OPEN ACCESS J. Zoo Biol. 04 (01) 2021. 29-34 DOI: 10.33687/zoobiol.004.01.4124

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Available Online at EScience Press
Journal of Zoo Biology
ISSN: 2706-9761 (Online), 2706-9753 (Print)
https://esciencepress.net/journals/JZB

Insecticidal Effects of Neem Leaf Extract, Inert Dust, and Entomopathogenic Fungi on Control of Khapra Beetle (*Trogoderma granarium*); A Stored Grain Pest

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ARTICLE INFO

Article History
Received: July 22, 2021
Revised: August 20, 2021
Accepted: September 28, 2021

Keywords
Khapra beetle
Neem leaf extract
Inert dust
Entomopathogenic fungi
Trogoderma granarium

ABSTRACT

Infestation by different insect pests of stored cereals and the storage of their products is a serious problem. Khapra beetle (*Trogoderma granarium*) is one of the most common, devastating insect pests of stored grains. It causes both nutritional and economical losses to stored cereals. Six different concentrations of three protectants (neem leaf extract, inert dust, and entomopathogenic fungus) were used for the bioassay in the present study. For neem leaf extract dose rates were 5%, 10%, 15%, 20%, 25% & 30% and leaves were collected from University of Agriculture Faisalabad, Pakistan. For DE formulation (Conern®) doses rates were 200ppm, 400ppm, 600ppm, 800ppm, 1000ppm & 1200 ppm/kg and 1g, 2g, 3g, 4g, 5g & 6g/ kg of wheat grains doses of *Beauveria bassiana* (Racer Bb) (1 g of Racer containing 10^8 conidia) imported from Agri Life, Medak District. Hyderabad, India was used. 30 larvae were used in each bioassay. Different doses of insecticides showed remarkable toxicity and significantly higher mortality rates in Khapra beetle. The toxic effect was found to be dependent on dose and duration. Summarizing, these insecticides could have likely practical utilizations in the protection of stored grain products against the attack by *Trogoderma granarium.*

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INTRODUCTION

Pakistan is an agricultural country, and the chief food of the people is wheat, which is grown on an estimated area of 8 acres. After fulfilling the feed requirement of people wheat is also exported to many countries worldwide. During the storage of wheat grains, about 5 to 10% damage must occur due to the attack of insect pests. This damage percentage sometimes increases at 50%, mostly in tropical regions due to the reason of favorable environment to insect pests, hot and humid summer. Khapra beetle (*Trogoderma granarium*) is the world's worst pest of stored grain product due to its quality deteriorating properties as technical barriers to trade (Khalique *et al.*, 2018). *T. granarium* and family Dermestidae beetles are the most destructive pests of stored product in the world (Burges 2008; Mark *et al.*, 2010). Grains are infested by the pest at all stages from their harvest to process until they are consumed. Khapra beetle prefers dry, hot conditions and is often found in stores of food and grains, seed processing plants, fodder production plants, dried milk factories, packing material stores, and kitchen pantries. The damage to wheat is about 0.287% at farm level and 0.24% loss of wheat by the attack of warehoused insect pests (Rauf *et al.,* 1990). In Pakistan, post-harvest losses of grain are reported at 10 to 15% (Ahmad, 1994). In Pakistan, every year, 2.6% of food grain production is lost in storage by the attack of store insect pests. Larvae of khapra beetle cause much damage. Larvae are voracious eaters and feed on cereal products, seeds, and grains. Khapra beetle hard to control because larvae of khapra beetle are stimulated into diapause in unfavorable extreme conditions. Diapausing larvae of khapra beetle are very tolerant to fumigation and other control methods. Larvae require very high doses for effective control. In many countries of the world, management of insect pests is mostly done by conventional synthetic insecticides such as malathion, pirmiphos-methyl, deltamethrin, chlopyrifos-methyl, sulfuryl fluoride, carbonyl sulfideand cyfluthrin (Hussain *et al.,* 2005; Zettler and Arthur, 2000). But their use is limited due to their persistence in food and have toxic effects on the environment (Daglish 2004). The unplanned and un-judicial fumigation of phosphine has become restricted as many stored product pests like *T. granarium* (Benhalima *et al.,* 2004). To cope with the problem of resistance in stored grain insect pests there is the supreme need to move alternate tactics (Nboyine *et al.,* 2015) of pest management, which are most eco-friendly, economical, and socially suitable (Tapondjou *et al.,* 2005). Botanical insecticides make IPM more feasible and also effective. It also decreases the dependence on artificial insect repellent products (Ma *et al.,* 2000; Zabel *et al.,* 2002). Experiments show that the reliability and efficiency of the biological control are successfully improved by combining different modes of action (Lemanceae and Alabouvette, 1993). Neem (*Azadirachta indica*) compounds are known as the best effective phytochemicals from the entomological repellent perception (Schmutterer, 1990). It is said that the bio-activity of *Azadirachta indica* is due to the contents of Azadiracht in the compound, which is a complex type of the limonoids compound (Nathan *et al.,* 2005). Some field and laboratory studies reveal that the neem extract is more compatible for biological control (Mitchell *et al.,* 2004). Diatomaceous earth (DE) is an effective insect repellent and also utmost efficacious in low humidity range (Lord, 2001) Entomopathogenic fungi are a widespread, an important component of terrestrial ecosystem. Entomopathogenic fungi are found throughout the world such as some species of e.g., *Beauveria bassiana* is present in tropical rainforests (Aung *et al.,* 2008). Various concentration-based combinations of entomopathogenic fungi with DE revealed significant insecticidal effects as related to solitary use of any toxic pesticide (Akbar *et al.,* 2004). So, the present study aimed to evaluate the insecticidal impact of neem leaf extract, DE, and Entomopathogenic fungi against *Trogoderma granarium*.

MATERIALS AND METHODS

The present experimental work was conducted at Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad.

Collection and Rearing of Insects

Damaged grain samples including heterogeneous insect culture of khapra beetles (*Trogoderma granarium*) were collected from different storage of Food Dept. Punjab is located at several places in district Faisalabad. A sterilized seed sample was taken in jars (capacity 2 kg) and collected insects culture were released in jars covered with a muslin cloth. The jars were kept in an incubator at 28 ± 2 °C and 65 ± 5°C relative humidity. After 3 days, adults were sieved out from the grains. Eggs and grains were put into jars and were kept at optimum growth condition to rear homogenous insect culture.

Insecticidal effect of leaf extract of *Azadirachta indica* against grubs of *Trogoderma granarium*

Bioassay

Crude extract from dried leaves of neem (*Azadirachta indica*) was prepared in acetone solvent using a Rotary shaker (IRMECO, OS 10) by dipping 50 g of powder in 250 ml acetone in conical flasks, by following the procedure was described by Sagheer *et al*., 2013. From filtration, acetone was evaporated using rotary evaporation. From the final extract, further dilutions were prepared. 5%, 10%, 15%, 20%, 25% and 30% dilution were prepared. The mortality of *Trogoderma granarium* larvae was checked using Petri-dishes. These dilution 5%, 10%, 15%, 20%, 25% and 30% were applied on Whatman filter paper. After treatment, filter papers were dried in the air. The dried filter paper was placed in Petri dishes, which were air tightly covered. Thirty larvae of *T. granarium* were released in each petri-dish. Data of dead insects were observed after 24, 48, and 72 hours. All the treatments were replicated three times and compared with the control group.

Insecticidal effect of different doses of commercial formulation (concern®) of diatomaceous earth against *Trogoderma granarium*

Bioassay

Six doses (200ppm, 400ppm, 600ppm, 800ppm, 1000ppm, and 1200ppm/kg of wheat) of diatomaceous earth were used which were applied in each plastic jar.

The required dose of DE was applied on sterilized wheat grains. Then DE was thoroughly mixed with grains by shaking for one minute. 30 larvae of *T. granarium* were released on treated grains. There was a control treatment also. All the treatments were replicated thrice. Data for mortality were observed after 7, 14, and 21 days after treatment.

Insecticidal potential of commercial formulation (IDA Bollaram-502325) of *Beauveria bassiana* against *Trogoderma granarium*

Bioassay

Six doses (1g, 2g, 3g, 4g, 5g & 6g/kg of wheat grain) of *Beauveria bassiana* were applied on sterilized grains. All samples were placed in plastic jars. 30 larvae of *T. granarium* were released on treated grains. The jars were covered and tightened with a muslin cloth. All the treatments were replicated trice, and one was a control treatment. Data on mortality were assessed after 7, 14, and 21 days exposure of two fungal concentrations.

Statistical Analyses

At the end of the bioassay, concerning data of recorded mortality was measured using Abbott's formula;

$$\text{Corrected Mortality (\%)} = \frac{\text{Mo(\%)} - \text{Mc(\%)}}{100 - \text{Mc(\%)}} \times 100$$

Mo is Observed mortality and Mc is Mortality in Control

The data about % corrected mortality of larva was analyzed statistically using the STATISTICA-8.0 software and data about different variables were compared by Tuckey-HSD test.

RESULTS AND DISCUSSION

The average percent mortality of *T. granarium* by applying of extract of *A. indica* at and different exposure times (24, 48 and 72 hours) is given in Table I. The acetone extract of *A. indica* shows that the mean mortality of test insects increases by increasing the concentration and exposure time. Maximum mortality (26.43%) was observed after 72 hours, and minimum mortality was found after 24 hours. The attributes of individual basil and neem extracts as insecticides were confirmed in literature (Keita *et al.,* 2001; Schmutterer, 1995; Satti *et al.,* 2010b). Maximum mortality (51.42%) occurred with 30% concentration after 72 hours and the lowest mortality (2.3%) was observed by 5% and 10% concentration after 24 hours exposure. Concluding results revealed that by using high concentrations of extract of *A. indica* for maximum time exposure, the toxicity of insecticide increased against the tested insect. Plant extracts and their combinations can be useful for the mean of conservation and integrity of the natural environment (Tapondjou *et al.,* 2001). The current result of plant extracts is similar to the result of the mortality of the said insect with *Azadirachta indica* up to 18 % (acetone extract of *A. indica*) (Ali *et al.,* 2017). Table 2 shows the larval % mortality of T. *granarium* at various concentrations (200, 400, 600, 800, 1000, and 1200ppm/kg of wheat) of DE and different time intervals (7, 14, and 21 days). Maximum mortality (74.24%) occurred with 1200ppm concentration after 21 days and the lowest mortality (7.22%) was observed with 200ppm concentration after 7 days exposure. Results revealed that by using a high concentration of DE for maximum time exposure, the toxicity of insecticide increased against the tested insect. Table 3 shows the % mortality of *T. granarium* at various concentrations (1, 2, 3, 4, 5, and 6 g/kg of wheat) of *B. bassiana* and different time intervals (7, 14, and 21 days). Maximum mortality (45.69%) occurred with 6g concentration after 21 days and the lowest mortality (24.09%) was observed with 1g concentration after 7 days exposure. Concluding results revealed that by using high concentrations of *B. bassiana* for a maximum time exposure, the toxicity of insecticide increased against the tested insect. In another study, Sookar *et al. (*2008) isolated *P. fumosoroseus, M. anisopliae*, and *B. bassiana* against *B. cucurbitae* and *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) and reported all were infective. Waqas *et al.,* 2014 determined maximum mortality rate by *B. bassiana* than the *M. anisopliae* on pests of stored grain; this might be because of the use of indigenous isolates as these types of isolates could be better modified or prepared to infect a specific host that lives in the same site (Batalla-Carrera *et al.,* 2013). In the light of the present study, it is recommended that we should provide attention to producing technical botanical insecticides that are harmless for health as well as an ecosystem, which will not only control the stored grain insect pests but also be safe for health and the environment because storage is our main objective.

Table 1. Means of % mortality of *T. granarium* (Everts) amongexposure period and concentration of *A. indica*.

Concentrations	Mortality percent means at different intervals			Overall Means
	24hrs	48hrs	72hrs	
5%	9.40±3.11 EF	15.19±1.26 DEF	14.28±2.47DEF	12.96±1.50 C
10%	2.34±1.17 F	2.53±3.34 F	7.14±3.77 EF	4.00±1.68D
15%	2.39±1.17 F	7.59±1.26 EF	18.57±2.47 DE	9.50±2.54CD
20%	9.40±1.17 EF	22.78±5.51CDE	30.0±3.77 BCD	20.73±3.59 B
25%	10.58±4.24 EF	27.84±3.79BCD	37.14±3.77ABC	25.19±4.36 B
30%	16.46±2.35 DEF	39.24±2.19 AB	51.42±1.42 A	35.71±5.22 A
Overall Means	8.42±1.46 C	19.20±3.20 B	26.43±3.76 A	

Table 2. Means regarding % mortality of *T. granarium* (Everts) among exposure period and concentrations of DE.

Concentrations	Mortality percent means at different intervals			Overall Means
	7days	14 days	21 days	
200ppm	7.22± 3.18H	10.80±2.34 GH	43.93±5.89 CD	20.65±6.67 E
400ppm	9.63± 2.08 GH	14.86±2.34 FGH	49.99±2.62 BC	24.83±6.44 DE
600ppm	12.04± 1.20 GH	24.32±2.70 EFG	53.02±1.51 BC	29.79±6.14 CD
800ppm	13.25± 2.08 GH	29.72±2.70 DEF	62.11±4.00 AB	35.03±7.33 BC
1000ppm	14.45±1.20 FGH	39.18± 2.34CDE	63.63±2.62 AB	39.09±7.17 AB
1200ppm	16.86±2.08 FGH	40.53±5.89 CDE	74.24±5.46 A	43.88±8.66 A
Overall Means	12.24±1.04 C	26.57±2.94B	57.82±2.72 A	

Table 3. Means regarding % corrected mortality of *T. granarium* (Everts)among exposure period and concentrations of *B. bassiana*.

Concentrations	Mortality percent means at different intervals			Overall Means
	7days	14 days	21 days	
1g	12.50± 3.00 G	19.51± 2.11 FG	40.26± 1.29 CD	24.09±4.31 D
2g	13.63± 3.00 G	23.16± 2.11 EFG	53.24± 2.24 BC	30.01±6.09 CD
3g	17.04± 3.00 FG	19.51± 2.11 FG	54.54± 3.43 BC	30.36±6.22 CD
4g	18.18± 3.93 FG	29.26± 2.43 DEF	61.04± 2.24 AB	36.16±6.59 BC
5g	20.45± 3.00 FG	36.58± 3.22 DE	66.23± 3.43 AB	41.09±6.89 AB
6g	20.45± 2.27 FG	43.90± 1.21 CD	72.72± 4.49 A	45.69±7.70 A
Overall Means	17.04±1.29 C	28.65±2.33 B	58.01±2.71 A	

CONCLUSION

Different acetonic dose rates of neem leaf extract, diatomaceous earth, and *B. bassiana* caused significant mortality against *T. granarium.* The presented data about extracts of plants and tested insecticides will serve as direction for proper planning of cost and selecting effective measures against *T. granarium* (Everts).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ACKNOWLEDGMENTS

I deem it my greatest pleasure to everybody who participated and helped us while doing this project from start to end.

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