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Shalini Shukla

Ph.D. Thesis of senior author,
Department of Zoology, DG
College, Kanpur, Uttar Pradesh,
India

Anupam Dubey

Biopesticides and Toxicological
Lab, Department of Zoology,
D.B.S. College, Kanpur, Uttar
Pradesh, India

BS Chandel

Biopesticides and Toxicological
Lab, Department of Zoology,
D.B.S. College, Kanpur, Uttar
Pradesh, India

Laboratory assessment and prospects of repellent potentiation of naturally occurring plant extractives against the lesser grain bore, *Rhizopertha dominica* Fabricius (Bostrichidae: Coleoptera)

Shalini Shukla, Anupam Dubey and BS Chandel

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Abstract

Prevention of food losses during postharvest storage is of paramount economic importance. The use of synthetic chemical insecticides is either not permitted or used restrictively because of the residue problem and health risks to consumers. In view of the above, there is a need for plants that may provide potential alternatives to the currently used insect control agents as they constitute a rich source of bioactive molecules. Available literature indicates that plant extractives could be source for new insecticides. This paper focuses on the current state of new biorational agents for repellent as grain protectants applied in insect-pest management. A screening five naturally occurring indigenous botanicals namely: *Acorus calamus* Linn. (rhizomes), *Annona squamosa* Linn. (unripe fruits) and *Vitex negundo* Linn. (leaves), *Gynendropsis gynendra* Linn., (seeds) and *Ocimum basilicum* Linn. (leaves) were formulated in distilled water. Triton X-100 at the rate of 0.5 percent as emulsifier and benzene at the rate of 5 percent as solvent were used were tested for their larval repellent biopotency against *Rhizopertha dominica* Fabricius and two control were also used in the present investigation. Over all, the highest *R. dominica* larval repellency was calculated *A. squamosa* extract showed the highest larval repellency (99.77 per cent) and placed on top followed by *O. basilicum* (99.54 per cent), *A. calamus* (99.38 per cent) *G. gynendra* (99.22 per cent), *V. negundo* (99.10 per cent) where as in control using Benzene was showed (99.38 per cent) and in absolute control was seen only 79.25 percent larval repellency and placed at bottom of merit.

Keywords: Grain protectants, *Annona squamosa*, *Ocimum basilicum* and *Annona squamosa*

1. Introduction

Agriculture is the major occupation of most of the people all over the world and more than 70% of Indian population depends on agriculture for their livelihood (Chandrasekar *et al.* 2005, Jeeva *et al.* 2005 and Kiruba *et al.* 2005) ^[1, 2, 3]. Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries (Talukder *et al.* 2004 and Dubey *et al.* 2008) ^[4, 5] Seventy per cent grain losses due to insect infestation are the most serious problem in storage, particularly in villages and towns of developing countries like India (Talukdar and Howse, 1993) ^[6]. It has been estimated that about 15-20% of the world agricultural production is lost every year due to insect infestation (Roy *et al.* 2005) ^[7]. In India losses caused by insects accounted for 6.5% of stored grain (Kumar 2009) ^[8]. Their attacks reduce both the quantity and quality of stored seed. *Rhizopertha dominica* Fab. (Coleoptera: Bostrichidae) is the most common and injurious to stored grains having an important position among the storage pests (Jacobson 2004) ^[9].

The test insect lesser grain borer, *Rhizopertha dominica* Fabricius, is a cosmopolitan pest of a wide variety of food granaries mainly cereals but also include grains from families Poaceae (e.g. rice, wheat, sorghum, oats, pearl, millet, malt, barley) and Fabaceae (e.g. chickpeas, peanuts, beans). The lesser grain borer is one of the great economic importance in the Indian sub-region of oriental region and many other zoogeographical regions of the world¹. The economically most important beetles infesting cereal grains on a worldwide scale, and its feeding can reduce kernels to the pericarp. Stored product *R. dominica* beetles are resistant to many grain protectants. *Rhizopertha dominica* females lay eggs on the surface and newly born larvae bore into the kernels, preferring breaks or the germ area where the covering testa is loose. On damaged kernels, first instar larvae mortality decreases and progeny production A

Corresponding Author:

Shalini Shukla

Ph.D. Thesis of senior author,
Department of Zoology, DG
College, Kanpur, Uttar Pradesh,
India

lot of research was conducted which dealt with the influence of the species and variety of the plant on the development of *R. dominica* and occurrence of the progeny.

Synthetic chemical pesticides have been used for many years to control stored grain pests (Salem *et al.* 2007) [10]. Fumigation of stored food grains with toxic gases is effective but not applicable at the farm level because the storage structures are not airtight. Furthermore, control of insects by insecticides has serious drawbacks, such as the toxic residues on stored grains, development of resistance by target species, pest resurgence and lethal effects on non-target organisms in addition to direct toxicity to users and health hazard (Adedire and Lajide 2003, Adedire *et al.* 2011, Ileke and Olotuah 2012, [11, 12, 13]. This situation indicates the need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms for pest control in storage.

Botanical insecticides are naturally occurring insecticides which are derived from plants (Golob *et al.*, 1999; Isman, 2000) [14, 15]. Botanical insecticides are used in several forms, such as powders, solvent extracts, essential oils and whole plants, these preparations have been investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Weaver and Subramanyam, 2000) [16]. The introduction of powdered leaves of *Salvia officinalis* L. and *Artemisia absinthium* L. to wheat grains was very effective in reducing population size and delaying development time of *R. dominica* (Klys, 2004) [17].

Compared to synthetic compounds they are less harmful to the environment, generally less expensive, and easily processed and used by farmers and small industries. Recently, there is a steady increase in the use of indigenous plant products as a cheaper and ecologically safer means of protecting stored products against infestation by insects (Ashamo and Odeyemi 2001, Akinkulolere *et al.* 2009) [18, 19] and this lead to the present study. The objectives of this study are to assess the larval feeding preferences for the varieties of wheat and to evaluate their susceptibility/resistance to *R. dominica* Fabr.

2. Materials and Methods

Material and methods

Examination of the influence of different wheat grain varieties ie; (x) on the emergence of the progeny of *R. dominica*, on stored wheat grains, as well as effect of their presence on chemical properties of grains were conducted in bio-pesticide and toxicological laboratory, Department of Zoology, D.B.S, College, Kanpur which is located in between latitudes 25.26° and 26.58° North and longitudes 19.31° and 84.34° East, Kanpur is situated at an elevation of about 127.117° metres above the mean sea level and has a semi-arid subtropical zone during, 2004-2005.

2.1 Tested insect and their rearing

The lesser grain borer, *Rhyzopertha dominica* Fabricius was reared on wheat kernels 450 g, and 100 adults were put in a glass jar (13 cm Diameter x 20 cm height) with the bottom covered with black. Adults of *R. dominica* of both sexes and 2-4-weeks old were used during the experiment with temperature (T) 29±1 °C and relative humidity (RH) 70±5% and a photoperiod of 16:8 (light/dark). Adults were allowed to oviposit for three days and were then removed in the bio-pesticide and toxicological laboratory, Department of Zoology, D.B.S, College, Kanpur.

Mixed wheat kernels, The lid of glass jar provided with a hole (3 cm Diameter) closed by a stainless steel wire mesh to allow gaseous exchange and checked daily. After three days of eggs hatches into larvae. The first instar larvae characterized by a terminal median spine.

2.2 Preparation of plant extracts

Fresh plant parts of *Acorus calamus* Linn., *Annona squamosa* Linn. (unripe fruites) and *Vitex negundo* Linn. (leaves), *Gynendropsis gynendra* Linn., (seeds) and *Ocimum basilicum* Linn. (leaves) extracts were used against *R. dominica* in the Biopesticide laboratory, Department of Zoology (Entomology), D.B.S.College, Kanpur affiliated to CSJM University, Kanpur, U.P. They were washed in running water and kept in laboratory for 7 days air drying. After drying they were made powder separately by an electric grinder. The extracts were prepared according to (Bapai and Chandel, 2020) [25] with minor modifications. For making extracts, 100 g of different plant powders were dissolved in 300 ml of petroleum ether solvent and stirred for 30 min. in a magnetic stirrer. The mixture was allowed to stand for 72 hours and shaking several intervals. It was filtered through a filter paper (Whatman no. 1) and to evaporate the solvents. The condensed extracts were preserved in tightly corked-labeled bottles and stored in a refrigerator until their further use.

Table 1: List of indigenous plant materials and their details

Scientific Name	Vernacular Name	Part Used	Faimly
<i>Acorus calamus</i> Linn.	Sweetflag	rhizome Asarone, choline, eugenol, ethyl ether, heptanoic acid, methylamine, saponin, tannic acid and trimethylamine	Acoraceae or Araceae Chander <i>et al.</i> (1990) [20].
<i>Annona squamosa</i> Linn.	Bullocks heart	Unripe fruites Anonaine, corydine, isocorydine and aporphine	Annonaceae Ashok kumar <i>et al.</i> (2010) [21].
<i>Gynendropsis gynendra</i> Linn.	Hulhul	Seeds Erpenolene, 1-α – terpenol, pentacosane, humulene, phytol	Capparidaceae Ndungu <i>et al.</i> (1995) [22]
<i>Ocimum canum</i> Sims.	Sweet Basil	Leaves Anethole, camphor, carvacrol, cineole, citral, esdragole, eugenol, linalool, safrole, saponin and thymol	Lamiaceae Mishra <i>et al.</i> (2012) [23].
<i>Vitex negundo</i> Linn.	Nirgundi	Leavesβ-caryophyllene, protocatechuic acid and oleanolic acid	Vebenaceae Rana <i>et al.</i> (2005) [24].

The calculated amount of various ingredients required to make different concentrations from the stock solution was prepared with the help of following formula and amount of ingredients taken are presented in the table (Table 2).

$$\text{Amount of Stock Solution} = \frac{\text{Amount required} \times \text{Concentration required}}{\text{Concentration of Stock Solution}}$$

Table 2: Preparation of different formulations of the selected plant materials

Concentration (%)	Amount of Stock Solution (ml)	Amount of Benzene (ml)	Amount of Emulsifiable Water (ml)	Total Amount (ml)
0.50	5.00	20.00	475.00	500.00
1.00	10.00	15.00	475.00	500.00
1.50	15.00	10.00	475.00	500.00

2.3 Collection of wheat grains

Only healthy sound and free from injury grains will take for study. Healthy wheat grains, *Triticum aestivum* (L.) were purchased from the local market of galla mandi, Kanpur, cleaned thoroughly and sun dried. The grains were cooled at 8-10% moisture level and stored at room temperature in air tight plastic bag for experimental use.

3. Experimental Protocol

The tests were carried out by placing 40 wheat kernels in glass containers (35 mm Ø; height 20 mm) with 10 first instars larvae, 0–24 h old. Such containers, closed with a net (120 mesh) to provide ventilation, were placed in an incubator at 29 ± 1 °C, 70 ± 5% R.H. and 16 h of light alternating with 8 h of darkness. Repellent test was conducted according to (Rajani and Chandel, 2016) [26] with minor modifications. The extracted materials were weighed and dissolved in petroleum for making different concentration (0.5, 1.0 and 2.0% along with control). Pilot experiments were done to obtain the appropriate concentrations of each extractive (0.5, 1.0 and 2.0%). Before applying extracts to the thorax of the insect, 10 minutes chilling were done with 4 °C in refrigerator. Then 1 µl of prepared solution was applied to the dorsal surface of each insect using a micropipette (volume digital micropipets, bio-rad, India).

3.1 Experimental Protocol

Repellent activity were studied at 0.5, 1.0 and 0, 1.5 per cent concentration treated for two minutes for testing. The repellent effect stored wheat, *Triticum aestivum* (L.) mixed in parts per 100 parts of seeds (W/W) and extract were used as food for the larvae of lesser grain borer, *Rhyzopertha dominica* Fabricius. Repellent test was conducted according to (Rajani and Chandel, 2016) [26] with minor modifications. The treated seed were left under electric fan for about half an hour, to make a dry film of the extracts on the seeds for each set of extract and one control. The treated seeds were kept in jar (23cm x 10cm) on moist filter paper. The untreated seeds were dipped in Benzene + emulsified water only. Ten larvae of *Rhyzopertha dominica* was introduced into a cage having treated stored wheat, *Triticum aestivum*. Ten starved *Rhyzopertha dominica* larvae were released in each jar along with control. Three replicates per treatments were maintained. The treated jars either repelled the insects or forced them to move from treated jars (T) to an empty jar(C) through the plastic pipe. The ones found in plastic pipe were considered repelled individuals. Total number of larvae reached to the food/repelled to untreated empty jars were recorded at an interval of 24 hours for each observation recorded and computed as per Abbott formula. (Abbott W. S. 1925) [27].

4. Statistical analysis

The observed data of repellent test was analyzed using following formula

$$\text{Percent Repellency} = \frac{A - B}{A} \times 100$$

Here,

A = Average Number of insects present on untreated portion

B = Average Number of insects present on treated portion

The data were corrected according to Abbott's (1987) formula: This observed value of 'F' is compared with the theoretical value of 'F' given by Finney, (1952) [19] for testing the significance [28].

5. Results and Discussions

5.1 Results

The results are summarized in Table-3 and Fig.1-4 which showed that all the plant extracts have proved to more or less repulsive against the emerging larvae of larvae of *Rhyzopertha dominica*. All selected plant extracts gave promising repellent activity the larvae of *Rhyzopertha dominica*. On the basis of mean per cent of larval repellency and their order of merit as per concentration of each extractives are described in the following ways and in the last final results also given below.

Table 3: Mean percent of *R. dominica* repellency against plant products on wheat seeds.

Extracts of plants	Average percentage of <i>R. dominica</i> repelled when extract mixed in parts per 100 parts of seeds (W/W)							
	0.5 per cent		1.0 per cent		1.5 per cent		Mean% Repellency	
	T ₁	TBV ₁	T ₂	TBV ₂	T ₃	TBV ₃	T	TBV
<i>A. calamus</i>	99.00	84.39 b	99.50	86.73 a	99.66	87.33 a	99.38	85.48
<i>A. squamosa</i>	99.66	87.33 a	99.66	87.33a	100.00	90.00 a	99.77	87.25
<i>G. gynendra.</i>	99.00	84.39 b	99.16	84.82 a	99.50	86.73 a	99.22	84.93
<i>O. basilicum</i>	99.3	85.35ab	99.66	87.33 a	99.66	87.33 a	99.54	86.11
<i>Vitex negundo</i>	98.83	84.39 b	99.16	84.82 a	99.33	85.38 a	99.10	84.56
Absolute control	79.00	62.77 c	79.00	62.77 b	79.77	62.77 b	79.25	62.87
Benezene control	99.33	85.38ab	99.33	85.38 a	99.50	86.73 a	99.38	84.35
S.E.	±1.33		±1.99		±2.55			
C.D. at 5%	2.85		4.09		5.48			

From table 3, it is evident that all the plant extracts at three doses repelled the lesser grain borers from 98.83 to 100.00 per cent, i.e., those adults *R. dominica* did not enter into the sacklets containing the treated wheat grains, while in the absolute control, this was about 79.00 per cent. Benzene proved to have a potent repellent effect keeping 99.00 per cent lesser grain borers away from sacklets

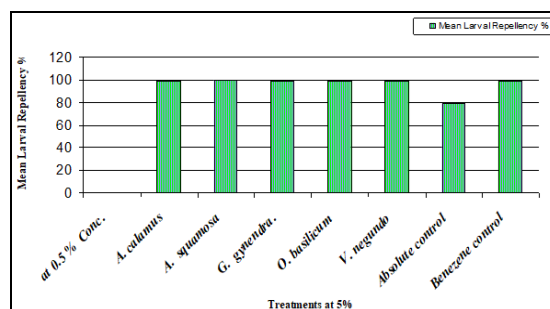


Fig 1: Mean% of *R. dominica* larvae repellency extract /100 parts of seeds (W/W)

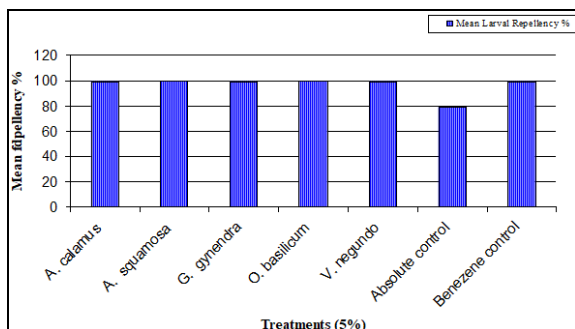


Fig 2: Mean% of *R. dominica* larvae repellency extract /100 parts of seeds (W/W)

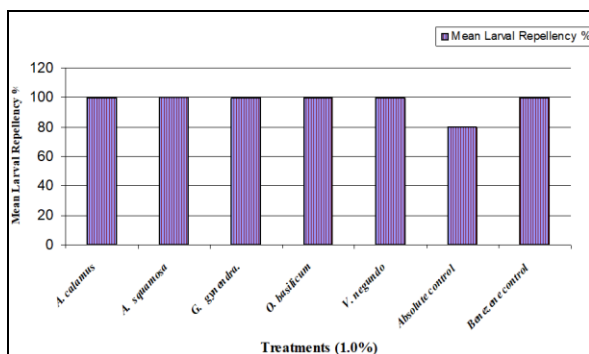


Fig 3: Mean% of *R. dominica* larvae repelled from 1.5% conc. extracts /100 parts of seeds (W/W)

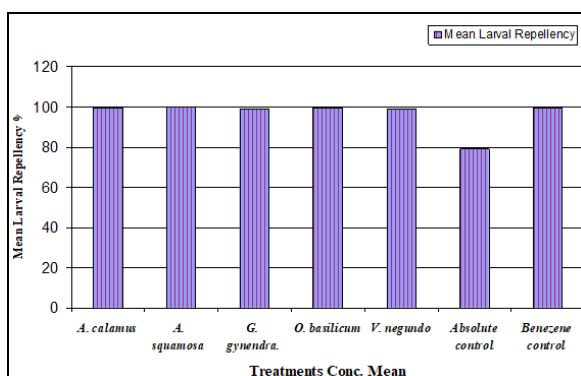


Fig 4: Mean% of *R. dominica* larvae repelled from plant extract /100 parts of seeds (W/W)

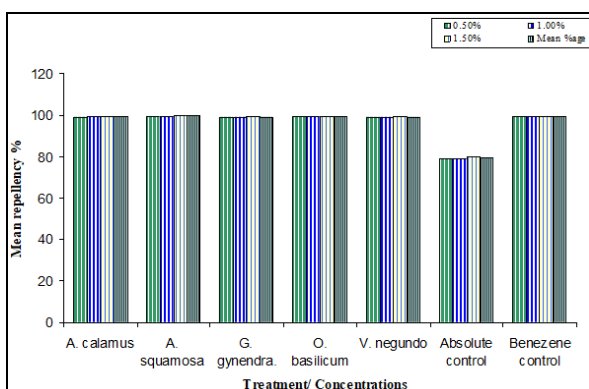


Fig 5: Over all mean% of *R. dominica* larvae repelled from plant extractives /100 parts of seeds (W/W)

In case of 0.5 per cent concentration of *A. squamosa* extract showed the highest larval repellency (99.66 per cent) and placed on top followed by *O. basilicum* (99.30 per cent), *A. calamus* and *G. gynendra* (99.00 per cent), *V. negundo* (98.83

per cent), and *O. basilicum* (99.30 per cent), whereas in Benzene control was showed (99.33 per cent) and in Absolute control was seen only 79.00 percent larval repellency and placed at bottom of merit.

In case of 1.0 per cent concentration of *A. squamosa* and *O. basilicum* extract showed the highest larval repellency (99.66 per cent) and both placed on top followed by *A. calamus* (99.50 per cent), *G. gynendra* and *V. negundo* (98.16 per cent) where as in control using Benzene was showed (99.33 per cent) and in absolute control was seen only 79.00 percent larval repellency and placed at bottom of merit.

In case of 1.5 per cent concentration of *A. squamosa* extract showed the highest larval repellency (100.00 per cent) and placed on top followed by), *A. calamus* and *O. basilicum* (99.66 per cent), *G. gynendra* (99.50 per cent), *V. negundo* (99.33 per cent) where as in control using Benzene was showed (99.50 per cent) and in absolute control was seen only 79.77 percent larval repellency and placed at bottom of merit.

Over all the cumulative results showed that the highest *R. dominica* larval repellency was calculated *A. squamosa* extract showed the highest larval repellency (99.77 per cent) and placed on top followed by *O. basilicum* (99.54 per cent), *A. calamus* (99.38 per cent) *G. gynendra* (99.22 per cent), *V. negundo* (99.10 per cent) where as in control using Benzene was showed (99.38 per cent) and in absolute control was seen only 79.25 percent larval repellency and placed at bottom of merit.

5.2 Discussions

it is evident that all the plant extracts at three doses repelled the lesser grain borers from 98.83 to 100.00 per cent, i.e., those adults *R. dominica* did not enter into the sacklets containing the treated wheat grains, while in the absolute control, this was about 79.00 per cent. Benzene proved to have a potent repellent effect keeping 99.00 per cent lesser grain borers away from sacklets. Plant essential oils and solvent extracts are the most studied botanical methods of controlling stored grain insect infestations reported by Bekele and Obeng-Ofori, 1996, Rao and Prakash, 2002, Park *et al.* 2003, Dubey *et al.* 2004, Talukde *et al.* 2004, Talukder *et al.* 2004, Mohal *et al.* 2006, Isman, 2006, Wang *et al.* 2006, Huang *et al.* 2007, Viglianco *et al.* 2008, Islam *et al.* 2009 and Bajpai, and Chandel, 2009 [29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40]

From very early times, different plant materials have been used as natural protectants of stored grains. Worldwide reports indicate that when plant extractives mixed with stored grains, reduce damage of stored product by stored grain insects-pest reported by Geng *et al.* 2011, Gandh *et al.* 2011, Ukeh and Umoetok, 2011, Wekes *et al.* 2011, Khemira *et al.* 2012, Lakshmi *et al.* 2012 and 2013, Singh *et al.* 2013 and Shah *et al.* 2013 [41, 42, 43, 44, 45, 46, 47, 48, 49]. In the support of the above findings Bekele *et al.* 1996 tested essential oil extract of *Ocimum suave* Wild at higher doses against storage insects of maize and found 100.0% mortality of *S. cerealella* and *R. dominica*. Moreover, the tested plant materials were highly repellent to *S. zeamais* and provided good protection [33]. Park *et al.* 2003 reported the promising repellent biopotential of *Acorus gramineus* rhizome isolated ingredient to stored grains insect pest. Talukder *et al.* 2009 reported that during the investigations on the toxic properties of combination of *Acorus calamus* L. rhizomes and *Thevetia nerifolia* Juss. seed extract, the lowest LC₅₀ values (43.27 µgcm⁻¹) were found against *S. oryzae* at 24 h after treatment (Talukder and

Khanam 2009) Lakshmi Soujanya *et al.* 2012 observed that leaf powders of *Vitex negundo* L., *Adathoda vasica* L., *Catharanthus roseus* L. and *L. camara* at 5% w/w were evaluated against *S. oryzae* in stored maize. All the treatments proved to be very toxic towards *S. oryzae* adults.

6. Conclusion

This study proved that the leaf extract of indigenous plants like *A.squamosa*, *O. basilicum*), *A. calamus* and *G. gynendra* can be used to protect stored grain pests. From table 3, it is evident that all the plant extracts at three doses ie, 0.5 percent, 1.0 percent and 1.5 percent concentrations repelled the lesser grain borers, *Rhyzopertha dominica* Fabricius larvae from 98.83 to 100.00 per cent. The adults *Rhyzopertha dominica* did not enter into the sacklets containing the treated wheat grains, while in the absolute control, this was about 79.00 per cent. Benzene proved to have a potent repellent effect keeping 99.00 per cent lesser grain borers, *Rhyzopertha dominica* away from sacklets.

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