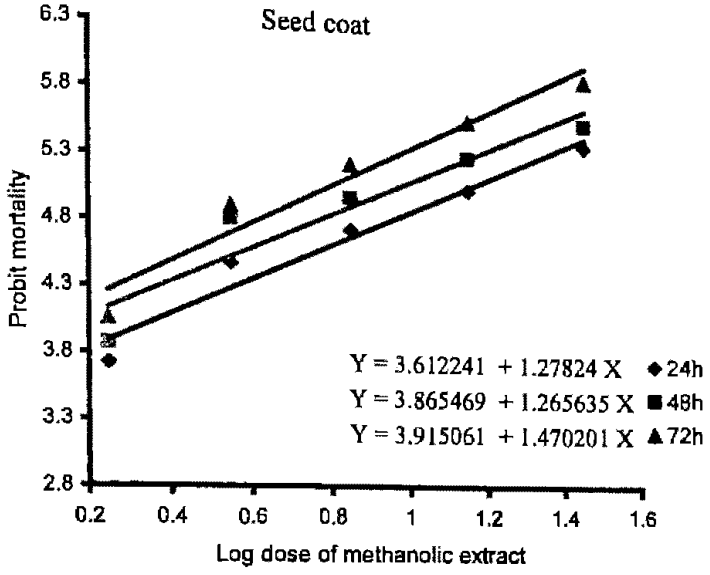


Figure 21: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Myristicus fragrans* seed coat extract in methanol after 24, 48 and 72 hours of exposure

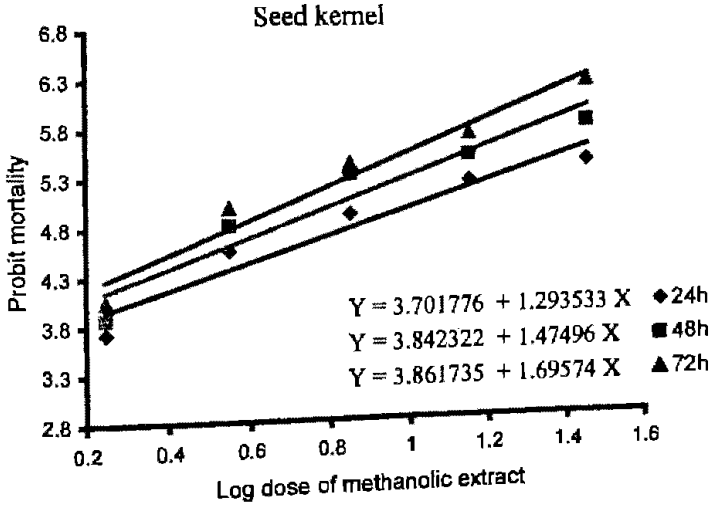


Figure 22: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Myristicus fragrans* seed kernel extract in methanol after 24, 48 and 72 hours of exposure

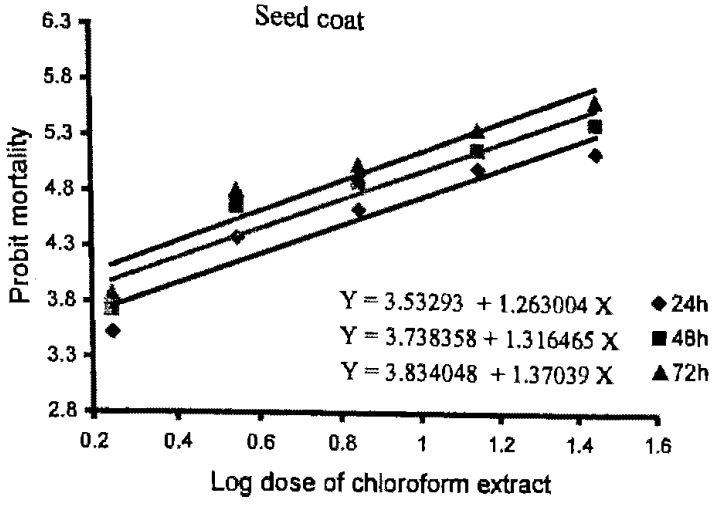


Figure 23: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Myristicus fragrans* seed coat extract in chloroform after 24, 48 and 72 hours of exposure

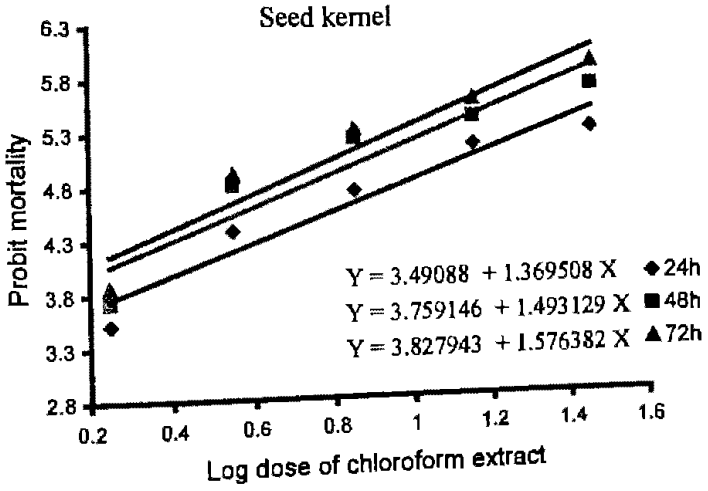
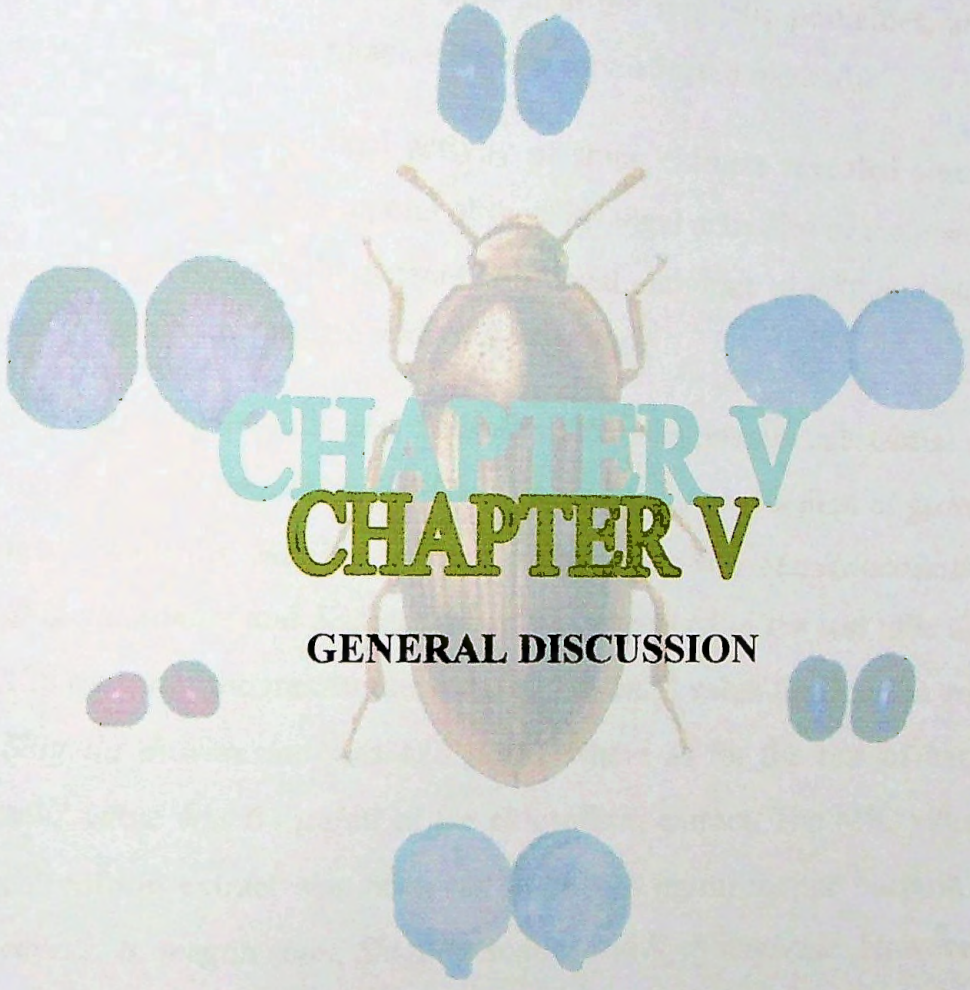


Figure 24: Regression line of probit mortality of adult *Alphitobius diaperinus* and log dose of *Myristicus fragrans* seed kernel extract in chloroform after 24, 48 and 72 hours of exposure



CHAPTER V

GENERAL DISCUSSION

GENERAL DISCUSSION

The use of phytochemicals as well as plant products such as powder, oil and extracts for the control of stored-product insect pests has agricultural importance and recently has received much more attention because these insecticidal compounds are safer than the synthetic pesticides, and can be easily obtained from plants with less sophisticated methods.

In vitro antifungal activity of some extracts revealed significant to moderate activity. As reports of the antifungal activities of the plant extracts are unavailable, so the present antifungal activities of different plants were not possible to compare with other findings.

The methanolic and chloroform extracts possess substantial minimum inhibitory concentration (MIC) value as 64 µg/ml. No sign of growth of the test organisms *Bacillus cereus*, *B. megaterium*, *Staphylococcus aureus*, *Shigella sonnei* and *S. dysenteriae* was observed in the test tube containing 128 µg/ml of the methanolic extract. The MIC value for *Bacillus cereus* and *Shigella dysenteriae* was 128 µg/ml where as for the rest of bacteria the MIC value was 64 µg/ml of the chloroform extract. The MIC value for the chloroform extract was observed to be 128 µg/ml for the bacteria *Bacillus cereus*, *B. megaterium*, *Shigella sonnei* and *S. dysenteriae*. However, it was 64 µg/ml of the chloroform extract for *S. aureus*.

The present results on the effect of oils on *A. diaperinus* are in agreement with earlier reports by Ali *et al.* 1983, Shelke *et al.* 1990, and Mirsa 1993. The authors suggested that the vegetable oils including those of mustard, til, coconut, sunflower, neem, nutmeg, karanja, mohua, bahera,

rape and dalda caused significant mortality of *Callosobruchus chinensis*, *C. maculatus*, *Lipophis erisimi* and other insects.

The essential oils of *E. citriodora* and *Ocimum basilicum* were very efficient against *C. maculatus* (Gakuru and Foua 1995). The essential oil extract of *Evodia rutaecarpa* had fumigant toxicity to *Tribolium castaneum* adults. The essential oil exhibit toxic, repellent and feeding deterrent activities against *T. castaneum* and *Sitiphilus zeamais* with different efficacies. *S. zeamais* adults were more susceptible than *T. castaneum* to contact toxicity. *T. castaneum* larvae were slightly more tolerant than the adults (Liu and Ho 1999). In fumigant toxicity, *T. castaneum* was more susceptible than *S. zeamais*. Nutmeg oil was much more potent against *S. zeamais* adults than *T. castaneum* as a contact insecticide than as a fumigant. Nutmeg oil was ovicidal to the eggs of *T. castaneum* (Huang *et al.* 1997). *T. castaneum* adults showed 10 and 100% mortality at concentrations of 18.9 and 33.9 mg/g food after three days, when the control mortality was 2%. *T. castaneum* adults were more susceptible than *S. zeamais* to the fumigant toxicity with *Cinnamomum aromaticum* extracted compound cinnmaldehyde (Huang and Ho 1998). Safrole possessed equal fumigant toxicity to adult *T. castaneum* and *S. zeamais*. Adults of *S. zeamais* were twice tolerant as *T. castaneum* to issosafrole. Issosafrole was more toxic than safrole to both the species (Huang *et al.* 1999). Adults of *S. zeamais* and *T. castaneum* showed similar susceptibilities to the contact toxicity of cardamomum oil extracted at the LD_{50} levels but *S. zeamais* was more susceptible than *T. castaneum* at LD_{50} level. *S. zeamais* were more than twice susceptible to fumigant toxicity to cardmomum oils. *T. castaneum* adults were much more susceptible than larvae (Huang *et al.* 2000).

It is known that *Murraya* contains monoterpene and sesquiterpene rich oils (Li *et al.* 1988) which exhibit growth disrupting activity against insects (Slama *et al.* 1974, Ghani 1998) Leaf powder and seed extract of *Calotropis procera* were insecticidal against *Rhizopertha dominica* (Sharma 1983a, Jacob and Sheila 1993), *C. chinensis* (Yadav and Bhatnagar 1987), *T. confusum* (Jahan *et al.* 1991) and *C. analis* (Naqvi and Perveen 1983).

Dwiveda and Kumar (1999) stated that a large number of plant extracts exhibited oviposition deterrent properties. Bhaduri *et al.* (1985) reported that plant extracts exhibit repellent, antifeedant or insecticidal properties. Presence of some repellent ingredients inhibits the laying of eggs by beetle on seed. In 3-4 days old eggs, the larvae are partially or fully formed, do not feed on the seed and thus they die due to starvation or when the 1st instar larvae come in contact with the extract coating of the seed, they die due to the insecticidal properties.

Bekele *et al.* (1996) reported that botanical pesticides are selective, environmentally safe and non-toxic to mammals, human being and other beneficial animals. Tiwari (1994) regarded plant materials to be safe for the mammals but toxic to the insects.

The results of antibacterial activity of oily extracts against a number of gram-positive and gram-negative bacteria showed mild to moderate toxic effects. When the concentration of the extract was increased, the zone of inhibition was found to be increased.

The brine shrimp lethality bioassay indicates the cytotoxicity as well as wide range of pharmacological activities, e.g. anticancer, and antiviral activities of oily extract of *Sida rhombifolia* (Islam *et al.* 2000). Methanolic

and chloroform extracts of experimental plants were found to be toxic against brine shrimp nauplii in the present investigation.

Many scientists isolated several compounds from these plant and their medicinal activities were reported but their insecticidal activities against *A. diaperinus* and brine shrimp nauplii were not reported. So our finding using extracted oil against stored product insect pest as well as brine shrimp nauplii seems to be a new work.

It is known that almost all the complex fractions of fixed vegetable oil yield fatty acid, etc. A significant effect of triglyceride oil fraction and oleic acid components on the adult mortality of *Z. subfasciatus* was reported by Hill and Schoonhoven (1981). The unsaturated fatty acids (linoic acid and oleic acid) present in *Annona. squamosa* seed oil (Parveen and Selman 1995a) have insecticidal properties (Kumar and Okonronkwo 1991).

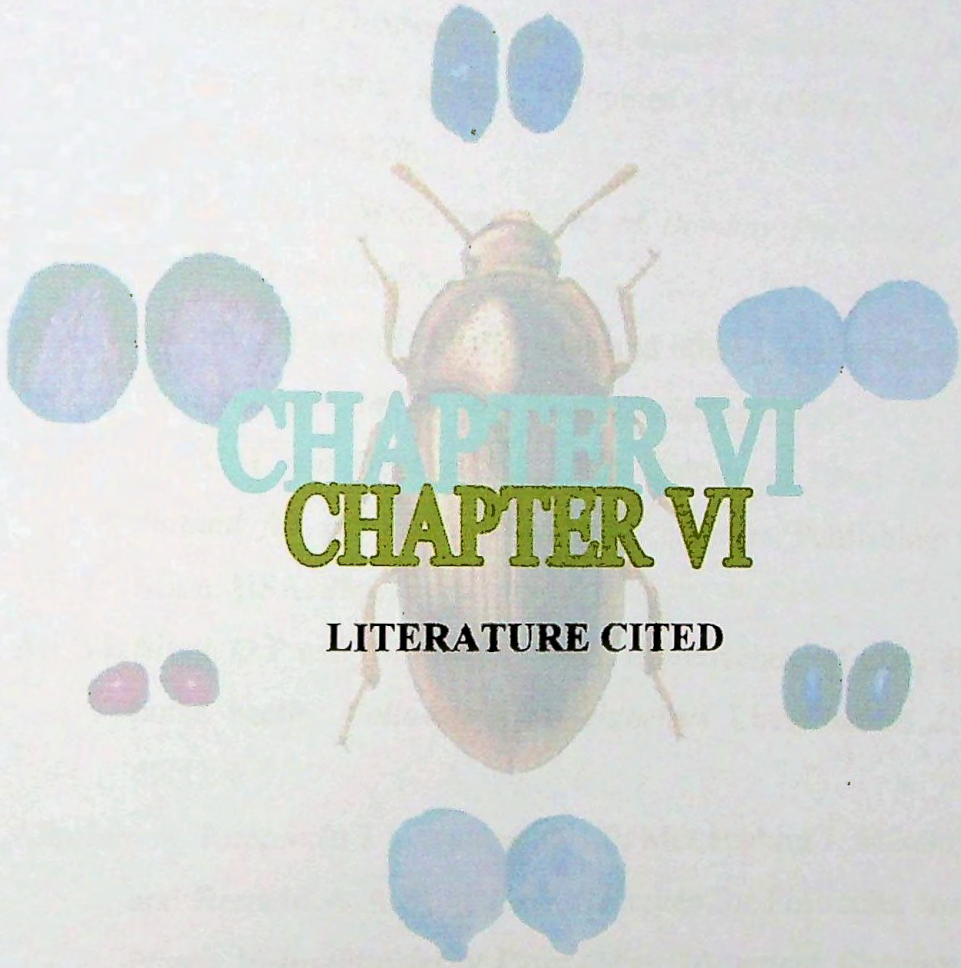
Different doses of plant materials have long been considered as human usage. The effects of plant products are shortlived and so frequent applications are requested to accomplish a reasonable degree of efficacy (Coats 1994). However, phytochemical have no harmful residues in the environment as do the synthetic pesticides. The environment friendly nature of botanicals is a great plus point in their pesticidal application. So the extracts, and plant-originated pesticides may be used in the IPM system with the lower doses of synthetic pesticides.

The present investigation reveals that the chloroform and methanolic extracts of plant seeds were highly active against the bacteria *Staphylococcus aureus*, and *Shigella dysenteriae* and the fungus, *Aspergillus flavus*. The MIC results indicated that the methanolic extract of the seed coat oil has the property of inhibiting bacterial growth even at low concentrations (64-128 µg/ml). This probably explains the use of the extract

of this plant in traditional medicines against a number of infections. So, detailed studies on this plant regarding their pharmacological and toxicological effects, and the bioactive compounds as well as their mechanism of action are needed.

The contact toxicity of the oil on the test insect in terms of LD_{50} (median lethal dose) of the extracts in two solvents from seed coat and seed kernels were not similar. In general, all seed kernel extracts showed a higher degree of toxicity on test insect, as compared to the seed coat extracts.

The results of the present investigation show that the seed oils of the experimental plants have antibacterial, antifugal and insecticidal properties. More comprehensive works are very much to be solicited for their effective use, especially in medicine and agriculture.



CHAPTER VI

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