



Insecticidal Activity of Mangrove *Rhizophora Mucronata* Using the Plant Parts on Selected Stored Grain Pest

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Abstract:

Mangroves are trees as well as shrubs, commonly called as halophytes since they grow in harsh environmental conditions such as high saline conditions. They constitute a unique ecosystem of coastal region which links fresh water and marine life. They can grow in such extreme environmental conditions due to their morphological and physiological adaptations, including complex root and salt filtration abilities to cope with inundation of salt water and wave action. They are well adapted to grow in anoxic conditions in intertidal zone or brackish water of tropical and subtropical coastal areas. They have their own ecosystem therefore their loss can directly show the negative impact on surrounding coastal ecosystem and associated ecosystems. Mangroves are of great importance to many people who live along tropical shorelines. In Southeast Asia mangroves have been managed as a sustained yield forest crop for more than a century. In recent years the ecological, environmental and socio-economic importance of mangroves has been emphasized by international agencies, governments, local authorities' non-government organization (NGOs), coastal communities and scientists.

Introduction:

Mangroves are trees as well as shrubs, commonly called as halophytes since they grow in harsh environmental conditions such as high saline conditions. They constitute a unique ecosystem of coastal region which links fresh water and marine life. They can grow in such extreme environmental conditions due to their morphological and physiological adaptations, including complex root and salt filtration abilities to cope with inundation of salt water and wave action. They are well adapted to grow in anoxic conditions in intertidal zone or brackish water of tropical and subtropical coastal areas. They have their own ecosystem therefore their loss can directly show the negative impact on surrounding coastal ecosystem and associated ecosystems. Mangroves are of great importance to many people who live along tropical shorelines. In Southeast Asia mangroves have been managed as

a sustained yield forest crop for more than a century. In recent years the ecological, environmental and socio-economic importance of mangroves has been emphasized by international agencies, governments, local authorities' non-government organization (NGOs), coastal communities and scientists.

Mangrove forests are home to a large variety of fish, crab, shrimp, and mollusk species. These fisheries form an essential source of food for thousands of coastal communities around the world. The forests also serve as nurseries for many fish species, including coral reef fish. This makes mangrove forests vitally important to coral reef and commercial fisheries as well (Duke, 2007). Plants establish a rich source of bioactive metabolites or compounds which can serve the purpose of an attractive insecticide an alternative of using synthetic insecticides for pest management, termed as botanical insecticides.

Several secondary metabolites present in plants serve as a defense mechanism against insect attacks and it has been demonstrated that the pesticidal properties of plant chemicals can be specific to particular target species, biodegradable to non-toxic products and potentially suitable for use in an integrated pest management (IPM) (Saira, 2017). There are many other plants which are existing and possessing insecticidal property against insect pests but are still unknown due to lack of knowledge.

Ancient and traditional methods have always used plant as an alternative method to prevent insects like in some countries burning orange peels prevents mosquitoes, stored grains were protected by adding neem leaves. Plants have always been beneficial to man since decades initially without proper knowledge and at present with abundant knowledge and awareness.

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. Most insecticidal compounds fall within four main classes the organochlorines, organophosphates, carbamates and pyrethroids. Out of these the major classes in use are organophosphates and carbamates (Ware et al, 1982; Dormon et al, 2000). Some compounds act as endocrine disrupters like methoxychlor, DDT and dieldrin. Carbamates, daminozib, piricarb and propamocarb has the potential of transactivation of estrogen receptors and therefore possess endocrine disrupting properties. Pyrethroids interfere with various ion channel in the nerve axon. These pesticides possess extreme toxicity and can harm natural ecosystem (Clark et al, 1982).

There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment (Rembold et al, 1983; Franzen et al 1993; FAO, 1982). The use of organochlorine insecticides has been banned in developed countries and alternative

methods of insect pest control are being investigated (FAO et al 2010).

Synthetic chemical insecticides such as organochlorine, organophosphorus, carbamates, pyrethrins and pyrethroids are currently used for larviciding purposes (Tiwary et al., 2007; Ali et al., 2013). However, their indiscriminate use resulted in several problems such as environmental pollution, insecticide resistance and toxic, hazardous affects which disrupt the human health and ecosystem (Devine, 2007; Bansal et al., 2011). They harm farm workers, even consumers, fish, birds, wildlife, disruption of natural biological control and pollution, extensive ground water contamination, potentially threatening human and environmental health (Nabil-el- Wakeil, 2011).

Natural insecticides can be chemical, mineral, or biological. The common goal of all is to kill, repel or otherwise interfere with the damaging behavior of insect pests. The use of medicinal plant is as old as man (Abhijit Kulkarni (2012).

The World Health Organization (WHO) has recognized and drawn attention of many countries to ever increasing interest of the public in the use of such plants for various curative purpose. These plants found in our environment enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox remedy (Akah and Nwabie, 1994). Since ancient times of civilization people have been relying on plants to overcome any invasions of parasites, household therapies to get rid of insect infestations, protection of crops in fields and agricultural arena.

Botanicals are a promising source of pest control compounds. The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment.

Plants are well known as an important source of many biologically active compounds. Plant derived natural products such as flavonoids, terpenes and alkaloids have received considerable attention due to their diverse properties. Consumption of natural products can not only cure the diseases but can be a very beneficial factor of reducing the risk of onset of any disease example consuming neem leaves is commonly observed to reduce blood sugar level, consumption of mangrove fruit can prevent onset of malaria (Jurenka, 2009; Newman and Cragg, 2007). Considering the large number of plants that are reputed to possess some form of insecticidal activity, it is a pity that only a few have been scientifically evaluated. A good example of a plant with reputed insect resistant properties is the mangrove tree *Rhizophora* spp (*Rhizophoraceae*) Lam. which grows in the salty muddy shores of the coast of East Africa and various parts of Asia. In general the mechanism of action of botanical insecticides in protecting plants from plant disturbing organisms directly inhibits the reproduction process of insect pests, especially female insects, reduces appetite, causes insects to resist food, destroys the development of eggs, larvae and pupa so that the breeding of insect pests is disrupted, and the Takahasi (1981) classified botanical insecticides based on the working of repellent group, rejecting the presence of insects example odour, prevent insects from eating sprayed plants, the reproduction of female insects and can disrupt the hormone system in insects body, the attractant group which can lure the presence of insects, control growth of bacteria and fungi and the botanical insecticide group that can decrease the preference of insects in accessing the food sources. (Victor, 2018).

Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, tannins (Nayak et al., 2014). They show different biological activities such as antifungal,

antibacterial, antifeedant, molluscicidal, and pesticidal properties (Varahala Rao and Naidu, 2009). As several distinct chemicals are present in the extracts of various parts of the mangrove plants, they have been used for centuries as a popular method for treating several health disorders (Nayak et al., 2014). Of late, research interest on mangrove plant exploration for medical usage and their utility in drugs for several ailments such as cancer, aids, etc. has been increased (Batsa and Periyasamy, 2013). However, only a few numbers of reports are available on their agricultural use, particularly their utilization in pest management practices (Jeyasankar et al., 2014; Kabar and Gichia, 2001).

Materials and Methods:

Methods Used for Collection of Mangrove and Its Extractions:

Mangrove species selected for studying the insecticidal effects was *Rhizophora mucronata* (Red Mangrove).

Location of mangrove species: For the collection of mangroves various places around Mumbai and Thane creek are selected. *Rhizophora* needs clean water to grow and hence Pollution may be the major reason of their decline. Apart from pollution actions like deforestation, constructions, shrimp farming, etc. contributes equally for the reduction in the number of mangroves.

Rhizophora species was collected from Godrej mangrove forest near Vikhroli, Mumbai. They are very well protected and conserved in Godrej Mangrove forest. Mangrove saplings are grown separately in their nursery and therefore this species is found only in this area in Mumbai. Collection of mangrove plant parts. The parts such as leaves and barks were collected from the selected Mangroves for the study of insecticidal property.

- i. Leaves: Leaves of species were collected for the study. Mangrove leaves has a unique adaptability to withstand in salty climate. It contains certain bioactive compounds.



Fig. 1: Pictures of Mangroves Rhizophora saplings

- ii. Barks: Barks from young plants were collected for the study. Young barks are tender and easily breakable. Rhizophora species was identified and authenticated from Godrej Mangrove Centre, Mumbai.



Fig.2: Pictures of Mangroves Rhizophora saplings



Fig.3: Pictures of Mangroves Rhizophora Extractions of leaf and bark.



Fig.4: Pictures of stored grain infestation.

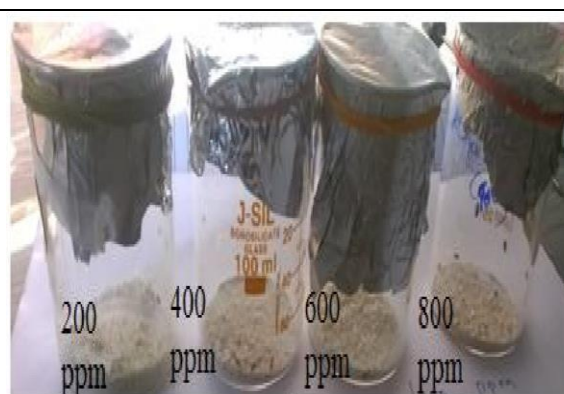


Fig.5: Pictures Insects treated with mangrove extracts and insecticidal effects of different concentration (ppm).

Extraction Of Phytochemicals:

Extractive values determine concentration or strength of active constituents present in given plant material in given solvent. The standard methods are used for extraction of phytochemicals from the different parts of the selected mangrove. Percentage of dry extract was calculated in terms of air-dried powder. Loss on drying is the loss in weight in percent resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions.

Preliminary Phytochemical Analysis:

Phytochemical studies of plant in different solvent extractions was analyzed. The presence of alkaloids, flavonoids, glycosides, fixed oils and terpenoids were confirmed by the qualitative preliminary phytochemical studies of each samples.

The following procedure is used for the extraction of phytochemicals.

i. Drying of plant parts:

These parts were thoroughly washed under running tap water immediately after collecting it.

ii. Preparation of fine dried powder of plant parts:

Then it was shade dried for 8 days to 10 days or more until all the parts were completely dried. It is very important and necessary step because dried plant parts can be powdered finely, using the electrical blender. Then the fine powder of all the selected parts of the selected Mangroves were stored separately in glass jars. They are then used for study the insecticidal property.

iii. Procedure for the Extraction:

Glasswares and chemicals required for the extraction: Solvent - 100% Methanol, Shaker, Bottles with tight lid used for overnight shaking for two days, Petri plates, Incubator, Micropipette, etc.

Procedure:

1. 5 gm of stored fine powdered of selected parts was dissolved in 50 ml of 100% methanol and kept for 48 hours in shaker for proper mixing and extracting the phytochemicals from the leaf and bark of all mangroves in the methanol.
2. After 48 hours the filtrate was collected through centrifugation and filtered through Whatman filter paper.
3. Filtrate was kept in petri-plates for evaporation of methanol at 37°C in an incubator or at Room Temperature. After the methanol was completely evaporated the extract was obtained. The extract obtained was of brown, dark brown and green coloured sticky consistency. It was weighed and then stored at 4°C in micropipette.

Stored grain pests

The pests which create a lot of nuisance and destroy the stored products. Most common pests found in field, stored food, go-downs and household kitchen. These insects are studied on for determining the insecticidal activity of mangroves. Adults and the larval stages of the pests are most destructive responsible for creating trouble.

Stored grain pests studied:

1. *Sitophilus oryzae*: The *Sitophilus oryzae* is commonly called Rice weevil belongs to order-*Coleoptera*. They infest rice, wheat, pulses and many other stored products.
2. *Tribolium confusum*: Commonly called confused flour beetle belongs to order-*Coleoptera*. Infest the flour like wheat flour, maida and many other flour.
3. *Tribolium castaneum*: Commonly called red flour beetle belongs to order-*Coleoptera*. Both the *Tribolium* species

show lot of resemblance. They also infest most of the flours.

4. *Bruchus*: There are many species of *Bruchus* that infest the grains belongs to order- *Coleoptera*. They infest chick peas, green moong, grams and many other pulses.
5. *Sitotroga*: Belongs to order – *Lepidoptera* and infests wheat.

Requirements for breeding of Pests:

Lab wares:

- i. Beakers- 500 ml beaker for rearing the insects present in the infested grains. Required for all pests.
- ii. Feed: 500 g of feed as required by respective pest (Mentioned above).
- iii. Muslin cloth: For covering the beakers in which pests are grown.
- iv. Sieve: For separating eggs, larvae, and adults.

Requirement of study of Insecticidal activity:

Chemicals and Glasswares:

Wheat flour, Mangrove extracts, Methanol, Distilled water, 200 ml Beakers, Test tubes, Pipettes (1ml, 5ml, and 10ml), Muslin cloth, Silver foil and tissue paper, Incubator, Weighing machine.

Preparation of concentration of extracts to study the insecticidal activity The extract was reconstituted in dist. water to obtain the dilutions of 200 ppm, 400 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm and 1000 ppm sub lethal concentrations. The insects such as *Sitophilus oryzae*, *Tribolium castenum*, *Tribolium confusum*, *Bruchus spp.* and *Sitotroga spp* were selected. Different sub lethal concentrations of the extract diluted was mixed with feed of the selected insects. The insects were fed and kept for 2 to 3 days. The effect was observed after 24 hrs to 48 hrs. (Akhtar, 2004; Bosque-Perez NA, .1990).

The mortality percent of the insect confirmed the insecticidal effect of Mangroves.

Testing procedure:

The selected insects were exposed to predetermined dosages of an insecticide (phytochemicals). The insects to be tested were free from any variations due to age, stage, sex, condition of nutrition, etc. The batches of insects were formed to ensure the random sample of the population. Approximately 10 to 20 insects were used to make one batch. The toxicity of an insecticide is usually determined using a dose response relationship. Five insects in each test series were used to detect the insecticidal effects. A control group for all the five insects were treated with distilled water only.

Application of the extracts on insects:

All the insects were treated with the leaves and barks extracts of mangrove of all species. Dilutions of different concentrations were prepared as mentioned in the earlier methods. The extract of all the three mangroves species was mixed with distilled water to prepare the dilutions. Dilutions from 100 ppm to 1000 ppm were prepared. Further it was applied on all the adults of the selected insects. After the insects are treated with different concentration of the insecticide, they were kept separately in another container. A mortality count was taken thereafter (Pradeepa, 2015).

Result:

Statistical analysis: The results obtained from the data collected were calculated to find the mortality percentage of the insects treated with mangrove extracts. Therefore, the insecticidal activity, antifeedant activity and the storage methods was experimented and studied on the selected stored grain pests on treatment with mangrove extracts. Toxicity was detected by finding the L.C50 value of the mangroves extract treated on insects with varied concentrations.

Biostatistical calculation was done by Anova (excel).

All the phases of their life cycle i.e., egg, larva, pupa and adult were observed in all the selected insects. Bruchus had a very short life cycle and the reproductive phase was very short due to which the population of the insects was

adequate. Both the Tribolium species, Tribolium confusum and Tribolium castaneum have lengthy life cycle and culturing these species was quite simple. Even culturing Sitophilus and maintaining its population was also equivalent to Tribolium species.

Table.1: Total volume of extract collected from different parts of the selected Mangroves

Plant Part of Mangrove	<i>Rhizophora mucronata</i>
Leaves	1.5 g
Barks	2.5 g

Table.2: Quantitative Tests for the detection of phytochemicals

Phytochemicals	Tests	<i>Rhizophora mucronata</i>
Protein	Ninhydrin Test	+
Phenols	Ferric Chloride Test	+
Flavonoids	Alkaline Reagent Test	+
Saponins	Froth Test	+
Glycosides	Killer Killian Test	+
Steroids	Salowski's Test	+
Terpenoids	Salowski's Test	+
Alkaloids	Mayer's Test	+
Tannins	Ferric Chloride Test	+

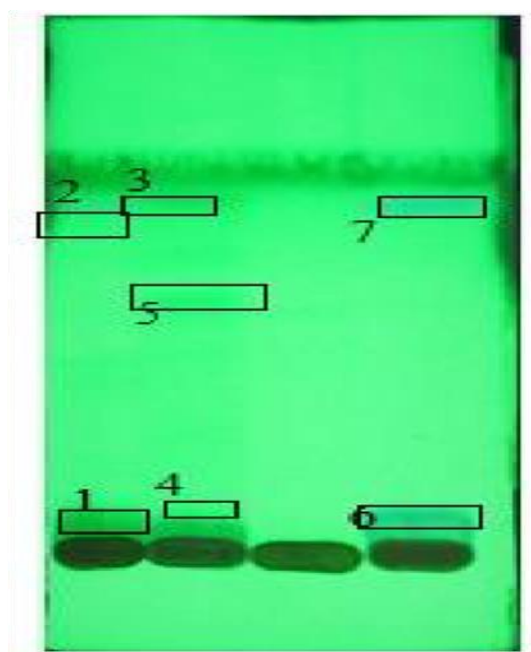


Fig.6: TLC bands observed U.V. Light at 245nm

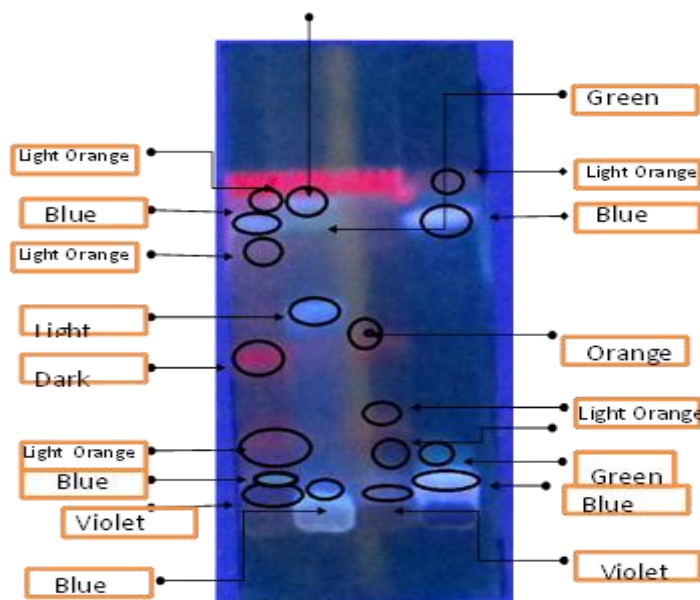


Fig.7: TLC plate with colour bands observed under U.V.Light at 345nm.

Table.3: Thin layer Chromatography result of *Rhizophora mucronata* with colored spots, R_f value and phytochemicals present.

Mangroves Species	No. of Spots	Color	R f values
1. <i>Rhizophora mucronata</i> (Leaf)	Spot 1	Light Violet	0.25 (Saponins)
	Spot 2	Light orange	0.38(lkaloids)
	Spot 3	Light orange	0.61 (Flavonoids)
	Spot 4	Light orange	0.83(Flavonoids)
	Spot 5	Light orange	0.92 (Flavonoids)
	Spot 6	-	-
	Spot 7	-	-
2. <i>Rhizophora mucronata</i> (Bark)	Spot 1	Blue	0.2 (Terpenoids)
	Spot 2	Grey	0.3 (Terpenoids)
	Spot 3	Light orange	0.38 (Alkaloids)
	Spot 4	Light orange	0.58 (Flavonoids)
	Spot 5	Blue	0.83 (Flavonoids)

Sitophilus oryzae (Rice Weevil):

Wheat flour was used to feed these insects. All the stages of Rice weevil were observed during culturing period. The insecticidal effect was observed on Rice weevil using the same feed in a separate container. 1g of feed was mixed with 1ml of each dilution from 100 ppm to 1000 ppm for all mangrove extracts. Rice weevil

showed 50% mortality at 800ppm after 48 hours of exposure.

Tribolium confusum (Confused Flour beetle):

These beetles are very destructive and can infest many stored grains within short duration. It was easy to culture these insects. Culturing of these was done using wheat flour, all-purpose flour, Barley. The insecticidal effect was

observed on Flour beetle using the same feed in a separate container. 1g of feed was mixed with 1ml of each dilution from 100 ppm to 1000ppm for all mangrove extracts. Confused Flour beetle showed 50% mortality from 700 ppm to 1000 ppm for mangrove leaves and barks after 48-72 hours as mentioned in tables given below.

***Tribolium castaneum* (Red Flour beetle):**

These beetles grow best in wheat flour and can also grow in many other stored products. The insecticidal effect was observed on Flour beetle using the same feed in a separate container. 1g of feed was mixed with 1ml of each dilution from 100 ppm to 1000 ppm for mangrove extracts. Confused Flour beetle showed 50% mortality on treatment with all mangrove extracts after 48-72 hours.

***Bruchus*:**

These beetles infest grams, chick peas, green moong dal, etc. These are voracious feeders. Adults are very destructive. Life cycle is

very short. Culturing these insects is difficult as they can hop and fly. All beetles cannot fly. They were fed with coarsely powdered gram flour which was treated with the mangrove extracts to check the insecticidal effect. 1g of feed was mixed with 1ml of each dilution from 100 ppm to 1000 ppm for all mangrove extracts. *Bruchus* showed 50% mortality with all mangrove extracts at different concentration of the extract.

***Sitotroga*:**

These insects grow in cereals like wheat, Barley, etc. and pulses like gram, moong, etc. 1g of feed was mixed with 1ml of each dilution from 100 ppm to 1000 ppm for all mangrove extracts. *Sitotroga* showed very low mortality with all mangrove extracts at different concentration of the extract. Culturing these insects was the most difficult task of this study. Very moderate number of insects could grow which was still inefficient to detect the insecticidal effect on all mangrove extracts.

Name of Mangrove: Leaf of *Rhizophora mucronata*:

Table.4: % Mortality of *Sitophilus oryzae* on treatment with leaf extract of *Rhizophora mucronata*

Concentration (ppm)	LOG 10 concentration	Dead	Total	Dilutions (ppm)	% mortality	Probit
200	2.301029996	1	10	200	10	3.72
400	2.602059991	2	10	400	20	4.1
600	2.77815125	3	10	600	30	4.48
800	2.903089987	6	10	800	60	5.25
1000	3	7	10	1000	70	5.52

LC₅₀ = 781 ppm.

Table.5: % Mortality of *Tribolium castaneum* on treatment with leaf extract of *Rhizophora mucronata*.

Concentration (ppm)	LOG 10 concentration	Dead	Total	%mortality	Probit
200	2.301029996	3	15	20	4.1
400	2.602059991	4	15	26	4.36
600	2.77815125	6	15	40	4.46
800	2.903089987	8	15	60	5.25
1000	3	10	15	70	5.52

LC₅₀ = 707.9 ppm.

Table.6: % Mortality of *Bruchus* on treatment with leaf extract of *Rhizophora mucronata*.

Dilutions(ppm)	LOG 10 concentration	% mortality	Probit
200	2.301029996	20	4.1
400	2.602059991	25	4.33
600	2.77815125	40	4.75
800	2.903089987	40	4.75
1000	3	60	5.25

LC₅₀ = 954 ppm

Table.7: % Mortality of *Tribolium confusum* on treatment with leaf extract of *Rhizophora mucronata*.

Dilutions(ppm)	LOG 10 concentration	% mortality	probit
200	2.301029996	20	4.1
400	2.602059991	30	4.48
600	2.77815125	45	4.87
800	2.903089987	50	5
1000	3	70	5.52

LC₅₀ = 660 ppm.

Table.8: % Mortality of *Sitotroga* on treatment with leaf extract of *Rhizophora mucronata*.

Dilutions(ppm)	LOG ₁₀ concentration	% Mortality	Probit
200	2.301029996	5	3.72
400	2.602059991	8	4.1
600	2.77815125	10	4.61
800	2.903089987	25	4.87
1000	3	30	5.13

LC₅₀ = 1600 ppm.

Name: Bark of *Rhizophora mucronata*.

Table.9: % Mortality of *Sitophilus oryzae* on treatment with Bark extract of *Rhizophora mucronata*.

Dilutions (ppm)	LOG 10 concentration	% Mortality	Probit
200	0.582063363	12	3.82
400	0.612783857	20	4.1
600	0.651278014	30	4.48
800	0.681241237	42	4.8
1000	0.764176132	66	5.81

L.C₅₀ = 891.2 ppm.

Table.10: % Mortality of *Tribolium confusum* on treatment with Bark extract of *Rhizophora mucronata*.

Dilutions (ppm)	LOG 10 concentration	Dead	Total	%mortality	Probit
200	2.301029996	4	15	26.6	4.36
400	2.602059991	4	15	26.6	4.36
600	2.77815125	6	15	40	4.76

800	2.903089987	8	15	50.3	5
1000	3	9	15	60	5.25

$LC_{50} = 851.13$ ppm.

Table.11: % Mortality of *Tribolium castaneum* on treatment with Bark extract of *Rhizophora mucronata*.

Dilutions (ppm)	LOG 10 concentration	% mortality	probit
200	2.301029996	15	3.96
400	2.602059991	22	4.23
600	2.77815125	40	4.76
800	2.903089987	58	5.2
1000	3	65	5.39

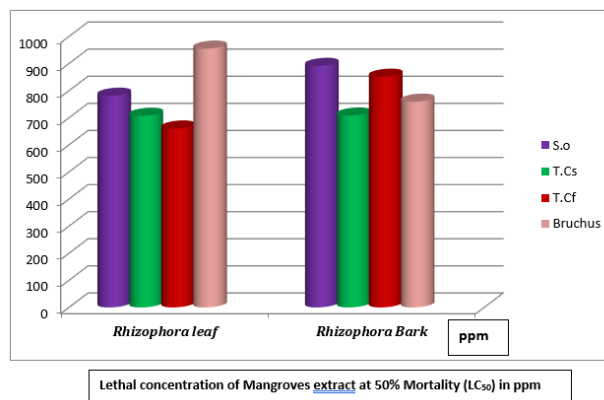
$LC_{50} = 707.94$ ppm.

Table.12: % Mortality of *Bruchus* on treatment with Bark extract of *Rhizophora mucronata*.

Dilutions (ppm)	LOG ₁₀ Concentration	% mortality	Probit
200	2.301029996	12	3.82
400	2.602059991	20	4.1
600	2.77815125	30	4.48
800	2.903089987	42	4.8
1000	3	66	5.81

$LC_{50} = 759$ ppm.

Fig.8: Graph showing lethal concentration (LC_{50} value) of Mangrove extracts on selected stored grain pests with 50% mortality. (*S. O* – *Sitophilus oryzae*, *T. Cs* – *Tribolium castaneum*, *T.Cf* – *Tribolium confusum*, *Bruchus*).



Conclusion:

Mangroves have countless significant role in environment and are advantageous in varied aspects. The present scenario demands the protection and conservation of mangroves and its habitat from getting devastated.

The following observations are done during the toxicity tests;

- After 24-48 hours of observation, the mortality % of the treated insects was

calculated for all the insects. After that with the help of probit table, LC_{50} (lethal concentration at 50%) of the leaves and barks extracts of all the mangrove plant parts were calculated.

- Among the mangrove species *Rhizophora* bark showed maximum effect on stored grain pest, medium effect by *Rhizophora* leaf.

- iii. It is concluded that the insecticidal property of selected mangrove tree is as follows:

Rhizophora mucronata bark is more effective than *Rhizophora mucronata* leaf.

- iv. Maximum insecticidal effect was observed on *Bruchus* followed by *Sitophilus oryzae*, *Tribolium castaneum* and least on *Tribolium confusum* and *Sitotroga*
- v. Selected stored grain pests effected by the insecticidal activity is observed as follows
Bruchus > *Sitophilus oryzae* > *Tribolium castaneum* > *Tribolium confusum* > *Sitotroga*

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