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Antimicrobial and insecticidal activities of Solanum torvum Sw. (Solanaceae) and Smilax zeylanica L. (Liliaceae) on Cryptolestes pusillus (Schon.) (Coleoptera: Cucujidae)

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ANTIMICROBIAL AND INSECTICIDAL ACTIVITIES OF *SOLANUM TORVUM* SW. (SOLANACEAE) AND *SMILAX ZEYLANICA* L. (LILIACEAE) ON *CRYPTOLESTES PUSILLUS* (SCHON.) (COLEOPTERA : CUCUJIDAE)

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by

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FEBRUARY 2009

DEDICATED
TO
MY BELOVED PARENTS

DECLARATION

I hereby declare that the thesis entitled **Antimicrobial and insecticidal activities of *Solanum torvum* Sw. (Solanaceae) and *Smilax zeylanica* L. (Liliaceae) on *Cryptolestes pusillus* (Schon.) (Coleoptera: Cucujidae)'** submitted to the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh, for the degree of **Master of Philosophy,** is the result of my own investigation and was carried out under the supervision of Professor Dr Md Wahedul Islam, Institute of Biological Sciences, Rajshahi University and Professor Dr Md Ataur Rahman Khan, Department of Zoology, Rajshahi University, Rajshahi, Bangladesh. The thesis contains no material previously published or submitted elsewhere for any other degree.

28.02.2009

Md Abdul Bari
Candidate

Rajshahi
February, 2009

CERTIFICATE

This is to certify that the contents reported in this thesis entitled ***Antimicrobial and insecticidal activities*** *of* ***Solanum torvum Sw. (Solanaceae) and Smilax zeylanica L. (Liliaceae) on Cryptolestes pusillus (Schon.) (Coleoptera: Cucujidae)*** *is the record of an original research work performed by Md Abdul Bari at the Integrated Pest Management (IPM) laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh, under our supervision for the degree of* ***Master of Philosophy****. It contains no material previously published or submitted elsewhere for any other degree. 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 **

The bioactivity and insecticidal activities of different parts of two plants, *viz.* leaf, stem, root and inflorescence of *Solanum torvum* and leaf, stem and root of *Smilax zeylanica* were assayed against gram positive and gram negative pathogenic bacteria, fungi, brine shrimps and the insect, red flat grain beetle, *Cryptolestes pusillus* were investigated.

Toxicidal activity of the chloroform and methanolic extracts of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* on some microbes (Gram-positive bacteria: *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylcoccus aureus*, *Sarcina lutea*, *Streptococcus-β–haemolyticus* and Gram negative bacteria: *Salmonella typhi*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella sonnei*, *Shigella boydii*, *Escherichia coli*, *Klebsiella species* (K.sp.), *Pseudomonas aeruginosa*, *Proteus* sp.; and fungi: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Vasin factum*, *Candida albicans*, *Mucor* sp., *Fusarium oxysporum* and *Colletotrichum falcatum*) 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, *C. pusillus* was determined. Methanolic extracts of root of *S. torvum* and *S. zeylanica* exhibited higher toxicity on the microbes, brine shrimp and the beetle.

All the 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 two test plants possessed strong residual toxic effects on *C. pusillus* that may have promising potential in controlling storage pest management programmes.

CONTENTS

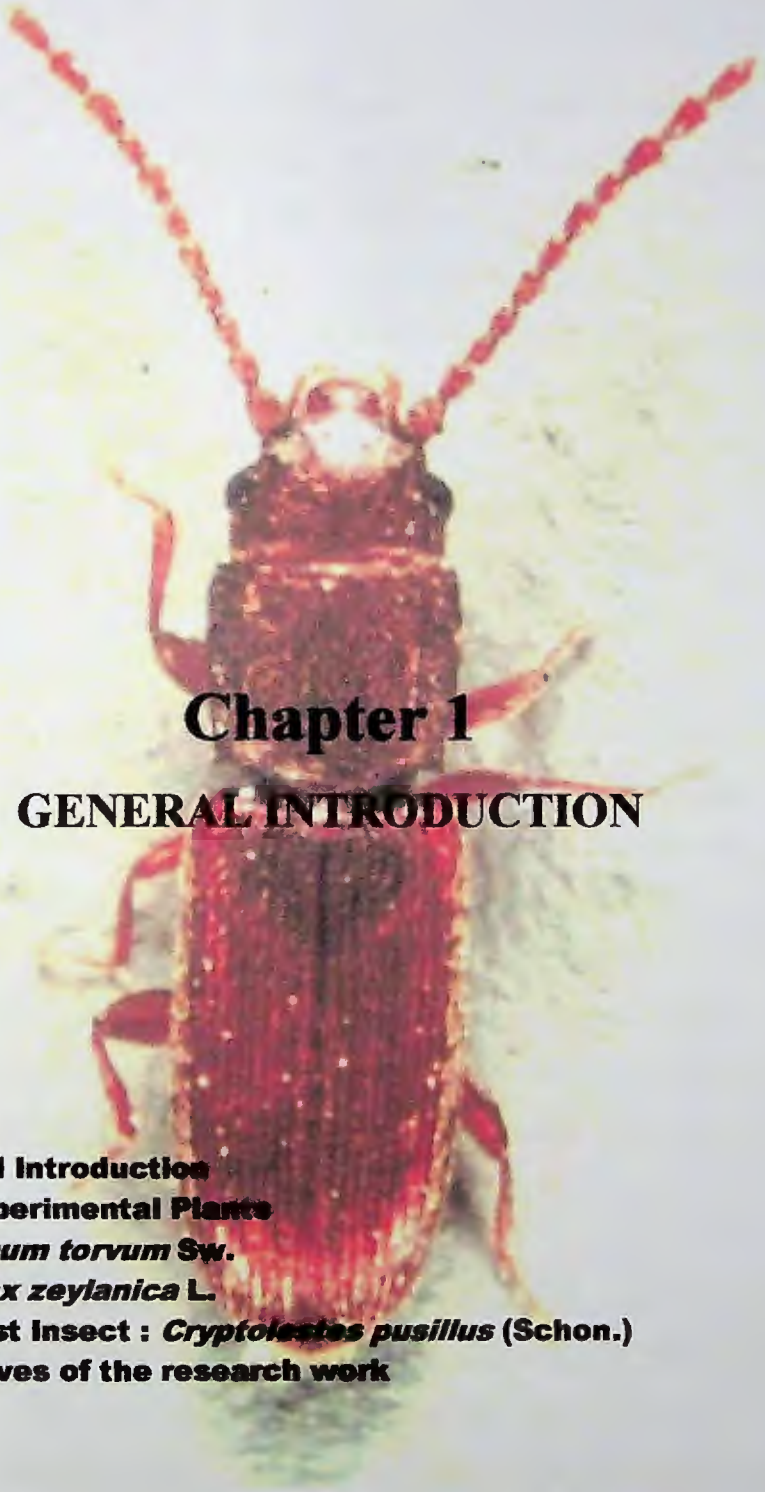
Abbreviations Used in the Text of this Dissertation

A	=	*Aspergillus*
B	=	*Bacillus*
C	=	*Cryptolestes*
C. V.	=	Co-efficient of variation
D. F.	=	Degrees of freedom
E	=	*Escherichia*
e. g.	=	Example given
et al.	=	And others
F	=	Variance ratio
i. e.	=	That is to say
viz.	=	Namely.
gm.	=	Gram (s)
L. D.	=	Lethal dose
L. C.	=	Lethal concentration
mg.	=	Milligram (s)
ml	=	Milliliter (s)
mm.	=	Millimeter (s)
µg	=	Microgram
µl	=	Microlitre
No.	=	Number of observation
P	=	Probability
ppm	=	Parts per million
P.R.C.	=	Percent reproduction control
S	=	*Shigella*
SD	=	Standard deviation
sp	=	Species
%	=	Percentage
χ^2	=	Chi-squared
^{0}C	=	Degrees Celsius

List of Plates

List of Tables

List of Figures



Chapter 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1. INTRODUCTION

The world is endowed with a rich wealth of medicinal plants. Medicinal plants are the local heritage with global importance. 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 care facilities.

The variety and sheer number of plants with therapeutic properties are 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 medicine. In the developing countries, about 80 % of the people depend upon the traditional system of medicine and 95 % of the industrial need of this is met through indiscriminate collection from the 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 there are only about 10,000 secondary plant metabolites whose major role in the plants against insect pests is defensive (Swain 1977). About 2,000 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, 225 are repellents, 32 growth regulators and 1 chemosterilant.

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 insects 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 of the world's supply of stored grains from insect damage ranges from 5-10 % of the world production (Burkholder 1990). In the 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 1985s 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 2000). Golob and Webley (1980) produced a bibliography summarizing 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.

Man has been fighting to protect his food from insect damage since his knowledge about their attack and losses to stored grains. Including the major concern of pest infestation during storage, the intensification of food production has led to several problems in the post harvest phase. This is further aggravated by the increased attention paid to maintenance of buffer stocks to provide food security for a country. The intensification of food production has led to several problems in the post harvest phase including pest infestation during storage. These problems are more acute in the tropics than in temperate zones because the environment in the former is more conducive to the growth and development of pests (Khan and Mannan 1991).

Post harvest losses of stored produce are generally expressed in terms of direct weight and nutrient loss (Howe 1965, Krishnamurthy 1975, Watters and Shuyler 1977).

Insects are the dominant group of storage pests but a second group of arthropods, the Acari or mites are also considered as a large group of important pests of stored food and other commodities. Other animal groups also contain pest species. So, pest control experts must often deal with simultaneous infestations by insects, mites, rodents and birds (Khan and Mannan 1991).

Among the insect pests of stored commodities, the beetles (Coleoptera) are the most dominant group followed by moths (Lepidoptera). More than 2000 species of field and storage pests annually destroy approximately one third of world's food production valued at about $100 billion among which the highest losses (43% of potential production) occur in developing Asian countries (Ahmed and Grainge 1986). Nearly 600 species of insects are associated with various stored products (Hinton 1945). The number of common storage insect pests in tropical regions is about 40 (Oudejans 1991).

According to Alam (1971), 5-8% of food grain seeds and different stored commodities are lost annually due to storage pests in Bangladesh. Pandey *et al.* (1980) estimated losses of 5.6 to 8.6 % for stored products in India. Morallo-Resjesus *et al.* (1991) reported that worldwide post harvest losses due to insects, microorganisms, rodents and birds are estimated to range from 10 to 20% annually.

Insect pests become important in stores when grains are kept for seed and consumption. Rice is the staple food of approximately half of the mankind and wheat is the second, though the global production of wheat is greater than that of

rice (Chandler 1979). Losses of rice and wheat in storage, therefore, affect the food availability of a large number of people.

One solution to this problem is to minimize post harvest losses due to pest attack. Both qualitative and quantitative losses of different grains are caused by many biological, chemical and physical factors, particularly by insect pests (Feakin 1970, Sinha and Muir 1973).

Farmers have been using plants or their parts in pest control for centuries. This method of pest control provide 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 various types of bioactivities against insects (Sighamony *et al.* 1984, Jayasinghe and Fujimoto 1990, Morallo-Rejesus *et al.* 1990 and 1993, Adalla *et a*l. 1993, Facknath and Kawol 1993, Kim *et al.* 1994, Naumann *et al.* 1994, Niber 1994, Rajuraman and Saxena 1994, Braverman and Chizov-Gingburg 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 prtoducts 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 plants contain some chemical constituents. Quantities of chemical constituents are usually found in the leaf, stem and root, occasionally in the

fleshy part of the fruit, inflorescence and flower. Many plant seeds have been used as a source of oil. There are many species of medicinal plants in our country. Of these two indigenous plants, Tit Begoon (*Solanum torvum* Sw.) and Kumarilata (*Smilax zeylanica* L.) belonging to the family Solanaceae and Liliaceae respectively have been considered as materials for the present investigation.

Solanum torvum Sw.

- *Solanum torvum* Sw. is a prickly, tomentose, erect shrub, 1.5-3 metre high, leaves having no prickles, white bell-shaped flowers and lobed fruits seated on the calyx.
- *S. torvum* is a common plant found throughout the Indian subcontinent. In Bangladesh, it is available in the dry regions and often occurs gregariously. It is commonly known as 'tit begoon' or 'hat begoon' or 'gota begoon'.
- Common people of Bangladesh, especially the tribes use the fruit of *S. torvum* as vegetables in their daily diet.

Smilax zeylanica L.

- *Smilax zeylanica* L. is a large woody climber with alternate, broadly ovate, stoutly petiolate, 3-5 nerved leaves, smooth stems having a few small distant prickles, tendrils and small greenish flowers in pedunculate many-flowered umbeis.
- *S. zeylanica* grows wild in Chittagong and sporadically in other areas throughout the country.
- *S. zeylanica* is commonly known as Kumarica, Kumarilata, Bulkumia, Bulkumialata in Bangla and Indian Sarsaparilla in English.

1.2.1. *SOLANUM TORVUM* SW.

Common name : Tit Begoon, Gota Begoon, Hat Begoon (Bangla.).

Propagation : Frugivorous birds eat the berry and spread the seeds.

Native range : It is widespread over the world although it is concentrated mainly in the tropics and subtropics. It is a common plant found throughout the Indian subcontinent.

Parts Used : Whole Plant.

Taxonomic position

Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Tracheophyta
Sub phylum	Eupphyllophytina
Infra phylum	Radiatopses
Class	Magnoliopsida
Subclass	Lamiidae
Order	Solanales
Family	Solanaceae
Genus	*Solanum* Linnaeus, 1753
Species	*S. torvum* Swartz, Prodr, 1788

Botanical Description

The family Solanaceae comprises about 80 genera and 3000 species, from which 1500 belong to the genus *Solanum*. This genus is widespread over the world although it is concentrated mainly in the tropics and subtropics. In Mexico, there are about 150 species. *Solanum torvum* are prickly, tomentose, erect shrubs, 1.5-3 meter high, leaves having no prickles, white bell-shaped flowers and lobed fruits seated on the calyx. Erect, much-branching perennial shrub up to 4 m in height. Stems densely tomentose, armed with scattered prickles. Leaves simple, alternate; blade ovate to elliptic in shape with an acute tip and rounded to oblique base, and mostly 5-20 cm long. Leaf margins shallowly and irregularly lobed, upper leaf surface scabrous, lower surface, petioles, and pedicels densely tomentose, petiole about one-quarter as long as the blade. Flowers in many-flowered corymbs borne at intervals on the stems. Calyx with five acute lobes, tomentose; corolla sympetalous, deeply divided into five acuminate lobes, white, and 12-18 mm long. Stamens five, yellow, epipetalous and erect. Fruit a many-seeded, green or yellow, glabrous, globose berry 10-15 mm in diameter (Whistler 1983).

Distribution

A major weed in pastures, roadsides and wasteland and also occurs in plantations, but not significantly in cultivated land. It prefers moist, fertile soil, but will tolerate drought. Infests pastures in solid stands that shade out undergrowth. Because of spines, interferes with passage of people and animals (Motooka *et al.* 2003). A weed of disturbed areas. Forms impenetrable thickets. In New Guinea, "a weed on grazing land; also at roadsides, forest edges and sometimes frequent among the regrowth on abandoned gardens. From near sea level to almost 2000 m" (Henty and Pritchard 1975). In New Caledonia, "espèce gênante dans les fourrés secondaires (MacKee 1994).

S. torvum is a common plant found throughout the Indian subcontinent. In Bangladesh it is common in dry regions and often occurs gregariously. It is locally known as tit begoon or gota begoon or hat begoon in Bengali and commonly known as turkey berry or susumber or gully-bean or thai eggplant or devil's fig. Common people of Bangladesh especially the tribes use the fruit of *S. torvum* as vegetables in their daily diet (Ghani 1998).

Medicinal Uses

The genus has toxic alkaloids which are distributed in all parts of the plant (Cronquist 1981). Several *Solanum* species contain free and glycosilated alkaloids, important substrates for the synthesis of steroidal hormones (Lewis and Liljegren 1970; Maití *et al.* 1979), thus making this species very important economically.

Leaves have been reported to contain the steroidal gluco-alkaloid, solasonine. In addition, they contain steroidal sapogenins, neochlorogenin, neosolaspigein and solaspigenine. They have also been found to contain triacontanol, tetratriacontanic acid, z-tritriacontanone, sitosterol, stigmasterol and campesterol. Fruits also contain the gluco-alkaloid, solasonine, sterolin (sitesterol-D-glucoside), protein, fat and minerals Chemical Abstracts (CA), 1949.

Plant parts are used as sedative, diuretic and digestive. They are also used in the treatment of coughs and colds (CA, 1949). Leaves are used as haemostatic. Extract of the fruits and leaves are said to be useful in case of liver and spleen enlargement and in the treatment of cough. Paste of root is used in cracks in feet. The fume of burning seeds is inhaled for toothache (Bhakuni 1962,1969). Due to the notable medicinal value of *S. torvum* (Belboukhari 2002, 2006), it

was considered to be of interest to carry out phytochemical and antimicrobial investigations of this species and the results leading to the antimicrobial screening are presented in this study.

A comparison was made between plants of *Solanum torvum* Sw. that grow in Chiapas, Mexico, and plants of the same species originating from India (Maiti, 1979). This was effected to establish either similarities or differences between these plants in total alkaloid contents and presence of solasodine, an important alkaloid for the partial synthesis of steroids. The total alkaloid content (0.12%) of the plants coming from Chiapas and India was the same. However, solasodine was found only in the plants of Chiapas. In addition, the total amount of glycoalkaloids (0.038%) and two glycosilated compounds derived from solasodine, solasonine (0.0043%) and solamargine (0.0028%) were determined.

The differences in climate conditions and location have an influence on the total alkaloid content of this species.. There are some species which contain a high amount of active glycoalkaloids, and it was interesting to know the relation between free alkaloids-glycosilated alkaloids. Therefore, the amount of glycoalkaloids and solasonine and solamargine (two glycosilated compounds of solasodine: Lewis *et al.* 1970), in *Solanum torvum*. These two compounds are the dominant steroidal glycoalkaloids in the genus *Solanum* (Ripperger 1995). Maití (1979) also reported the presence of solasodine in other species of *Solanum*.

Plate 1 : Tit begoon, *Solanum torvum* Sw. with flowers

Plate 2 : Tit begoon, *Solanum torvum* Sw. without flowers

1.2.2. *SMILAX ZEYLANICA* L.

Common name : Kumarilata, Kumarica, Kukur, Dainey, Bulkumia, Bulkumialata (Bangla.), Indian Sarsaparilla (English).

Propagation : By seeds.

Native range : Grows wild in Chittagong and sporadically in other areas.

Parts used : Roots and leaves (mainly).

Taxonomic position

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Caryophyllidae
Order	Plumbaginales
Family	Plumbaginaceae
Genus	*Smilax*
Species	*S. zeylanica* L.

Botanical Description

A large woody climber with alternate, broadly ovate, stoutly petiolate, 3-5-nerved leaves, smooth stems having a few small distant prickles, tendrils and small greenish flowers in pedunculate many-flowered umbels. A prickly, tendril climber. Tendril usually two, arising on either side of the leaf stalk. Prickles short, bent, scattered on the stem. Leaves alternate, broadly egg-shaped to elliptic, 6-12 × 4-8 cm, base rounded to rarely slightly heart-shaped with a narrow leaf sheath, apex rounded to acute, sometimes notched, margin entire, hairless, leathery; nerves 3-7, arising from the base of the leaf; leaf stalks 1-3 cm long, twisted. Male and female flowers are separated, seen in the same plant, arranged in axillary's 1-3 branched umbels, greenish white, about 6 mm long, 8 mm across, distinctly stalked. Berries globose, about 8 mm across, shiny, smooth, hairless, green turning red and becoming black when ripe.

Distribution

S. zeylanica grows wild in Chittagong and sporadically in other areas of the country. It is a common plant found throughout the Indian subcontinent. In Bangladesh, it is common in dry regions and often occurs gregariously. It is locally known as kumarica, bulkumialata in Bangla., Indian Sarsaparilla in English and commonly known as kumarilata or kumarica or kukur or dainey. Common people of Bangladesh especially the tribes use the roots and rhizomes of *S. zeylanica* as tonic in their daily treatment.

Medicinal uses

The plant contains 1-3% steroidal saponins, phytosterols, starch, resin, sarsapic acid and minerals (Chevallier 1996). Leaves and root contain diosgenin (Kar and Sen 1984). Roots also contain large amounts of tannin, saponin, 31-norcycloartenol and beta-sitosterol, a heteroside, parillin, a complex phenolic acid and potassium nitrate. The saponin, on hydrolysis, yields the sapogenins, sarsasapogenin, asperagenin and another steroid sapogenin. The roots also contain coumarin, smilasperic acid, a volatile oil, a crystallisable principle hemidesmine, and rutin (Said 1996).

Roots are used as a substitute for the Official drug, Sarsaparilla, in the treatment of venereal diseases; decoction is applied for rheumatism, pains in the lower extremities, sores, swelling and abscesses, and used in dysentery (Chopra *et al.* 1956). Juice of tender stems is used in weakness, and, with turmeric, used as a blood purifier, juice made from roots are used in blood dysentry and abscess.

The rhizomes are bitter, acrid, thermogenic, anodyne, anti-inflamatory, digestive, laxative, depurative, aphrodisiac, diuretic, sudorific, febrifuge and tonic. They are useful in syphilis, leprosy, skin diseases, epilepsy, insanity, scrofula, vitiated conditions of vata, flatulence, dyspepsia, colic, neuralgia, constipation, helminthiasis, psoriasis, fever, strangury, seminal weakness and general debility.

Plate 3 : Kumarilata, *Smilax zeylanica* L. without flowers

Plate 4 : Kumarilata, *Smilax zeylanica* L. with flowers

1.3. THE TEST INSECT : *CRYPTOLESTES PUSILLUS* (SCHON.)

Pest status, distribution and damage

Cryptolestes pusillus (Schon.) is a very common destructive insect pest of stored grain (Davies 1949, Pajni and Bedi 1974, Barker 1976). It is popularly known as the **Red Flat Grain Beetle.** The pest utilises beans, cassava, cocoa, cowpeas, groundnuts, maize, rice, sorghum, wheat and wheat flour as their hosts.

Howe and Lefkovitch first recorded this pest (1858). *C. pusillus* is cosmopolitan in distribution. Its status as a major pest of stored grains in the United Kingdom has been described by Freeman (1952). *C. pusillus* occurs in stored wheat, wheat flour, rice (husked), bran, corn meal and other stored products including pulses. It virtually feeds on all kinds of stored grains and milled cereal products and causes immense damage to stored wheat, flour and other stored commodities in all the tropical and subtropical countries of the world including Bangladesh (Dhaliwal 1976, Hossain *et al.* 1986, Kirkpatrick and Cagle 1978). The damage is caused both by the larval and adult stages (Cotton 1963).

In case of heavy infestation, the colour of flour is turned into pinkish with a disagreeable odour and a disgusting taste. The viscous and elastic properties of the flour are adversely affected and thus making it unsuitable for human consumption. The contamination involves accumulation of exuviae, egg shells, pupal cases, living or dead insects or insect parts, noxious and persistent odours, webbing of food and the faecal material of the beetles (Mondal 1985, Khan and Mannan 1991). Such losses may cause severe economic problems.

The quantitative assessment of loss is really a difficult task because of extremely variable and complex traditions that influence storage practice and also the lack of proper "loss survey" techniques.

Taxonomic notes of *C. pusillus*

Cryptolestes pusillus belongs to the family Cucujidae. The family Cucujidae is also known as the Laemophloeidae. This name was proposed by Schoenherr (1817) for a new genus of *Cucujus*. In 1899, Ganglbauer again described *C. pusillus* but later on recognized that it was the same species previously described as *pusillus* by Schoenherr (1817). *C. pusillus* has two synonyms such as *Laemophloeus pusillus* and *Laemophloeus mintus*. In the United Kingdom, *C. pusillus* is called the **Flat Grain Beetle.**

Biology of *C. pusillus*

- The adult *C. pusillus* is reddish-brown in colour with a flattened body. Males are normally smaller in size than the females having especially long and slender antennae.
- In the present experiment the larval, pre-pupal and pupal stages ranged from 19-21, 3-4, and 4-5 days respectively and it took about 30-34 days for egg to adult emergence.
- The biology of *C. pusillus* was run at 30±1°C and 70 % RH as outlined by Ahmed *et al.* (1994).
- The egg appears shiny white when first deposited but as development proceeds, it becomes pale yellowish. The incubation period ranged from 2.5 to 3.2 days with an average of 2.79 ± 0.21 days.
- Four distinct larval instars were observed in *C. pusillus*.

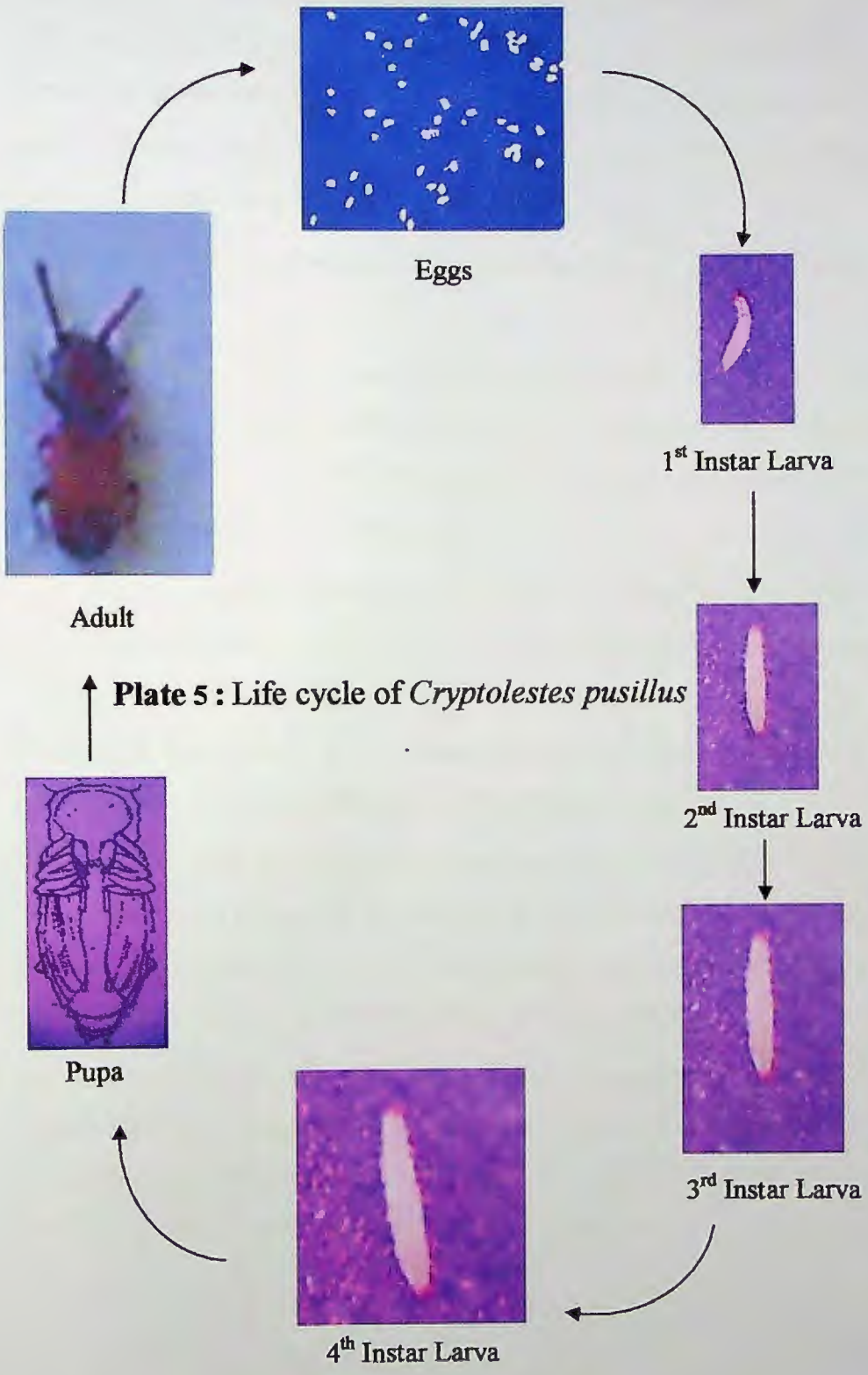


Plate 5 : Life cycle of *Cryptolestes pusillus*

- The 1st instar larva measures 0.51-0.75 mm in length (average 0.68 mm) and 0.08-0.14 mm (average 0.10 ± 0.01 mm) in head-capsule width. It has distinct ovoid or rounded head with 3-jointed antennae. Eye brownish, oval, each with 3 elongated bristles on the inner sides. The larva has 11 post-cephalic segments (3 thoracic, 8 abdominal) and 3 pairs of thoracic legs. The 1st instar larva is active, restless and moves forward by contracting and expanding its body.
- The 2nd and 3rd instar larvae are more or less same as 1st instar but larger in size.
- The fourth instar or mature larval body is milky white except the head and anal regions which appear brownish. The length of mature larva ranged from 1.85 to 3.75 mm (average 2.85 ± 0.65mm) and 0.35 to 0.55 mm width (average 0.45 ± 0.06 mm).
- Two pale brownish longitudinal lines proceed mid-dorsally starting from the 1st thoracic segment ending at the 7th abdominal segment. Antennae 3- jointed, tip of each antenna has an elongated hair and 2 short bristles.
- Mouthparts are distinct. After passing through 4 larval instars and a period of 20 ± 1.25 days, the larva was transformed to pre pupa.
- The pre-pupa is elongated and larval characters disappeared. The mature larva after 3 days of cocoon formation within the loosely attached food material was transformed to the pre-pupal stage. The length of pre-pupae was 2.06±0.16 mm and 0.31±0.02 mm in head-capsule width.
- The pupa is of exarate type. Six elongated hairs are present at the frontal region. Brownish circular eyes are visible. The pupal stage lasted for 4.2±0.50 days. The freshly formed pupa was light yellowish which gradually turned to pale reddish before adult emergence.

- The male pupa is smaller in size and is devoid of the finger-like lobes but female pupa is larger having characteristic finger-like lobes, the gonapophyses. The male pupa measured 1.56±0.02 mm in length while a female pupa measured 1.74± 0.04 mm.

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 of human and wild life.

The potentials for developing antimicrobials into medicines seem to be very much promising from the point of both drug development and phytomedicines.

Pest management

The concept of pest management grew out of the discontent with the purely insecticidal approach to pest control of the 1950s. Although the term was coined in the early 1960s, pest management had its roots in the earlier theories of biological control. Since its inception, pest management gained momentum and has become the major pest control philosophy for agricultural pests in developed countries. Australian entomologists I R Clark and P W Geier clearly outlined the principles of pest management in 1961. They suggested the term

"protective population management" or "pest management" for their ideas. Pest management was ordered to be used wherever practicable by presidential degree in 1977 and is mandated by California law to be used when feasible. Considering the economic benefit, methods of preventing losses of storage devices have been gradually developed.

Methods of pest management

There are various kinds of control measures such as chemical control, physical control, mechanical control, radiation control and biological control that are recommended and can be practised.

Use of botanicals

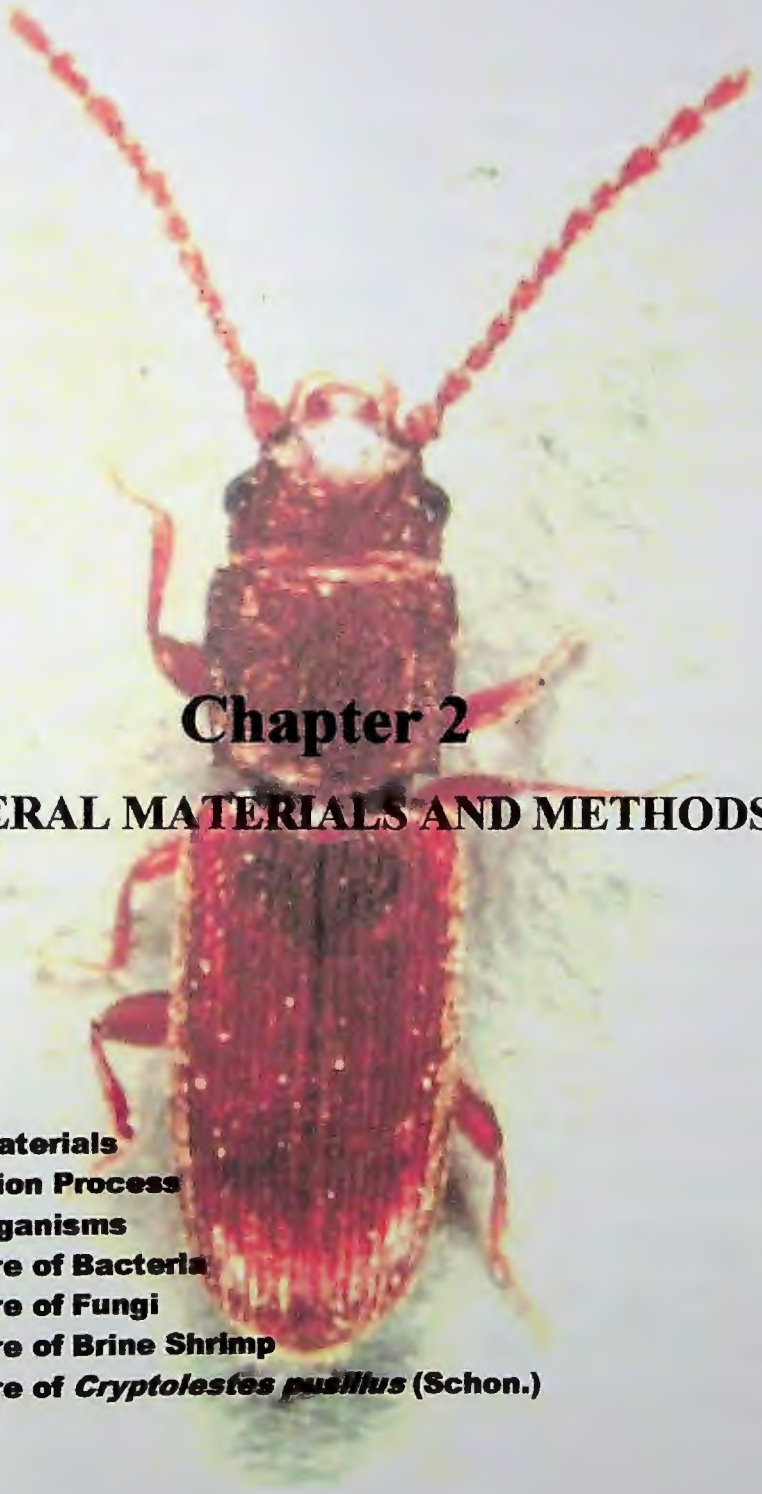
Botanicals (Plant products or oils) represent a very promising group of pesticides because of their apparent safety to mammals. Several works on botanicals as pest control agents have been made (Jacobson and Crosby 1967, Verma and Pandey 1978, Kabir *et al.* 1984, Rajasekaran and Kumaraswami 1985, Weaver *et al.* 1997). Sunita (2006) reported the effectiveness of neem extract (*Azadirachta indica* A. Juss.) in controlling the pest, *C. ferrugineus*.

Although, all of these techniques (measures) are very much effective for controlling the pests, there are many problems behind these specially the resistance problem to insecticides. The problem of resistance to pesticides provides a major reason for the development of improved pest management strategies that are less completely dependent upon pesticide use (Khan and Mannan 1991).

Today, there are more insect species of pest status than ever before and the pest control costs have been raised strikingly. Moreover, the insecticides are now threat to human life and creating the environmental hazards in terms of global context. Consequently, non-chemical methods of pest control have drawn much attraction and accordingly, the biological control of pests is an encouraging and popular option.

1.4. OBJECTIVES OF THE RESEARCH WORK

The aim of the present research work was to investigate the effectiveness of different parts of two plants, *viz.* leaf, stem, root and inflorescence of Tit begoon (*Solanum torvum* Sw.) and leaf, stem and root of Kumarilata (*Smilax zeylanica* L.) against bacteria, fungus, brine shrimp and the insect, *Cryptolestes pusillus* (Schon.). Selection of the plants as chemicals in the experiment was based on their effectiveness against other stored product insect pests. The main objective of the investigation was also to study the effects of these extracts on behavioural and biological aspects of *C. pusillus* as well as their toxicity causing the mortality of adults.



Chapter 2

GENERAL MATERIALS AND METHODS

2.1. Plant Materials
2.2. Extraction Process
2.3. Test Organisms
2.3.1. Culture of Bacteria
2.3.2. Culture of Fungi
2.3.3. Culture of Brine Shrimp
2.3.4. Culture of *Cryptolestes pusillus* (Schon.)

GENERAL MATERIALS AND METHODS

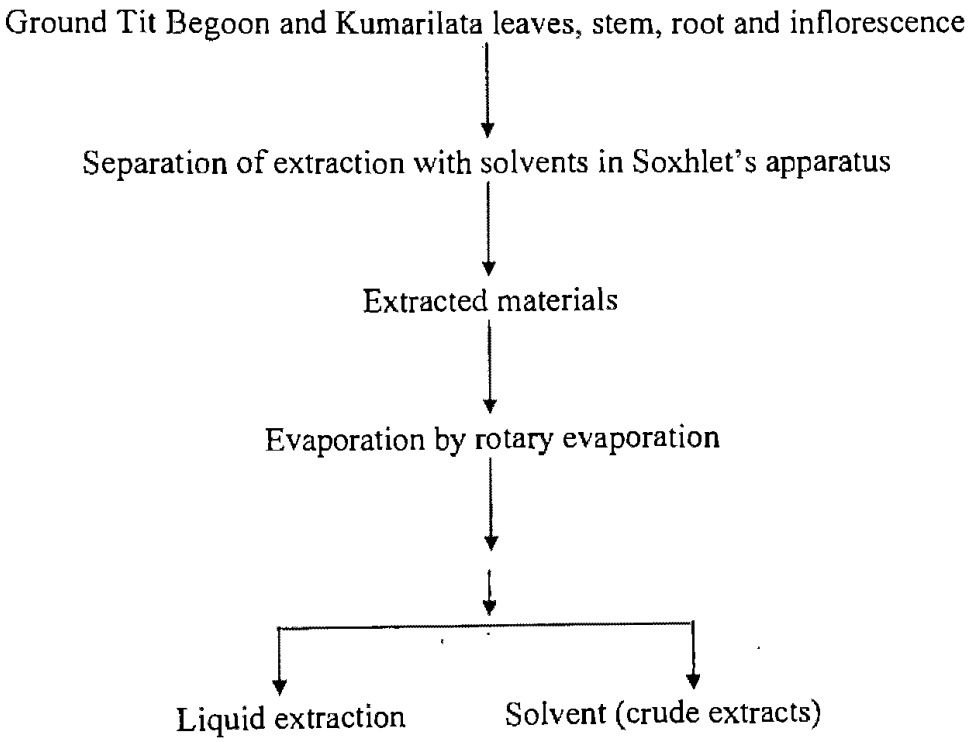
2.1. PLANT MATERIALS

Two plants, *viz.* Tit begoon (*Solanum torvum* Sw.) and Kumarilata (*Smilax zeylanica* L.) have been considered for this investigation. The whole test plants were collected from the Botanical Gardens and Rajshahi University Campus. Collected plants were stored and their leaf, stem, root and inflorescence were separated. Leaf, stem, root and inflorescence were chopped into small pieces and dried under reduced pressure (Vaccum drier) at room temperature 28 ± 2^{0} C. Different plant parts (leaf, stem, root and inflorescence) 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.* leaf, stem, root and inflorescence of *Solanum torvum* and leaf, stem and root of *S. zeylanica* were extracted separately. Sufficient amounts of dust of each plant part were extracted in two different organic solvents, *viz.* chloroform ($CHCl_3$) and methanol (MeOH) 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.

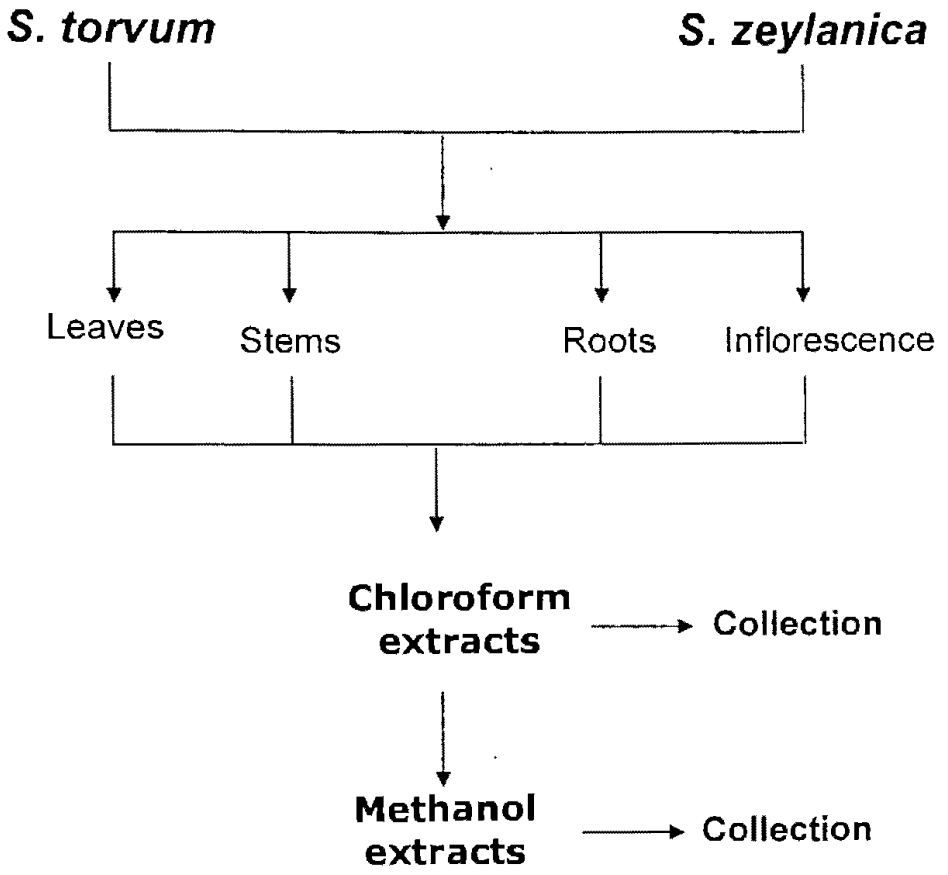
The extracts were collected separately and the mixed solvent was removed from the extracts with a vaccum rotary evaporator under reduced pressure. The extracts of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* were labeled and stored in a refrigerator at 20^0 C. The scheme for a liquid extraction method to produce standardized and enriched different parts of plant extracts is outlined bellow :



OUT LINE OF EXTRACTION PROCEDURE

- Scheme for a liquid extraction method to produce standardized and enriched *S. torvum* leaves, stem, root and inflorescence extracts.

- Collection of extracts in chloroform and methanol solvents from the plant materials of *S. torvum* and *S. zeylanica*



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 Red Flat grain beetle, *Cryptolestes pusillus* (Schon.).

2.3.1. CULTURE OF BACTERIA

Collection of bacteria : The microorganisms used in the present study were 15 (Six gram positive and Nine gram negative) human pathogenic bacteria and eight pathogenic fungi were collected from the Department of Pharmacy, University of Rajshahi, Bangladesh. The pure culture was previously collected from the Microbiological Laboratory, Department of Microbiology and Institute of Nutrition and Food Science (INFS), University of Dhaka, International Centre for Diarrhoea Diseases Research Bangladesh (ICDDRB), Dhaka, Bangladesh.

Table 1 : List of test pathogenic bacteria

Serial No.	Name of test organism	Strain number
Gram positive bacteria		
I	*Bacillus cereus*	-
II	*Bacillus megaterium*	QL-38
III	*Bacillus subtilis*	QL-40
IV	*Staphylococcus aureus*	ATCC-259233
V	*Sarcina lutea*	QL-166
VI	*Streptococcus-β-haemolyticus*	CRL
Gram negative bacteria		
VII	*Salmonella typhi*	-
VIII	*Shigella dysenteriae*	AL-35587
IX	*Shigella shiga*	ATCC-26107
X	*Shigella sonnei*	AJ-8992
XI	*Shigella boydii*	AL-17313
XII	*Escherichia coli*	FPFC-1407
XIII	*Klebsiella* sp.	-
XIV	*Pseudomonas aeruginosa*	CRL
XV	*Proteus* sp.	-

The following apparatus and reagents were used in the present investigation :

i. Filter paper discs (5mm diameter)

ii. Test tubes

iii. Petri dishes (20 cm and 6 cm diameter)

iv. Sterile forceps

v. Inoculating loop

vi. Bunsen burner

vii. Micropipette (10-100 µl)

viii. Laminar Air Flow Unit (Model No. NU- 126-300E)

ix. Autoclave (ALP Co. Ltd. KT-301, Tokyo)

x. Shaking Incubator (DK-SI 010 and Max-^{Q} / 4000)

xi. Incubator (OSK, 9639 A, Japan)

xii. Electronic Balance (ADAM ^{R} PW-124)

xiii. Soxhlet's apparatus (Type: EV-16)

xiv. Vaccum Rotary Evaporator (Rota vapor R-215)

xv. Punch machine

xvi. Beakerr

xvii. Nutrient agar media (DIFCO)

xviii. Alcohol (95 %)

xix. Methanol

xx. 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 121^0 C and a pressure of 15-lbs./sq. in. for 20 minutes. Blank or control discs were first kept in a covered Petridish and then subjected to dry heat sterilization at 180^0 C for one hour. Later they were kept in a laminar hood under 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. Adams and Roe medium.

Preparation of subcultures : 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 121^0 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 Air Flow Unit). The inoculated slants were then incubated at 37^0 C for 24-48 hours 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 20 ml autoclaved medium with the help of an inoculating loop in an aseptic area. The test tube was shaken in an shaking incubator 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 clockwise 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 4^0 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 or blank discs.

A. Sample discs : Sterilized filter paper discs 5 mm in diameter (BBL, Cocksville USA) 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 werer left for a few minutes for complete removal of solvent.

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

C. Control or 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 1 mg and 4 mg of each crude extracts (leaf, stem, root and inflorescence of *S. torvum*, and leaf, stem and root of *S. zeylanica*) in 200 µl of solvents to get a concentration 50 and 200 µg/10µl. 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 15 mm 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 4^0 C to obtain maximum diffusion of the test material. Finally, the plates were incubated at 37^0 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 pathogenic 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, Rajshahi, Bangladesh. The fungi were :

I. *Aspergillus fumigatus*,

II. *A. niger*,

III. *A. flavus*,

IV. *Vasin factum*,

V. *Mucor* sp.,

VI. *Candida albicans*,

VII. *Fusarium oxysporum*, and

VIII. *Colletotrichum falcatum*

Necessary Equipment :

i. Glass Petridish (20 cm diameter)

ii. Test tube (8-10 inch)

iii. Conical flask (1000 and 500 ml)

iv. Niddle 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 glass wares were cleaned and washed with recommended cleaning solution, which was prepared in the laboratory as follows :

Potassium dichromate	100 gm
Acid/Sulphuric	500 gm
Water	1000 gm

Glass wares were sterilized in an autoclave for 15 to 20 minutes at 250^0 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 th addition of agar. Most fungi will grow in media highly rich 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 (Alexopolos and Beneke 1962). Fungi were cultured on potato Dextrose Agar (PDA) media.

Composition : Composition of the potato Dextrose Agar (PDA) medium used was as follows :

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

Sliced potatoes were cooked in a beaker containing 500 ml 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 agar melt, 20 gm of sucrose was dissolved and restored to 1000 ml 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 121^0 C and 15 Ups/sq.m pressure. Glassware 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 sample, 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 instruments and surrounding area were cleaned with absolute alcohol. Room heater was placed in order to prevent any pathogen contamination from outside.

2.3.3. CULTURE OF BRINE SHRIMP

Collection of Eggs : *Artemia salina* Leach eggs were collected from the Department of Pharmacy, University of Rajshahi, 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 (10 and 100 µl)

v. Glass vials (10 ml)

vi. Magnifying glass

Procedure

Preparation of Simulated Seawater : Since the lethality test involves the culture the brine shrimp nauplii, the nauplii should growing seawater. Seawater nature contains 3.8 % sodium chloride (NaCl) 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. Shrimps eggs were added to the divided tank. A constant oxyzen supply and a constant temperature (around 37^0 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. Toxicity was studied using the larvae of brine shrimp nauplii, *Artemia salina* L (Meyer *et al.* 1982 and McLaughlin 1992). For each crude extracts 0.32 mg were initially dissolved in 100 µl of pure Dimethylsulfoxide (DMSO) to make hydrophilic before adding 1.9 ml of distilled water to get a concentration of 320 µg/ 2 ml for each extract which was used as stock solution. Sample extract solutions of 160, 80, 40, 20 and 10 ppm were made from the stock solution using by a serial tube dilution technique (Reiner 1982) and were placed in five different vials. Ten nauplii were then placed in each vials. 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 sea water were taken.

Counting of Nauplii : After 24 hours of incubation, the vials were observed and the number of survivors in each vials were counted and noted. The experiments were replicated thrice along with a standard, Ampicillin trihydrate. From this experiment, 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 no control mortality was observed during the exposure periods.

2.3.4. CULTURE OF THE INSECT (*CRYPTOLESTES PUSILLUS*)

Necessary Equipment

For maintaining the culture of the beetles, *C. pusillus* the following laboratory equipments 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 equipment mentioned above were sterilized by keeping in an oven for about six hours at 120^0 C (Khan 1981).

Origin of the beetles

The adults of *C. pusillus* were collected from the culture maintained at the Applied Zoology Division, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Rajshahi, Bangladesh.

Food medium

The adults of *C. pusillus* were mass reared on a standard mixture of whole wheat flour and powdered brewer's yeast in the ratio of 19:1 (Khalequzzaman *et al.* 1994) throughout the experiments.

Culture of beetles

This is a common pest of stored grains all over the tropical and sub-tropical countries of the world including Bangladesh. 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 petridishes (6cm diameter). Several sub-cultures were maintained, each containing 100 beetles in 60 gm of food in a Petridish.

Collection of eggs

After mating, the female beetle of *C. pusillus* laid eggs on the food medium. The eggs were deposited singly and were not cemented by the secretions of this insect to the substratum. Generally, eggs were dropped loosely in farinaceous material. A mated single female laid 3-6 eggs per day. 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 then placed in a petridish and incubated at room temperature. The incubation period ranged from 2-3 days.

Transfer of larvae to the food

The larvae hatched out in 2-3 days which were collected by a fine sable hair brush. Newly hatched larvae were transferred to the petridishes (6 cm diameter) containing wheat flour with the aid of a hair brush.

One hundred larvae were used for each food and the experiment was replicated thrice. The 1st instars larva was active, restless and moved forward by repeated contracting and expanding its body.

Collection of pupae and adults

After passing through 4 larval instars and a period of 19-21 days the larva was transformed to prepupa. The mature larva after 3 days of cocoon formation within the loosely attached food material was transformed to the pupal stage. The pupal stage lasted for 4-5 days. The freshly formed pupa was light yellowish which gradually turned to pale reddish before adult emergence. The young pupae were collected with a fine sable hair brush and transferred to petridishes for the adult emergence. The adult *C. pusillus* is a flattened, oblong and reddish-brown beetle. The total developmental period took 30-35 days.

Plate 6 : Dust of different parts of *S. torvum* and *S. zeylanica*

Notes: A = Inflorescence of *S. torvum* B = Root of *S. torvum*

C = Stem of *S. torvum* D = Leaf of *S. torvum*

E = Root of *S. zeylanica* F = Stem of *S. zeylanica*

G = Leaf of *S. zeylanica*

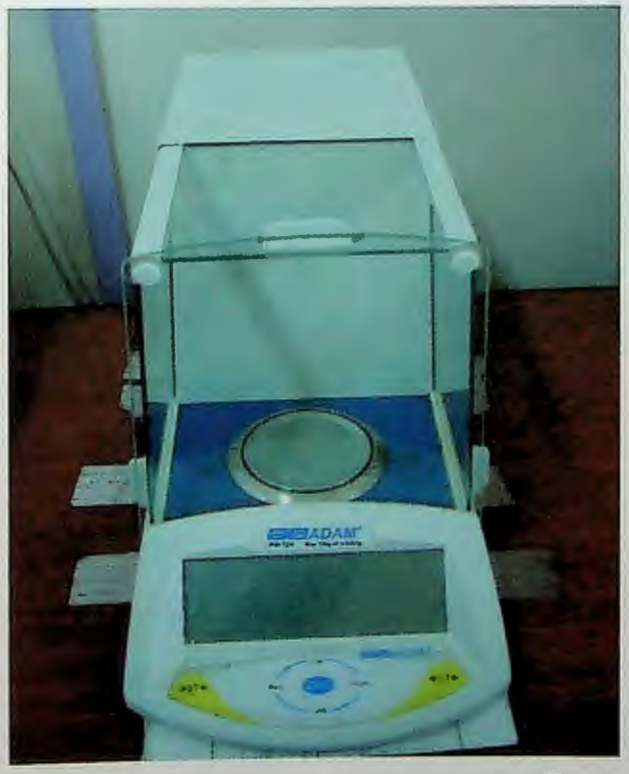


Plate 7 : An Electronic Balance (ADAM [R] PW-124)

Plate 8 : Extract preparation with chloroform

Plate 9 : Extract preparation with methanol

Plate 10 : Chloroform extracts of *S. torvum* and *S. zeylanica*

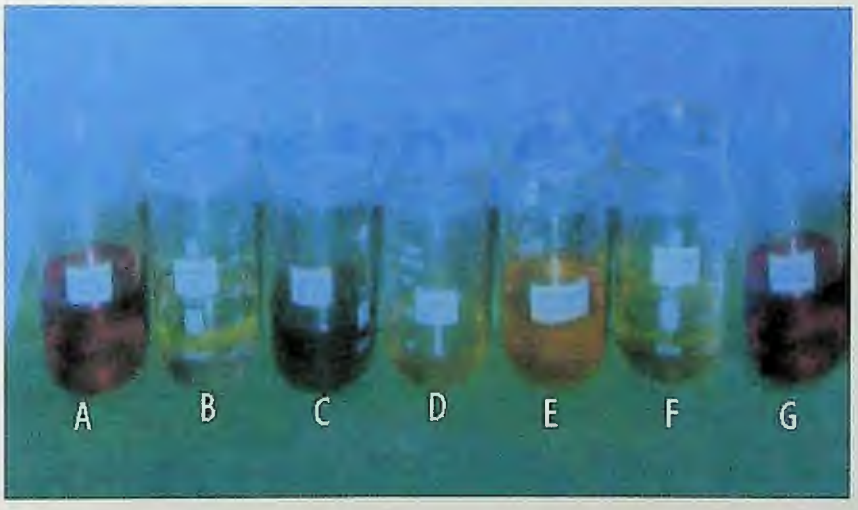


Plate 11 : Methanol extracts of *S. torvum* and *S. zeylanica*

Notes: A = Inflorescence of *S. torvum* B = Root of *S. torvum*

C = Stem of *S. torvum* D = Leaf of *S. torvum*

E = Root of *S. zeylanica* F = Stem of *S. zeylanica*

G = Leaf of *S. zeylanica*

Plate 12 : Shaker (Max-^{Q} / 4000)

Plate 13 : Shaking Incubator (DK-SI 010)

Plate 14 : Plant parts extraction in a Soxhlet's apparatus (Type: EV-16)

Plate 15 : Evaporation of mixed solvent from extraction by a Vaccum Rotary Evaporator (Rota vapor R-215)

Plate 16 : Laminar Air Flow Unit (Model No. NU- 126-300E)

Plate 17 : Autoclave (ALP Co. Ltd. KT-301, Tokyo)

Plate 18 : Adult red flat grain beetle, ***Cryptolestes pusillus*** **(Schon.)**

Plate 19 : Mass culture of ***Cryptolestes pusillus*** **(Schon.)**

Chapter 3

BIOLOGICAL ACTIVITIES OF DIFFERENT PLANT PART EXTRACTS

BIOLOGICAL ACTIVITIES OF DIFFERENT PLANT PART EXTRACTS

3.1. INTRODUCTION

Medicinal plants are the local heritage with global importance. 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 spices, as natural colouring agent, as antimicrobial properties and as insecticides. Plant based antimicrobials represent a vast untapped sources of medicines. Continued and further exploration of plant antimicrobials needs to occur (Farnsworth *et al.* 1991). The potentials for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines (Iwu *et 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 microorganisms 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 different parts of two plants, *i. e.* leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* was determined.

Materials and Methods

The antibacterial activity of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* were performed using the disc diffusion assay method of Calabrasi and Chabnep (1996). The Gram-positive bacteria: *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylcoccus aureus, Sarcina lutea, Streptococcus-β –haemolyticus* and Gram negative bacteria : *Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigella sonnei, Shigella boydii, Escherichia coli, Klebsiella species* (K.sp.), *Pseudomonas aeruginosa, Proteus* sp. were used as the test organisms to determine the medicinal effect of the target plants. The initials stocks were collected from the Microbiology Laboratory, Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh.

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 the 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 test tubes were then plugged with cotton and sterilized in an autoclave at 121^0 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 37^0 C for 18-24 hours 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 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 1982). The minimum inhibitory concentration of root and of *S. torvum* and stem of *S. zeylanica* were determined against gram positive (*Bacillus cereus, Bacillus subtilis, Streptococcus-β-haemolyticus*) and gram negative (*Salmonella typhi* and *Shigella dysenteriae*) bacteria. Nutrient agar and nutrient broth (DIFCO) were used as bacteriological media.

Preparation of the solution : The extract of root of *S. torvum* and stem of *S. zeylanica* 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 inocula : The test organisms were grown overnight at 37.5^0 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 as 1, 2, 3, 4, 5, 6, 7, 8, 9 and the rest three were assigned as C_M 9 (medium), C_S (medium + sample) and C_I (medium + inoculums).

2. To each of twelve test tubes, 1 ml of the sample solution was added and mixed well.

3. Then to the first test tube, 1 ml of the sample solution was added and mix well.

4. One ml content from the first test tube was transferred to the second test tube, was mixed uniformly and again1 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 37.5^0 C for 12 to 18 hrs.

The same procedure was also applied to determine the minimum inhibitory concentration against Gram positive (*Bacillus cereus, Bacillus subtilis, Streptococcus-β-haemolyticus*) and Gram negative (*Salmonella typhi* and *Shigella dysenteriae*) bacteria for each of the sample. The results are given in **Tables** 3 and 4.

3.3. ANTI FUNGAL ACTIVITY

The same procedure as used for the antibacterial activity test was performed for the antifungal activity of the extract of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica*. The only difference was that the period of incubation was 48 hrs at room temperature. Eight (Three) identified fungi collected from the Department of Pharmacy, University of Rajshahi were used for the test of antifungal activity. The fungi are listed bellow:

Table 2 : List of Test Pathogenic Fungi

I. *Aspergillus fumigatus*,
II. *ASpergillus niger*,
III. *ASpergillus flavus*,
IV. *Vasin factum*,
V. *Mucor* sp.,
VI. *Candida albicans*,
VII. *Fusarium oxysporum*, and
VIII. *Colletotrichum falcatum*

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

3.4. BRINE SHRIMP TOXICITY BIOASSAY

Toxicity of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* 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/l) to one side of the divided tank. Constant temperature (35 ± 2^0 C), sufficient light and air supply were maintained to give the sufficient aeration. After 48 hrs, mature nauplii were collected and used for the experiment.

Preparation of the sample solution : One milligram of each of the sample of crude extract and standard antibiotic Ampicillin tryhydrate 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 concentration 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 20 µl DMSO was added to each vial.

Counting of Nauplii and Data Analysis : After 24 hrs. 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, extract of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* separately.

3.5. RESULTS AND DISCUSSION

The biological activities of the extract of different parts of two plants *viz.* leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* were assayed against gram positive and gram negative pathogenic bacteria, fungi and brine shrimps. The results of these investigations are discussed bellow under separate headings.

Antibacterial activity

The antibacterial activities of the crude extracts of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* were determined at a concentration of 50 and 200 µg/disc against a total 6 gram positive and 9 gram negative human 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. At 50 µg/disc of crude extracts of root most of the test microorganisms of gram positive bacteria showed clear zone of growth of inhibition except *Sarcina lutea*. Out of nine gram negative bacteria only *Salmonella typhi* and *Shigella dysenteriae* shows clear zone of inhibition at both the concentration of chloroform and methanolic crude extracts of root. The crude extracts of both solvents, the root exhibited bigger and more prominent clear zone a 200 µg/disc against *Streptococcus-β-haemolyticus*. The chloroform and methanol extracts of the stem of *S. zeylanica* showed significant antibacterial activities against *Bacillus cereus* and *Salmonella typhi*. (**Tables** 3, 4) and (**Plates** 20 to 34).

Table 3 : Antibacterial activity of Chloroform and methanolic extracts of leaf, stem, root and inflorescence of ***S. torvum*** and standard Ciprofloxacin

Test Organisms	Diameter of zone of inhabitation (in mm)																
	Chloroform extract								Methanol extract								Ciprofloxacin 30 (µg/disc)
	Leaf		Stem		Root		Inflor*		Leaf		Stem		Root		Inflor*		
µg/disc $^{-1}$ →	50	200	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
Gram positive bacteria																	
Bacillus cereus	-	-	-	09	06	14	07	13	-	-	-	19	08	22	-	21	33
B. megaterium	-	-	-	-	07	12	-	-	-	-	-	-	08	19	-	-	35
B. subtilis	-	-	-	-	07	18	-	-	-	-	-	-	08	21	-	-	34
Staphylococcus aureus	-	-	07	09	07	13	07	12	-	-	07	19	07	22	07	21	34
Sarcina lutea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33
Streptococcus-β-haemolyticus	-	-	-	-	09	21	-	-	-	-	-	-	09	24	-	-	34
Gram negative bacteria																	
Salmonella typhi	-	11	07	14	07	19	07	13	-	16	07	20	07	21	07	21	35
Shigella dysenteriae	-	-	-	-	07	18	-	17	-	-	-	-	07	20	-	18	34
S. shiga	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
S. sonnei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33
S. boydii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35
Klebsiella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35
Proteus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34

Notes : Inflor* = Inflorescence, - = Not active

Table 4 : Antibacterial activity of Chloroform and methanolic extracts of leaf, stem and root of ***S. zeylanica*** and standard Ciprofloxacin

Test Organisms	Diameter of zone of inhibition (in mm)												
	Chloroform extract						Methanol extract						Ciprofloxacin 30 (µg/disc)
	Leaf		Stem		Root		Leaf		Stem		Root		
µg/disc $^{-1}$ →	50	200	50	200	50	200	50	200	50	200	50	200	
Gram (+) bacteria													
Bacillus cereus	-	-	07	17	07	14	-	-	09	21	08	19	33
B. megaterium	-	-	-	-	-	-	-	-	-	-	-	-	35
B. subtilis	-	-	06	13	07	-	-	-	08	20	-	-	34
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	34
Sarcina lutea	-	-	-	-	-	-	-	-	-	-	-	-	33
Streptococcus-β-haemolyticus	-	-	07	12	07	12	-	-	08	20	-	-	34
Gram (-) bacteria													
Salmonella typhi	-	11	07	20	07	17	-	16	08	23	07	21	35
Shigella dysenteriae	-	-	-	12	07	18	-	-	-	19	07	18	34
S. shiga	-	-	-	-	-	-	-	-	-	-	-	-	34
S. sonnei	-	-	07	-	-	-	-	-	09	-	-	-	33
S. boydii	-	-	-	-	-	-	-	-	-	-	-	-	34
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	35
Klebsiella sp.	-	-	-	-	-	-	-	-	-	-	-	-	34
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	35
Proteus sp.	-	-	-	-	-	-	-	-	-	-	-	-	34

Note : - = Not active

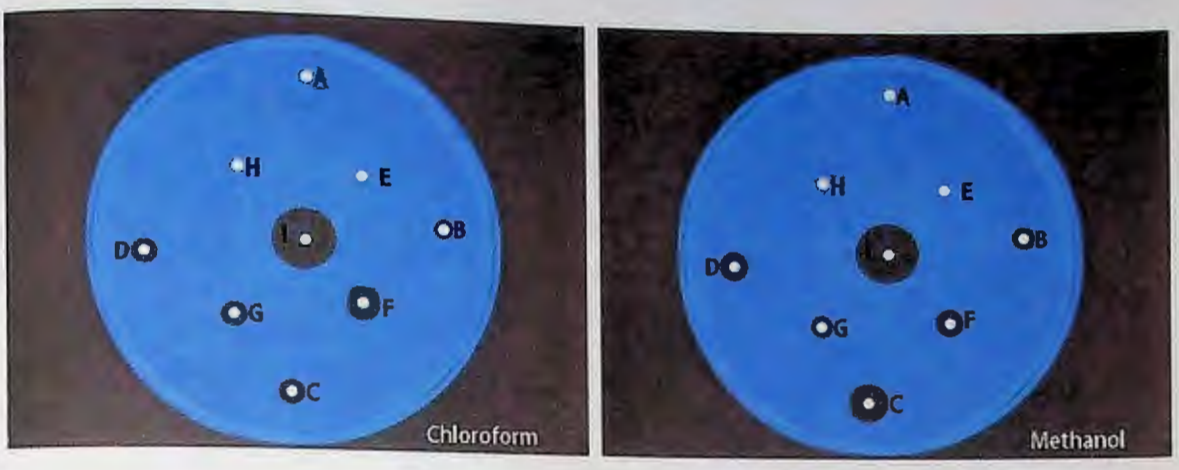


Plate 20 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Bacillus cereus*

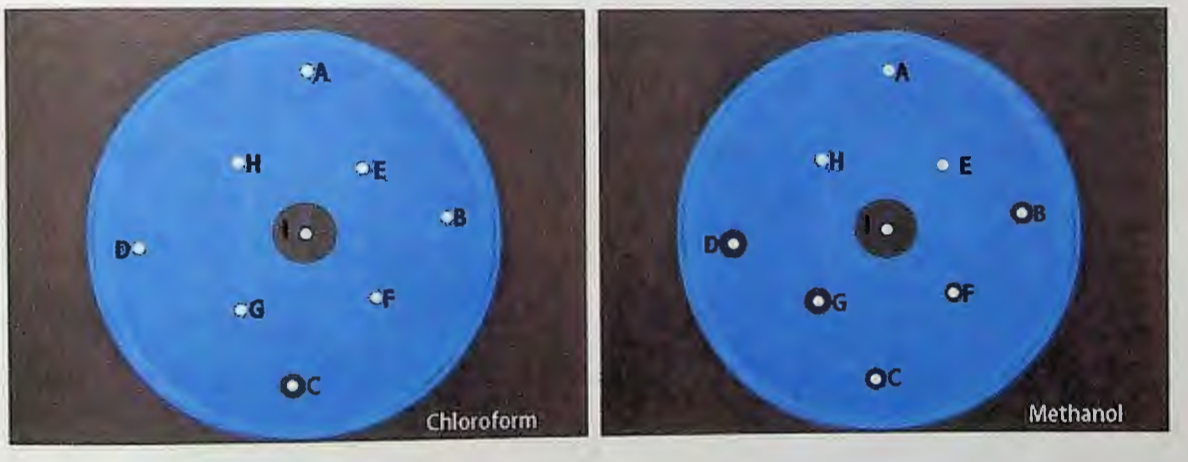


Plate 21 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Bacillus megaterium*

Notes : A - Tit begoon leaf
B - Tit begoon stem
C - Tit begoon root
D - Tit begoon inflorescence
E - Kumarilata leaf
F - Kumarilata stem
G - Kumarilata root
H - Solvent, and I - Ciprofloxacin (Control)

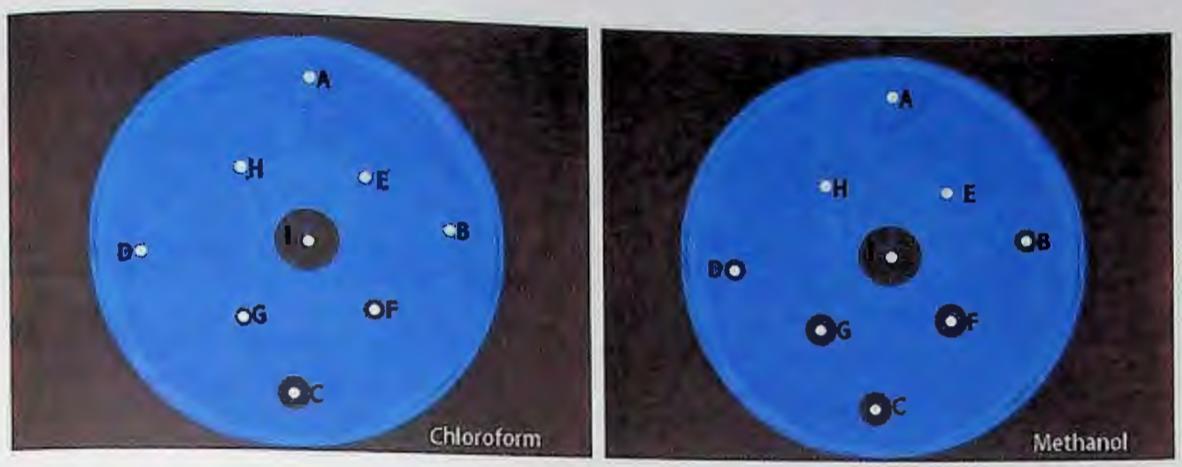


Plate 22 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Bacillus subtilis*

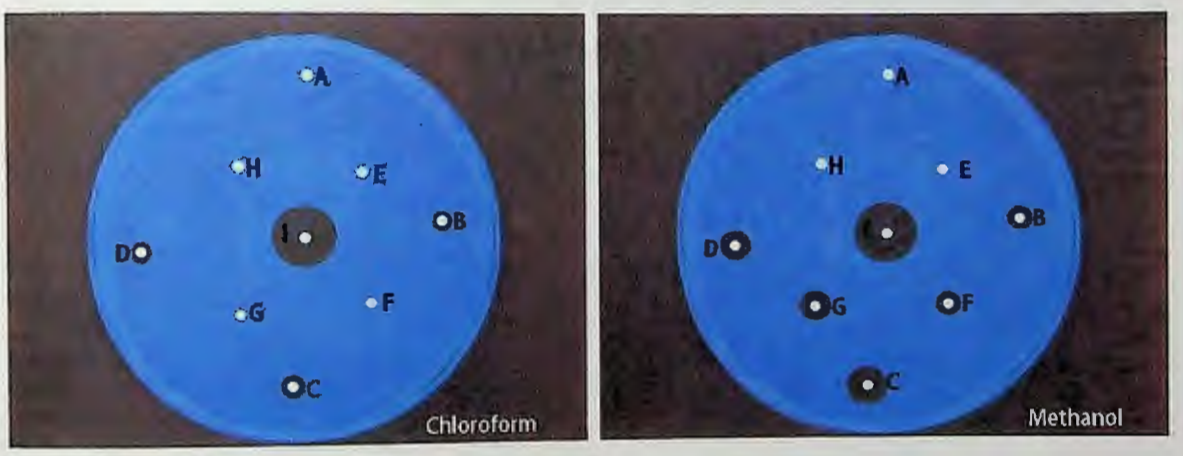


Plate 23 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Staphylococcus aureus*

Notes : A - Tit begoon leaf
B - Tit begoon stem
C - Tit begoon root
D - Tit begoon inflorescence
E - Kumarilata leaf
F - Kumarilata stem
G - Kumarilata root
H - Solvent, and I - Ciprofloxacin (Control)

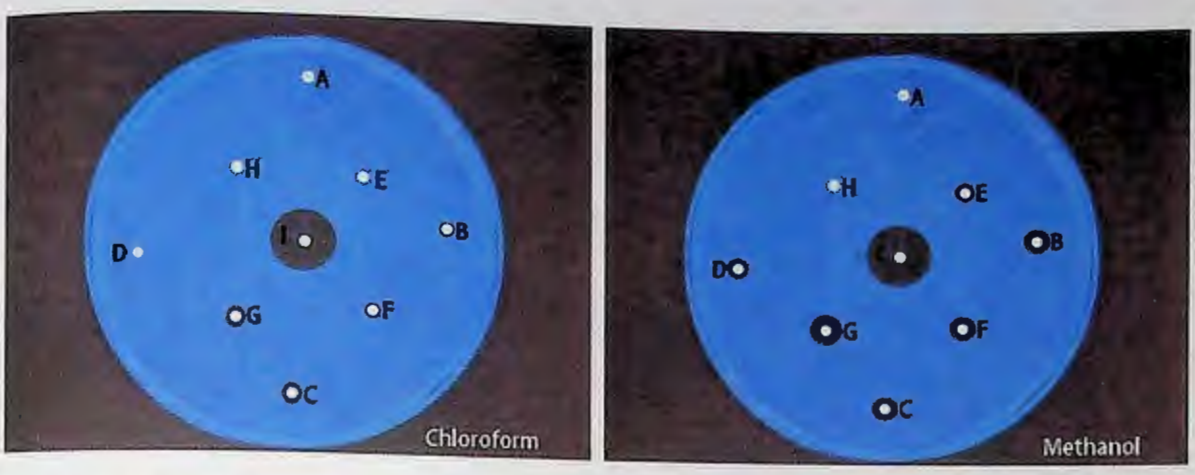


Plate 24 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Sarcina lutea*

Plate 25 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Streptococcus.-β-haemolyticus*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

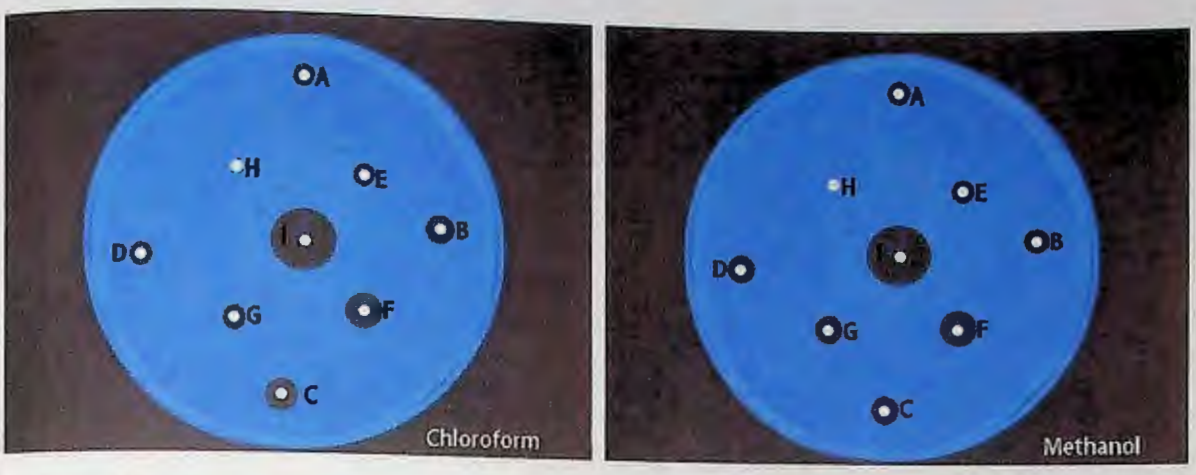


Plate 26 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Salmonella typhi*

Plate 27 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Shigella dysenteriae*

Notes : A - Tit begoon leaf
B - Tit begoon stem
C - Tit begoon root
D - Tit begoon inflorescence
E - Kumarilata leaf
F - Kumarilata stem
G - Kumarilata root
H - Solvent, and I - Ciprofloxacin (Control)

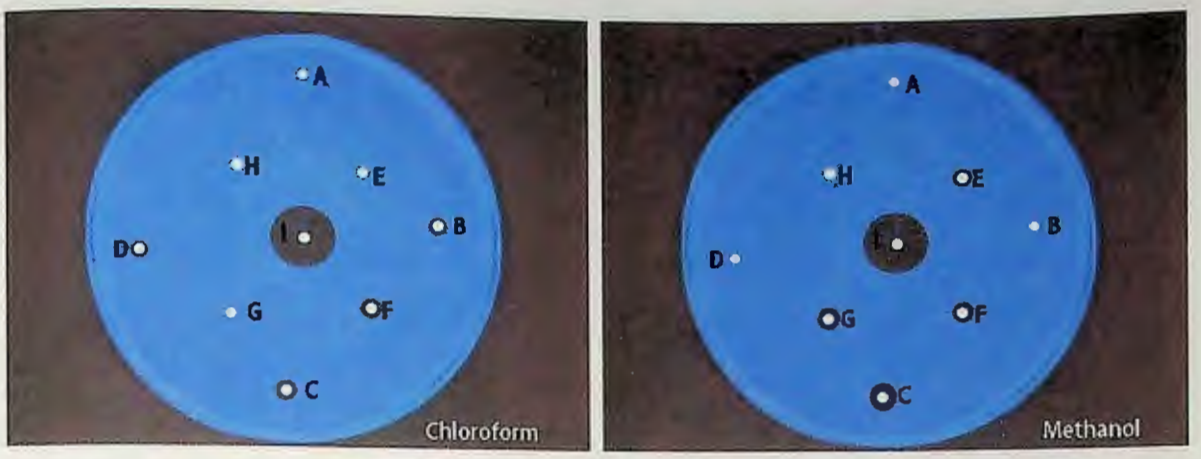


Plate 28 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Shigella shiga*

Plate 29 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Shigella sonnei*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

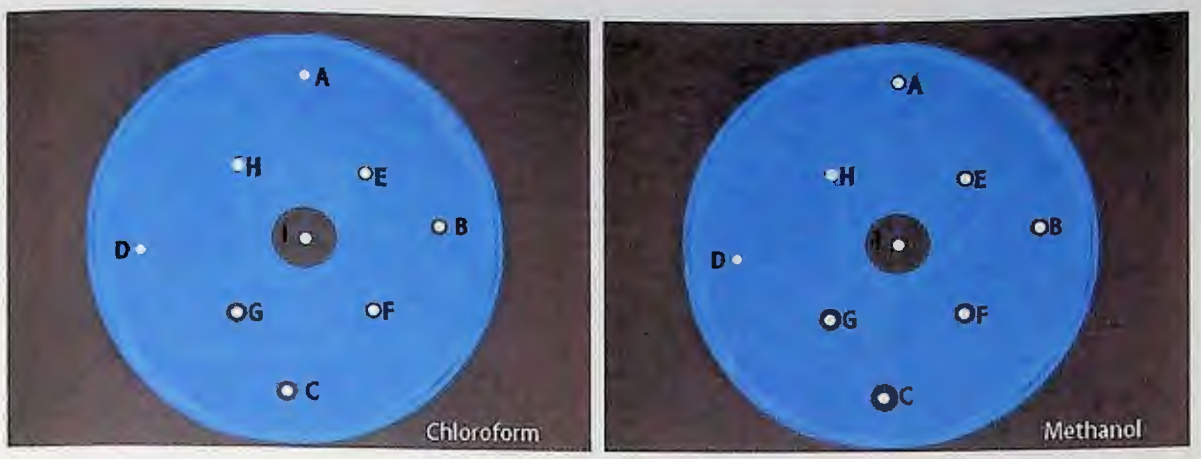


Plate 30 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Shigella boydii*

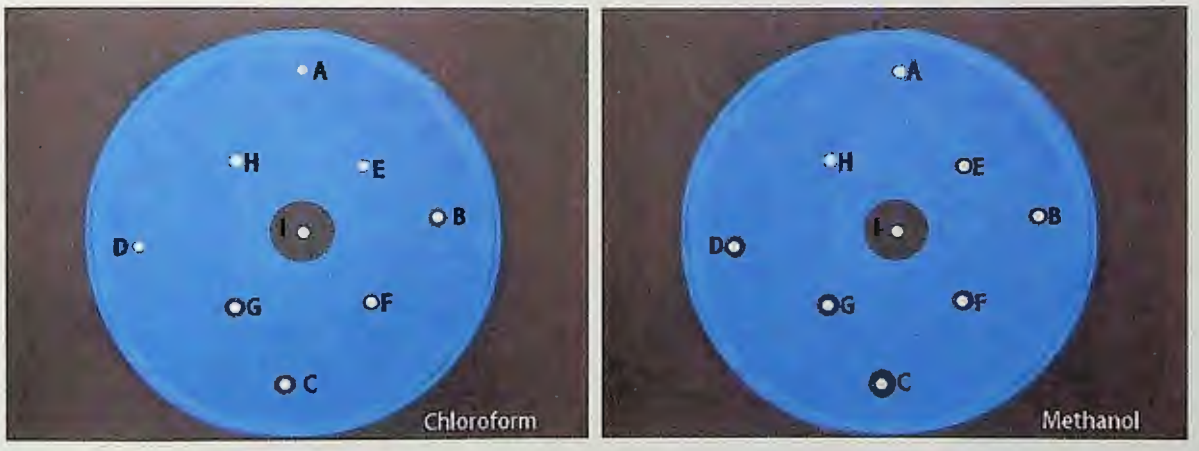


Plate 31 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Escherichia coli*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

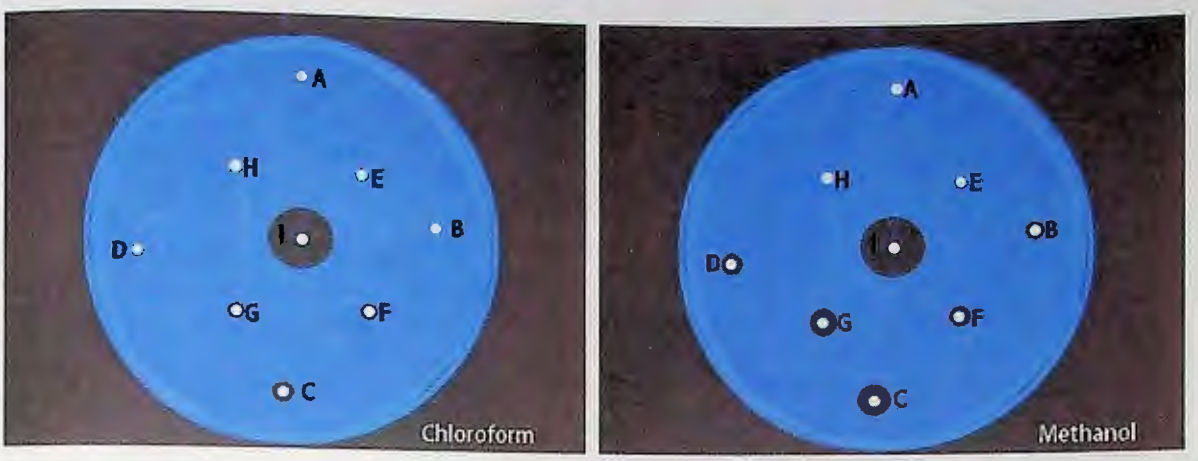


Plate 32 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Klebsiella* sp.

Plate 33 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Pseudomonas aeruginosa*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

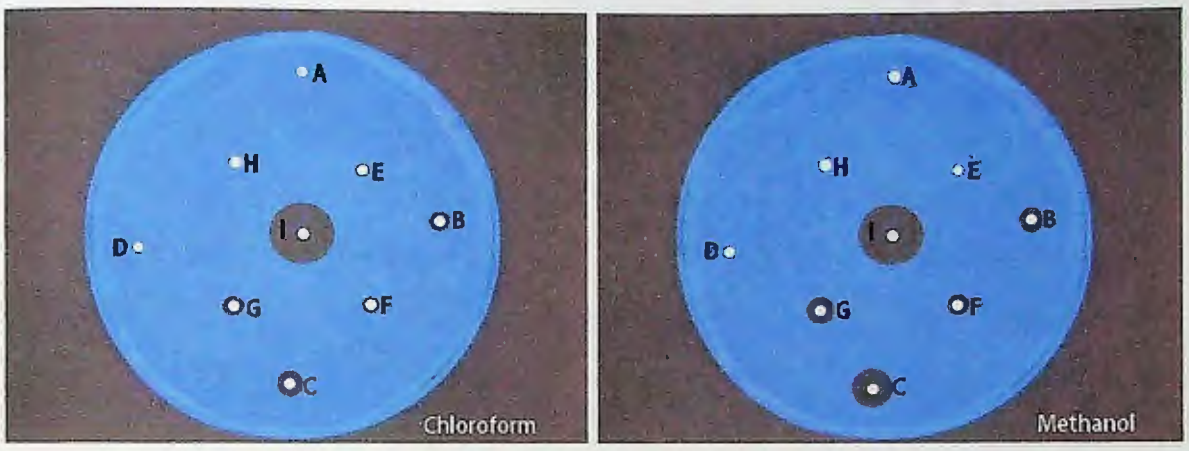


Plate 34 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Ciprofloxacin (30µg/disc) against *Proteus* sp.

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

Minimum Inhibitory Concentration (MIC)

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 (MIC) of the methanolic crude extracts of root of *S. torvum* and stem of *S. zeylanica* were determined against *Bacillus cereus, Bacillus subtilis, Streptococcus-β-haemolyticus, Salmonella typhi* and *Shigella dysenteriae* using by a serial tube dilution technique (Reiner 1982) and the results has been tabulated in **Tables** 5, 6.

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 broth medium (1 ml) in each test tube and incubated at 37.5^0 C for 24 hrs.

No sign of growth of the test organisms *Bacillus cereus, Bacillus subtilis, Streptococcus-β-haemolyticus, Salmonella typhi* and *Shigella 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 methanolic crude extracts of root of *S. torvum.*

The minimum inhibitory concentration (MIC) for the chloroform extract was observed to be 128μg/ml for *Bacillus cereus, Streptococcus-β-haemolyticus* and *Shigella dysenteriae.* However, for the rest of bacteria the MIC was 64 μg/ml of the chloroform extract of stem of *S. zeylanica.*

No inhibition was observed in the test tube containing sample lower than the concentrations. Three control tests were performed using nutrient broth 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 MIC results are presented in **Tables** 5 and 6.

Table 5 : Minimum inhibitory concentrations of methanolic root extract of ***Solanum torvum*** against five pathogenic bacteria

Test tube No.	Nutrient broth or potato dextrose broth medium added (ml)	Diluted solution of methanol extract from root(µg/ml)	Inocula added (µl)	*Bacillus cereus*	*Bacillus subtilis*	*Streptococcus-β-haemolyticus*	*Salmonella typhi*	*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 6 : Minimum inhibitory concentrations of chloroform stem extract of ***Smilax zeylanica*** against five pathogenic bacteria

Test tube no.	Nutrient broth or potato dextrose broth medium added (ml)	Diluted solution of chloroform extract from stem (µg/ml)	Inocula added (µl)	*Bacillus cereus*	*Bacillus subtilis*	*Streptococcus-β-haemolyticus*	*Salmonella typhi*	*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	128	64	128

Notes : + = growth, - = 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 leaf and inflorescence but prominent zone of inhibition was observed for stem at 200 µg/disc and for root extract at both 50 and 200 µg/disc of both chloroform and methanol extract against *Vasin factum, Aspergillus fumigatus* and *Candida albicans.* At 200 µg/disc the root extract of *S. torvum* exhibits bigger and more clear prominent zone against *V. factum* (**Table 7**). Methanolic extracts of stem exhibited promising antifungal activities against *A. fumigatus, V. factum* and *C. albicans* at 200 µg/disc than the other extracts tested. The antifungal activity was determined after 48 hrs. of incubation at room temperature (30^0 C) and the results are shown in **Tables** 7 and 8 and **Plates** 35 – 42.

Table 7 : Antifungal activity of Chloroform and methanolic extracts of leaf, stem, root and inflorescence of ***S. torvum*** and standard Nystatin

Test Organisms	Diameter of zone of inhabitation (in mm)																
	Chloroform extract								Methanol extract								Nystatin 50 (µg/disc)
	Leaf		Stem		Root		Inflor*		Leaf		Stem		Root		Inflor*		
µg/disc →	50	200	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
Aspergillus fumigatus	-	-	-	09	07	10	-	-	-	-	-	12	07	13	-	-	28
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29
A. flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
Vasin factum	-	-	-	-	09	13	-	-	-	-	-	-	11	15	-	-	28
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
Candida albicans	-	-	-	08	07	11	-	-	-	-	07	11	09	13	-	-	29
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
Colletotrichum falcatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29

Notes : Inflor* = Inflorescence , - = Not active

Table 8 : Antifungal activity of Chloroform and methanolic extracts of leaf, stem and root of ***S. zeylanica*** and standard Nystatin

Test Organisms	Diameter of zone of inhibition (in mm)												
	Chloroform extract						Methanol extract						Nystatin 50(µg/disc)
	Leaf		Stem		Root		Leaf		Stem		Root		
µg/disc →	50	200	50	200	50	200	50	200	50	200	50	200	
Aspergillus fumigatus	07	11	07	13	-	-	08	15	09	17	-	-	28
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	29
A. flavus	-	-	-	-	-	-	-	-	-	-	-	-	27
Vasin factum	-	-	07	12	-	-	-	-	08	13	-	-	28
Mucor sp.	-	-	-	-	-	-	-	-	-	-	-	-	28
Candida albicans	07	12	-	10	07	11	08	12	09	14	08	12	29
Fusarium oxysporum	07	12	-	-	-	-	07	12	-	-	-	-	28
Colletotrichum falcatum	-	-	-	-	-	-	-	-	-	-	-	-	29

Note : - = Not active

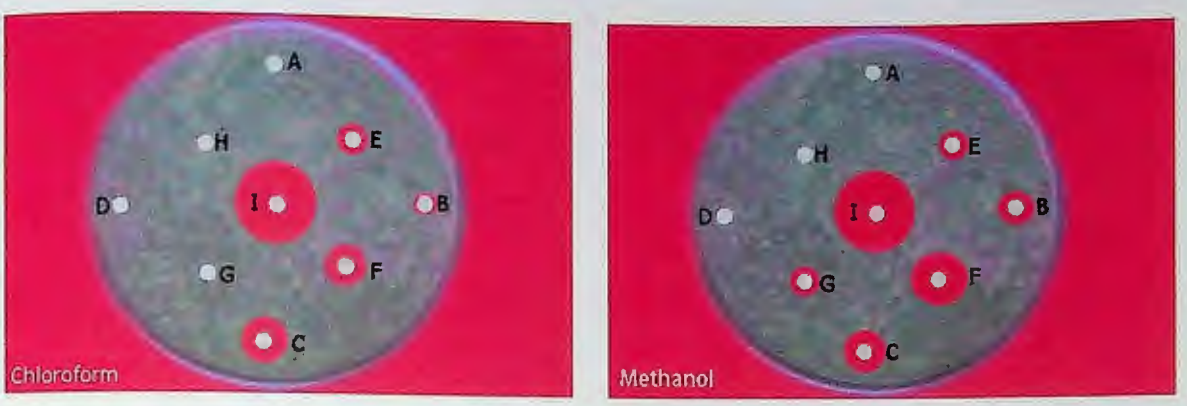


Plate 35 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Aspergillus fumigatus*

Plate 36 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Aspergillus niger*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

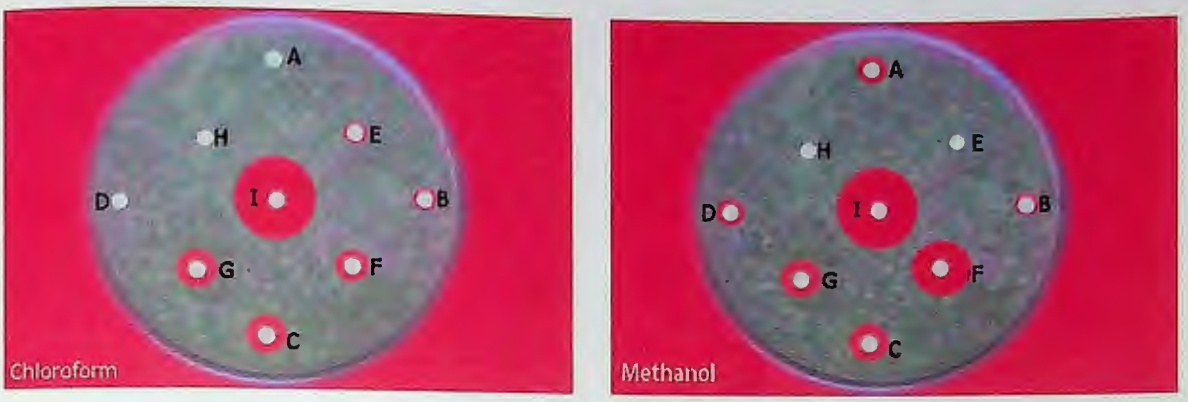


Plate 37 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Aspergillus flavus*

Plate 38 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Vasin factum*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

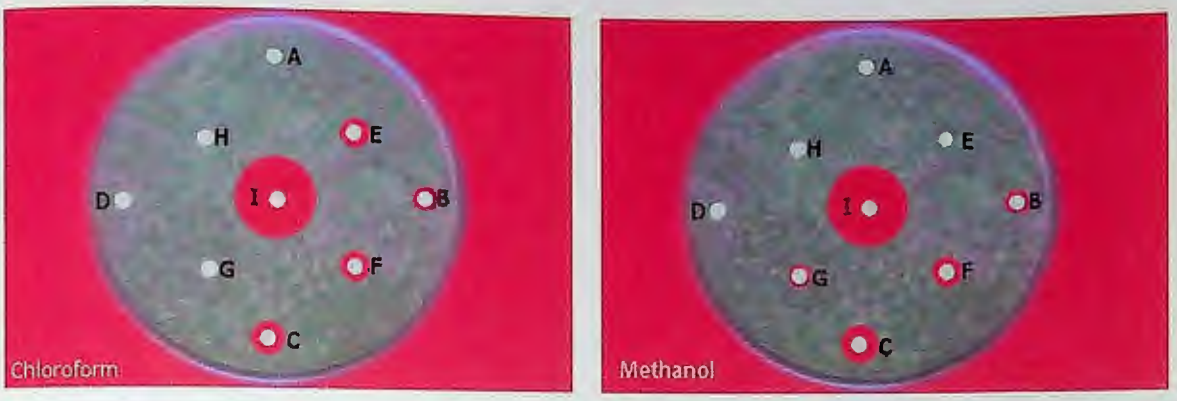


Plate 39 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Mucor* sp.

Plate 40 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Candida albicans*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

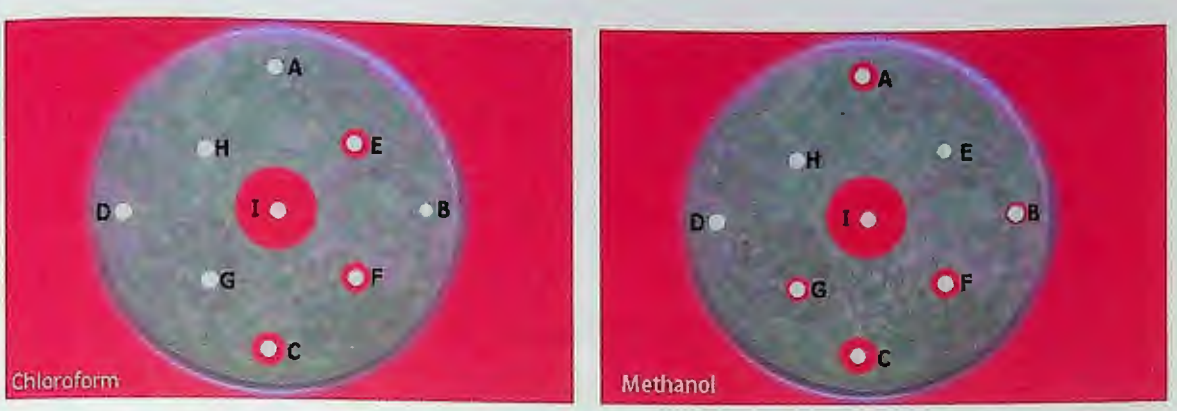


Plate 41 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Fusarium oxysporum*

Plate 42 : Effect of chloroform and methanolic extracts from different parts of tit begoon and kumarilata and standard Nystatin (50µg/disc) against *Colletotrichum falcatum*

Notes : A - Tit begoon leaf B - Tit begoon stem
C - Tit begoon root D - Tit begoon inflorescence
E - Kumarilata leaf F - Kumarilata stem
G - Kumarilata root H - Solvent, and I - Ciprofloxacin (Control)

Brine shrimp toxicity bioassay

The results of the brine shrimp toxicity bioassay are shown in the **Tables** 9 and 10. 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 crude extracts. Regression lines drawn by plotting log concentration of the tested extracts against probit mortality reveal linear correlation between dose and mortality. The LC_{50} values of the crude extracts (leaf, inflorescence, stem and root of *S. torvum* and leaf, stem and root of *S. zeylanica*) and standard ampicillin trihydrate are given in **Tables** 9 and 10.

It is evident that all the crude extracts were found to be lethal to brine shrimps nauplii indicating that the extracts are biologically active. The chloroform extract was more active with lower LC_{50} values whereas the methanol extracts were comparatively less active with higher LC_{50} values.

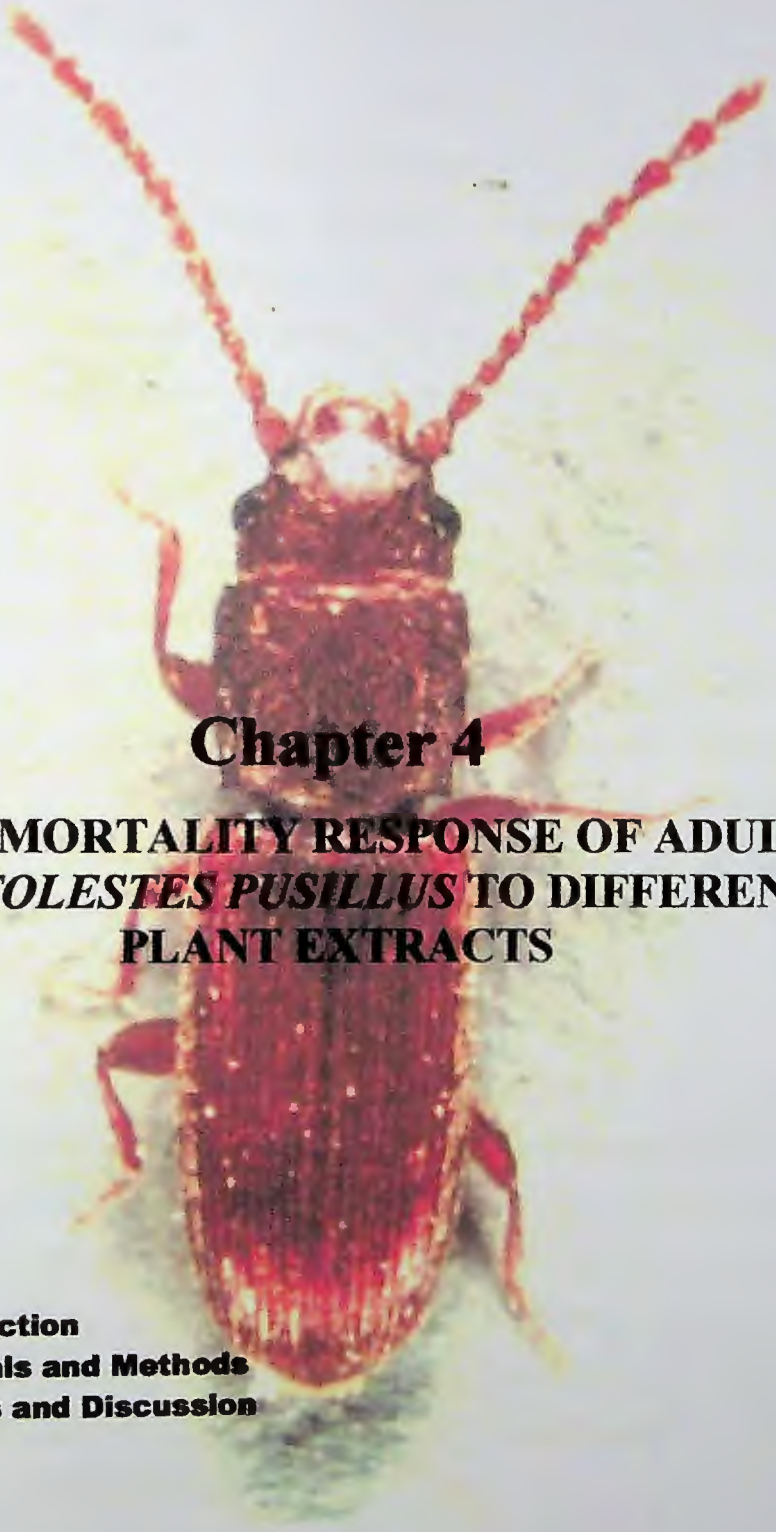
Brine shrimp toxicity bioassay is a recent development in the bioassay for the bioactive compounds (Chaterjee 1975, Mayer *et al.* 1982, Alkofahi *et al.* 1989, Pelcjar *et al.* 1986, McLaughlin 1988 and 1992, Persoone 1980). The brine shrimp assay has advantages of being rapid (24 hrs.), inexpensive 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 degree of cytotoxicity and pharmacological activities of the fractionated extracts.

Table 9 : Ampicillin trihydrate, leaf, stem, root and inflorescence of *Solanum torvum* extract toxicity bioassay on brine shrimp nauplii

Solvents	Extracts	LC_{50} (ppm)	95 % Confidence limits (ppm)		Regression equations	χ^2 Values
			Lower	Upper		
	Control (20µg DMSO)	0	0	0	0	0
Chloroform	Ampicillin	16.1813	7.1674	36.5316	Y = 4.0595 + .7778 X	0.0407
	Leaf	124.290	62.7717	246.101	Y = 2.7964 + 1.0520 X	0.4602
	Stem	92.2536	52.5535	161.9441	Y = 2.8572 + 1.0904 X	1.1852
	Root	35.4629	23.6360	53.2076	Y = 3.1316 + 1.2055 X	0.5496
	Inflorescence	119.138	56.7722	250.0173	Y = 3.0370 + 0.9455 X	0.2640
Methanol	Ampicillin	16.1813	7.1674	36.5316	Y = 4.0595 + 0.7778 X	0.0407
	Leaf	497.538	72.7571	3402.348	Y = 3.0297 + 0.7305 X	0.1321
	Stem	325.7122	73.6927	1439.605	Y = 3.0300 + 0.7839 X	I .1919
	Root	203.5926	46.0256	900.5836	Y = 3.5833 + 0.6135 X	0.3034
	Inflorescence	453.1791	67.4574	3044.455	Y = 3.1279 + 0.7047 X	0.1864

Table 10 : Ampicillin trihydrate, leaf, stem and root of ***Smilax zeylanica*** extracts toxicity bioassay on brine shrimp nauplii

Solvents	Extracts	LC_{50} (ppm)	95 % Confidence limits (ppm)		Regression equations	χ^2 Values
			Lower	Upper		
Chloroform	Control (20µg DMSO)	0	0	0	0	0
	Ampicillin	16.1813	7.1674	36.5316	Y = 4.0595 + 0.7778 X	0.0407
	Leaf	80.1584	47.0795	136.479	Y = 2.9429 + 1.0803 X	2.0852
	Stem	45.0024	30.5341	66.3263	Y = 2.9063 + 1.2664 X	0.1880
	Root	58.5008	35.6916	95.8865	Y = 3.1864 + 1.0262 X	1.6726
Methanol	Ampicillin	16.1813	7.1674	36.5316	Y = 4.0595 + 0.7778 X	0.0407
	Leaf	271.494	84.0231	877.249	Y = 2.7320 + 0.9318 X	0.1592
	Stem	117.331	52.9931	259.781	Y = 3.1918 + 0.8737 X	0.2672
	Root	158.425	69.2173	362.607	Y = 2.8300 + 0.9864 X	0.1091



Chapter 4

DOSE-MORTALITY RESPONSE OF ADULT *CRYPTOLESTES PUSILLUS* TO DIFFERENT PLANT EXTRACTS

DOSE-MORTALITY RESPONSE OF ADULT *CRYPTOLESTES PUSILLUS* TO DIFFERENT PLANT EXTRACTS

4.1. INTRODUCTION

The use of natural pesticides in agricultural and horticultural industries has increased in recent years. These bio-pesticides offer very 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).

Various methodologies have been used to determine the effectiveness of plant materials and their extracts. Almost all trials were laboratory-based and of short duration and therefore, do not necessarily reflect responses which would be observed under real farm conditions. Toxicity of solvent extracts of *Acorus calamus* L. to some grain pests (Paul *et al* 1965, Rahman and Schmidt 1999), McDonald *et al.* (1970) and Morton (1976) reports preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects.

Extracts of plant material rely on the solubility of the active components. Two solvents *viz.* chloroform and methanol are being used for the extraction. Extracts may contain active components that can be used for insect bioassay. Moreover, information on the weight or yield of the active component, from a particular extraction technique its concentration on final application to the commodity is very important. Although serial dilutions of the extract of different plant materials have frequently used to insect bioassay but there is no report on such type of work on tit begoon and kumarilata.

The specific objectives of this part of study are :

- To find the standard plant that contains optimum level of antimicroibial component,
- To find the standard plant that contains optimum level of pesticidal component,
- To standardize best solvent system for the extraction of maximum amount of pesticidal component,
- To standardize the optimum dose to kill the test insect.

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 *C. pusillus* the surface film method was used. The concentrations used were 0.424, 0.848, 1.696, 3.393 and 6.791 mg/cm^2. The doses were calculated by measuring the dry weight of the crude extracts applied in the Petridish divided by the surface area.

The adult mortality was recorded 24, 48 and 72 hrs. 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).

$$P_t \frac{Po - Pc}{100 - Pc} \times 100$$

Where,

P_t = Corrected mortality,

P_0 = Observed mortality and

P_c = Control mortality.

The observed data was then subjected to Probit analysis according to Finney (1974) and Busvine (1971) using a software developed in the Department of Agriculture and Environmental Science, University of Newcastle-Upon-Tyne, UK.

Different parts of two plants, *viz.* leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* were extracted separately with chloroform and methanol. The extract condensed using rotary evaporator and weighed. The yield of the extracts according to solvents and parts of the plant are given in **Table** 11. The outputs of the extract varied due to the types of explants and extraction media. Among the different types of plant parts, extract out put was the lowest for root and highest for leaf powder in both cases.

Table 11 : Extract from leaf, stem, root and inflorescence of ***S. torvum*** and leaf, stem and root of ***S. zeylanica*** with different solvents

Plant parts	Weight of dust (gm)	Weight of Chloroform extracts (gm)	Weight of Methanol extracts (gm)
Solanum torvum			
Leaf	250	25.72	24.78
Stem	250	12.25	13.55
Root	250	14.16	12.55
Inflor*	250	13.16	21.27
Smilax zeylanica			
Leaf	250	23.28	18.51
Stem	250	11.91	13.07
Root	250	10.95	12.63

Note : Inflor* = Inflorescence

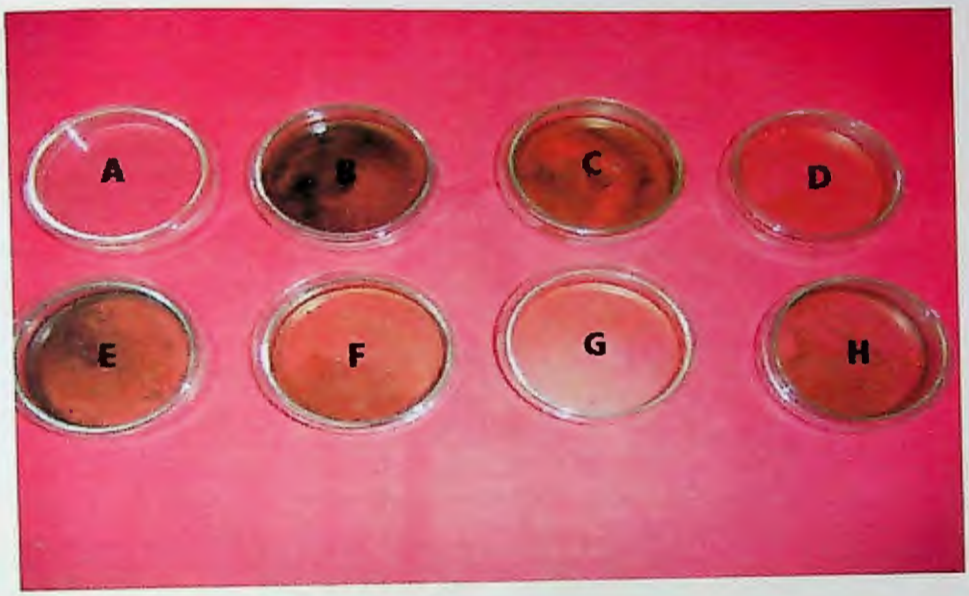


Plate 43 : Different extract of test plants

Notes: A = Control (Solvent)
B = Leaf of *S. torvum*
C = Stem of *S. torvum*
D = Root of *S. torvum*
E = Inflorescence of *S. torvum*
F = Root of *S. zeylanica*
G = Stem of *S. zeylanica*
H = Leaf of *S. zeylanica*

Plate 44a : Leaf of *S. torvum*

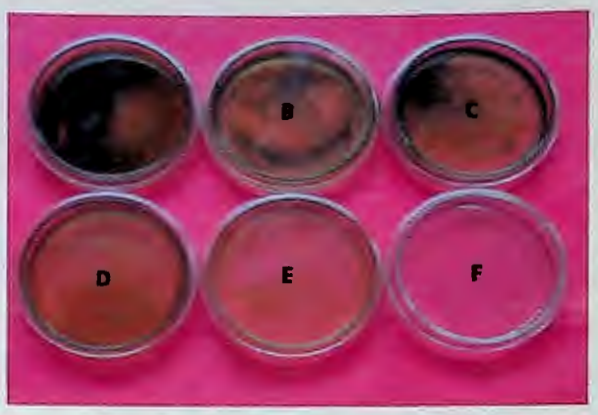


Plate 44b : Stem of *S. torvum*

Plate 44c : Root of *S. torvum*

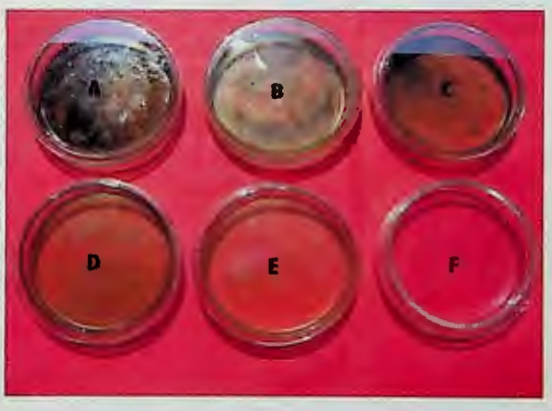


Plate 44d : Inflorescence of *S. torvum*

Plate 44 : Dose Mortality response of *C. pusillus* adult with leaf, stem, root and inflorescence extracts of *Solanum torvum*

Notes : A = 0.424 mg/cm^2 B = 0.848 mg/cm^2
C = 1.696 mg/cm^2 D = 3.393 mg/cm^2
E = 6.791 mg/cm^2 F = Control (Solvent)

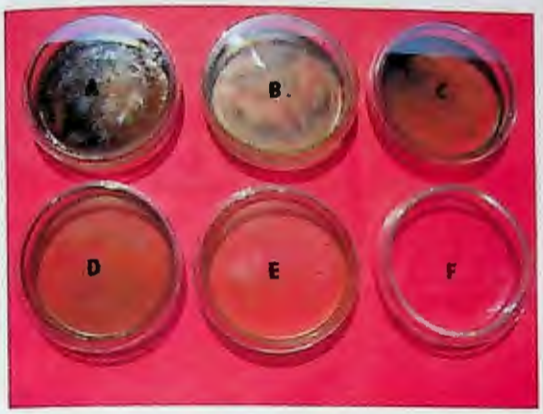


Plate 45a : Leaf of *S. zeylanica*

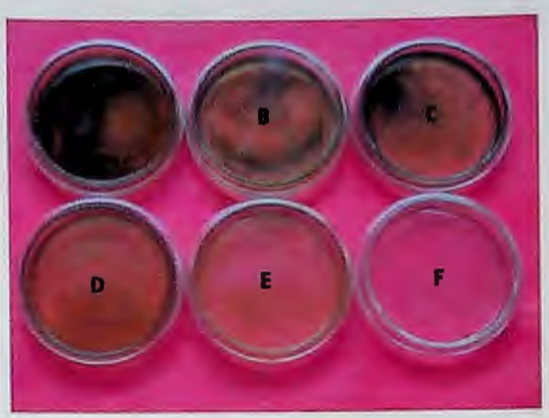


Plate 45b : Stem of *S. zeylanica*

Plate 45c : Root of *S. zeylanica*

Plate 45 : Dose Mortality response of *C. pusillus* adult with leaf, stem and root extracts of *Smilax zeylanica*

Notes : A = 0.424 mg/cm^2 B = 0.848 mg/cm^2
C = 1.696 mg/cm^2 D = 3.393 mg/cm^2
E = 6.791 mg/cm^2 F = Control (Solvent)

4.3. RESULTS AND DISCUSSION

The LD_{50} values, 95% confidence limits, regression equation (Y), chi-square (χ^2) values and probit regression lines are shown in **Tables** 12, 13, 14 and 15 and in **Figures** 1 to 14. The methanolic extracts were found to be more toxic to the beetles than the chloroform extract.

The effect of different doses of the leaf, stem, root and inflorescence extract of *Solanum torvum* on the mortality of adult *Cryptolestes pusillus* were found to be toxic. However, the degree of toxicity was found to be different with extraction solvents and χ^2 values with different degree of freedoms indicate no heterogeneity for all the dose-mortality tests done with leaf, stem, root and inflorescence extract of *S. torvum* on *C. pusillus*.

The calculated LD_{50} values of chloroform extracts of *S. torvum* on *C. pusillus* were 96.96219, 38.97169, 24.93003 mg/cm^2 for leaf, 14.74552, 8.87479, 7.09454 mg/cm^2 for stem, 4.22744, 3.30933, 3.07992 mg/cm^2 for root and 10.12795, 10.33345, 5.79846 mg/cm^2 for inflorescence extract respectively. Whereas, the LD_{50} values of methanolic extract were 13.82274, 24.06804, 17.33238 mg/cm^2 for leaf, 12.87178, 8.80765, 6.32185 mg/cm^2 for stem, 5.80144, 4.24446, 3.98838 mg/cm^2 for root and 11.88017, 13.18793, 10.83418 mg/cm^2 for inflorescence extract after 24, 48 and 72 hrs. of treatment, respectively.

The effect of different doses of the leaf, stem and root extracts of *S. zeylanica* on the mortality of adult *C. pusillus* were found to be toxic. The calculated LD_{50} values of chloroform extracts of *S. zeylanica* on *C. pusillus* were 71.20013, 152.0579, 67.22268 mg/cm^2 for leaf, 14.4869, 11.41479, 7.92375 mg/cm^2 for stem and 6.42685, 4.67162, 2.40395 mg/cm^2 for root extract.

Whereas, the LD_{50} values of methanolic extracts were 39.79373, 19.38654, 22.0639 mg/cm^2 for leaf, 14.26849, 12.68115, 10.84893 mg/cm^2 for stem and 12.57753, 5.83150, 4.32224 mg/cm^2 for root extract after 24, 48 and 72 hrs. of treatment, respectively.

All the crude extracts of the tested plant 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 (*Datura metal*) and *Sapindus mukarasis* against many stored-product insect pests (Khanam *et al.* 1990, 1991, Khalequzzaman and Rahman 1992, Khalequzzaman and Islam 1992, Malek and Wilkins 1993, Talukder and Howse 1995). Lethal and sublethal effects of withanolides from *Salpichora origanifolia* and analogues on *Ceratitis* capitata (Bado *et. al.* 2004) and Mungkornasawakul *et. al.* (2004) reports on phytochemical and larvicidal studies on *Stemona curtisii.*

The results described above reveal the contact toxicity of the extracts from all parts of the two test plants. The degree of insecticidal properties according to the solvents are MeOH > $CHCl_3$; according to the exposure periods 72 > 48 > 24 hrs.; according to the plant parts are root > stem > inflorescence > leaf in case of *S. torvum* and root > stem > leaf in case of *S. zeylanica.*

Table 12 : LD_{50}, 95% confidence limits and regression equations of chloroform extracts of leaf, stem, root and inflorescence of ***S. torvum*** against *C. pusillus* adults

Extract	Exposure Period (h)	LD_{50} mg/cm^2	95 % Confidence limits		Regression equations	χ^2 Values (df)
			Lower	Upper		
Leaf	24	96.96219	0.31830	29537.14	Y = 3.314013 + .8486786 X	0.030507 (1)
	48	38.97169	3.08185	492.8177	Y = 2.399718 + 1.00368 X	0.659575 (2)
	72	24.93003	3.33487	186.3659	Y = 2.69141 + .9632279 X	0.306548 (2)
Stem	24	14.74552	5.45300	39.87351	Y = 1.846821 + 1.453976 X	1.899467 (3)
	48	8.87479	4.56353	17.259	Y = 2.0038 + 1.537965 X	2.071652 (3)
	72	7.09454	4.0500	12.42777	Y = 2.10214 + 1.56562 X	2.799713 (3)
Root	24	4.22744	2.90134	6.15964	Y = 2.197787 + 1.723296 X	3.08654 (3)
	48	3.30933	2.32157	4.71735	Y = 2.535989 + 1.621337 X	1.984274 (3)
	72	3.07992	2.13117	4.45104	Y = 2.737086 + 1.520223 X	2.056633 (3)
Inflor*	24	10.12795	4.69491	21.8482	Y = 3.137575 + 1.852198 X	0.057011 (1)
	48	10.33345	4.07574	26.19897	Y = 2.407402 + 1.287131X	0.135551 (2)
	72	5.79846	3.40374	9.87799	Y = 2.436443 + 1.45383 X	0.471082 (3)

Note : Inflor* = Inflorescence

Table 13 : LD_{50}, 95% confidence limits and regression equations of methanolic extracts of leaf, stem, root and inflorescence of ***S. torvum*** against *C. pusillus* adults

Extract	Exposure Period (h)	LD_{50} mg/cm^2	95 % Confidence limits		Regression equations	χ^2 Values (df)
			Lower	Upper		
Leaf	24	13.82274	4.95244	38.58061	Y = 2.197027 + 1.309437 X	0.735264 (3)
	48	24.06804	3.87235	149.5912	Y = 2.968472 + .8530667 X	0.041384 (3)
	72	17.33238	4.17771	71.90791	Y = 2.870949 + .9509542 X	0.145495 (3)
Stem	24	12.87178	3.91956	42.27072	Y = 2.934388 + .9791309 X	0.507011 (3)
	48	8.80765	3.53587	21.93935	Y = 2.960327 + 1.048751 X	0.599503 (3)
	72	6.32185	3.05152	13.09703	Y = 3.036117 + 1.090535 X	1.292585 (3)
Root	24	5.80144	3.21886	10.45613	Y = 2.709249 + 1.298953 X	1.402052 (3)
	48	4.24446	2.42486	7.42947	Y = 3.133204 + 1.146806 X	.880344 (3)
	72	3.98838	2.32911	6.82972	Y = 3.152604 + 1.154048 X	.713155 (3)
Inflor*	24	11.88017	5.31638	26.54779	Y = 2.678689 + 2.159716 X	0.045719 (1)
	48	13.18793	5.16066	33.70142	Y = 1.568701 + 1.618402 X	0.031538 (2)
	72	10.83418	4.89190	23.99465	Y = 1.658211 + 1.642321 X	0.046993 (2)

Note : Inflor* = Inflorescence

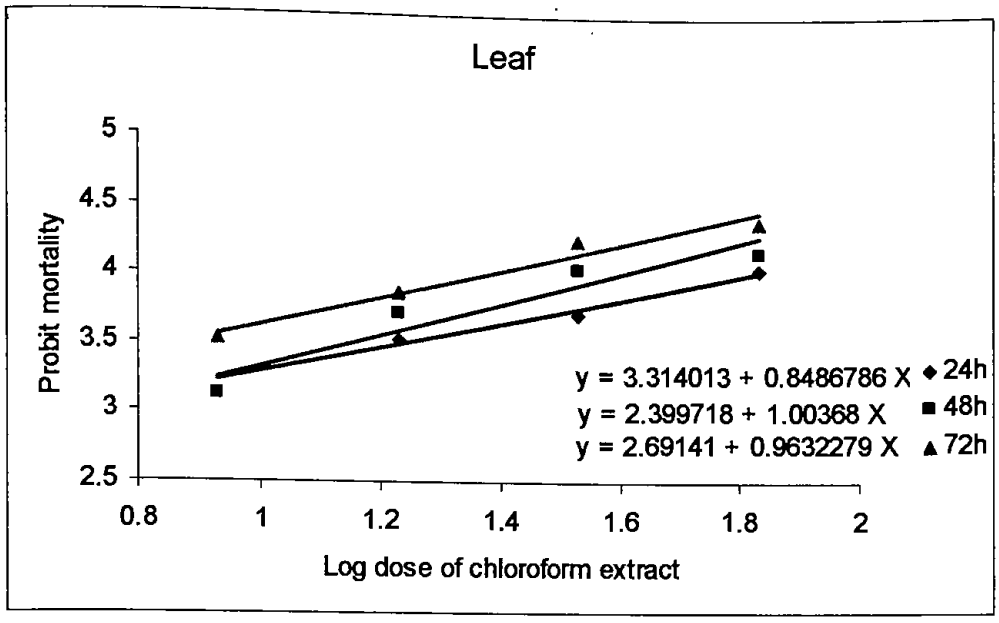


Figure 1 : Regression line of probit mortality of adult *C. pusillus* and log dose of leaf extract of *S. torvum* in chloroform after 24, 48 and 72 hours of exposure

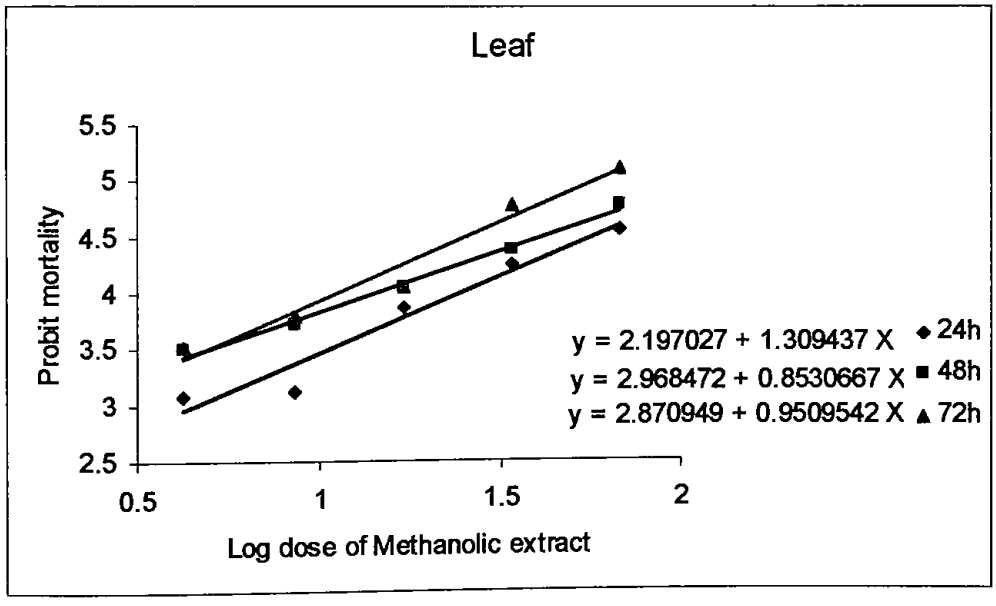


Figure 2 : Regression line of probit mortality of adult *C. pusillus* and log dose of leaf extract of *S. torvum* in methanol after 24, 48 and 72 hours of exposure

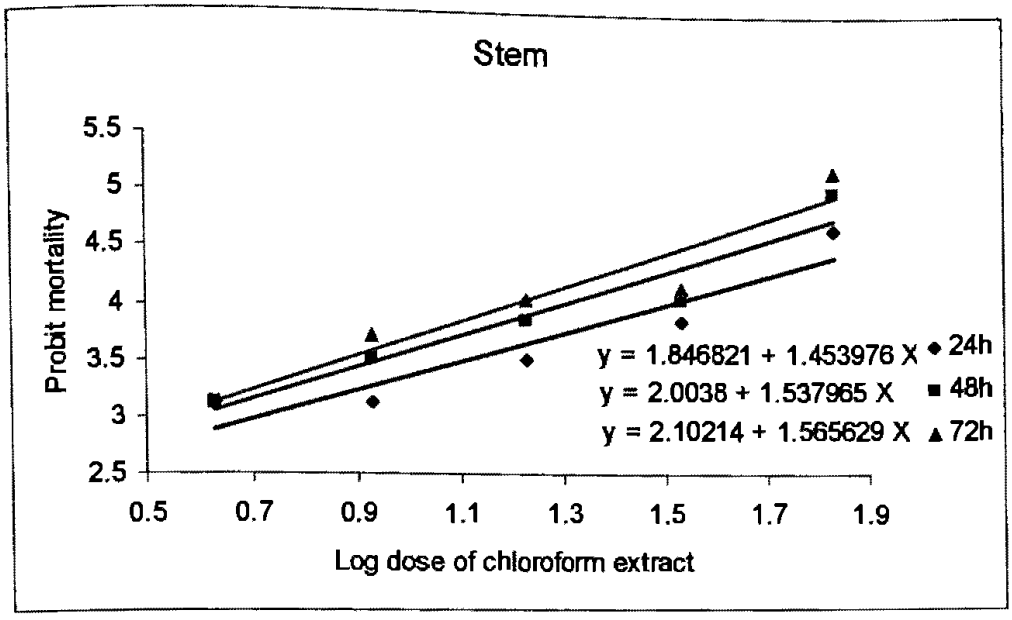


Figure 3 : Regression line of probit mortality of adult *C. pusillus* and log dose of stem extract of *S. torvum* in chloroform after 24, 48 and 72 hours of exposure

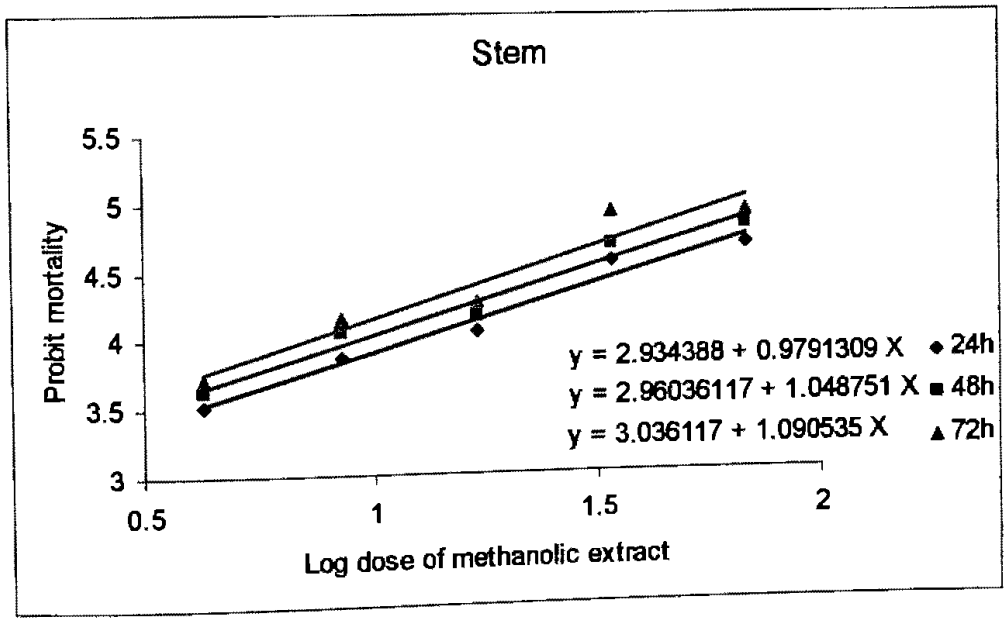


Figure 4 : Regression line of probit mortality of adult *C. pusillus* and log dose of stem extract of *S. torvum* in methanol after 24, 48 and 72 hours of exposure

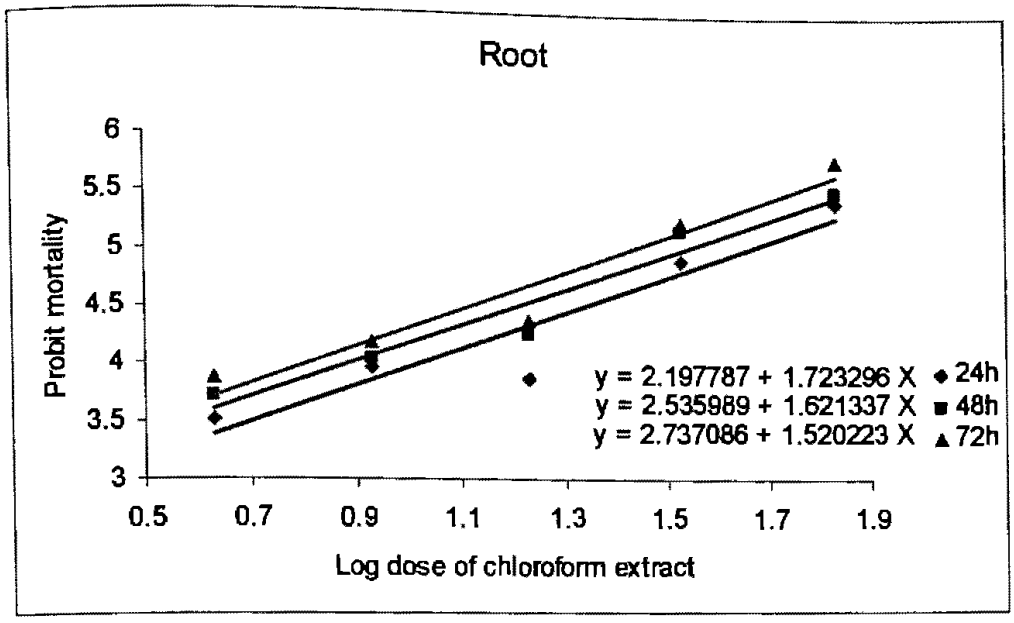


Figure 5 : Regression line of probit mortality of adult *C. pusillus* and log dose of root extract of *S. torvum* in chloroform after 24, 48 and 72 hours of exposure

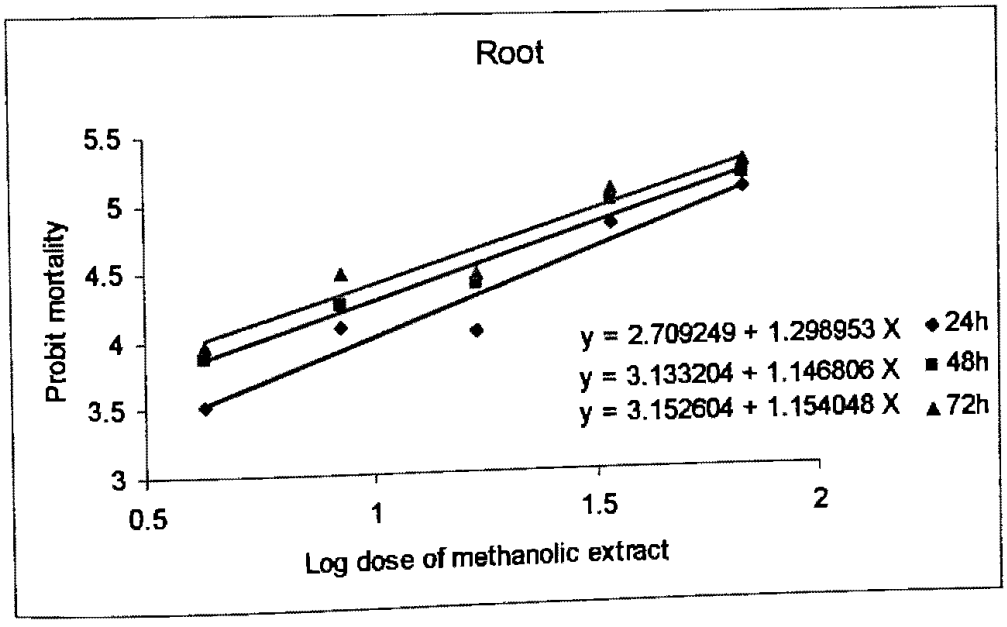


Figure 6 : Regression line of probit mortality of adult *C. pusillus* and log dose of root extract of *S. torvum* in methanol after 24, 48 and 72 hours of exposure

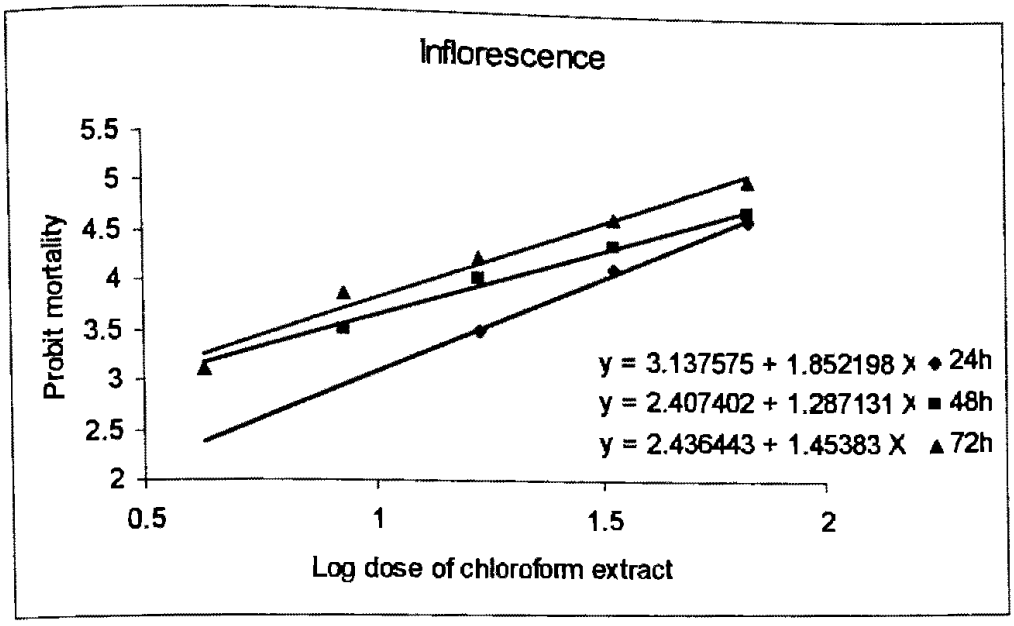


Figure 7 : Regression line of probit mortality of adult *C. pusillus* and log dose of inflorescence extract of *S. torvum* in chloroform after 24, 48 and 72 hours of exposure

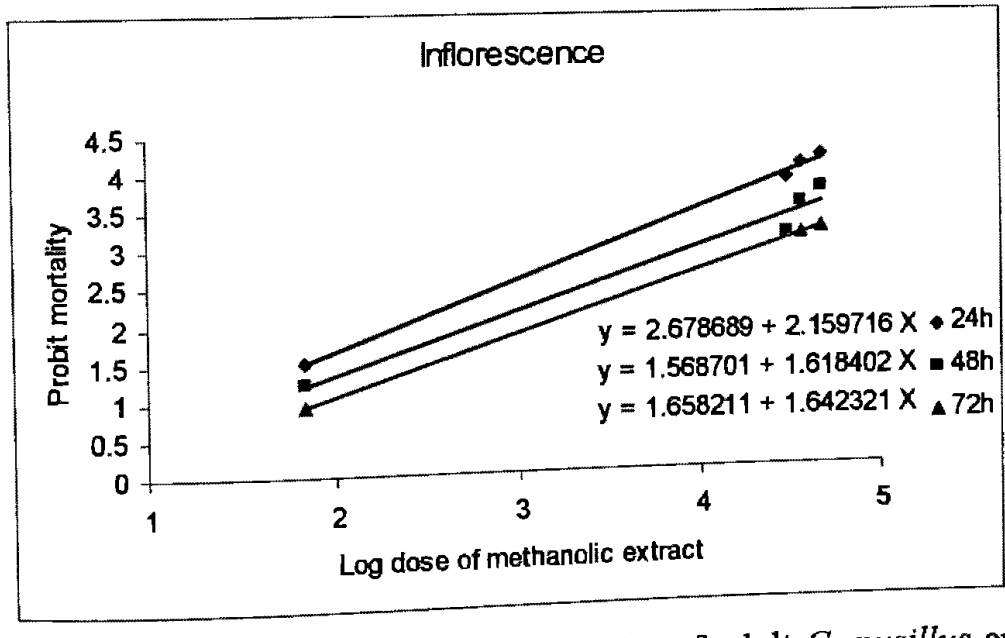


Figure 8 : Regression line of probit mortality of adult *C. pusillus* and log dose of inflorescence extract of *S. torvum* in methanol after 24, 48 and 72 hours of exposure

Table 14 : LD_{50}, 95% confidence limits and regression equations of chloroform extracts of leaf, stem and root of ***S. zeylanica*** against *C. pusillus* adults

Extract	Exposure Period (h)	LD_{50} mg/cm^2	95 % Confidence limits		Regression equations	χ^2 Values (df)
			Lower	Upper		
Leaf	24	71.20013	3.69477	1372.061	Y = 2.213098 + 9770098 X	1.680163 (3)
	48	152.0579	1.65199	13996.18	Y = 2.900118 + .6599236 X	0.695751 (3)
	72	67.22268	3.15118	1434.026	Y = 2.80302 + .7770002 X	0.457111 (3)
Stem	24	14.4869	3.76158	55.79305	Y = 3.426211 + 1.355575 X	0.013346 (1)
	48	11.41479	4.82010	27.03209	Y = 2.173187 + 1.373928 X	0.376934 (3)
	72	7.92375	3.99673	15.70928	Y = 2.423197 + 1.356976 X	1.215176 (3)
Root	24	6.42685	3.21548	12.84547	Y = 2.895109 + 1.164211 X	1.319775 (3)
	48	4.67162	2.55664	8.53622	Y = 3.133003 + 1.118319 X	2.266853 (3)
	72	2.40395	1.67246	3.45536	Y = 3.039315 + 1.419834 X	0.731632 (3)

Note : Inflor* = Inflorescence

Table 15 : LD_{50}, 95% confidence limits and regression equations of methanolic extracts of leaf, stem and root of ***S. zeylanica*** against *C. pusillus* adults

Extract	Exposure Period (h)	LD_{50} mg/cm^2	95 % Confidence limits		Regression equation	χ^2 Values (df)
			Lower	Upper		
Leaf	24	39.79373	1.66651	950.2098	Y = 3.285974 + 1.07139 X	0.0059227 (1)
	48	19.38654	4.57667	82.12035	Y = 2.053177 + 1.288228 X	0.1713014 (2)
	72	22.0639	5.04688	96.45862	Y = 2.430505 + 1.09635 X	0.1615448 (3)
Stem	24	14.26849	4.76160	42.75662	Y = 1.985843 + 1.399084 X	0.6349506 (2)
	48	12.68115	4.91774	32.70026	Y = 2.184573 + 1.338666 X	1.072644 (3)
	72	10.84893	4.15482	28.32832	Y = 2.676095 + 1.141751 X	1.213344 (3)
Root	24	12.57753	3.93860	40.16506	Y = 2.916273 + 0 .9924421 X	1.565948 (3)
	48	5.83150	3.06911	11.08023	Y = 2.901175 + 1.18861 X	2.020481 (3)
	72	4.32224	2.56378	7.28679	Y = 2.96496 + 1.244133 X	1.363483 (3)

Note : Inflor* = Inflorescence

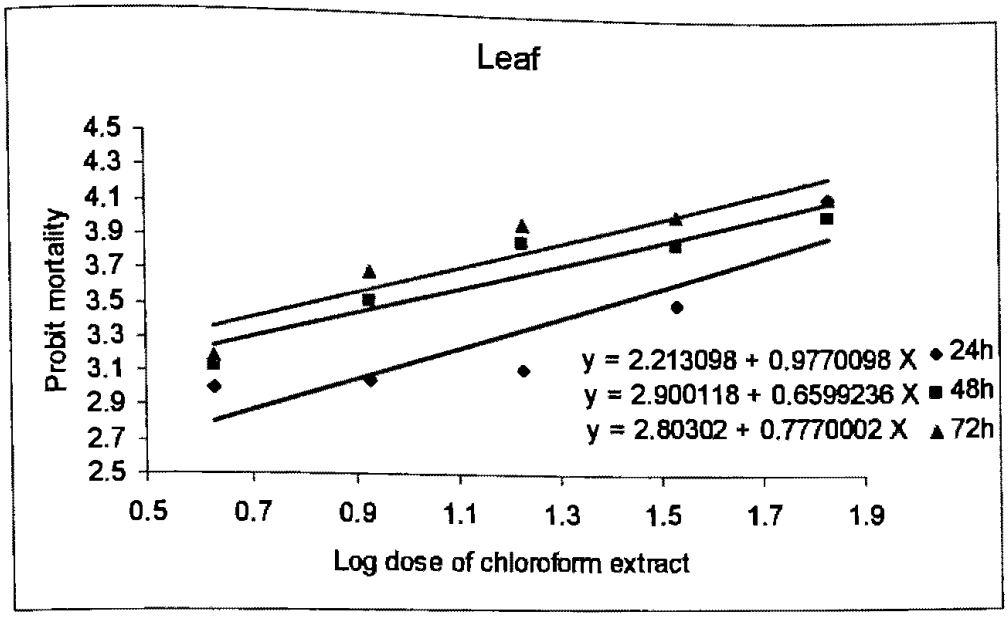


Figure 9 : Regression line of probit mortality of adult *C. pusillus* and log dose of leaf extract of *S. zeylanica* in chloroform after 24, 48 and 72 hours of exposure

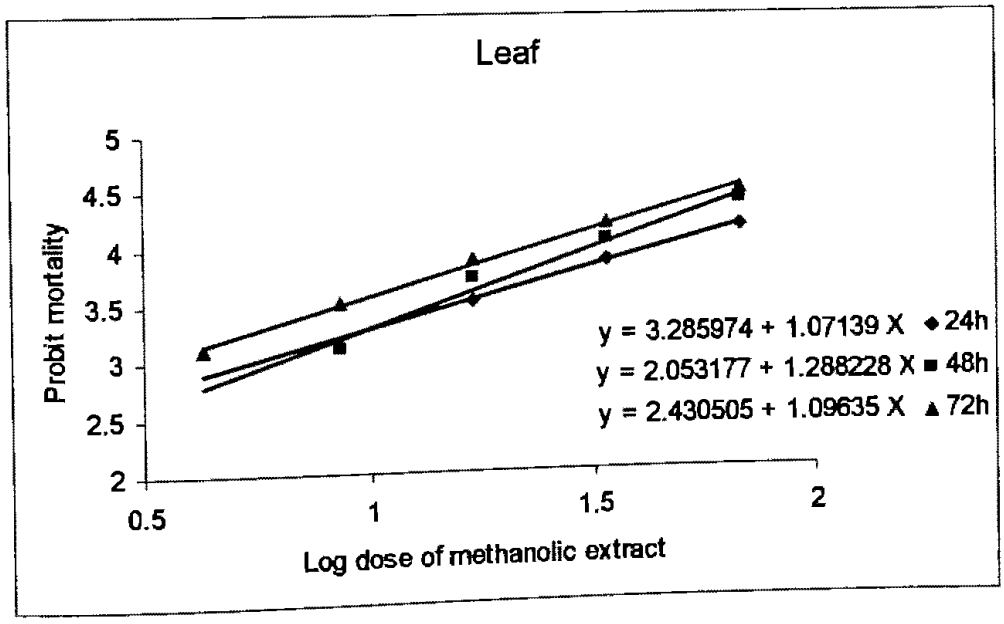


Figure 10 : Regression line of probit mortality of adult *C. pusillus* and log dose of leaf extract of *S. zeylanica* in methanol after 24, 48 and 72 hours of exposure

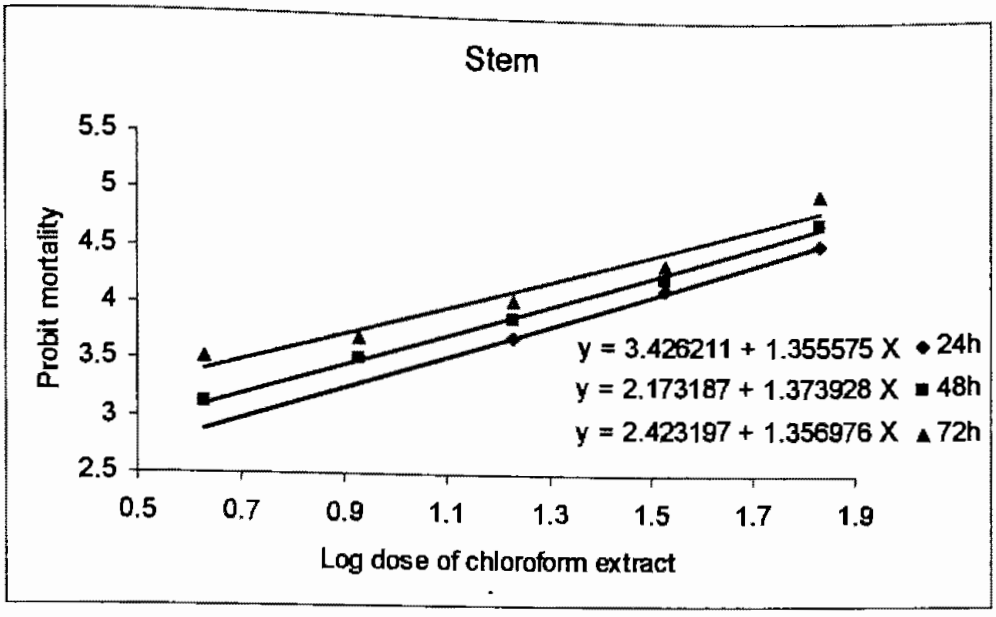


Figure 11 : Regression line of probit mortality of adult *C. pusillus* and log dose of stem extract of *S. zeylanica* in chloroform after 24, 48 and 72 hours of exposure

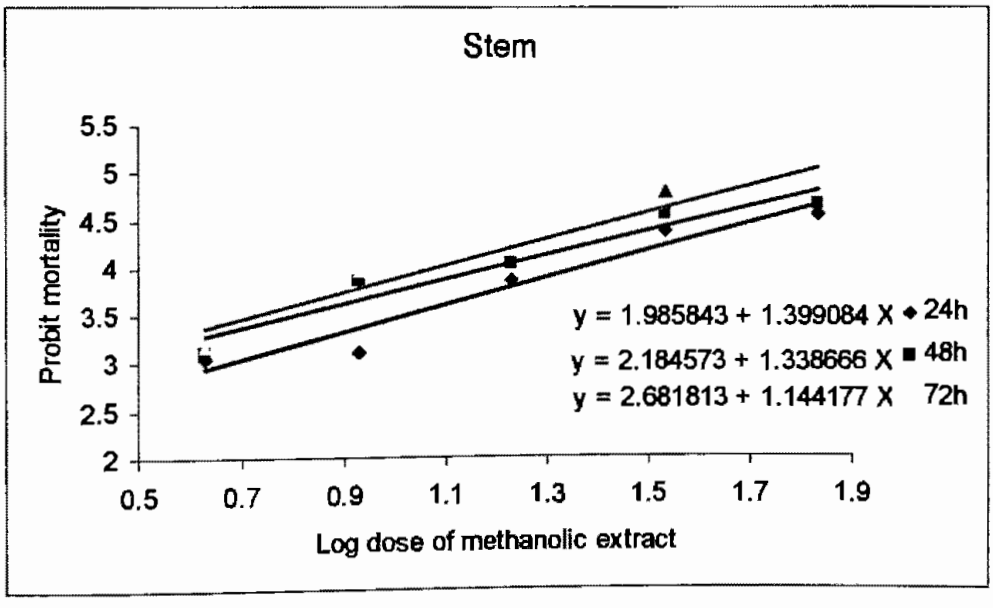


Figure 12 : Regression line of probit mortality of adult *C. pusillus* and log dose of stem extract of *S. zeylanica* in methanol after 24, 48 and 72 hours of exposure

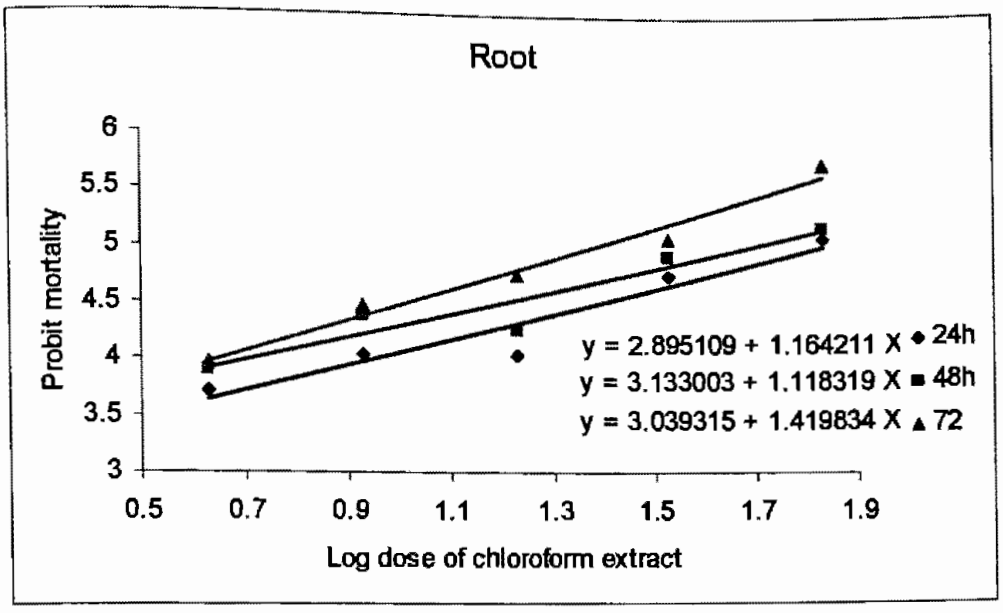


Figure 13 : Regression line of probit mortality of adult *C. pusillus* and log dose of root extract of *S. zeylanica* in chloroform after 24, 48 and 72 hours of exposure

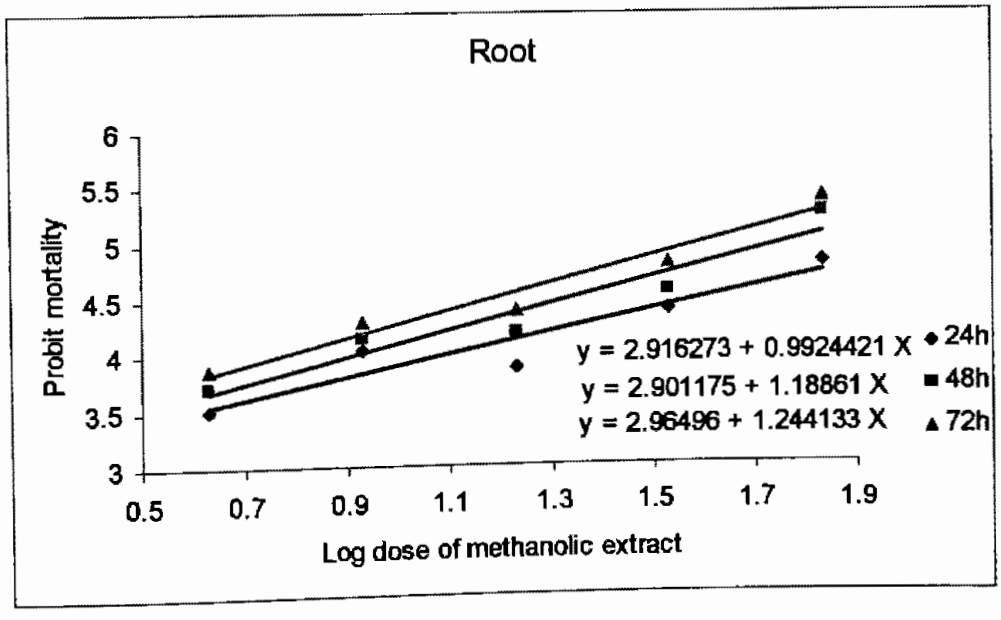
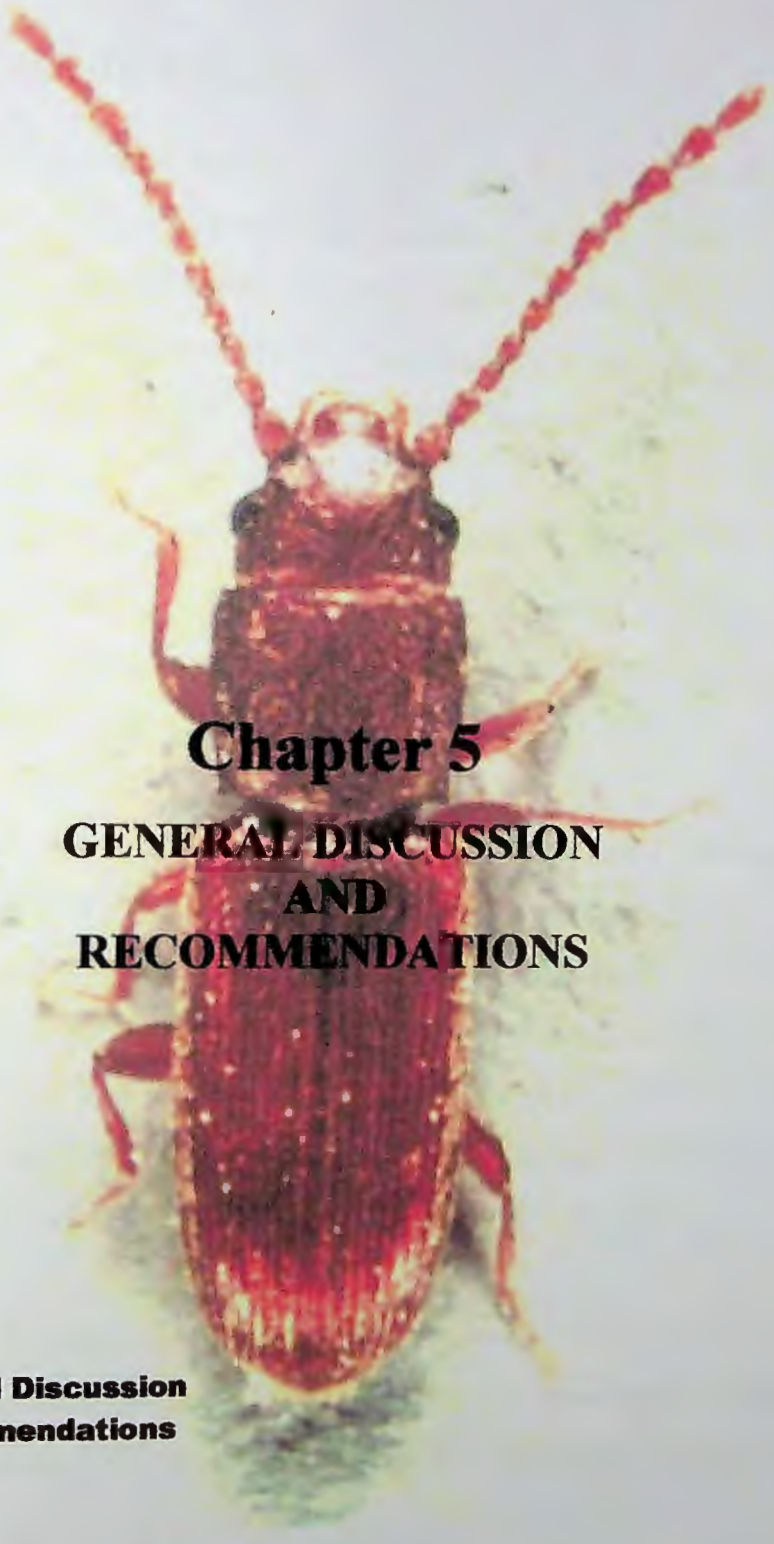


Figure 14 : Regression line of probit mortality of adult *C. pusillus* and log dose of root extract of *S. zeylanica* in methanol after 24, 48 and 72 hours of exposure



Chapter 5

GENERAL DISCUSSION AND RECOMMENDATIONS

GENERAL DISCUSSION AND RECOMMENDATIONS

5.1. GENERAL DISCUSSION

To use of phytochemicals as well as plant products such as powder, dust, oil and crude extracts, for the control of stored-product insect pests has much agricultural importance and currently 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 chloroform and methanol extracts possess substantial minimum inhibitory concentration (MIC) value as 64µg/ml. No sign of growth of the test organisms *Bacillus cereus, Bacillus subtilis, Streptococcus-β-haemolyticus, Salmonella typhi* and *Shigella dysenteriae* was observed in the test tube containing 128 µg/ml of the methanolic 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 methanolic crude extracts of root of *S. torvum*.

The MIC for the chloroform extract was observed to be 128µg/ml for *Bacillus cereus, Streptococcus-β-haemolyticus* and *Shigella dysenteriae*. However, for the rest of bacteria the MIC was 64 µg/ml of the chloroform extract of stem of *S. zeylanica*.

No inhibition was observed in the test tube containing sample lower than the concentrations. Three control tests were performed using nutrient broth 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 present results on the effect of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* on adult *C. pusillus* are in agreement with earlier reports by Ali *et al.* 1983, Shelke *et al.* 1990, and Mirsa 1993.

Many researchers work on the chemical composition of different plants, e.g. *Sophora*, *Solanum hispidium*, *Nephelium maingayi*, *Ambrosia artemisiifolia* L. and *Helichrysum amorginum*. Abe *et. al.* (2004) reported trypanocidal constituents in plants. Triterpenoid saponins from the root of *Sophora* (Byun *et. al.* 2004), Saponins from the brak of *N. maingayi* (Ito *et. al.* 2004), Antimycotic spirostanol saponins from *S. hispidium* leaves (Gonzalez *et. al.* 2004). It is already reported the chemical composition and antimicrobial activity of *A. artemisiifolia* and *H. amorginum* cultivated in Greece (Chalchat *et. al.* 2004, Chinou *et. al.* 2004). Cos *et. al.* (2004) stated plant substances as anti-HIV agents selected according to their putative mechanism of action.

Some other scientists report on antimicrobial activities of different medicinal plants e.g. Antimicrobial evaluation of some medicinal plants for their anti-enteric potentials against multi-drug resistant *Salmonella typhi* (Rani and Khullar 2004). Antimicrobial activity of selective Peruvian medicinal plants (Rojas *et. al.* 2003). Antimicrobial activity of crude extracts of *Cassia alata* (Selvamani and Latha 2004), identification of the antibacterial component of an ethanolic extract of the Australian medicinal plant, *Eremophila duttonii* (Shah *et. al.* 2004), antifungal constituents of *Melicope borbonica* (Simonsen *et. al.* 2004), antimicrobial activity of *Mahonia aquifolium* (Slobodnikova *et. al.* 2004).

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, antifeedent 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 crude extract and thus they die due to starvation or when the 1st instar larvae come in contact with the extract, they die due to the insecticidal properties.

Tiwari (1994) regarded plant materials to be safe for the mammals but toxic to the insects. Lale (1995), Rajendran and Sriranjini (2008) studied on the use of plant products in the managements of stored product pests. Bekele *et al.* (1996) reported that botanical pesticides are selective, environmentally safe and non-toxic to mammals, human being and other beneficial animals. Ahn *et al.* (1998) reported on the insecticidal and acaricidal activity of Casvacrol and β-thujaplicine derived from *Thujopsis dolabrata* vas.

The results of antibacterial activity of all the crude 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 toxicity bioassay indicates the cytotoxicity as well as wide range of pharmacological activities, e.g. anticancer, and antiviral activities of oily extract of *Sida rbombifolia* (Islam *et al.* 2000). Chloroform and methanolic extracts of experimental plants were found to be toxic against brine shrimp nauplii in the present investigation.

Many researchers isolated several compounds from these plants and their medicinal activities were reported but their insecticidal activities against adult *C. pusillus* and brine shrimp nauplii were not reported. So, the findings of our investigation using extracts of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* on stored product insect pest as well as brine shrimp nauplii seems to be a new work.

Different doses of plant materials have long been considered as human usage. The effects of plant products are short lived 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 eco 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 research reveals that the chloroform and methanolic extracts of tested plants were highly active against the bacteria *Bacillus cereus*, *Salmonella typhi* and *Streptococcus*-β-*haemolyticus* and the fungus, *Aspergillus fumigatus* and *Candida albicans*. The MIC results indicated that, the methanolic extract of root of *S. torvum* and the chloroform extracts of the stem of *S. zeylanica* has prosperity to inhibit bacterial growth even at low concentration (64-128 µg/ml).

This probably explains the use of extract of this plant in traditional medicines against a number of infections. So, consequently, a detailed further study of this plant, in order to determine their pharmacological and toxicological effects, bioactive compounds as well as their mechanism of action are needed.

The contact toxicity of the extracts on the test insect in terms of LD_{50} in two solvents from the two experimental plants were not similar. In general, all extracts showed a higher degree of toxicity on the test insect.

The results of the present investigation show that the effect of the extracts of leaf, stem, root and inflorescence of *S. torvum* and leaf, stem and root of *S. zeylanica* have antibacterial, antifungal and insecticidal activities. More comprehensive research is very much to be solicited for their effective use, especially in the field of medicine and agriculture.

5.2. RECOMMENDATIONS

The chloroform and methanolic extracts of the test plants *viz. S. torvum* and *S. zeylanica* showed significant antimicrobial (antibacterial and antifungal) activities. against *Bacillus cereus* and *Salmonella typhi*, *Streptococcus*-β-*haemolyticus* was compared with Ciprofloxacin and *Aspergillus fumigatus* and *Candida albicans*, *Vasin factum* were comparable with Nystatin when tested at a concentration of 200 µg/disc respectively. The Minimum Inhibitory Concentration of methanolic extract were found to be 64 µg/ml against *B. subtilis* and *S. typhi,* and 128 µg/ml against *B. cereus* , *Streptococcus-β-haemolyticus* and *S. sonnei.*

Tiwari (1994) regarded plant materials are safe for the mammals but toxic to the insects. The use of phytochemicals is a promising and an authentic method among the Integrated Pest Management systems for the control of stored grain pests. *S. torvum* and *S. zeyla*nica have the potentiality to manage the red flat grain beetle, *C. pusillus.*

The present investigation advocates the suitability and efficacy of the extracts of *S. torvum* and *S. zeylanica* to check the population of the red flat grain beetle, These extracts of the tested plants can be used effectively, and will play significant roles in the management of *C. pusillus* as botanical agents, which is very important from both environmental as well as Integrated Pest Management points of view.

C. pusillus is a cosmopolitan and noxious pest that causes enormous damage to different stored commodities including wheat and flour. In Bangladesh, chemical pesticides are indiscriminately used to protect stored cereals and pulses that cause ecological imbalance as well as health hazards to humans. The following recommendations can be made for controlling *C. pusillus* by these botanical agents.

- The above plants may be cultivated in the large scale and the active ingredients extracted industrially and directly applied with lower doses than synthetic pesticides. This indigenous technology seems to be economically feasible and non-toxic.
- The stringent requirements of safety for humans and the environment are now needed for all new pesticides, especially for those used in food stores. In this regards the plant extracts used in the present study were found quite potential and match the planning of the new pesticides.
- Botanicals act quickly to stop feeding of insect pests and often cause immediate paralysis of feeding and are easily extracted and formulated and cheaper than other inorganic and chemical insecticides. These are easily applicable and pests can not grow resistance against botanicals as rapidly as the synthetic pesticides.

- Creation of awareness among the people for protecting their stored commodities by the use of botanicals which keeping the environment sound and will also saves a lot of foreign currency.
- Intensive training, workshop and seminars for farmers, warehousemen, traders, processors, extension workers, pest control technicians and quarantine officers on storage and pest management principles. Practice of using biocontrol agents for controlling stored grain pests to be also encouraged.
- Farmers should be made familiar with the techniques for collecting, identifying, extraction procedure and preserving different beneficial indigenous medicinal plants of economic importance.
- As there is no available facility in our country to determine the structural elucidation and insecticidal characterization of plant materials hence, no such information can be presented in the Thesis. So, further research is needed in this context.
- The chloroform and methanolic extracts of the test plants *viz. S. torvum* and *S. zeylanica* showed significant antimicrobial activities. The extracts from different parts of the test plants showed good cytotoxic effects. So, it is highly recommended that, future concerted efforts should be directed towards their phytochemistry as well as pharmacological effects.

Chapter 6

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LITERATURE CITED

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