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ARTICLE

Bioefficacy of Ethanolic Leaves Extract of *Azadirachta indica* Against Stored Product Insect Pest, *Tribolium castaneum* (Herbst.)

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ABSTRACT

Tribolium castaneum is a species of beetle in the family tenebrionidae, the darkling beetle. Conventional insecticides used for controling stored product pests are expensive and are proven to have side effects hence the need to have an alternative biopesticides from plants which are proven to be effective against many insects. *Azadrachta indica* has been used for the control many insects, literature review show no report of its effects on *Tribolium castaneum*. This study is aimed at evaluating the bioinsecticidal activity of ethanolic leaves extract of *Azadirachta indica* against stored *Tribolium castaneum*. Different concentrations (60, 70, 80, 90, and 100%) of ethanolic leaves extract of *A. indica* was applied on the filter paper and were allowed to dry. Control was done by applying water only on the filter paper. For the determination of the percentage mortality, 15 adults of the insects was taken into the Petri dishes embedded with the filter paper covered with a lid. The *A. indica* extracts at different concentrations tested showed significant insecticidal activity against *T. castaneum*. Secondary metabolites screening indicated that the *A. indica* extract have secondary metabolites that are related to insecticidal activity. The study has shown that the ethanolic leaves extract *A. indica* is effective in managing stored product pest.

1. Introduction

Tribolium castaneum is a species of beetle in the family tenebrionidae, the darkling beetle. It is a worldwide pest of store product, particularly food grain, found in most tropical and subtropical countries in the world. it is a pest of stored maize and a variety of stored product. This insect pest feed on internally on maize grains. It is a pest that affects many stored products and can be a major pest in anthropogenic structures that is used for the storage of grains products such as biscuits industry and flour mills [1].

T. castaneum is very common and most dangerous pest that affects stored products worldwide and is usually found in stored grains, warehouses and mills. The presence *Triboilium castaneum* in maize flour and other stored products results in decrease in nutritive value, economic damage and contamination [2]. *Tribolium castaneum* attacks the germ part or embrayo portion of grains and also affects the quality and quantity of grains. This pest has a long interaction with stored products and has been found in interaction with a number of commodities such as

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beans, nuts, peas, flour and grains [3].

Insect's beetles of the genus *Tribolium*, especially the red flour beetle, *TriboIium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum*, are major pests of most stored-food products and are mostly found in cereal processing facilities such as flour mills. [4, 5].

The Red beetle has been reported to be highly mobile both as adults and larvae in patches of flour and broken grains [6, 7]. One factor that has made the insect population high in any substrate it colonizes is that its female insects can lay eggs almost throughout their life span [8]. This study is therefore designed to study Bioinsecticidal Activity of Ethanolic Leaves Extract of *Azadirachta indica* Against Stored Product Insect Pest, *Tribolium castaneum* (Herbst).

2. Materials and Methods

2.1 Study Area and Insect Collection

The study was conducted in the Biological Sciences Laboratory, Bauchi State University, Gadau. *Tribolium castaneum* was collected from Azare main market, Azare Bauchi State between the months of January – March, 2018.

2.2 Insect Rearing

Tribolium castaneum collected was stored in a plastic jars in the laboratory having the same temperature and relative humidity. Flour of maize was utilized as media for *T. castaneum*. Adults insects was sieved out and one hundred insects was put in each of the plastic jars with 300gm of maize flour. They insects were allowed to mate and lay eggs.

2.3 Collection and Extraction of Plant Material

Leaves of *Azadirachta indica* (Neem) was collected from Bauchi State University, Gadau Main campus. The leaves were authenticated by an acknowledged botanist in the Department of biological Sciences,Bauchi State University, Gadau. Leaves of *Azadirachta indica* was washed thoroughly and dried under the shade. The leaves was then crushed into powder using Grinder to get fine powder and then sieved with a fine mesh sieve. Extraction of the plant material was done by combining 50 g of ground sample and 100 ml of ethanol and shake well for 4 hours with Rotary Shaker followed by filtration with whatman filter paper. The leaves extract was then put into the Rotary evaporator to have 100% stock solution as described by [9] and [10].

2.4 Determination of Insecticidal Activity

Different concentrations (10, 30, 80, 90, and 100%) of ethanol leaves extract of *A. indica* was put onto the filter paper and then allowed to dry some time. The control treatment was treated with water only. The percentage mortality of the insects was examined by adding 15 adults of the insects into the petri dishes with whatman's filter paper and covered. Maize flour was put into the petri dishes to reduce mortality due to starvation. Percentage mortality of adults insects was recorded after period of 2, 4, 6 and 8 hours after treatment. Completely Randomized Design was followed with three replicates.

2.5 Qualitative Phytochemical Screening

The leaves extract of *A. indica* was tested for the presence phytochemicals such as tannins, saponins, flavonoids, phenolics and alkaloids using a method described by [11]

2.6 Data Analysis

Data analysis was carried out with MINITAB software version 9. The results is expressed as mean ± standard error of mean. The value of $P \leq 0.05$ were considered to be significant.

3. Results

3.1 Insecticidal Activity of Ethanolic Extract of *A. indica* against *Tribolium castaneum*

The ethanolic leaves extract of *Azadrachta indica* proved to activity against *Tribolium castaneum* in all the tested treatments. This is because of the number of dead *T. castaneum* found in the experimental and control groups. Different concentrations of ethanolic leaves extract of *A. indica* showed that 70%, 80% 90% and 100% ethanolic extract significantly higher *T. castaneum* mortalities than 60% and the control (untreated group) at two and four hours after spraying ($P<0.05$). However, at six and eight hours after spraying, different concentration of ethanolic extract of *A. indica* induced significantly higher mortalities than the control (Table 1).

Table 1. Insecticidal activity of ethanolic leaves extract of *A. indica* *Tribolium castaneum*

DOSE	60%	70%	80%	90 %	100%	CONTROL
2 HRS	0.00 ± 0.00^{a}	0.33 ±0.58^{a}	0.00 ± 0.00^{a}	0.67 ±0.58^{a}	3.00 ±1.00^{b}	0.00 ± 0.00^{a}
4 HRS	0.00 ± 0.00^{a}	0.33 ±0.58^{a}	1.53 ± 0.58^{b}	2.00 ± 0.00^{b}	1.33 ±0.58^{b}	0.00 ± 0.00^{a}
6 HRS	0.67 ±0.58^{a}	0.67 ±0.58^{a}	1.00 ± 0.00^{a}	1.33 ± 0.58^{a}	2.67 ±1.16^{b}	0.00 ± 0.00^{a}
8 HRS	1.00 ± 0.00^{ab}	1.00 ±1.00^{ab}	1.33 ± 0.58^{ab}	1.67 ± 1.16^{a}	1.00 ±0.00^{ab}	0.00 ± 0.00^{b}

Note: Values are expressed as Mean ± SEM of three replicates.

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3.2 Qualitative Phytochemical Screening

Some secondary metabolites were present in the ethanolic leaves extract of *A. indica*. The extract contained alkaloids, Phenolic, flavonoids, and tannins while saponins were absent (Table 2).

Table 2. Qualitative Analyses of *A. indica* leaves

Phytochemical	Result
Alkaloid	+
Phenolic compound	+
Flavonoids	+
Saponins	-
Tannins	+

Key: + = Present – = Absent

3.3 Discussion

This study was aimed at evaluating the insecticidal activity of ethanolic extract of *A. indica* stored products pest *Tribolium castaneum*. Extracts from many plants have been reported to have insecticidal effect against many insect pests [12]. Other researchers reported that plants consist of a cheaper alternative to chemicals insecticides due to biodegradable nature and less toxic to non-target organisms [13].

The ethanolic extract of *A. indica* produced insecticidal activity against Triboilum castaneum in a manner that is dose dependent. The result of the present study is in agreement with the effects of other plants extracts tested against various insects. Haile and Raja [12] reported insecticidal effects of *Croton macrostachys* and *Schinus molle* extracts on cabbage aphids *Brevicoryne brassicae*. Furthermore, [14] reported insecticidal activity of neem extract (*Azadirachta indica*) on several insects pests.

The effects of ethanolic leaves extract of *A. indica* against *Tribolium castaneum* would possibly due to the present of one or more groups of secondary metabolites found in the extract. Screening of phytochemicals revealed that the ethanolic leaves extract of *A. indica* have tannins, flavonoids, phenolics and alkaloids (Table 2). These metabolites acts in regulating interactions between the environments and the plants. Furthermore, they have been reported to show insecticidal effects on many insects [15, 16].

Flavonoids have been shown an excellent insecticidal effects against various insects pest. For example a research conducted by [17] revealed that flavonoids from Ricinus communis posses a good insecticidal and antimicrobial effects on C. chinensis. Alkaloids are natural metabolites which play a vital role in the ecology of organisms which synthesize them. Alkaloids along with other phytochemicals are part of the plant defenses against phytophagous animals [18, 19, 20]. The insecticidal activity may be due to the combine effects of phenolic compound [21]. Therefore, the appearance of flavonoids, alkaloids and phenolic compounds in the ethanolic extract of *A. indica* may be attributed to its insecticidal activity.

3.4 Conclusion

The findings of this study has revealed the insecticidal activity of ethanolic extract of *A. indica*. The significant reduction in the number of *T. castaneum* from two to eight hours of treatment with various doses of the ethanolic leaves extracts showed that the extract have some elements of insecticidal activity. From the present study, it is possible to conclude that ethanolic extract of *A. indica* may contain similar components to organic chemicals used to control *Tribolium castaneum*.

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