

**REPELLENT AND TOXICOLOGICAL IMPACT OF AQUEOUS EXTRACTS OF LEAVES OF PLANTS STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA AGAINST SITOPHILUS ORYZAE****Anjali P¹, Jeevakarunya V² and Pawlin Vasanthi Joseph³**¹& ²PG Student and ³HOD and Associate Professor

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ABSTRACT: Experiments were conducted for the evaluation of contact and repellent activities of aqueous based plant extracts of *Strychnos nux-vomica* and *Justicia adhatoda* against the insect pest of stored grain products, rice weevil: *Sitophilus oryzae*. Various concentrations, (5, 10, 15 and 20%) of the plant extracts were applied on the filter papers in the bioassay experiments and after the release of adult beetles, mortality was evaluated after fixed interval (24, 48, and 72 hours). Repellency was also checked using area preference method after a fixed period (24, 48, and 72 hours). The result showed that the plants that used in the study *Strychnos nux-vomica* and *Justicia adhatoda* leaves extracts have high potency to control the pest due to the presence of active phytochemical compounds. The percentage mortality increases on the increasing order of plant extract concentration (5% > 10% > 15% > 20%) and with time of exposure (24hrs > 48hrs > 72hrs). The percentage mortality observed was high at 72 hours in 20 % in *Strychnos nux vomica* when compared to *Justicia adhatoda*. The percentage repellency increases on the increasing order of plant extract concentration (5% > 10% > 15% > 20%) and decreases with increasing exposure of time. The percentage repellency observed highest in *Strychnos nux-vomica* when compared to *Justicia adhatoda*. Results revealed that plant extracts are suitable for the formulation of biopesticides for the management of insect attacking stored products.

Keywords: *Sitophilus Oryzae*, Percentage Mortality, Percentage Repellency, Plant Extracts, Phytochemical Analysis, Insecticidal Activity.

I. INTRODUCTION

Plants are the natural laboratories where a great number of chemicals which are naturally synthesized may be considered as the most important sources of active chemical compounds. Different parts of plants are used in protecting crops from the attack of pests.

Strychnos nux-vomica L., belongs to the family Loganiaceae, also known as nux vomica, poison nut, snake wood, strychnine tree, Quaker buttons. The species is indigenous to India and is distributed in moist deciduous forests throughout tropical India [1]. Alkaloid strychnine from seed extract of *Strychnos nux-vomica* was used for the eradication of rodents during the early nineties in the United States and other European countries [2]. Seed, leaf and stem extract possess insecticide and nematicide properties. Ethyl acetate leaf extract at 200 ppm is lethal to third instar larvae of Mosquito *Culex quinquefasciatus*; a vector for *Wuchereria bancrofti* responsible for filariasis [3].

Justicia adhatoda is a species of plant in the Acanthaceae family. It is commonly known as Vasaka or Malabar nut [4]. Plants have evolved a wide variety of chemical compounds which are known as secondary metabolites where flavonoids, volatiles oil, tannins, saponines, glycosides, alkaloids and resins [5] for protecting plants against pests. Therefore, these compounds were used in the production of pesticides. *Adhatodavasicia* (L.) (Acanthaceae) is an insecticidal plant [6]. Leaf extract has anti-feeding activity against *Spodoptera littoralis* [7].

Sitophilus oryzae (Coleoptera: Curculionidae) is commonly known as rice weevil. It is one of the most important destructive primary pests attacking many common stored cereals including rice, wheat, maize and split peas and it enjoys worldwide distribution. Stored and milled rice grains are prone to attack by *Sitophilus oryzae* and the latter grains are mostly preferred causing heavy economic losses and both the adults and larvae feed on the carbohydrates in rice grains causing weight loss and contamination [8]. In absence of control, the stored grains can be destroyed even up to 100% [9].

Wide varieties of synthetic pesticides are available today. These synthetic pesticides are not as good as they are highly expensive, harmful & toxic to both mankind as well as the entire ecosystem. Instead of that, we can shift to plant based products which are cheap, biodegradable and environmentally friendly. A study on the repellency of hexane, ethyl acetate and methanol plant extracts from the leaves of *Strychnos nux-vomica* L., *Lepidium sativum* L. and *Azadirachta indica*, A. Juss against rice weevil *Sitophilus oryzae* showed that all the plant extracts were repellent at doses between 78.4 and 235.8 µg/cm and hexane and ethyl acetate extracts of *A. indica* followed by *L. sativum* were found to be the most effective compared to the extracts of *S. nux-vomica* [10]. The fumigant toxicity and repellent activity of hexane, diethyl ether and methanol extracts of *T. asiatica* leaves and fruits were studied against *C. maculatus*, *S. oryzae* and *T. castaneum*. Results suggested that Diethyl ether fruit extract of *T. asiatica* can be used



as an eco-friendly fumigant and repellent against *S. oryzae* and *T. castaneum* [11]. An experiment on insecticidal effect of two medicinal plants *Polygonum hydropiper* L. and *Abrus precatorius* L. leaves against the rice weevil *Sitophilus oryzae* L. The mortality of adults increased with increasing dose concentrations from 1000 ppm to 5000 ppm each solution, with an exposure time of 72 hours [12]. Therefore, the present study is based on the leaf extract of *Strychnos Nux-vomica* as well as *Justicia adhatoda* in aqueous solvent, could be used as a plant-based agent to control *Sitophilus oryzae*.

II. MATERIALS AND METHODS

The experimental setup includes one control and four experimental boxes marked as 5%, 10%, 15%, 20%, kept as triplet for different hours (24, 48, and 72) with plant extract of different concentration on a dry filter paper placed in the boxes, *Sitophilus oryzae* was introduced and feed was provided to avoid mortality due to starvation in the box.

A. Insect Collection

Mass Collection of rice weevil, *Sitophilus oryzae*, was made from the house granaries by hand picking and sieving method.

B. Plant Collection

Plant material, leaves of *Strychnos nux-vomica* and *Justicia adhatoda* were collected from local areas of Thrissur District in Kerala. The leaves of the plant were collected in the middle of December 2020. The collected plants were washed with clean tap water and were shade dried. After drying, the plant materials were powdered by using an electrical grinder and then sieved with a fine mesh sieve. Powders of the leaves were used for extraction in aqueous solution. Fifty grams of powder was used for extraction. One fourth of powder used for phytochemical analysis and remaining powder kept in reserve for the experiment.

C. Preparation of Plant extracts

The extraction was made by mixing 50 g of ground sieved sample and 100 ml of distilled water and shaking was ensured for 24 hours now and then. After 24 hours, filtration was made with the help of filter paper. This extract is now used as stock solution and store it in fridge. Prepare different concentrations of stock – 5% is 5ml of stock and 95ml of Distilled water, likewise prepare for 10%, 15% and 20%.

D. Bioassay for Percentage Mortality

Different concentrations of extracts are applied on the filter paper, allowing the paper to dry for a reasonable period of time. Then place it in a box or petri plate. Control is maintained by treating the filter paper with distilled water only. Introduced 10 beetles into the box and Small amount of rice was provided to decrease chances of mortality due to starvation. Close the box and mortality of adult beetles was being recorded after 24, 48 and 72 hours. Experiment was replicated three times and Completely Randomized Design (Factorial) was followed [13].

$$\text{Percentage Mortality} = \frac{\text{No. of dead Pests}}{\text{Total No. of Pests}} \times 100$$

E. Bioassay for Percentage Repellency

Repellency of the plant extracts was checked against the *Sitophilus oryzae* by using the area preference method [14] in which filter paper was cut into two equal halves. Different concentrations were made on the one half. After drying, the treated paper was stapled together and was adjusted in the Petri dishes. Maintain one control box for each hour. Ten adult beetles of *Sitophilus oryzae* were released in the centre of both halves. Repellency data was taken after a period of 24, 48 and 72 hours by observing how many beetles avoid staying on the plant extract smeared half of filter paper and are found in distilled water half. Diet will be provided on both sides (treated and untreated end of filter paper) to decrease mortality due to starvation.

$$\text{Percentage Repellency} = \frac{\text{No. of Pests in untreated half}}{\text{Total No. of Pests}} \times 10$$

F. STATISTICAL ANALYSIS

Results of the study are presented as mean \pm standard deviation for 10 experimental insects (*Sitophilus oryzae*) per treatment group. Significant differences between groups were determined by one way analysis of variance (ANOVA).

G. PHYTOCHEMICAL ANALYSIS OF PLANT SPECIMENS

Preliminary qualitative screening for phytochemicals, of these plant species (*Strychnos nux vomica* and *Justicia adhatoda*) was carried out with the following methods.



- **Test for Alkaloids**
2ml of plant extract was treated with Wagner's reagent. Brown (or) Red colour precipitate indicates the presence of alkaloids.
- **Test for Flavonoids**
0.5g of plant powder was added with 10 ml of distilled water 3-5ml of dilute ammonia solution were added to the side of the test tube, then added 1 ml of con. Sulphuric acid. Yellow colour indicates the presence of flavonoids.
- **Test for amino acids**
To 2-3 ml of purified plant extract add 2-5 drops of Ninhydrin solution. The samples were kept in a boiling water bath for 1-2 minutes. Purple color indicates the presence of amino acids.
- **Test for tannins**
2ml of purified plant extract was taken in a test tube and add 2 drops of 5% ferric Chloride solution and the presence was indicated by yellow color.
- **Test for reducing sugar**
To 2ml of purified extract add 2ml of Fehling's solution. The solution was kept in water bath for 40 degree Celsius. Formation of brick red precipitate at the bottom of the test tube indicates the presence of reducing sugars.
- **Test for steroids**
1ml of purified extract was placed in a test tube, 2ml of acetic anhydride followed by addition of 3-5 drops of chloroform. Then 2 drops of concentrated Sulphuric acid were added along the side portions of the test tube drop by drop. Formation of blue or green color indicates the presence of steroids.
- **Test for Phenols**
To 1ml of purified sample add 2ml of distilled water. Then 2-3 drops of 10% aqueous Ferric Chloride solution was added. The presence of phenol was indicated by the formation of blue or green color.
- **Test for Saponins**
2ml of purified plant extract was taken and added with 5 ml of distilled water. The mixture was vigorously shaken. The presence of saponins was indicated by the formation of persistent foam
- **Test for phytosterols**
To 1 ml of purified plant extract add 2 ml of chloroform and a few drops of acetic anhydride and then add equal volume of Con. H₂SO₄ Formation of bluish green color indicates the presence of phytosterols.
- **Test for Cardiac Glycosides**
To 1 ml of plant extract add a few drops of Concentrated H₂SO₄. The formation of red color indicates the presence of Cardiac glycosides

III. RESULTS

A. Phytochemical analysis

- **Strychnosnux-vomica**

The phytochemical analysis of Strychnosnux-vomica shows the presence of alkaloids, tannins, steroids, phenols, phytosterols, and cardiac glycosides and absence of flavonoids, amino acids, reducing sugars, and saponins (Table-1).

- **Justicia adhatoda**

The phytochemical analysis of Justicia adhatoda shows the presence of alkaloids, flavonoids, amino acids, reducing sugars and saponins and absence of tannins, steroids, phenols, phytosterols and cardiac glycosides (Table-1).



TABLE: 1 PHYTOCHEMICAL ANALYSIS OF STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA

SL NO.	PHYTOCHEMICAL COMPOUNDS	STRYCHNOS NUX-VOMICA	JUSTICIA ADHATODA
1.	Alkaloids	+	+
2.	Flavonoids	-	+
3.	Amino acids	-	+
4.	Tannins	+	-
5.	Reducing Sugars	+	+
6.	Steroids	+	-
7.	Phenols	+	-
8.	Saponins	-	+
9.	Phytosterols	+	-
10.	Cardiac Glycosides	+	-
+; Indicates the presence of secondary metabolites. -; Indicates the absence of secondary metabolites.			

B. Percentage Mortality

The experiment showed a relation of pest mortality level at three-time intervals (24hr, 48hr, and 72hr) in 4 different concentrations (5%, 10%, 15%, and 20%). The results (Table- 2) indicate variation in mortality among the plant extracts tested against the selected pest. The percentage mortality of rice weevil; *Sitophilus oryzae* in *Strychnosnux-vomica* is high at 72 hours for all the four concentrations. At 5% concentration it is 10.00 ± 0.00 , for 10% it is 23 ± 0.47 , for 15% it is 40 ± 0.81 , and for 20% it is 46.6 ± 0.47 . The lowest percentage mortality is seen at 24 hour, 5% (3.3 ± 0.47), 10% (10.00 ± 0.00), 15% (16.6 ± 0.47) and 20% (33.3 ± 0.47). Quick knockdown effect was highest in the case of 20% concentration and lowest in the case of 5% concentration of *Strychnosnux-vomica*. The analysis of variance for mortality of the *Strychnosnux-vomica* leaves extracts against *Sitophilus oryzae* of different hours for different concentrations is significant at 5% level.

TABLE: 2 PERCENTAGE MORTALITY RICE WEEVILS; SITOPHILUS ORYZAE IN STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA AQUEOUS LEAVES EXTRACT

NAME OF PLANT EXTRACT	CONC. (%)	NO. OF INSECTS USED	MORTALITY (%)		
			24HOUR	48HOUR	72HOUR
STRYCHNOS NUX-VOMICA	5	10	3.33 ± 0.47	6.6 ± 0.47	10 ± 0.00
	10	10	10.0 ± 0.00	13 ± 0.47	23 ± 0.47
	15	10	16.6 ± 0.47	23.3 ± 0.47	40 ± 0.81
	20	10	33.3 ± 0.47	43.3 ± 0.47	46.6 ± 0.47
CONTROL	-	10	-	-	-



JUSTICIA ADHATODA	5	10	0.00 ± 0.00	6.6 ± 0.47	16.6± 0.47
	10	10	10.0 ± 0.00	16.6± 0.47	26.6± 0.47
	15	10	23.3 ± 0.47	26.6± 0.47	36.6± 0.47
	20	10	26.6 ± 0.47	33.3± 0.94	40.0± 0.81
CONTROL	-	10	-	-	-

(Values are Mean ± Standard Deviation)

Likewise, the percentage mortality of rice weevil; *Sitophilus oryzae* in *Justicia adhatoda* is high at 72 hours for all the four concentrations (Table-2). At 5%, it is 16.6±0.47, for 10%, it is 26.6±0.47, for 15%, it is 36.6±0.47 and for 20%, it is 40.0 ± 0.81. The lowest percentage mortality is seen at 24hour for all four concentrations; 5% (0.00 ± 0.00), 10% (10.0 ± 0.00) 15% (23.3 ± 0.47) and 20% (26.6 ± 0.47). Quick knockdown effect was highest in 20% and lowest in 5% concentration of *Justicia adhatoda*. The analysis of variance for mortality of the *Justicia adhatoda* leaves extract against *Sitophilus oryzae* of different hours for different concentrations is significant at 5% level.

TABLE: 3 ANALYSIS OF VARIANCE (ANOVA) FOR MORTALITY AT 24, 48 AND 72 HOURS FOR DIFFERENT CONCENTRATIONS OF STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA LEAVES EXTRACT AGAINST SITOPHILUS ORYZAE

NAME OF PLANT	HOURS	F VALUE	P VALUE	F CRITICAL
STRYCHNOS NUX-VOMICA	24	19.88	0.0004	4.066
	48	21.58	0.0003	4.066
	72	19.73	0.0004	4.066
JUSTICIA ADHTODA	24	27.33	0.0001	4.066
	48	7.00	0.0125	4.066
	72	6.66	0.0143	4.066

(P< 0.05 is significant.)

The results indicate (Table-2) that the mortality values significantly increased depending on the increasing order of plant extract concentration (5%>10%>15%>20%) and with time of exposure (24hrs>48hrs>72hrs). When compared to the effect of aqueous plant extracts on the mortality of *S. oryzae*, *Strychnosnux-vomica* is more potent bioinsecticide as compared to *Justicia adhatoda*.

C. Percentage Repellency

Repellency against the plant extracts was also observed. The percentage repellency of rice weevil; *Sitophilus oryzae* in *Strychnosnux-vomica* is high at 24 hours for all the four concentrations (Table-2). At 5%, concentration is 63.3±0.47, for 10%, it is 70±0.81, for 15%, it is 73.3±0.47, and for 20%, it is 86.6±0.47. The lowest percentage mortality (Table-2) is seen at 72 hour, 5% (50±0.81), 10 % (63.3±1.24), 15% (70±0.81) and 20% (73.3±0.94).

TABLE: 4 PERCENTAGE REPELLENCY OF STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA AQUEOUS LEAVES EXTRACTS AGAINST RICE WEEVIL; SITOPHILUS ORYZAE

NAME OF PLANT EXTRACT	CONC. (%)	NO.OF INSECT S USED	REPELLENCY (%)		
			24Hrs.	48Hrs.	72Hrs.
STRYCHNOS NUX-VOMICA	5	10	63.3±0.47	56.6 ±0.47	53.3± 0.47
	10	10	70.0±0.81	66.6 ±0.47	63.3± 0.47
	15	10	73.3±0.47	70.0 ±0.00	66.6± 0.47
	20	10	86.6± 0.47	80.0 ±0.81	76.6± 0.47
CONTROL	-	10	-	-	-



JUSTICIA ADHATODA	5	10	60.0± 0.81	56.6± 0.47	53.3± 0.47
	10	10	70.0± 0.81	63.3± 0.47	56.6± 0.47
	15	10	73.3± 0.47	66.6± 0.47	63.3± 0.47
	20	10	83.3± 0.47	76.6± 0.47	73.3± 0.47
CONTROL	-	10	-	-	-

(Values are Mean ± Standard Deviation)

Likewise, the highest repellency was observed in the case of Strychnosnux-vomica at 20% while the lowest was induced by Strychnosnux-vomica at 5% concentration. The analysis of variance for repellency of the Strychnosnux-vomica leaves extracts against Sitophilus oryzae of different hours for different concentrations is significant at 5% level.

TABLE: 5 ANALYSIS OF VARIANCE (ANOVA) FOR REPELLENCY AT 24, 48 AND 72 HOURS FOR DIFFERENT CONCENTRATIONS OF STRYCHNOS NUX-VOMICA AND JUSTICIA ADHATODA LEAVES EXTRACT AGAINST SITOPHILUS ORYZAE

NAME OF PLANT	HOURS	F VALUE	P VALUE	F CRITICAL
STRYCHNOS NUX-VOMICA	24	6.666	0.014	4.066
	48	5.777	0.021	4.066
	72	8.333	0.007	4.066
JUSTICIA ADHTODA	24	4.16	0.047	4.066
	48	6.25	0.017	4.066
	72	7.00	0.012	4.066

(P< 0.05 is significant.

Similarly, the percentage repellency of rice weevil; Sitophilus oryzae in Justicia adhatoda is high at 24hours for all the four concentrations. At 5%, it is 60±0.81, for 10%, it is 70±0.81, for 15%, it is 73.3±0.47 and for 20%, it is 83.3±0.47. The lowest percentage repellency is seen at 72 hours for all four concentrations; 5% (53.3±0.47), 10% (56.6±0.47), 15% (63.3 ± 0.47) and 20% (73.3±0.47). At 5%, repellency was lower while at 20% a greater deterrence forced the insects to remain in the untreated half. The analysis of variance for repellency of the Justicia adhatoda leaves extract against Sitophilus oryzae of different hours for different concentrations is significant at 5% level.

Results depicted that the repellence tended to decrease with the passage of time and the highest was observed after 24hour while the lowest was observed after 72 hours of treatments. Likewise, the repellency values significantly increased depending on the increasing order of plant extract concentration (5%>10%>15%>20%). Plant extracts showed a significant repellency against Sitophilus oryzae.

IV. DISCUSSION

Several studies focused on the extraction of the active materials from leaves and seed of different plants [15]. It was found that ethyl extract of StrychnosNux-vomica showed the highest mortality i.e., 96% in 500ppm concentration, due to the presence of strychnine and brucine (Alkaloids) [16]. Likewise, the aqueous extract of Strychnosnux-vomica showed maximum percentage mortality i.e., 46.6% in 20% concentration may be due to the presence of alkaloids in it is justified. Since, StrychnosNux Vomica is used in medicine, it contains eco-friendly chemicals with zero harm to the non-target organisms. In this work Justicia adhatoda showed 40% maximum percentage mortality of Sitophilus oryzae in aqueous extract at 20% concentration. It was similar to the study carried out by Neeru and Sonu (2015) [17] who evaluated the insecticidal activity of the plant Justicia adhatoda against two adult insects Ticks and Termites. This initial study has shown that the ethanolic extract of the leaves of this plant does have a good potential for use against these two insect pests. The crude extract has shown mortality percentages of 70% in the case of Ticks and 90% in the case of Termites after exposure. In the present study the percentage mortality is low in aqueous extract may be due to the inability of water as a solvent to extract active materials.

A number of plant products and extracts were tested against Sitophilus oryzae with the use of various research techniques and with variable efficacy [18]. An investigation on the repellency of essential oils of P. nigrum and E. camaldulensis against T. castaneum under laboratory conditions. E. camaldulensis was recorded more effective as compared to P. nigrum, showing



significantly higher repellency at all concentrations [19]. Although the present study recorded significant repellency of *Strychnos nux-vomica* and *Justicia adhatoda* aqueous leaves extract at 24 hours against *S. oryzae* L. That might be due to its relative resistance but it decreased on the passage of time. This confirms the findings of several studies which demonstrated the highly lethal/repellent effect of some of these species against stored-product beetles [20]. The study showed repellent effects of both plants and, it can be said that both of these compounds are good candidates for practical applications in preventing the occurrence of this pest in storage areas, as well as in eliminating *S. oryzae* by scaring cereals and food products from stored grain. Detering *S. oryzae* from products will reduce not only quantitative but also qualitative losses caused by contamination of the stored cereal grain, for example through dead individuals or excrement. The *Strychnos nux-vomica* and *Justicia adhatoda* could be used as repellents and insecticides as one element in an integrated pest management program against *S. oryzae*.

Plants in the family Cruciferae, such as Brassica species produce glucosinolates as secondary metabolites that have shown anti-insect activity against coleopterans, including weevils and beetles. The economic impact on stored grain insect management of the glucosinolate breakdown products was reported [21]. In the present study the presence of secondary metabolites such as alkaloids, tannins, steroids, phenols, phytosterols, and cardiac glycosides in *Strychnos nux-vomica* and the presence of alkaloids, flavonoids, amino acids, reducing sugars and saponins in *Justicia adhatoda* could be attributes to the major reason for insecticidal and repellent effect of the plants.

V. CONCLUSION

The present study used plant-based extracts as a protectant to protect the stored grain products from insect attack. Both the plants used in the present study (*Strychnos nux-vomica* and *Justicia adhatoda*) possess insecticidal properties. The use of bio pesticides as a supplement has emerged as a promising alternative to chemical pesticides and their demand is rising steadily in all parts of the world. Therefore, this study has provided some information about the potentials of “bio pesticides for pest control” and if fully exploited, could serve as a very effective alternative method for pest control as well as good component of integrated pest management.

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