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Bio-control effect of neem (*Azadirachta indica* A. Juss, 1830) leaf and seed powders against adult bean weevil (*Acanthoscelides obtectus* Say, 1831) mortality

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Abstract
A laboratory study with the objective of investigating the effects of neem seed and leaf powder against adult bean weevil was conducted at an animal research laboratory. The study had one factor neem seed powder at rates of 2, 4 and 6% w/w and control group and another factor of neem leaf powder at rates of 2, 4 and 6% w/w and control group. The treatments were applied with three replications. Data were collected on dead adult weevils only. The dead adult weevils were counted within 7, 21 and 42 days after the infested bean was treated by neem seed powder and leaf powder. The collected data were subjected to one way ANOVA procedures. Results showed that there were significant differences ($p<0.05$) in the mean percent mortality of *A. obtectus* among the different concentration of seed and leaf powder with time of storage. The highest mean mortality percent were recorded at the 6% w/w concentration. The 2, 4 and 6% w/w concentration rates of neem seed powder killed about 68.39, 73.6 and 87.15% of adult weevils, respectively throughout the storage period. Whereas the leaf powder treatment killed 66.17, 70.28 and 82.57% adult weevils respectively. It is concluded that the application 6% w/w of both neem seed and leaf powder are effective in the control of bean weevil and could be used as an alternative control option in integrated stored pest management strategies by small scale farmers.

Keywords: Bean weevil, bio-control, neem, powder

Introduction

Stored grains such as bean, wheat, rice, maize and sorghum are the main components of basic food in many countries. Stored grains are destroyed by a number of insect pests. Stored grain pests invade grains to meet their food and shelter needs, resulting in quantitative and qualitative losses. Since the harvest of grains occurs seasonally while the market demand is relatively unceasing, the storage step has a critical role in the global economy. In this sense, the deterioration of stored products is a problem for agriculture, mainly in tropical regions where the seeds are often subjected to high temperatures and humidity. In addition to chemical and physical factors, the stored grains can also be damaged by biological agents such as insect pests. The insects belonging to the orders Coleoptera (beetles) causes most of the damage to stored grains (Napoleão *et al*., 2015; Srivastava and Subramanian, 2016) [1, 2].

Bean weevils are originally native to Central America, however grain shipments at the end of the 19th century introduced the species to Europe where it subsequently spread around the globe. It is now found in Europe, Asia, North and South America, Africa, Australia and elsewhere (Saqpunaru *et al*., 2006; Karakas, 2021) [3, 4].

The bean weevil, *Acanthoscelides obtectus* Say (Bruchidae), causes significant damage to haricot-bean and bean. Larvae usually eat the pot contents completely, decreasing the yield by 50-60%. Partially damaged grains lose their germinating power and taste quality. The pest damages grain in both field and storehouses, becoming rather harmful. Control measures include keeping the temperature below zero in storehouses, fumigation, insecticide treatments in fields (Grewal and Atwal, 1967; Salha *et al*., 2009; Karakas, 2021) [5, 6, 4].

Insecticides used for chemical control have been considered the most effective and accessible means to control insect pests of stored products. These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for vertebrates especially for mammalian health (Karakas, 2016) [7].

The adverse effects of synthetic pesticides have amplified the need for effective and biodegradable pesticides. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. Among various kinds of natural substances that have received particular attention as natural agents for insect management are essential oils and extracts from aromatic plants (Karakas, 2017) [8].

The Indian neem tree, *Azadirachta indica* A. Juss (Meliaceae), is a promising source of botanical insecticides. Due to their relative selectivity, neem products can be recommended for many integrated pest management programs (Biswas *et al*., 2002). It is generally believed that bioactivity of neem is due to the azadirachtin (AZA) (complex limonoids) content (Butterworth and Morgan, 1971) [9].

Research has shown that neem extract is effective against nearly 200 species of insects. It is significant that some of these pests are resistant to pesticides, or are inherently difficult to control with conventional pesticides. Among such insects are floral thrips, diamondback moth and several leaf miners. Most neem products belong to the category of medium-to broad-spectrum pesticides, i.e., they are effective over a wide range of pests (Bhattacharyya *et al*., 2007) [10]. According to Jagannathan *et al*., (2015) [11] neem tree extracts has been used against household pests, storage pests and crop pests of field. Neem has been produced as fumigant used as a pesticide and disinfectant in many

countries on a commercial basis by farmers and agriculturists. This 100% natural product is nontoxic and environmentally friendly. It assumes more importance in developing countries where millions of deaths are reported every year due to the accidental intake of synthetic pest fumigants.

Therefore, the present study was initiated to find and recommend possibly the most effective neem leaf and seed powders against adults of *A. obtectus* in the stored bean grains under laboratory conditions.

Materials and Methods

Test insect

The experiment was conducted at Ankara University, Science Faculty, Animal Physiology Laboratory. In this study, *A. obtectus* laboratory stock, which is being maintained since 2019, on the dry bean seeds under laboratory conditions (28 ± 2 °C and 65 ± 5% RH). Newly emerged adult *A. obtectus* were transferred from stock culture to the new culture containers (425 ml) and oviposition was provided for 48 hours. After this time, adult individuals were removed from the culture and new adult emergence were provided and these individuals were selected for the experiment.

Test plant

In laboratory practice, bio-control effect of leaf and seed powders from neem, *A. indica* was tested against adult bean weevil, *A. obtectus*. Neem was obtained from a local sapling company of Yalova.

Bioassay

Firstly, the collected neem leaves and neem seed were grounded by pestle and mortar separately at the laboratory.

There was a factor neem seed powder and neem leaf powder in proportions (2, 4, and 6% w/w) in the experiment. Treatments were arranged in a three-replicate completely randomized design.

Two hundred fifty grams of clean bean grain was placed in 1000 mL glass jar and thoroughly 40 adult weevils were added into it. After the infestation of weevils occurred in the jars different rates (2, 4 and 6% w/w) of neem seed and (2, 4 and 6% w/w) leaf powder were applied in three replications. The application of treatment was taken under areas were no direct sun light affect as neem may lose its insecticidal properties if applied under direct sunlight.

Application I (Neem seed powder)

2% w/w neem seed powder+250 g bean+40 adult weevils
4% w/w neem seed powder+250 g bean+40 adult weevils
6% w/w neem seed powder+250 g bean+40 adult weevils
0% control (250 g bean+40 adult weevils)

Application II (Neem leaf powder)

2% w/w neem leaf powder+250 g bean+40 adult weevils
4% w/w neem leaf powder+250 g bean+40 adult weevils
6% w/w neem leaf powder+250 g bean+40 adult weevils
0% control (250 g bean+40 adult weevils)

Data were collected on dead adult weevils only. The dead adult weevils were counted within 7, 21 and 42 days after the infested bean was treated by neem leaf powder and neem seed powder. Dead weevils were recorded from the treated and the control jars. The first data was taken on the 8th day after the treatment application while the two later observations were taken on the 18th and 33th days after the first observation.

Statistical data

The collected data were subjected to one way analysis of variance (ANOVA) using the General Linear Model procedures of SAS. Comparison of treatment means was performed using Fisher's least significant difference test at $p<0.05$ probability level.

Results

The analysis of variance showed that there were significant differences ($p<0.05$) in the mean percent mortality of bean weevil among the different concentration of neem leaf powder and neem seed powder with time of storage (Table 1 and 2).

The treatment of neem seed powder (2, 4 and 6% w/w) showed different mortality potential on bean weevils. After 7 days of treatment application, the 6% w/w neem seed powder concentration was significantly different from the 4, 2 and 0% while the 4 and 2% neem seed powder were statistically equal to each other.

Table 1: Effect of neem seed powder on mortality of adult bean weevils within period of treatment application

Concentration (%) Application I	Days / Mortality		
	7	21	42
6	30.71^{a}	27.01^{a}	29.43^{a}
4	22.63^{b}	24.00^{a}	26.97^{ba}
2	20.69^{b}	23.33^{a}	24.37^{b}
0*	1.31^{c}	1.27^{b}	0.71^{c}
LSD	6.69	7.13	7.01
CV	13.13	15.01	11.67

Means along the same column with different superscripts are significantly different ($p<0.05$), LSD: Least Significant Difference, CV: Coefficient of Variation, *: Control Group

Table 2: Effect of neem leaf powder on mortality of adult bean weevils within period of treatment application

Concentration (%) Application II	Days / Mortality		
	7	21	42
6	27.33^{a}	27.71^{a}	27.53^{a}
4	21.32^{b}	24.93^{a}	24.03^{ba}
2	19.57^{b}	23.71^{a}	22.89^{b}
0*	1.21^{c}	1.11^{b}	0.40^{c}
LSD	5.89	6.99	11.43
CV	12.11	12.87	7.69

Means along the same column with different superscripts are significantly different ($p<0.05$), LSD: Least Significant Difference, CV: Coefficient of Variation, *: Control Group

And after 21 days of treatment application, the 6, 4 and 2% neem seed powder concentration were significantly equal to with each other while they were statistically different from the control group. Likewise, after 42 days of treatment application, the 4% concentration was significantly equal to with the 6% concentration and the 2% concentration while the 6, 2 and 0% were statistically different. Consequently, the highest mean mortality percent were recorded at the 6% w/w concentration while the least was recorded at the control group. Hence, the 2, 4 and 6% w/w concentration rates of neem seed powder killed about 68.39, 73.6 and

87.15% adult bean weevils, respectively throughout the storage period (Table 1).

The analysis of variance for the neem leaf powder concentration also showed statistical difference in the mortality of adult bean weevils. After 7 days of treatment application, the leaf powder concentration at rates of 6, 4 and 2% w/w and control were statistically different from each other while after 21 days of treatment application the 6, 4 and 2% w/w were statistically similar to each other and statistically different with the control group. Besides, after 42 days of treatment application, the 4% concentration was statistically equal to with the 6 and 2% while the 6, 2 and 0% were statistically different to each other.

Therefore, the neem leaf powder treatment with 2, 4 and 6% w/w concentration killed 66.17, 70.28 and 82.57% adult bean weevils, respectively. As the case with the neem seed powder treatment, still high rate of mortality was recorded at the 6% w/w of neem leaf powder treatment while the least was recorded at the control group (Table 2).

Comparing the neem seed and leaf powder treatment, the highest mortality rate was recorded at the neem seed powder (87.15%). However, this high amount recorded at the neem seed powder is by far below than that considered the minimum effective concentration.

Discussion

Results of the present study show that the active ingredient of Neem's seed and leaf parts, azadirachtin, caused high mortality of *A. obtectus* on the one hand and completely hindered or significantly reduced progeny emergence on the other hand, indicating its potential use in the management of bean weevil.

The inhibition of *S. zeamais* progeny emergence and maize grain damage as a result of treatment with Neem was probably due to the huge array of azadirachtin activities on the insect's hormone system. It has been proved that azadirachtin disrupts or inhibits development of insect eggs, larvae or pupae, preventing the moulting of larvae or nymphs, disrupting mating and sexual communication, deterring females from laying eggs, sterilising adults, poisoning larvae, thus preventing adult maturation by inhibiting the formation of chitin, the essential substance for the insect to form an exoskeleton (Schmutterer, 1990; Mordue and Blackwell, 1993; Karnavar, 1987; Murugan *et al*., 1999) [12, 13, 14, 15].

Comparing the findings of this study with other findings, this study was conducted by Ileke and Oni (2011) [16], Khaliq *et al*., (2014) [17]. Ileke and Oni (2011) [16], in their study on the toxicity of some herbal powders on maize weevil, *Sitophilus zeamais*, and stored wheat grains, *Triticum aestivum*, found that the mortality rate of adult *S. zeamais* increased from 2.5-25%.

Moreover, Khaliq *et al*., (2014) [17] found that in licorice, *Glycyrrhiza glabra*, the mortality rate of maize weevil at 5% concentration was 35.81% and the mortality rate due to 24 hour exposure to the same plant was 35.55%.

From the same study, Khaliq *et al*., (2014) [17] found 5% w / w mortality rate 55.01% and 63.56% from chicory, *Chicorium intybus* and chebulic myrobalan, *Terminalia chebula* for the same plants, respectively, and 46.11% and 48.88% due to 24 hour exposure.

However, the current study mortality rate was lower than found in the study by Yohannes *et al*., (2014) [18]. Yohannes *et al*., (2014) [18] found that after 10 days of exposure, corn seed treated with 10g of Mentha and Schinus leaf powder recorded 96.6% mortality, followed by Melia (93.3%) and Phytolacca (90%).

However, lower values recorded from this study do not mean that neem seed and leaf powder are not effective against death of adult maize weevils. The result is due to the lower concentration level used in the current study.

The insecticidal effect of the leaf and seed powder may increase or decrease over time, as the significant effect of the treatment depends on the active ingredient of each ratio and the amount of dust in contact with each pest weevil.

Conclusion

The need for a stable and safe food supply for the growing world population has led to the discovery of neem tree as a bio-pesticide. With increasing knowledge on the use of bio-pesticides, it will gradually replace the traditional chemical pesticides currently in use. One of the problems with the use of chemical pesticides has been their effects on 'non-target' organisms. Often proven to be harmful to various beneficial species in the ecosystem. However, neem extracts lack these effects.

References

1. Napoleão TH, Agra-Neto AC, Belmonte BR, Pontual EV, Paiva PMG. Biology, ecology and strategies for control of stored-grain beetles: A review. In: Beetles: Biodiversity, Ecology and Role in the Environment. Ed. Camilla Stack. Nova Science Publishers, Inc. Chapte,2015:5:105-121.
2. Srivastava C, Subramanian S. Storage insect pests and their damage symptoms: An overview. Journal of Grain Storage Research, 2016, 53-58.
3. Saqpunaru T, Filipescu C, Georgescu T, Bild YC. Bioecology and control of bean weevil (*Acanthoscelides obtectus* Say.). Cercetari Agronomice în Moldova,2006:39(2):5-12.
4. Karakas M. The effect of temperature and relative humidity on the development of the bean weevil, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae),2021:6(5):16-20.
5. Grewal SS, Atwal AS. The influence of temperature and humidity on the development of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae). Journal of Agricultural Research,1967:6:353 -358.
6. Salha H, Kalinovic I, Ivezic M, Rozman V, Liska A. Application of low temperatures for pests control in stored maize. 7th Croatian congress of cereal technologists Flour – Bread, 2009, 608-616. Opatija, Hrvatska.
7. Karakas M. Toxic, repellent and antifeedant effects of two aromatic plant extracts on the wheat granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae). International Journal of Entomology Research,2016:1(6):24-28.
8. Karakas M. Use of aromatic plant extracts as bio-insecticides for the control of stored-product insect, *Sitophilus granarius*. International Journal of Entomology Research,2017:2(1):27-29.
9. Butterworth JH, Morgan ED. Investigation of the locust feeding inhibition of the seeds of the neem tree, *Azadirachta indica.* Journal of Insect. Physiology,1971:17:969-977.

10. Bhattacharyya N, Chutia M, Sarma S. Neem (*Azadirachta indica* A. Juss), a potent bio-pesticide and medicinal plant: A review. Journal of Plant Science,2007:2:251-259.
11. Jagannathan R, Ramesh RV, Kalyanakumar V. A review on neem derivatives and their agricultural applications. International Journal of Pharmacy and Technology,2015:6(3):3010-3016.
12. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology,1990:35:271–297.
13. Mordue AJ, Blackwell A. Azadirachtin: an update. Journal of Insect Physiology,1993:39:903–924.
14. Karnavar GK. Influence of azadirachtin, on insect nutrition and reproduction. Proceedings Indian Academic Science (Animal Science),1987:96:341–347.
15. Murugan K, Senthil KV, Jeyabalan D, Babu R, Senthil NS, Sivaramakrishnan S. Interactive effect of neem products on the control of pulse beetle, *Callosobruchus maculatus* (F). Neem Newsletter,1999:15:41–44.
16. Ileke KD, Oni MO. Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Motschulsky) [Coleopteran: Curculiondae] on stored wheat grains (*Triticum aestivum*). African Journal of Agricultural. Research,2011:6:3043-3048.
17. Khaliq A. Nawaz A, Ahmad MH, Sagheer M. Assessment of insecticidal potential of medicinal plant extracts for control of maize weevil, *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae). Basic Research Journal of Agricultural Science and Review,2014:3:100-104.
18. Yohannes A, Asayew G, Melaku G, Derbew M, Kedir S, Raja N. Evaluation of certain plant leaf powders and aqueous extracts against maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Asian Journal of. Agricultural. Science,2014:6:83-88