

Phytochemical analysis and biopesticidal activity of selected indigenous plant powders against the red flour beetle - *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

Agriculture in India has been around since the beginning of civilization. The agricultural sector acts as the bedrock of the Indian economy with 46 percent of the work force engaged in this sector. As one of the major cereals, spice, and fruit producing nations of the world, the agriculture sector ensures food security of the country and supplies raw material to agro based industries. *Tribolium castaneum* is one of the major pests of stored grains. The present investigations were carried out to evaluate the efficacy of botanicals: Custard Apple (*Annona squamosa* L.), Banyan (*Ficus benghalensis* L.), Night-flowering jasmine (*Nyctanthes arbor-tristis* L.) and Hibiscus (*Hibiscus rosa-sinesis* L.) leaf powder and Amla (*Phyllanthus emblica* L.) powder, on percent mortality and weight loss. The research was carried out at Department of Zoology, Vidyasagar College, Kolkata, West Bengal in 2025-26. The experiment was conducted in doses of 0.5gm, 1gm, 1.5gm, 2gm, 2.5gm & 3gm at 7,14,21 and 28 days of exposure. In the research, it was concluded that *Nyctanthes arbor-tristis* leaf powder has the most effective treatment (86.67%), whereas, *Phyllanthus emblica* and *Ficus benghalensis* both powder (75.53%) was the least effective in comparison to control against adult mortality owing to *T. castaneum*. Similarly, the most effective treatment for weight loss of wheat grains is that *Nyctanthes arbor-tristis* that recorded minimum weight loss (0.43%) and least effective treatment that recorded more weight loss is *Ficus benghalensis* (0.91%). So, *Nyctanthes arbor-tristis* powder could be used safely for controlling *Tribolium castaneum*. *Hibiscus rosa-sinesis* powder also had a significant insecticidal activity. Hence due to being eco-friendly, cheap and easy availability of these plants, their powders can be the promising substitutes of chemical insecticides in pest management programs.

Keywords: Stored grain pest, biopesticide, red flour beetle, night-flowering jasmine

Introduction

Stored grain pests refer to insects and other pests that affect the stored grains resulting in post-harvest losses. Stored grain pests can be categorized based on their behavior when attacking stored grains; some insects will attack whole and healthy grains (e.g., rice weevil and lesser grain borer) while others attack cracked and unhealthy grains (e.g., red flour beetle and saw-toothed grain beetle). Stored grain pests can also include insects that affect the quality, nutritional value, and marketability of stored grains.

Among these pests, *Tribolium castaneum*, is the worst and has managed to spread all over the world, damaging food products such as wheat flour and cereals. Both the adults and grubs cause serious damage to all kinds of grains including flour and dried fruits.

Tribolium castaneum, occupies grains and milled products made from grains. The habitat of this species has close ties with human activity, particularly storage areas for food, in grain mills, granaries, storage facilities, and kitchen areas where cereal grains, flour, and other milled grains are kept. It is commonly found in grains like wheat, rice, corn, and barley, along with their milled products such as flour and bran. It usually resides in cracks, crevices, and dust in storage containers, walls, and floors, thus making detection and management challenging.

It is an internationally recognized pest of stored grain products, its high rate of reproduction, places it among the most important stored-product pests in terms of biological significance^[1].

It is crucial to control the insect in order to avoid any losses from stored food and ensure food quality. There are many methods of *T. castaneum* management that include both physical, chemical and biological measures, and utilization of plant products.

Plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants^[2, 3, 4]. The insecticidal and acaricidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control^[5]. These plant derivatives act as repellents, antifeedants, or even deadly or lethal substances which inhibit insect reproduction^[6]. Especially remarkable are tropical plants from which hundreds of secondary metabolites with insecticidal properties have been extracted. They are environmentally less harmful than synthetic pesticides and acting in many insects in different ways^[7, 8, 9]. To minimize use of pesticides and to avoid pollution of the environment, natural antifeedant, deterrent and repellent substances have been searched for pest control during recent times^[10, 11, 12]. The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. Yang and Tang^[13] reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and

environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Among the advantages of using such insecticides is their biodegradability and less likelihood of becoming resistant. These insecticides have thus become widely used in IPM.

In many areas of the world locally available plant materials are widely used to protect stored product against damage by insect infestation ^[14, 15]. Botanical products are environmentally safe, less hazardous, economic and easily available. Botanicals like Garlic, Bonkalmi, Turmeric, Bazna, Bishkatali, Custard apple, Datura, Durba, Eucalyptus, Ghora-neem, Hijal, Karanja, Mahogoni, Marigold, Neem, Nishinda, Pithraj, and many others may be grown by farmers with minimum expense.

Neem for the existence of azadirachtin, inhibits insect feeding, development and may inhibit oviposition ^[6], nicotine (derived from *Nicotiana tabacum*), effective against insects causing the uncontrolled nerve firing and masking acetylcholine ^[16], *Eucalyptus globulus* leaves have metabolites, used as fumigant ^[17], turmeric plant produces curcumin, acts as a repellent and poison for pests ^[18], the garlic plant secretes sulfur compounds with insecticidal properties ^[19].

Currently, there exists great interest in the utilization of indigenous plants of the particular geographical region in place of artificial pesticides as powders. Farmers usually incorporate powdered leaves, seeds, and rhizomes of indigenous plants into their grains during storage to protect them from insect pests.

The present experiment was, therefore, undertaken to evaluate the toxic effect of leaf powder of Custard Apple (*Annona squamosa*), Night flowering jasmine (*Nyctanthes arbor-tristis*), Hibiscus (*Hibiscus rosa-sinensis*), Banyan (*Ficus benghalensis*) and the dried fruit powder of Amla; Indian Gooseberry (*Phyllanthus emblica*) on the development and mortality of *T. castaneum*.

Material & Methods

Experimental Site

The experiment was done by completely randomized design (CRD) method using three replications of five treatments with untreated as control at the entomology lab of Vidyasagar College Salt Lake Campus, Kolkata during 2025-26. The treatment details are as follows.

Treatment Details

A total of five treatments were included in this research which could be obtained easily in the surrounding regions. Different five treatments such as Custard Apple (*Annona squamosa* Linnaeus), Banyan (*Ficus benghalensis* Linnaeus), Night-flowering jasmine (*Nyctanthes arbor-tristis* Linnaeus) and Hibiscus (*Hibiscus rosa-sinensis* Linnaeus) leaf powder and Amla (*Phyllanthus emblica* Linnaeus) powder were selected and collected from the local regions of Kolkata and were kept at room temperature.

Insect Culture: Rearing of Test Insect *Tribolium castaneum*

To rear the insect, clean, un-infested wheat was purchased from the local market. Colonies of red flour beetles were collected from naturally infested flour from the warehouse and was mass reared in room temperature in laboratory. The red flour beetles were put in transparent box of plastic. Moreover, the storage box was covered with muslin cloth to allow circulation ^[20].

Collection of Botanicals

Leaves & young twigs of 4 different botanicals (*Annona squamosa* L., *Ficus benghalensis* L., *Nyctanthes arbor-tristis* L. and *Hibiscus rosa-sinensis* L.) were collected as sample botanicals from Kolkata, Bankura and Burdwan district, and fruits (*Phyllanthus emblica* L.) from local market of Kolkata, West Bengal.

Preparation of Botanicals

After collecting the leaves from local regions and then air dried under shade. The dried leaves were ground to a fine powder with the help of grinding machine. The powder was passed through sieve to get the fine powder of uniform particle size in all cases.

Mixing of Grain Protectants

100gm of healthy and uninfested healthy wheat grains were taken in plastic jars and different botanicals were mixed in according to required quantities (0.5,1,1.5,2,2.5,3 gm). Freshly emerged 15 beetles are released in the jars. Jars were covered with muslin cloth and tied with rubber bands. Observations were recorded at 7, 14, 21 and 28 days for mortality. Similarly, observations were made for Grain weight loss for the mentioned days also.

Phytochemical Analysis of the selected botanicals

Phytochemical analysis is the qualitative and quantitative analysis of presence of secondary metabolites in plants. These studies are conducted to identify phytochemicals in the Acetone, Ethanol, and Water etc. extracts of the test plants, and the phytochemicals are detected using color tests.

The procedure of Phytochemical tests of the mentioned botanicals is described below.

Preparation for phytochemical tests

The powder of the mentioned plant products along with the three solvents were mixed and kept in different conical flasks in a ratio of 1:3. The flasks were stirred 4-5 times and after that was kept still for 30 minutes. Then the contents were filtered through filter paper in different beakers. After that, the different tests for different secondary metabolites were performed, which is explained below.

a. Test for Flavonoids

One milliliter of the extractive was added to one ml of concentrated sulphuric acid and formation of orange colour confirmed the presence of flavonoid ^[21]

b. Test for phenol

One ml of the extractive was treated with 3 % ferric chloride and the appearance of deep blue or green colour showed the presence of phenol ^[21]

c. Test for quinone

One milliliter of the extractive was treated with 5 ml of Hydro chloric acid. Formation of yellow precipitate indicated the presence of quinone ^[21]

d. Test for terpenoid

Two milliliters of the extractive were treated with two ml of acetic acid and three ml of concentrated sulphuric acid. Deep red colour development showed the presence of terpenoid ^[22]

e. Test for coumarin

Two milliliters of the extractive were taken and 3 ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarin [22]

f. Test for alkaloid

One ml of the extractive was added with six drops of Mayer's reagent. The formation of yellowish cream precipitate indicated the presence of alkaloid [23, 24]

g. Test for glycoside

0.5 gm of extractive was taken and dissolved in two ml glacial acetic acid. One drop of ferric chloride solution and

one ml of concentrated sulphuric acid were added. Brown ring at the interface indicated the presence of glycoside.

h. Test for saponin

One ml of extractive was mixed with five ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponin [23, 24]

i. Test for tannin

One ml of extractive was treated with three drops of 1% lead acetate solution. Formation of yellow or red precipitate indicated presence of tannin.

Table 1: Treatment details

Treatment no.	Treatment	Dosage per 100 gm of wheat grains	Plant Part Used	References
T1	Custard apple powder	0.5,1,1.5,2,2.5,3	Leaf	Tripathi and Patel (2018)
T2	Night flowering jasmine powder	0.5,1,1.5,2,2.5,3	Leaf	-
T3	Banyan powder	0.5,1,1.5,2,2.5,3	Leaf	-
T4	Hibiscus powder	0.5,1,1.5,2,2.5,3	Leaf	-
T5	Indian gooseberry powder	0.5,1,1.5,2,2.5,3	Fruit	-
T6	Control	-	-	-

▪ Control studies

Control studies for *Tribolium castaneum* mortality using botanical powder, an Untreated Control had been set up., a Vehicle Control, and a Positive Reference Control. These ensure test validity, correct for natural death, and allow for the calculation of Abbott's formula for mortality.

Untreated Control (Negative Control)

- **Setup:** Infested grains (with same 15 beetles) without any botanical powder.
- **Purpose:** Ensures the base survival rate of *Tribolium castaneum* under laboratory conditions. High mortality here invalidates the test.
- **Recorded Observations**

$$\text{Percentage of Mortality} = \frac{\text{number of dead beetles}}{\text{number of beetles tested}} \times 100$$

$$\text{Percentage of Weight Loss in Treated Grain} = \frac{\text{initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

▪ Statistical Analysis

The data averaged into respective parameter requisite was subjected to suitable transformation. After analysis, data was accommodated in the table as per the needs of objectives for interpretation of results [25]

Results

a. Efficacy of Different Plant Powders and Insecticide on Percent Adult Mortality of *Tribolium castaneum*

The efficacy of different botanicals on mortality of *Tribolium castaneum* was tested. Freshly emerged 15 beetles are released in the plastic jars. The mortality of released adults was recorded at 7, 14, 21 and 28 days after treatment. The results are shown below in tabular forms in Table 2 and in graphical forms in Fig 1.

Table 2: Effect of different botanicals on adult mortality of *Tribolium castaneum*

Dose (gm)	7days	14days	21days	28days	Dose (gm)	7days	14days	21days	28days
0.5	6.67%	20%	42.2%	57.8%	0.5	11.13%	28.87%	44.47%	53.33%
1	13.33%	24.47%	44.47%	62%	1	8.87%	22.2%	40%	57.8%
1.5	13.33%	28.87%	42.2%	60%	1.5	15.53%	35.53%	51.13%	68.87%
2	15.53%	35.53%	51.13%	66.67%	2	20%	35.53%	53.33%	71.13%
2.5	20%	37.87%	55.53%	75.53%	2.5	26%	42.2%	62.2%	77.8%
3	31.13%	44.47%	60%	77.8%	3	37.8%	48.87%	73.33%	86.67%

Table 2: Percentage mortality of *Tribolium castaneum* under treatment of *Annosa squamosa*, *Nyctanthes arbor-tristis*, *Ficus benghalensis* and *Hibiscus rosa-sinensis* leaf powder and *Phyllanthus emblica* fruit powder in different doses (gm) over different time interval.

Dose (gm)	7days	14days	21days	28days	Dose (gm)	7days	14days	21days	28days	Dose (gm)	7days	14days	21days	28days
0.5	10%	17.8%	28.87%	51.13%	0.5	4.47%	15.53%	35.53%	48.87%	0.5	11.13%	22.2%	40%	51.13%
1	11.13%	24.47%	33.33%	53.33%	1	6.67%	17.8%	28.87%	46.67%	1	8.87%	24.47%	35.53%	51.13%
1.5	15.53%	31.13%	44.47%	57.8%	1.5	13.33%	28.87%	46.67%	60%	1.5	13.33%	28.87%	51.13%	66.67%
2	17.8%	33.33%	48.87%	64.47%	2	17.8%	31.13%	53.33%	64.47%	2	17.8%	35.53%	53.33%	73.33%
2.5	20%	37.8%	51.13%	71.13%	2.5	22.2%	37.8%	53.33%	66.67%	2.5	17.8%	35.53%	53.33%	71.13%
3	24.47%	42.2%	55.53%	75.53%	3	24.47%	40%	62.2%	82.2%	3	22.2%	35.53%	53.33%	75.53%

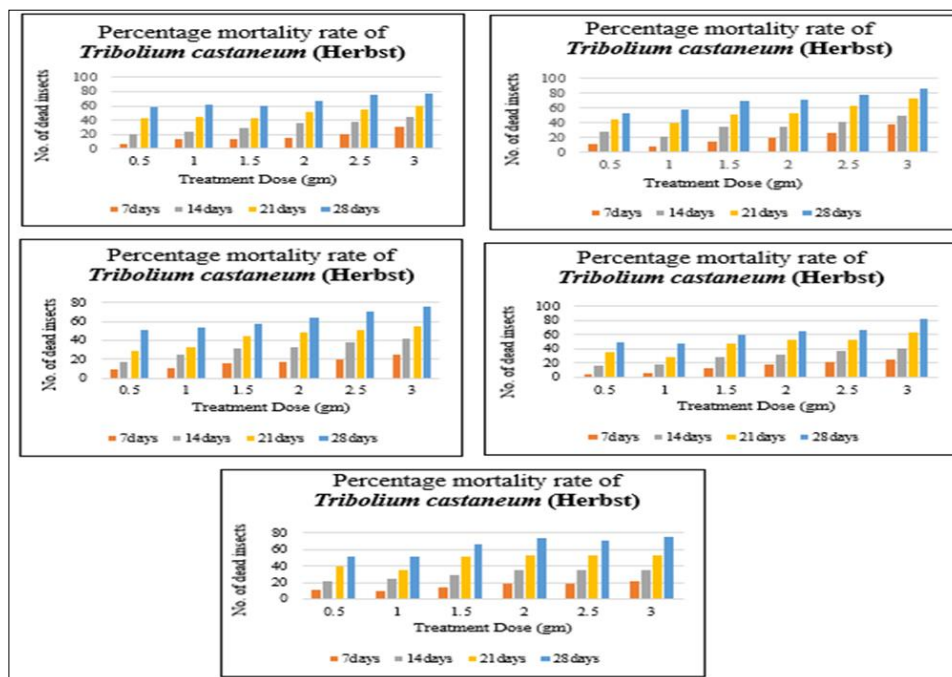


Fig 1: Graphical Representation of Percentage mortality of *Tribolium castaneum* under treatment of *Annosa squamosa*, *Nyctanthes arbor-tristis*, *Ficus benghalensis* and *Hibiscus rosa-sinensis* leaf powder and *Phyllanthus emblica* fruit powder in different doses (gm) over different time interval

b. Efficacy of Different Plant Powders and Insecticide on mean weight loss of wheat grain

The efficacy of different botanicals in inducing weight loss of *Tribolium castaneum* was evaluated at the same

observation intervals as mortality. The mean results are shown below in tabular form in Table 3 and in graphical form in Fig 2.

Table 3: Effect of different botanicals on weight loss of wheat grain

Dose (gm)	<i>Annona squamosa</i>	<i>Nyctanthes arbor-tristis</i>	<i>Ficus benghalensis</i>	<i>Hibiscus rosa-sinensis</i>	<i>Phyllanthus emblica</i>
0.5	0.5	0.37	0.71	0.36	0.39
1	0.52	0.38	0.62	0.36	0.45
1.5	0.58	0.39	0.91	0.46	0.51
2	0.55	0.39	0.89	0.35	0.48
2.5	0.56	0.43	0.85	0.39	0.43
3	0.62	0.4	0.88	0.34	0.48

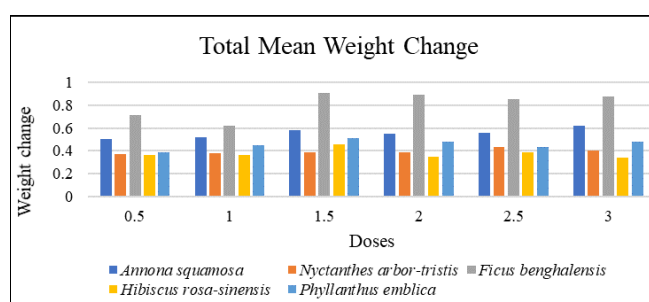


Fig 2: Graphical Representation of Mean Weight change of *Tribolium castaneum* under treatment of *Annosa squamosa*, *Nyctanthes arbor-tristis*, *Ficus benghalensis* and *Hibiscus rosa-sinensis* leaf powder and *Phyllanthus emblica* fruit powder in different doses (gm) over different time interval.

sinensis leaf powder and *Phyllanthus emblica* fruit powder in different doses (gm) over different time interval.

c. Presence of Phytochemicals

The phytochemical tests are performed to know about the major secondary metabolites present in the selected plant products. The presence of these metabolites with a spectrum of anti-insect properties helps to develop phyto-insecticides. Here the results are given in tabular form for all the plant products and solvents mentioned earlier in Table 4 and the pictures of the tests are attached below in Fig 3 & 4 respectively.

Table 4: Phytochemical Test Results

Plant	Solvent	Chemicals Present								
		Glycoside	Phenol	Tannin	Alkaloid	Terpenoid	Quinone	Flavonoid	Coumarin	Saponin
Custard Apple; leaf (<i>Annona squamosa</i> L.)	Distilled Water	+	+	+	+	-	-	+	+	+
	Acetone	+	+	+	+	+	+	+	-	-
	Ethanol	+	+	+	+	+	+	+	+	-

Note: (+) Presence of phytochemical constituent; (-) Absence of phytochemical constituent

Plant	Solvent	Chemicals Present								
		Glycoside	Phenol	Tannin	Alkaloid	Terpenoid	Quinone	Flavonoid	Coumarin	Saponin

Night flowering jasmine; leaf (<i>Nyctanthes arbor-tristis</i> L.)	Distilled Water	+	+	+	+	+	+	+	+	+
	Acetone	+	+	+	+	+	-	+	+	-
	Ethanol	+	+	+	+	+	-	+	+	+

Plant	Solvent	Chemicals Present								
		Glycoside	Phenol	Tanin	Alkaloid	Terpenoid	Quinone	Flavonoid	Coumarin	Saponin
Hibiscus Leaf; (<i>Hibiscus rosa-sinensis</i> L.)	Distilled Water	+	+	+	+	+	+	+	-	+
	Acetone	+	+	+	+	+	-	+	+	+
	Ethanol	+	+	+	+	+	-	+	+	+

Plant	Solvent	Chemicals Present								
		Glycoside	Phenol	Tannin	Alkaloid	Terpenoid	Quinone	Flavonoid	Coumarin	Saponin
Banyan Leaf (<i>Ficus benghalensis</i> L.)	Distilled Water	+	+	+	-	-	+	+	+	-
	Acetone	+	+	+	-	+	+	+	+	-
	Ethanol	+	+	+	-	-	-	+	+	+

Plant	Solvent	Chemicals Present								
		Glycoside	Phenol	Tanin	Alkaloid	Terpenoid	Quinone	Flavonoid	Coumarin	Saponin
Indian Gooseberry; Fruit (<i>Phyllanthus emblica</i> L.)	Distilled Water	+	+	+	-	+	-	+	-	-
	Acetone	+	+	+	+	-	-	+	+	-
	Ethanol	+	+	+	+	+	+	+	+	-

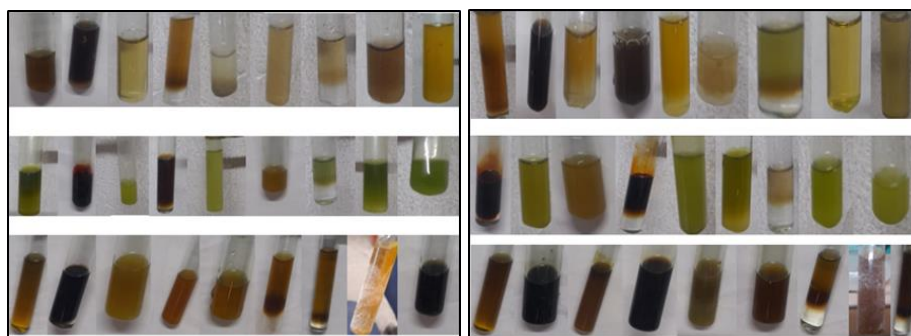


Fig 3: Phytochemical results of *Annona squamosa* L. and *Nyctanthes arbor-tristis* L. in water, acetone and ethanol; Flavonoid, Phenol, Quinone, Terpenoid, Coumarin, Alkaloid, Glycoside, Saponin, Tannin

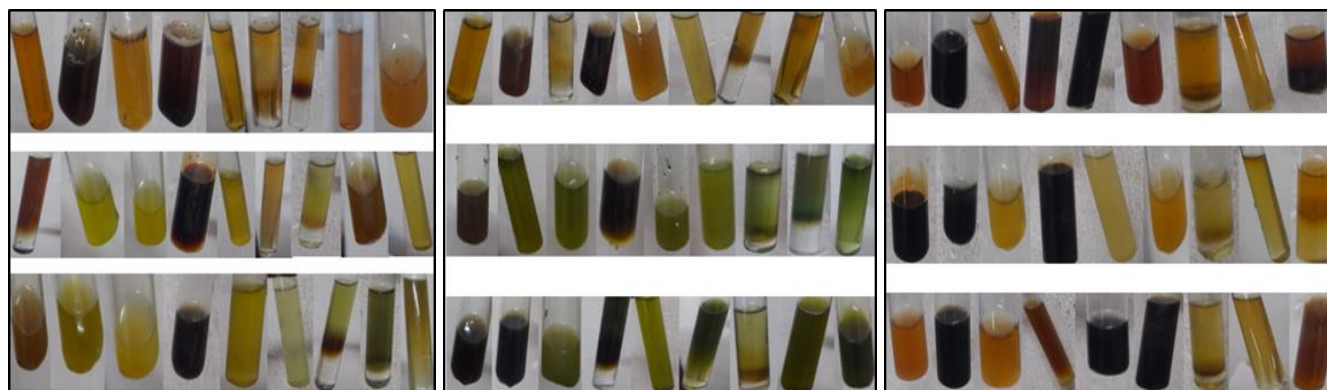


Fig 4: Phytochemical results of *Ficus benghalensis* L., *Hibiscus rosa-sinensis* L. and *Phyllanthus emblica* L. in water, acetone and ethanol; Flavonoid, Phenol, Quinone, Terpenoid, Coumarin, Alkaloid, Glycoside, Saponin, Tannin

Discussion

In the research, from data of Table 2 and graphs in Fig 1, it can be said that, night flowering jasmine leaf powder was the most effective treatment (86.67%) then hibiscus (82.2%) and custard apple (77.8%), whereas, Indian gooseberry and banyan both powder (75.53%) was the least effective in comparison to control against adult mortality owing to *T. castaneum*.

Similarly, from data of Table 3 and the graph in Fig 2, it was observed that, the most effective treatment for weight loss of wheat grains is that night flowering jasmine that recorded minimum weight loss (0.43%) and least effective

treatment that recorded more weight loss is banyan (0.91%), while the hibiscus shows weight loss of about 0.46%; custard apple 0.62% but Indian gooseberry shows 0.51%. For control in this study, no such mortality had been observed also weight loss caused 1.78 gm after 28 days exposure.

From data of Table 4 and Fig 3 & 4, the presence of chemicals in these plants are listed.

- *Annona squamosa* L. shows positive results to most of the secondary metabolites; but, terpenoid and quinone are negative when the powder is in water. Also, for acetone coumarin and saponin are noticed to be absent.

And lastly, for ethanol only presence of saponins is not found.

- For, *Nyctanthes arbor-tristis* L. in water all of the metabolites are present. But, quinone and saponin for acetone is absent, also, only quinone is not present in ethanol.
- In case of, *Ficus benghalensis* L., only alkaloid is absent in all of the solvents. Except that, in water saponin, terpenoid is absent. In acetone saponin and in acetone terpenoid is absent.
- For *Hibiscus rosa-sinensis* L. all secondary metabolites are present except, coumarin in water medium and quinone in both acetone and ethanol.
- In *Phyllanthus emblica* L. mostly all metabolites are present except alkaloid, quinone, coumarin and saponin in water medium; terpenoid, quinone and saponin in acetone and lastly in ethanol only saponin; are absent.

The progressive increase in mortality throughout the experimental period agrees with earlier reports that botanical powders often exert their effects gradually through desiccation, feeding inhibition, respiratory interference and physiological disruption rather than immediate knockdown action [26]. Such delayed action is characteristic of many plant-based insecticides used in stored-product protection.

Conclusion

After analyzing the present investigations, it is hereby concluded that the powder of dried leaves of *Annona squamosa* L., *Ficus benghalensis* L., *Nyctanthes arbor-tristis* L. and *Hibiscus rosa-sinensis* L. and dried fruit powder of *Phyllanthus emblica* L. possess significant insecticidal activity and have the potential to be used as an alternative to synthetic insecticides in granaries against *Tribolium castaneum* and possibly against other stored grain insect pests. The powder of *Nyctanthes arbor-tristis* L. leaves is the best among the plants evaluated in present studies and can be employed as an integral part of integrated pest management strategies to reduce the harmful impacts of the chemical pesticides. Considering the insecticidal activity and abundant availability in wild rural and urban areas there is a great potential for further investigation and practical use in pest management programs. They can also be made a part of integrated pest management as it will be helpful in reducing the fruit, leaves and wastes which is capable of polluting the environment. Additionally, all of them are extensively grown and relished in sub-tropical India therefore, there will always be a high availability of these botanicals.

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