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Research Paper

PESTICIDAL ACTIVITIES OF *MORINGA OLEIFERA* SEED OIL EXTRACT TO *TRIBOLIUM CASTANEUM* AND *TRIBOLIUM CONFUSUM* ON MILLED MAIZE

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Abstract

A study of Pesticidal activities of *Moringa oleifera* seed oil extract to Rust-Red Beetles, *Tribolium castaneum* and Confused Flour Beetles, *Tribolium confusum* on milled maize was conducted in the research Laboratory of Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Southeastern Nigeria. This study was to investigate the mortality and repellency effects of *Moriga oleifera* seed oil on the stored product pests. The study was carried out under ambient temperature of 28^0c-32^0c and relative humidity of 72%-80%. The study was conducted using 5 concentration levels of 20%, 10%, 5% 2.5% and 1.25% including a control which corresponded to 200µl/ml, 100µl/ml, 50µl/ml, 25µl/ml, 12.5µl/ml, of the moringa seed oil per milliliter. Mortality and repellency data were recorded and probit analysis carried out to determine the LD_{50} values. LD_{50} were determined to be 1.78µl/ml, 1.67µl/ml, 1.67µl/ml, and 1.9µl/ml, for direct application on *Tribolium castaneum*, direct application on *Tribolium confusum*, residual filter paper treatment on *Tribolium castaneum* and residual filter paper treatment on *Tribolium confusum* respectively. There were no significant differences in mortality and repellency effects recorded within and between the groups ($P \geq 0.05$). The studies showed that moriga seed oil has poor mortality and repellency effects to the insect pests as not more than 6.6% mortality or repellency effects were recorded in any of the treatments.
Key words: Pesticidal, *Moringa*, *Tribolium,* Milled Maize.

INTRODUCTION

Milled maize suffers heavy losses during storage due to insect pests' infestation. FAO estimated that 10 to 25% of the world's harvested food is destroyed annually by insects and rodent pests [2]. Insect pests cause damage to stored grains and processed products by reducing their dry weight and nutritional value [11].

A major problem of stored flour products in the tropics is infestation of these damage insects. Most important among flour products pests are the Red Flour Beetle, *Tribolium* *castaneum* and the Confused Flour Beetle, *Tribolium confusum* [6]. Red flour beetles attack stored grain products such as flour, cereals, pasta, biscuits, beans, nuts and others. Confused flour beetle also attack the same products, both causing loss and damage. They may cause allergic response but are not known to spread diseases and cause no damage to structure or furniture. These flour products are important to man as they are sources of proteins and carbohydrate [6].

In the past decades, control measures have been taking against these pests. Such measures include, the use of synthetic insecticides formulated as dusts. But such products are highly cost and have residual effects on flour products meant for human consumption. Pest resistance and the deleterious effects of these formulated pesticides on non-target organisms pose challenges to man [8]. These limitations lead many scientists into research the recent decades.

Prakash and Rao[10] noted that plant products having considerable potential as insecticide compounds are gaining attention in recent years, particularly for the management of stored product pests as they are environmentally safe and do not leave residues in the stored product. Many studies have been conducted to show that plants or vegetable oils from palm kernel and coconut are effective in controlling insect pests [1][3]. Therefore, this is research targeted at discovering botanicals that may replace the synthetic insecticides [4].

This research is aimed at demonstrating the pesticidal activities of *Moringa oleifera* seed oil extract on *Tribolium castaneum* and *Tribolium confusum*.

The specific objectives of the study are;

1. To test the insecticidal activity of Moringa oil on the two stored product insect pests.
2. Determine the level of efficacy of solvent extracts of Moringa seed oil as pesticide on rust red and confused flour beetles infesting milled maize in the laboratory.
3. Determine the level of toxicity (LD_{50}) on impregnated filter paper surface and treated milled maize.
4. Determine the repellency effect of Moringa seed oil extract to the flour beetles.

MATERIALS AND METHODS

Area study

The research was carried out in the laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State, Southeastern Nigeria.

Insect culture

Flour of milled maize infested with Adults of *Tribolium confusum* and *Tribolium castaneum* were obtained from the Eke-Awka market in Awka, Anambra state, southeastern Nigeria. The infested flour was used to set up the experimental insect cultures in plastic jars. Nets were used as covers to allow air. It was kept under ambient temperature of 28^{OC}–32^{OC} and relative humidity of 72%-80%. The cultures were left for 6 weeks to develop until needed for treatment. Before the treatment, the cultures were opened for about 10mins, so as to allow adult insects to fly off remaining only larvae. The experimental milled maize flour was sieved in order to remove the flour dust to obtain the desired insect larvae needed for the experiment.

Source of moringa seeds

Some packets of dried Moringa seeds were obtained from the distributor. The capsules were aired under the shade for 7 days. The seeds were ground with mortar and sieved. Fine powdery product obtained was used for oil extraction.

Hexanoic extraction of moringa seed oil
Soxhlet Extractor was used to obtain a 100% pure oil extract of the Moringa seed. The components of the apparatus are; round bottom flask, Soxhlet Extractor, thimble, condenser for water inlet and outlet and a heating mantle.

Procedure
50g of powdered seed of Moringa was weighed and placed into a thimble of the Soxhlet Extractor apparatus. 250ml of n-Hexane was poured into 50ml round bottom flask from external source. The apparatus was set up, controlled and was allowed for 2 hours. When it was noticed that the solute – solvent mixture in the extractor, after many refluxes became clearer and the 50g of ground declining, another packet containing 50g of powdered product was subsequently placed in the thimble and the process repeated several times until the whole blended Moringa seeds got exhausted. Then it was stopped. The thimble chamber was opened and the remaining chaff of the seeds removed. The filtrate in the round bottom flask was heated to boiling with water bath so that the solvent evaporated while only the desired oil extract remained. The oil extract appeared amber in colour.

Serial dilution of the moringa oil
The oil extract obtained was serially diluted with acetone to obtain graded concentrations of 200µl/ml (20% dilution), 100µl/ml (10% dilution), 50µl/ml (5% dilution), 25µl/ml (2.5% dilution) and 12.5µl/ml (1.25% dilution) including a control.

Treatments of the flour beetles
10 larvae of the beetle were introduced into each of the five separate experimental jars containing uninfected milled maize. Each was treated with the serially diluted Moringa seed oil respectively. Each treatment was replicated four times.

Residual application on filter paper
The five different concentrations (200µl, 100µl, 50µl, 25µl, 12.5µl) plus a control of the oil extract were selected and tested to determine the LD_{50} value. One milliliter aliquots of the different concentrations of the oil extract of each treatment was replicated four times. The oil was absorbed by the filter papers and left for 30 minutes to allow the acetone to evaporate. Ten larvae of the flour beetles of mixed sexes were exposed to the treatment for 3 days. Records of mortality were taken after every 24 hours. Insect larvae were counted dead when they refuse to move parts of the body in response to external stimuli.

RESULTS
The results of the research on the pesticidal activities of moringa seed oil in both direct and residual application, the repellency effects on these case study agric-stored product insects are shown below.
Table 1 shows the same number of mortality occurring in the first and second concentration of moringa oil, 200µl and 100µl respectively while no mortality occurred in the last three concentrations 50µl, 25µl and 12.5µl respectively. These records where taking within the first three days. Analysis of Variance showed no significant differences in mortality within and between the treatments ($P \geq 0.05$). The probit/mortality graph of direct treatment and the mortality effect of moringa oil are shown in figure 1 below. LD_{50} was 1.78 µl/ml.

In table 2, the highest number of mortality occurred in the highest concentration, 200µl, while same number of mortality occurred in the second, third and fourth concentrations, 100µl, 50µl and 25µl respectively. No mortality occurred in the lowest concentration and control. There were no significant differences in the mortality between and within the concentrations ($P \geq 0.05$). The probit/mortality graph indicating LD_{50} of moringa oil concentration in *Tribolium confusum* by direct treatment is shown below in figure 2. LD_{50} was 1.67µl/ml.
Table 3 shows the mortality response of *Tribolium castaneum.* Concentration 200µl has just 3.3% mortality same as 100µl and 50µl. The lower concentrations recorded no mortality including the control set up. There were no significant differences in mortality within and between the groups ($P \geq 0.05$). The graph of probit/mortality response is shown in figure 3 with LD_{50} of moringa oil to *Tribolium castanium* on filter paper to be 1.67µl/ml.
Table 4 shows the mortality response of *Tribolium confusum* to moringa oil on impregnated filter paper. 6.67% mortality was observed in the concentration, 200µl while 3.33% were observed in 100µl, 50µl and 25µl respectively. No mortality was recorded in the least concentration and the control. There were no significant differences in mortality within and between the groups. The graph of probit/mortality response is shown in figure 4 below. LD_{50} of moringa oil to *Tribolium confusum* on filter was 1.9µl/ml.
Table 5 shows 5% repellency occurring in 200µl and 100µl concentrations respectively within the first three days. Also 2.5% repellency is seen in 50µl and 25µl concentrations respectively. The lowest concentration and control were not seen to repel any of flour beetles. There were no significant differences in repellency on the different concentrations within and between groups ($P \geq 0.05$).
Table 6 showed the highest repellency of 5% at the highest concentration. Same number of 2.5% repellency occurred at 100µl and 50µl respectively. None was recorded on 25µl, 12.5µl and control. There were no significant differences in repellency on the different concentrations within and between groups ($P \geq 0.05$).

Table 1: Average mortality of *Tribolium castaneum* treated with various concentrations of moringa oil (Direct application).

Dose in µl/ml	Total mortality	Average mortality	Log dose	% observed mortality	Probit value
200	1	0.025	2.33	2.50	3.04
100	1	0.025	2.00	2.50	3.04
50	0	0.00	1.63	0.00	-
25	0	0.00	1.33	0.00	-
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

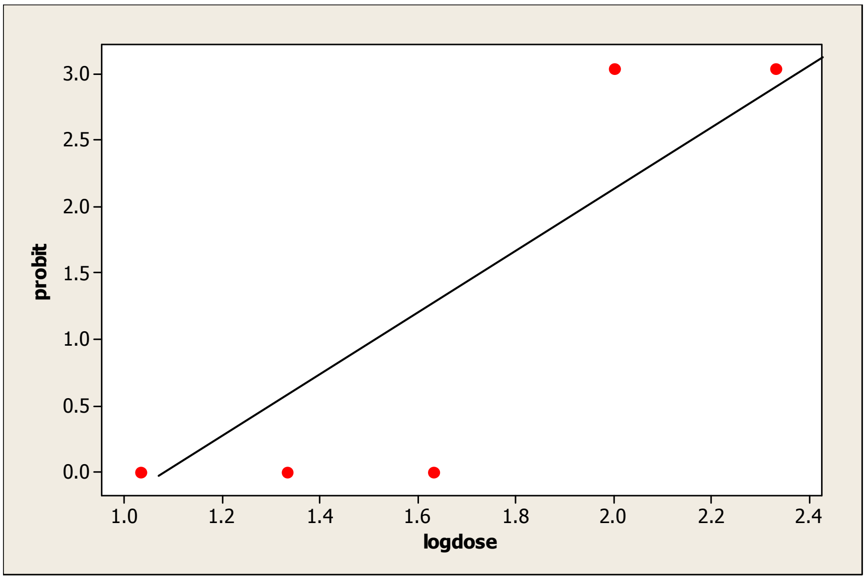

Fig. 1: probit/mortality graph of direct treatment of moringa oil on Tribolium Castaneum. Probit = - 3.52 + 2.84 logdose, $L.D_{50}$= 1.78

Table 2: Average mortality of *Tribolium confusum* treated with various concentration of moringa seed oil (Direct application).

Dose in µl/ml	Total mortality	Average mortality	Log dose	% observed mortality	Probit value
200	2	0.05	2.33	5.00	3.36
100	1	0.025	2.00	2.50	3.04
50	1	0.025	1.63	2.50	3.04
25	1	0.025	1.33	2.50	3.04
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

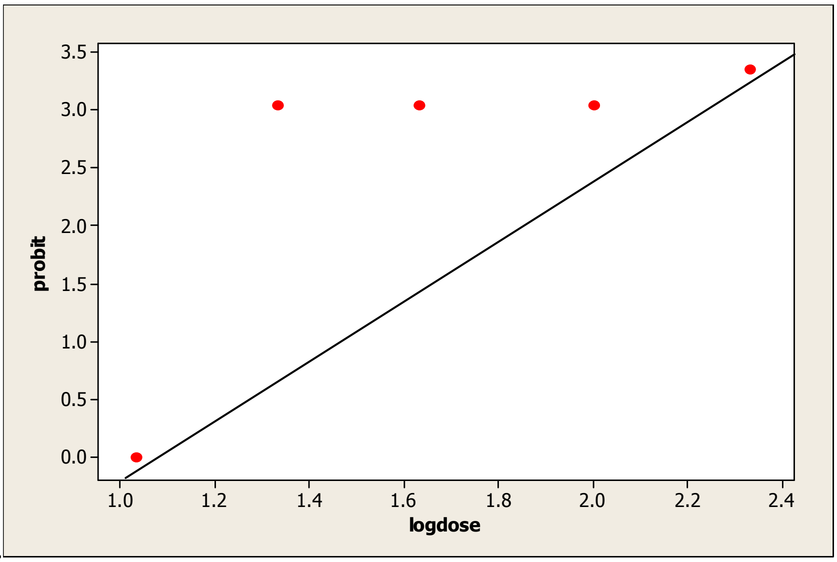

Fig. 2: probit/mortality graph of direct treatment of moringa oil on Tribolium Confusum. Probit = - 0.83 + 2.00 logdose, L.D50= 1.67.

Table 3: Average mortality of *Tribolium castaneum* treated with various concentration of moringa seed oil in impregnated filter paper (Residual application).

Dose in μl/ml	Total mortality	Average mortality	Log dose	% observed mortality	Probit value
200	1	0.033	2.33	3.33	3.16
100	1	0.033	2.00	3.33	3.16
50	1	0.033	1.63	3.33	3.16
25	0	0.00	1.33	0.00	-
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

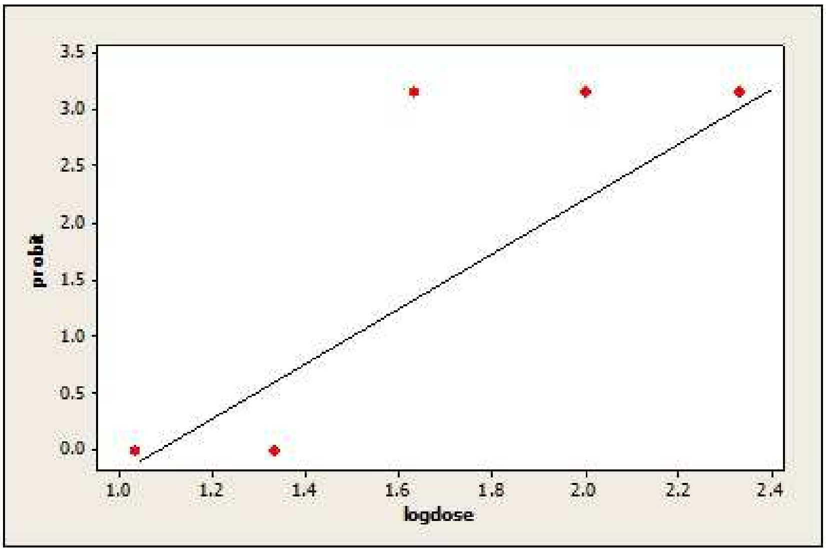

Fig. 3: probit/mortality graph of residual treatment of moringa oil on *Tribolium Castaneum* on impregnated filter paper. Probit = - 2.86 + 2.86 logdose, L.D50= 1.67.

Table 4: Average mortality of *Tribolium confusum* treated with various concentration of moringa seed oil on impregnated filter paper (Residual application).

Dose in μl/ml	Total mortality	Average mortality	Log dose	% observed mortality	Probit value
200	2	0.066	2.33	6.67	3.49
100	1	0.033	2.00	3.33	3.16
50	1	0.033	1.63	3.33	3.16
25	1	0.033	1.33	3.33	3.16
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

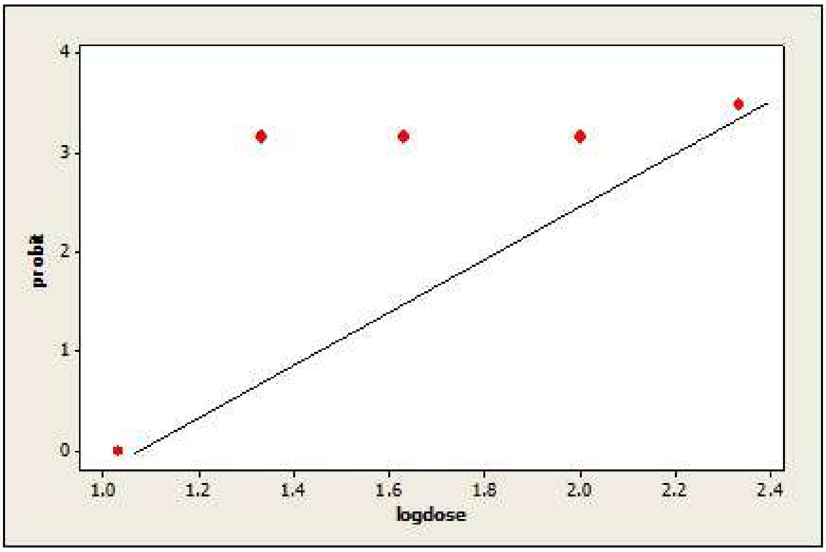

Fig. 4: probit/mortality graph of residual treatment of moringa oil on Tribolium Confusum on impregnated filter paper. Probit = - 0.86 + 2.08, logdose LD_{50}= 1.9µl/ml.

Table 5: Average repellency of *Tribolium castaneum* treated with various concentrations of moringa seed oil.

Dose in µl/ml	Total repellency	Average repellency	Log dose	% observed repellency	Probit value
200	2	0.050	2.33	5.00	3.36
100	2	0.050	2.00	5.00	3.36
50	1	0.025	1.63	2.50	3.04
25	1	0.025	1.33	2.50	3.04
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

Table 6: Repellency of *Tribolium confusum* treated with various concentration of moringa seed oil.

Dose in µl/ml	Total repellency	Average repellency	Log dose	% observed repellency	Probit value
200	2	0.050	2.33	5.00	3.36
100	1	0.025	2.00	2.50	3.04
50	1	0.025	1.63	2.50	3.04
25	0	0.00	1.33	0.00	-
12.5	0	0.00	1.03	0.00	-
Control	0	0.00		0.00	-

DISCUSSION

This study was to investigate the pesticidal activities of *Moringa oleifera* seed oil extract in the control of *Tribolium confusum* and *Tribolium castaneum* which are pests of milled maize and other flour products. The scope of the study also covers the repellency effect of the Moringa oil on the above mentioned insect pests. The observations from the results after 3 days (72 hours) period of revealed moringa seed oil activities to have a less significant mortality and repellency effect to *Tribolium castaneum* and *Tribolium confusum*.

Ali and Mohammed [1] noted that the value of maximum mortality of insect pests differs from plant to plant. As observed in their study, their results reached its maximum mortality value on *T.confusum*. In the case of leaf extracts of *Anethum graveolens*, it was 56.67% at 4.5 hrs exposure, *Apium graveolens*: 93.33% at 5 hrs, *Eucalyptus glauca*: 90% at 2 hrs, *Malva parviflora* : 96.67% at 3 hrs, *Mentha longifolia*: 93.33% at 4 hrs, and *Zingiber officinalis* 100% at 2 hrs. Mortality was noted with different plants and exposure times.

In this study, the value of maximum mortality of the insect pests to Moringa oleifera seed oil extract if far below the findings of the above cited authors. The different concentrations of moringa oil used were 200µl, 100µl, 50µl, 25µl, 12.5µl respectively. In the direct application of the moringa seed oil to *Tribolium castaneum,* little mortality was only recorded at the two higher concentrations, being 2.5% in each. None was recorded in others including the control. Such was seen on the same treatment on *Tribolium confusum.* 5% mortality occurred in 200µl/ml concentration while 2.5% occurred in 100µl/ml, 50µl/ml and 25µl/ml respectively. No mortality occurred in 12.5µl/ml and the control. The regression equation was used to determine the LD_{50} of both treatments being 1.78µl/ml and 1.67µl/ml respectively.

In the residual application at the various concentration levels*, i*n the first 3 days, mortalities were recorded at the 3 higher concentrations, while non occurred in control. They have uniform mortality of 3.3% in each case. For *Tribolium confusum* on residual application, mortality occurred in 4 concentrations being the highest in mortality studies of this research. 6.6%, 3.3%, 3.3%, 3.3% mortality was recorded in 200µl/ml, 100µl/ml, 50µl/ml and 25µl/ml respectively. In any of the control set up, the LD_{50} of the moringa oil on *Tribolium castaneum* on residual treatment is 1.67µl/ml while that of *Tribolium confusum* is 1.9µl/ml. complete mortality was not recorded in any concentration level.

The findings of this study were in sharp contrast to Okonkwo [7] who noted that 100% mortality of adults of *C. maculates* where achieved in 24 hours on filter paper treatment with moringa oil.

Tribolium species are classified among the least susceptible insect pests of stored-product and often more difficult to kill than other stored-product beetles, though the order of toxicity often varies depending on the particular insecticide [5].

The repellency studies followed a similar course. Within the first 3 days, 5% of *Tribolium castaneum* were repelled on 200µl/ml and 100µl/ml concentrations while 2.5% occurred in 50µl/ml and 25µl/ml. None occurred in the lowest concentration and control. Similar observation was made on *Tribolium confusum,* recording 5% in 200µl/ml and 2.5% 100µl/ml and 50µl/ml only while no repellency occurred in others. The result agreed with the findings of Pugazhvendan et al,[9] who tested the repellent activity of plant powders of A. mexicana, *T. purpurea* and *Prosopis juliflora* against *T. castaneum*. All the plant powder showed repellent activity at various levels except *P.juliflora* at 0.5mg concentration.

Not much has been done by other researchers to investigate the pesticidal activities of *Moringa oleifera* seed oil to *Tribolium confusum* and *Tribolium castaneum* on milled maize, so that these findings can be compared. Also, not much is known about moringa as a pesticide. Yet, the observations in this study showed that moringa seed oil has poor pesticidal effects to these insects, if compared with its insecticidal action on other insects [7].

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