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Repellent bioactivity of naturally occurring indigenous *Neem, Azadirachta indica* A. Juss formulations on wheat, *Triticum aestivum* Linn. (Poaceae) against stored lesser grain borer, *Rhyzopertha dominica* (F.)

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Abstract
Various synthetic insecticides used to control stored pests were applied during the storage period, providing wheat grain protection. At the same time, the hazardous synthetic chemicals effects have several negative consequences for humans and the environment. To discover an eco-friendly alternate method, the repellent activities of different solvent neem formulations ie; Azadirachtin, Neemazal, Neem seed kernel extract (NSKE), deoiled neem seed kernel suspension (DNSKPS), neem leaf extract (NLE), Benezene control and absolute control gave 99.77, 99.54, 99.38, 99.38, 99.22, 99.10, 79.25 per cent repellent respectively, 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. So it can be concluded that the *Azadirachta indica* A. Juss formulations will substantially replace the application of chemical pesticides in the near future to stored-insect pest management.

Keywords: Azadirachtin, repellent, neem leaf extract, DNSKPS and Neemazal

1. Introduction
More than twenty thousand insect-pest species of field and storage have been reported to destroy about one-third of the world's food production annually among them, highest losses (43 per cent) happen in the developing countries of oriental region. The major pests of stored grain and pulses of the Indian subcontinent have been categorized into two groups namely, on one hand, primary pests are capable of penetrating and infesting intact kernel of grain and have immature stages develop within kernel of grain, i.e., lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L.), granary weevil, *Sitophilus granaries* (L.), *Khapra* beetle, *Trogoderma granarium* (Everts) and the pulse beetle *Callosobruchus chinensis* (L.), while on other hand, the secondary pests feed on as broken kernels, debris and grain damaged by primary pests, *viz*., rust-red flour beetle, *Tribolium castaneum* (Herbst), rusty grain beetle, *Cryptolestes ferrugineus* (L.), sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), mites, *Liposcelis corrodens*. Generally, the immature stages of the secondary pest species are found external to the grain.

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is a major pest that damages grains in oriental, Indo-China and Ethiopian regions of world, whereas *R. dominica* is a worldwide insect pest that feeds on stored seeds (Campbell *et al.* 1976)[1]. Gravid females lay eggs either singly or in groups in the grain quantity, with several eggs observing together, forming a bundle. First instars are energetic and can be recognized by a terminal median spine at the dorsal surface of the last abdominal segment. First instars come in kernels and carry on immature improvement within grains. There is a number of research on the wheat kernel-huge number by *R. dominica* first instars lesser grain borer can successfully infest an uncontaminated wheat kerne [1]. There is 8% humidity content is critical for first instars to bore into whole and sound sorghum kernels; the germ is the first point of record on sound kernels.

R. dominica causes cost-effective losses to stored cereals. The destruction occurs due to weight failing by generating frass from injured grains decline of nutrient contents and causing damages and changes in grain physicochemical properties (Jilani and Saxena, 1990) [2]. There are 70–80|% population uses traditional treatments, mainly of the plant source, to treat diseases (Ignatowicz and Wesolowska (1996) [3]. The use of indigenous plants in nutrition products increases the body's natural immunity against several diseases. The botanist is tiresome to develop ecofriendly botanical pesticides in the substitution of harmfully affect the environment and human health. The use of eco-friendly bio-pesticides is acceptance increased. Plant extracts and essential oils have long been used to kill or prevent insects in stored goods (Shah *et al*., 2016) [4]. Plant extracts of several plants demonstrate significant toxic, fumigant and repellent effects on the adult of *R. dominica* showing that plant had bioactive compounds (Cheraghi *et al*. 2016 [5].

Biorational insecticides are good choices for bio-chemical insecticides and proved their repellent efficiency in controlling insect pests. The effective control of *R. dominica* and other stored-grain pests, with insignificant insecticide use involves an integrated management method related to sanitation, monitoring. Plant extracts and essential oils have long been used as repellent or prevent insects in stored goods (Ahmed and Grainge1986)[6]. Plant extracts of several plants demonstrate significant toxic, fumigant and repellent effects on the adult of *R. dominica* (Schmutterer 1990; Khan and Marwat, 2004, Isman 2006, Boeke *et al*. 2013) [7, 8, 9, 10]. Plant components having insecticidal qualities have been reported to be used all over the world because they are handy, less costly, highly effective, and safer for humans and their surroundings. However, these insecticides are frequently related to hazardous residues for the consumer and the environment (Joshi *et al.*, 2019) [11]. Hence,

there is an awareness in finding an alternative way for stored products. Plants in the family Meliacea are aromatic herbs with cosmetics and medicinal preparations (Gitahi *et al.,* 2021) [12]. With these backgrounds, this present study was aimed to assess the neem derivatives and products controlling potential against the lesser grain borer, *R. dominica* (Opiyo *et al.,* 2021) [13].

The aims of this investigations was to evaluate the repellent bioefficacy of certain neem formulations to the pest lesser grain borer, *R. dominica* and the damage it produced to cereals during the storage period.

2. Materials and Methods

2.1 Preparation of plant extracts

Fresh Neem seed kernel, neem (*Azadirachta indica* L.), plant parts ie; seed kernel, leaf extractives, Deoiled Neem Seed Kernel Powder Suspension, neem products like Azadirachtin, Neemazal formulations were used against *R. dominica* in the plant products laboratory, Department of Botany, D.B.S. College, C.S.J.M. University Kanpur. The neem materials were collected around the college and university campus. They were washed in running water and kept in laboratory for shade air drying. After drying they were made powder separately by an electric grinder. The extracts were prepared according to (Chandel and Sengar 2018) [14] with minor modifications by using petroleum ether as solvents and soxhlet apparatus. The extractives were 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 and makes their formulation ie; 0.5, 1.0 and 1.5 per cent were prepared at room temperature to eliminate the solvent.

Table 1: List of neem products and neem extractives used to control of *R. dominica* oviposition on wheat seeds

Sr. No.	Extracts of plants	Particulars
1.	*Azadirachtin*	Neem Product
2.	Neem seed kernel Extracts	NSKE
3.	*Neemazal*	Neem Product
4.	Deoiled Neem Seed Kernel Powder Suspension	DNSKPS
5.	Neem leaf extracts	NLE
6.	Absolute control	Control
7	Benezene control	Control

2.2 Collection of wheat grains

Healthy wheat, *Triticum aestivum* (Linn.) grains were purchased from the local market of Kanpur Galla mandi, Kanpur Nagar cleaned thoroughly and sun-dried. The grains were cooled at 8-10% moisture level and stored at room temperature in airtight plastic bag for experimental use.

2.3 Mass rearing of lesser grain borers

The lesser grain borer**,** *R. dominica* Fabr. was mass-reared in the laboratory at ambient room temperature (28±0.5 ºC) in glass jars (47 cm height × 4cm dia). Approximately 200 adults were released in each glass jar containing 500 g of wheat grains and the mouth was closed with a piece of cloth fastened with rubber band to prevent contamination and escape of insect. After oviposition, the adults were separated from the grains by sieving and seeds along with eggs were left in the container for emergence of next generation. The newly emerged adults (1-7-days- old) were collected and again allowed for oviposition with new grains in different containers to maintain a stock culture of the test insect. The process was containing for getting enough pest throughout the study.

2.4 Repellent Bioassey: In bioassays, unsexed beetles aged 2-4 weeks were employed. The filter paper strips were exposed to the Neem extractives and neem products and of different concentrations of 100, 200, 300 and 400 ppm against the *R.dominica*. After the filter paper was kept in the plastic containers into the arms of the olfactometer. Then the control filter paper with Neem Azal was used. After the attachment of all the plastic vials with the arms, ten pairs of newly emerged adults of *R.dominca* were introduced into the olfactometer and values were calculated by using formula.

$$\frac{Nt-Nc}{Nt+Nc} X\ 100$$

Where EPI = Excess Proportion Index

Nt = the number of insects in the treated sample; Nc = the number of insects in the control sample side.

Table 2: Mean percentage of *R. dominica* repellency against neem products on wheat seeds

Neem products	Average percentage of *R. dominica* 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 % age	
	T_1	TBV_1	T_2	TBV_2	T_3	TBV_3	T	TBV
Azadirachtin	99.00	84.39 b	99.50	86.73 a	99.66	87.33 a	99.38	85.48
NSKE	99.66	87.33 a	99.66	87.33a	100.0	90.00 a	99.77	87.25
Neemazal	99.00	84.39 b	99.16	84.82 a	99.50	86.73 a	99.22	84.93
DNSKPS	99.3	85.35ab	99.66	87.33 a	99.66	87.33 a	99.54	86.11
NLE	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 2, it is evident that all the *neem* products 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.

Table 3: Mean percentage of *R. dominica* repellency against neem products on wheat, *Triticum aestivum* Linn. Seeds

Neem products	**Mean percentage of *R. dominica* repelled when extract mixed in parts per 100 parts of seeds (W/W)**			
Neem products	0.5 per cent	1.0 per cent	1.5 per cent	Mean %
Azadirachtin	99.00	99.50	99.66	99.38
NSKE	99.66	99.66	**100.0**	99.77
Neemazal	99.00	99.16	99.50	99.22
DNSKPS	99.3	99.66	99.66	99.54
NLE	98.83	99.16	99.33	99.10
Absolute control	79.00	79.00	79.77	79.25
Benezene control	99.33	99.33	99.50	99.38
S.E.	±1.33	±1.99	±2.55	
C.D. at 5%	2.85	4.09	5.48	

Table 4: Mean percentage of *R. dominica* repellency against neem products on wheat, *Triticum aestivum* Linn. seeds based on TBV

Neem products	**Mean % TBV of *R. dominica* repelled when extract mixed in parts per 100 parts of seeds (W/W)**			
Neem products	5% TBV	1.0% TBV	1.5% TBV	Mean% TBV
Azadirachtin	84.39 b	86.73 a	87.33 a	85.48
NSKE	87.33 a	87.33a	90.00 a	87.25
Neemazal	84.39 b	84.82 a	86.73 a	84.93
DNSKPS	85.35ab	87.33 a	87.33 a	86.11
NLE	84.39 b	84.82 a	85.38 a	84.56
Absolute control	62.77 c	62.77 b	62.77 b	62.87
Benzene control	85.38ab	85.38 a	86.73 a	84.35
S.E.	±1.33	±1.99	±2.55	
C.D. at 5%	2.85	4.09	5.48	

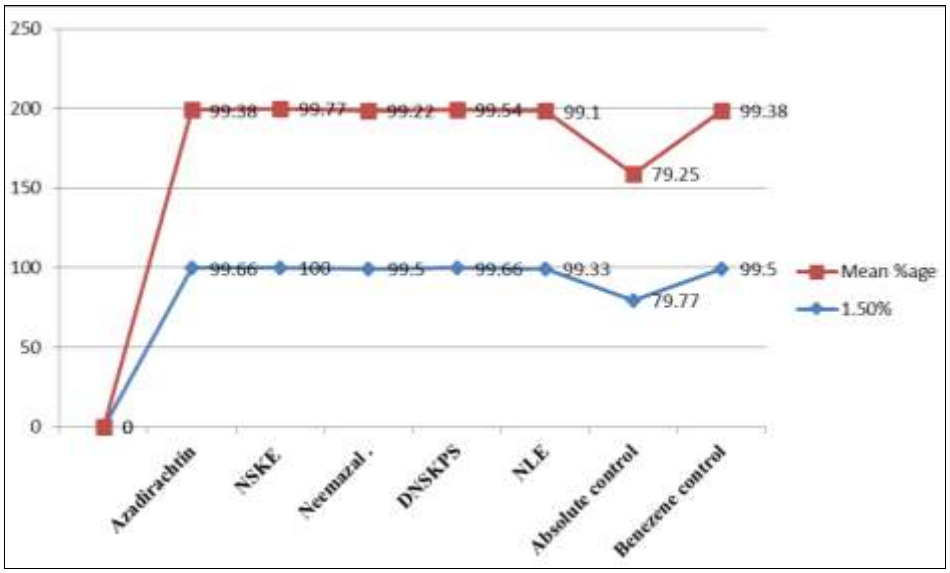

Fig 1: Mean percentage of *R. dominica* repellency against neem products on wheat, *Triticum aestivum* Linn., seeds compared with 0.5 per cent.

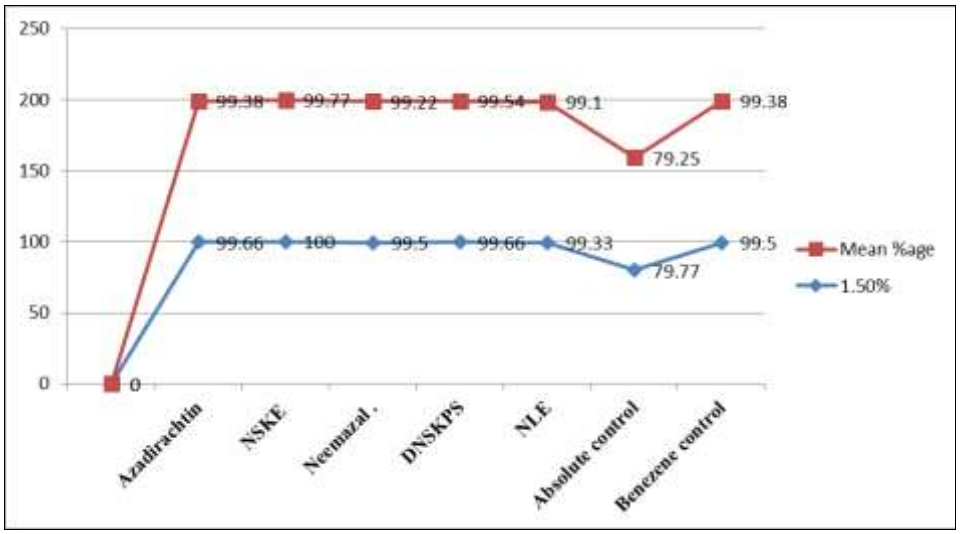

Fig 2: Mean percentage of *R. dominica* repellency against neem products on wheat, *Triticum aestivum* Linn. seeds compared with 1.0 per cent.

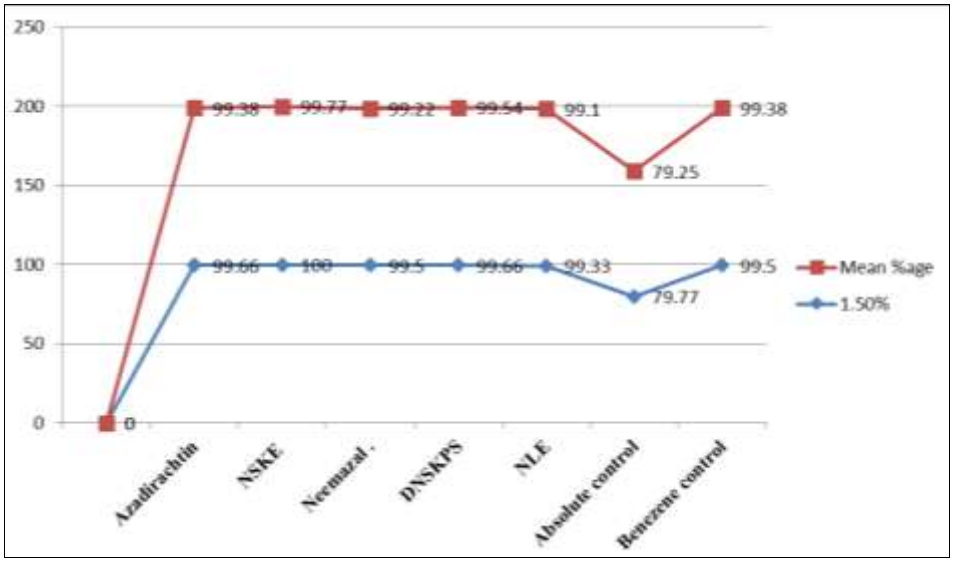

Fig 3: Mean percentage of *R. dominica* repellency against neem products on wheat *Triticum aestivum* Linn., seeds compared with 1.5 per cent.

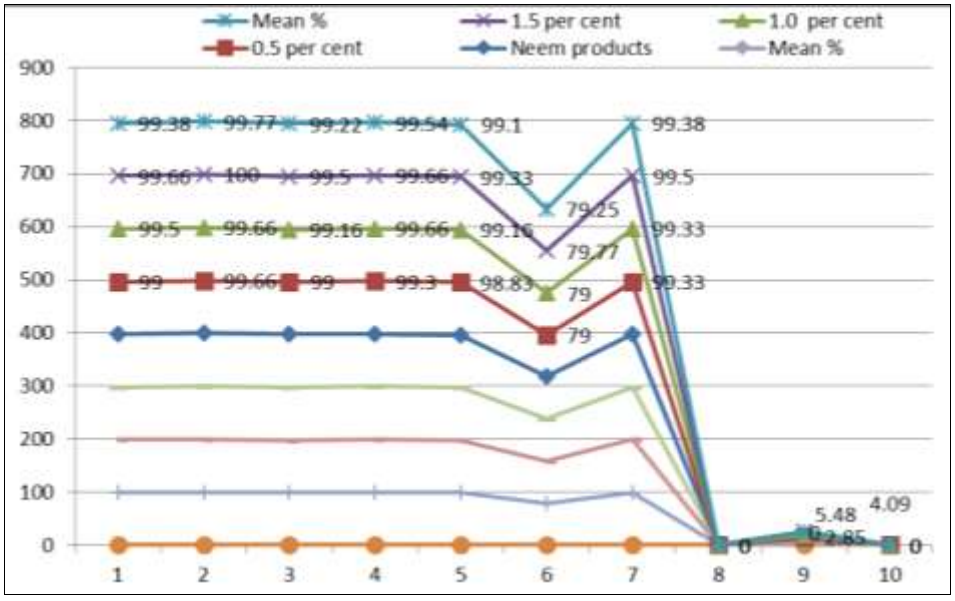

Fig 4: Overall Mean percentage of *R. dominica* repellency against neem products on wheat *Triticum aestivum* Linn.

3. Result and Discussions

Data depicted from Table 2, that all the *neem* products 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. it is evident from table 3 of TBV that all the *neem* products at three doses repelled the lesser grain borers from 84.39b to 90.00 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 62.77 c per cent. Benzene proved to have a potent repellent effect keeping 85.38 ab per cent lesser grain borers away from sacklets.

In the support of above findings various workers reported the repellent biopotency of herbal materials for the insect pest control (Ambika Devi and Mohandas, 1982, Pandey *et al.*1985, Ahmed and Grainge, 1986, Mohiuddin *et al.*1987, Zanno *et al.*1987, Xie *et al.*1995, Talukder and Howse, 1995, 2000, Wong *et al.*2005) [15-23].

Pandey *et al.* (1976) studied the powder of sweat flag (rhizomes), *kaner* (drupes), *adhatoda* and *Sadabahar* (leaves), petroleum ether extract of garlic, onion and *neem* oil for their repellent effects against *C. chinensis* infesting gram seeds. Among them, garlic extract acted as feeding deterrent and protected stored grain for up to 135 days. Only 1.65 per cent of seeds were damaged during this time, as compared with 95.56 per cent of the controls [24].

Rhyzopertha dominica is a dynamic pest that attacks various stored grains, rice, wheat, sorghum, and tubers (Jilani *et al.*1988) [25]. They cause quantity losses, also deteriorating the quality during storage due to their feeding activities. Bowrey *et al.* (1984) tested *neem*, lineseed, mustard, castor and mahua cakes, which were tested for their effect on egg laying, infestation and repellent action against *Sitophilus oryzae* Linn. The result showed that the powder of *neem* cake was most effective in reducing and minimizing the damage followed by lineseed, mustard, mahua and castor cakes. The repellent property was maximum in both *neem* and lineseed cake powder followed by mustard, caster and mahua, respectively [26]. Rout (1986) conducted an experiment to test the comparative efficacy of *neem*, *A. indica* and *Annona squamosa* (seed powder) 5 percent (w/w) admixed with stored wheat, significantly reduced the damage by *S. oryzae* [27]. Tipping *et al.* (1987) tested the effect of *neem* kernel extract on *S. zeamais.* Significant repellency was observed in the 0.1 percent concentration of *neem* kernel extract [28]. Makanjuola (1989) evaluated seed

extract of *neem*, *A. indica* 80.00 per cent concentration reduced grain damage of *C. maculatus* on cowpea and *S. zeamaise* on maize seed [29]. Similarly, the oil of neem extractives and their products were produced significant repellent activity to number of insect pest (Jotwani *et al*. (1965), Girish and Jain (1974), Roomi and Ariquiddin (1977), Singh and Kataria (1984), Jilani and Saxena (1990), Chander and Nagender (1999), Tripathi *et al*. (2000), Negbenebor and Nura. (2020) [30-37]. Finally, it can be concluded that the natural plant extracts can verify the best alternatives to chemical repellents.

4. Conclusion

Conclusively, our experiments reveal that the *neem* products 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 79.25 per cent. Benzene proved to have a potent repellent effect keeping 99.00 per cent lesser grain borers away from sacklets. So it concluded that natural products, primarily neem-based chemicals offer an excellent alternative to synthetic pesticides.

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