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INSECTICIDAL POTENTIAL OF COMMERICAL NEEM-BASED INSECTICIDE, NIMBECIDINE EC AGAINST THE RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM*) IN THE LABORATORY

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Abstract

The red flour beetle, *Tribolium castaneum* is an important secondary pest of that feeds on a wide range of stored products worldwide. The current study was conducted to investigate the insecticidal potential of Nimbecidine EC oil derived from the seeds of the neem tree *Azadirachta indica* A. Juss. against *T. castaneum* using contact efficacy, flour treatment, female fecundity and population growth assays. The Nimbecidine EC was applied at three dosages (1, 3, 5 and 10% (v/v). The highest dosage of 3.0% of Nimbecidine EC oil tested killed 25.6% of larval stages within 6 days h on contact bioassay. Efficacy of Nimbecidine EC, varied according to the beetle's developmental stage. Early instar larvae were more susceptible compared with and adults at 6 days post-treatment. Nimbecidine EC caused about 63% reduction in total fecundity of the female adults. The results demonstrate that Nimbecidine EC can be used as a part of IPM programme to manage storage insect pests, however, further studies under commercial storage conditions are required.

Key words: Nimbecidine EC, female fecundity, IPM, neem, *T. castaneum*

Introduction

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is considered as one of the most damaging pest of stored products in the world, including Iraq due to its high infestation potential (Zettler and Cuperus, 1990). Both the larvae and adults cause damage. The greatest damage can be occurred during the hot and humid monsoon season as a result of rapid increasing its population. The larvae are negatively phototactic and are always found hidden in food (Nadeem *et al*., 2012).

Synthetic insecticides are still the main way to control *T. castaneum*. However, the extensive usage of chemical insecticides has been associated with many problems (Kavallieratos *et al*., 2017). These problems include mainly; insect resistance development coupled with the resurgence of treated primary insects, toxicity to man, animals and other non-target organisms, and environmental contamination (Koureas *et al*., 2012). Lessard *et al.,* (1998) reported resistance in *T. castaneum* against synthetic pyrethroids. These problems have encouraged the development of alternative methods of managing this insect pest in storage.

Botanical insecticides were used as attractive alternatives to insecticides for insect pest management, because botanicals are less harmful to the environment due to their biodegradation (Isman, 2006). Among the studied of commercial botanical insecticides, several studies have been reported the efficacy of neem oil as a potential insecticidal (Aarthi and Murugan, 2010), antifeedant, growth retardant (Gubara, 1983), and repellent against stored insect pests (Nasir *et al*., 1987). For example, Das *et al*., (2006) reported that Nimbicidine ® commercial neem-based insecticide significantly inhibited egg hatching, pupation and adult emergence of *T. castaneum*. Nadeem *et al*., (2012), found that neem seed extract caused 64.44% mortality of *T. castaneum* at a concentration of 10% after 72h.

Thus, the current study was aimed to evaluate the toxicity of different concentrations of Nimbecidine EC commercial neem-based insecticide against fourth instar larvae of *T. castaneum*, to investigate the insecticidal activities of Nimbecidine EC against different instar larvae, pupae and adults of *T. castaneum* and to investigate sub-lethal effects of Nimbecidine EC on the fecundity of individual *T. castaneum* adults, and population growth.

Meterials and Methods

Tribolium castaneum rearing

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The stock culture of *T. castaneum* originated from wheat grain stores in Najaf, Iraq, in 2014, and was maintained at the Entomology Laboratory, Faculty of Agriculture, University of Kufa, Najaf, Iraq. Individuals of *T. castaneum* were cultured in a controlled environmental room at 28 ± 1_C and 55–65% RH under continued darkness. Wheat flour sterilized at 60°C for 60-90 min. was used as food. Insects (50 male and female pairs) were reared on wheat flour placed in 500 ml glass jars covered with a muslin cloth and rubber bands. After 1 week of oviposition period, the adults were removed and the progeny that emerged subsequently were used for the different assays. Insects were re-cultured after every 10 weeks.

Nimbecidine EC

Nimbecidine EC is a commercial formulation of Azadirachtin EC (0.03%) (T. Stanes & Company Limited) was used. Nimbecidine EC is an extract of neem seed, which acts as repellent, antifeedant and growth regulator. The concentrations used in the present experiment were 1, 3, 5 and 10%. To obtain these concentrations, 1, 3, 5 or 10 ml of Nimbecidine EC was diluted in 100 ml of distilled water and 0.7 ml of emulsifier (Palmolive Ultra soap, Henkel Deutschland), where a preliminary experiment confirmed that this amount of emulsifier in water had on negative effect on all stages of *T. castaneum*. The suspensions were then agitating for 20 minutes on a magnetic stirrer, and were shaken again before they were used in experiments.

Bioassay

Three concentrations of Nimbecidine EC (1, 3, 5 and 10%) were tested. One to three days old *T. castaneum* fourth instar larvae were first transferred into Petri dishes (9 cm diameter) to refrigerator at 4°C for 10 min to reduce their activity to enable topical treatment to be carried out (Mohammed *et al*., 2019). For each concentration, Whatman-No. 1 filter paper (9 cm diameter) containing 50 fourth larval instars was placed individually in 9-cm diameter Petri dish and sprayed with 1 ml of suspension using a 750 ml trigger water sprayer (Ampulla, Hyde, UK). The application was made with a distance of 15 cm between the sprayer nozzle and Petri dish and they were kept at room temperature for 30 min to dry. Treated insects in each Petri dish were transferred using a camel hair brush to a new 9-cm Petri dish containing 2g of wheat flour placed on the bottom, a lid with 1-cm hole covered with nylon mesh, then they were sealed with Parafilm to prevent them from escaping and incubated at 28 ± 1°C and 55–65% RH for 6 days. Five petri dish replicates were used for each concentration and control. Insect mortality was recorded after 1, 2, 4 and 6 days. Individuals were presumed dead if they remained immobile and did not respond to three probings with a blunt dissecting probe after a 5 min recovery period (Adarkwah *et al*., 2010).

Effect of *T. castaneum* developmental stages on the insecticidal efficacy of Nimbecidine EC

Nimbecidine EC at a concentration of 10% was selected based on the level of *T. castaneum* mortality in the bioassay experiment. Different developmental stages of *T. castaneum* (second instars, third instars, fourth instars, fifth instars, pupa and adults) were obtained from the laboratory using the method described by Nadeem *et al*., (2011). Each developmental stage was replicated five times. The experimental protocol and assessment were identical to the bioassay described above.

***T. castaneum* mortality in wheat flour**

The effect of Nimbecidine EC-treated wheat flour on the mortality of fourth instar larvae and adults of *T. castaneum* was assessed in the laboratory. 10 ml of diluted Nimbecidine EC was mixed with 1 kg of wheat flour and stirred continuously on a mechanical roller for 15 min to ensure even spread of the material over the surface of the wheat flour. Then, the mixture was left for 3 days to ensure saturating the flour with neem oil. Three to five old-day *T. castaneum* adults of mixed sex (10 individuals per jar) were released in individual small glass jars with 5 g of treated wheat flour, covered with muslin cloth, and kept at the same conditions described above. In addition, one to three days old *T. castaneum* fourth instar larvae (10 individuals per jar) were released in individual small glass jars with 5g of treated wheat flour. In control treatment, wheat flour was mixed with distilled water only. Each treatment was replicated five times. Insect mortality was recorded after 1, 2, 4 and 6 days.

Effect of Nimbecidine EC on the fecundity of *T. castaneum*

To determine the sublethal effect of Nimbecidine EC on the fecundity of *T. castaneum* adult females, male-female (3–5 day old) pairs (each pair is a replicate) were introduced into each 9-cm Petri dishes containing Whatman No. 1 filter papers were sprayed with 1 ml of a concentration of 10%. Control treatment was sprayed with distilled water only. Each treatment was replicated 10 times. After 24 h, each female was transferred to individual sterile Petri dish with 1g of wheat flour covered with muslin cloth, secured using a rubber band and were kept at 28 ± 1°C and 65 ± 5% RH. The number of eggs produced by each female was recorded after 5 days using microscope eyepiece camera.

Effect of Nimbecidine EC on *T. castaneum*

population growth

Male female *T. castaneum* pairs (3-5 days-old) were (5 pairs per jar) were released in individual glass jars with 50 g of treated wheat flour, covered with muslin cloth, and kept at the same conditions described above for 40 days. In control treatment, wheat flour was mixed with distilled water only. Each treatment was replicated five times. The numbers of live adults, pupae and larvae were recorded.

Statistical analysis

The analysis was performed using GenStat software (VSN International 2016). Cumulative mortality was corrected for natural death in the control using Abbott's formula (Abbott, 1925). Normality of data distribution was assessed using the Shapiro-Wilk test. Corrected mortalities were logit transformed when necessary to meet the assumption of normality. For bioassay, one-factor repeated measurement ANOVA was used to determine the effect of different concentrations of Nimbecidine EC on the mortality of *T. castaneum*. One-factor repeated measurement was used to determine the effect of developmental stage on the mortality of *T. castaneum*. The effect of Nimbecidine EC on the fecundity of females was analyzed using one-way ANOVA. One-factor repeated measurement ANOVA was also used to determine the effect of Nimbecidine EC on the population build-up of *T. castaneum*. Mean comparisons were performed using LSD test at 5% level of significance ($P < .05$).

Results and Discussion

Bioassay

Table 1 shows the corrected mortality of *T. castaneum* later larval after exposure to different concentrations of Nimbecidine EC oil. The corrected mortality was concentration dependent with the highest concentration of 10% causing 25.6% mortality of *T. castaneum* after 6 days, which was significantly higher than 7.6% with the lowest concentration of 1% ($F4, 279 = 373.59$, $P < 0.001$). The effect of time after exposure on the percentage of corrected mortality was significant ($F4, 279 = 373.59$, $P < 0.001$), with a significant mortality rate recorded for all concentrations 6 days after treatment (Table 1). The interactions between developmental stages and time after treatment were significantly different ($P < 0.001$). *Tribolium castaneum* mortality in the control treatment was 3%.

The toxic effect of Nimbecidine EC was found to be dose and exposure time dependent. These results are in agreement with Athanassiou *et al.,* (2005) who assessed Neem Azal at 50, 100 and 200 ppm against *Rhyzopertha dominica* (F.), *Sitophilus oryzae* L. and *T. confusum* and found that mortality of the stored insects increased with increase in dose of neem oil and time of exposure. The effect of neem in controlling *T. castaneum* can be related to bitter compounds of neem that often have an antifeedant effect and can interfere with hormonal processes in insects (Schmutterer, 1990) or it may be related to lack of oxygen as a result of closing the spiracles with oil (Hall and Harman, 1991). The results obtained in the present study are consistent with the findings of several researchers who had demonstrated the toxic of different commercial formulations of neem against a wide range of stored insect pests (Isman 2006; Obeng-Ofori 2007; Mustafa *et al*., 2014). Adarkwah *et al*., (2010) found that Calneem® oil is provided a good protection of wheat grains and flour from weight loss caused by red flour beetle *T. castaneum*.

Effect of *T. castaneum* developmental stages on the insecticidal efficacy of Nimbecidine EC

The developmental stage of *T. castaneum* treated with Nimbecidine EC oil had a significant effect on the corrected mortality ($F_{3, 79} = 53.88$, $P < 0.001$), with the highest mortality 30% for the early instar larvae, compared with 19.8% for adults at 6 days after treatment (Table 2). The effect of time after exposure on the percentage of corrected mortality was significant ($F_{3, 79} = 130.99$, $P < 0.001$), with a significant mortality rate recorded for all developmental stages 6 days after treatment. The interactions between developmental stages and time after treatment were significantly different ($P < 0.001$). The mortality rate in control treatments ranged between 2 and 3.6%.

The variation in developmental stage susceptibility of *T. castaneum* to Nimbecidine EC neem oil may be explained by biochemical changes during host cuticle and/

Table 1: Effect of different concentrations Nimbecidine EC on the corrected mortality (means ± SE) of larval stages of *T. castaneum* after 1, 2, 4 and 6 days.

Concent-ration	Corrected mortality (mean ±SE)			
	1 day	2 days	4 days	6 days
1%	0 ±0.0aA	0.8 ± 0.1dA	4.8 ± 0.2dB	7.6 ± 0.5dC
3%	0 ± 0.0aA	2.8 ± 0.3cB	8.8 ± 0.4cC	12.4 ± 0.4cD
5%	0 ± 0.0aA	4 ± 0.1bB	10.4 ± 1.1bC	17.6 ± 0.9bD
10%	0 ± 0.0aA	9.6 ± 0.6aB	19.6 ± 0.8aC	25.6 ± 1.3aD

Means in the same column followed by different lowercase letters indicate significant differences; means in the same rows followed by different uppercase letters indicate significant differences in the values at $P < .05$, using LSD test.

or inhibiting the release of prothoracicotropic hormones and allatotropins by azadirachtin, which is the major component in neem, thereby affecting metamorphosis in *T. castaneum* (Banken and Stark 1997) This confirms the findings of Adarkwah *et al*., (2010) who reported low mortality of late larvae and pupae exposed to 0.5% Calneem® neem oil compared to early larvae of *T. castaneum*.

T. castaneum mortality in wheat flour

Mortality of *T. castaneum* larval instars and adults was affected by treatment and time of exposure (treatment: $F_{1, 39} = 22.57$, $P < 0.001$; time: $F_{3, 39} = 56.59$, $P < 0.001$). After 6 days, all treatments caused significantly higher mortality than control treatment (Table 3). For all treatments, mortality increased with increasing exposure time. The interaction between treatment and time of exposure was significantly different ($P < 0.001$).

Effect of Nimbecidine EC on the fecundity of *T. castaneum*

The fecundity of *T. castaneum* adult females was affected by Nimbecidine EC treatment ($F_{1,19} = 28.3$, $P < 0.001$). Beetles exposed to distilled water only (control) produced 18.9 ± 2.7 eggs, which was higher than those exposed to Nimbecidine EC at a concentration of 10% (7 ± 0.8 eggs). Lale and Mustapha (2000) reported significant reduction in egg laying in *Callosobruchus maculatus* treated with neem seed oil. In addition, neem oil dosages applied at lower dosages of 0.5–3.0% significantly reduced progeny production in *T. castaneum*, compared to the control. In our study, Nimbecidine EC reduced the total number of eggs laid per female of *T. castaneum* by about 63% compared to untreated females.

Table 2: Corrected mortality (means ± SE) of early larvae, later larvae, pupae and adults of *T. castaneum* treated with to Nimbecidine EC at a concentration of 10% after 1, 2, 4 and 6 days.

Treatment	Corrected mortality (mean ±SE)			
	1 day	2 days	4 days	6 days
Early larvae	2 ±0.1aA	14 ± 1.0aB	24 ± 2.2aC	30 ± 3.4aD
Later larvae	2 ± 0.1aA	4 ± 0.8bB	12 ± 1.3bC	25 ± 2.1bD
Pupae	0 ± 0.0bA	7.6 ± 0.2cB	18.2 ± 1.1bC	24.1 ± 1.5bC
Adults	0 ± 0.0bA	7.7 ± 0.3cB	13.1 ± 0.5cC	19.8 ± 0.8cD

Means in the same column followed by different lowercase letters indicate significant differences; means in the same rows followed by different uppercase letters indicate significant differences in the values at $P < .05$, using LSD test.

Table 3: Corrected mortality (means ± SE) of larval stage of *T. castaneum* exposed to Nimbecidine EC-treated wheat flour treated with to Nimbecidine EC at a concentration of 10% after 1, 2, 4 and 6 days.

Treatment	Corrected mortality (mean ±SE)			
	1 day	2 days	4 days	6 days
Larval stage	2 ±0.1aA	14 ± 1.0aB	24 ± 2.2aC	30 ± 3.4aD
Adult	2 ± 0.1aA	4 ± 0.8bB	12 ± 1.3bC	26 ± 2.1bD

Means in the same column followed by different lowercase letters indicate significant differences; means in the same rows followed by different uppercase letters indicate significant differences in the values at $P < .05$, using LSD test.

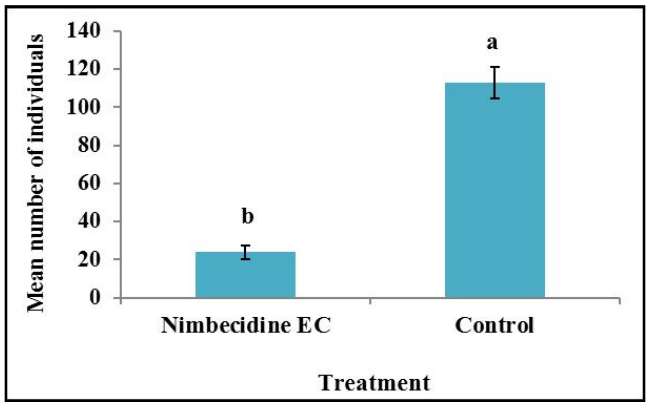

Fig. 1: Population growth level of *T. granarium* exposed to Nimbecidine EC-treated wheat flour, compared to those exposed to untreated wheat flour. Different letters above columns indicate significant differences in the values at $P < .05$, using LSD test.

Effect of Nimbecidine EC on *T. castaneum* population growth

The treatment of wheat flour with Nimbecidine EC significantly reduced the population growth of *T. castaneum*, compared to untreated wheat flour (control) ($F_{1, 19} = 41.34$, $P < 0.001$). The number of *T. castaneum* individuals in control with was larger than those exposed to wheat flour treating with Nimbecidine EC (Fig. 1).

The larger reduction in the population size of *T. castaneum* could not be explained by any of the known modes of action of neem: antifeedant effects and growth regulator effects (Saxena *et al.,* 1988). Antifeedant effects of neem on stored insect pests have been extensively studied. Saxena (1987) reported neem treated grain can disrupt insect feeding by making the treated grain unattractive or unpalatable which affect insect growth, survival and reproduction. Moreover, the reduction may due the negative impact of Nimbecidine EC on the fecundity of *T. castaneum* adult females and/or the possible repellency of neem.

It can be concluded that Nimbecidine EC might be useful as an alternative to synthetic insecticides for the control of *T. castaneum*. However, toxic effect of both plant extracts on *T. castaneum* was found to be dose and exposure time dependent. Further

experiments are required to be carried out in commercial storage conditions.

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