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Natural Enemies and the Effect of Neem Kernel Oil on Small Faba |Bean Beetle (Bruchidius incarnatus under Conditions of Northern State, Sudan

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Abstract
The lowest number of predators was found in Latty while the highest was found in EL Burgage. The parasites found were: egg and larval parasites. The predators found were a mite and an assassin bug. All concentrations of neem kernel oil reduced significantly number of eggs laid, percentages of eggs hatched, percentages of adult emergence and percentages of adult survival, while they are increased percentage of adult mortality. Number of eggs laid, percentages of eggs hatched, percentages of adult emergence and percentages of adult survival significantly decreased with increase of oil concentrations while percentage of adult mortality significantly increased.

Keywords: Mite, Concentration, Emergence, Survival and Mortality.

Introduction

Faba bean (*Vicia faba* L.) belongs to the family Leguminosae. Faba bean (*V. faba* L.) is one of the earliest domesticated food legumes in the world and it is a much appreciated food legume in the Middle-East, the Mediterranean region, China and Ethiopia. It is the most important legume crop and it is grown as an irrigated winter crop mainly in the northern part of Sudan (Northern State and Nile River State) and at higher elevation of Jebel-Marra (Salih, 1995 and Salih *et al*., 1995). The crop has a wide range of uses: as feed of poultry, the human consumption and as a lignocellulosic biomass to produce bioethanol and biogas. In addition, the crop contributes to soil fertility through biological nitrogen fixation. Faba bean crop is valued and considered a cash crop in Egypt and Sudan (Muehlbauer and Tullu1997; Duc and Marget, 2002 and Petersson *et al.*, 2007). In store, faba bean seeds are subject to attack by many coleopterous insects of the family Bruchidae such as *Bruchidius incarnatus* (Boh) which is an important pest of faba bean (Siddig, 1981; Cardona *et al*., 1985 and Ragaa, 2003). Most bruchid beetles are destructive to the seeds of leguminous crops which are vital source of protein for communities unable to obtain adequate

supplies of meat or fish (Amal, 2004). The damage caused by insects of store products can take many forms. The attack on the endosperm results in a loss of weight, a reduction in the seed ability to germinate, a reduction in the nutrients and deterioration of their quality. Ultimately, the specific gravity of seed decreases, lowering the market value of the product (Ragaa, 2003). The success of synthetic pesticides during the past decades had led to their wide spread acceptance for use against various groups of agricultural and public health pests. However, after 3-4 decades of extensive use, many adverse effects, such as resurgence of treated pests, toxicity to mammals and non-target organisms especially beneficial insects, environmental pollution, development of resistance, … etc were found to be associated with these chemicals (Fageer, 1999). In this context, botanical insecticides such as neem oil can offer a better alternative to synthetic pesticides, enabling safer control of pest populations. Among the botanical insecticides currently marketed, neem oil is one of the least toxic to humans and shows very low toxicity to beneficial organisms, so it is, therefore, very promising for the control of many pests. The oil is considered a contact insecticide (Cox, 2002). It has a broad spectrum of action, inhibiting feeding, affecting hormone function in juvenile stages, reducing ecdysone, deregulating growth, altering development and reproduction, suppressing fertility, sterilizing, repelling oviposition, and disrupting molting processes (Brahmachari, 2004).

These experiments were carried out with the main objectives of: the occurrence of faba bean beetle associated with natural enemies and to determine the effect of neem kernel oil on small faba bean beetle.

Materials and Method

The experiments were conducted for the years 2013-2015 at Dongola- Northern State-Sudan- located within latitude 16° and 22° N and longitude 20° and 32° E (Mukhtar *et al.,* (2013). For rearing and culture techniques the original stock culture of *B. incarnatus* was derived from a stock culture maintained on faba bean at Dongola, Sudan at room temperature since 2013. Ten pairs of adults were introduced to 2 kg kilner jars furnished with 2kg of faba bean (Beladi variety). The faba bean used in the culture was from the 2013/2014 season that was initially fumigated in storage in Dongola with phostoxin. Every three months the culture was renewed maintaining the adults emerging from the same lot to prevent crowding and mould growth. Only during the winter months when the temperature was low, it was necessary to place the cultures in an incubator maintained at 30°C. Temperature and R.H. were continuously recorded using a thermohygrograph.

Field survey:- To determine the natural enemies of small faba bean beetle in the Northern State this trial was carried out. Hence, the Northern State was divided into three areas, the three areas of the Northern State were surveyed. These were El Borgag, Dongola, and Latty. In each of three areas five pairs of small faba bean beetle were put in a glass jar (440 ml) contains 250 g faba bean Beladi variety. The jar was locked tight lock with wire dictate, this treatment was replicated four times in each area. Jars were checked every week for a year to collect and record names of the predators and parasites of the insect which mentioned above and their incomplete stages. The collected natural enemies were put in bottles containing chloroform to kill them. Predators and parasites that have not been identified were sent to Entomology Department – Faculty of Agriculture – Shambat - Khartoum University for identification.

Laboratory experiment:- To study the effect of neem kernel oil on small faba bean beetle (*B. incarnates* (Boh)), the insects were

reared under laboratory conditions at room temperature, the relative humidity was maintained at 70 - 75 RH. by using sodium chloride solution and container full of water. Beladi variety of faba bean which is used for testing was kept in freezer for one week to be sure that it is clean from infestation. To prepare neem kernel oil about 200 grams of yellow neem seeds (ripe fruits) were gathered from Dongola area in season 2015. Then the outer layer of pulp was removed. The seeds were cleaned and spreaded out in the shade on paper sheets to dry for 3-4 days. The seeds coats were break away and the kernels inside were exposed. Then, the kernels were pounded by a mortar (Moulinex type AW5 No- 05118) until they were become a fine powder. The powder was kept in light-proof container, a tin with appropriate tight lid. The powder of seed kernel was then extracted by 400 mls N. Hexane using a soxhlet apparatus. Then the solvent was evaporated under reduced pressure using a rotary evaporator. The obtained crude oil was kept in an amber glass bottle, tightly wrapped with aluminum foil and stored in laboratory until need for experiment (Shazali, 1990). To apply experimental procedures, neem kernel oil (NKO) with concentrations of 0.5, 1.00, 2.00, 3.00, 4.00 and 5 ml/kg were pipetted on undamaged faba bean seeds in large storage jars (440 ml). The treated seeds were then shaken well for ten minutes to have a good coverage of the seed surface. About 250 gm of treated seeds from each concentration of NKO, and the control (untreated seeds) with four replications were put in glass jars (440 ml). *B. incarnatus* insects were obtained from cultures maintained on faba bean at 30°C and 70% RH. Every treatment was infested with 3 pairs of insects (0 -24hours old). Then the jars covered with muslin cloth and immediately after treatment. The adults left to lay eggs and after two weeks the adults were removed and the number of eggs laid on each seed, and those that had hatched were counted and recorded. Eggs that turned white and opaque were judged to be hatched. Seeds were then returned to their jars and the insects were allowed to develop. The number of insects emerged were counted and removed every day until no adult emerged in five consecutive days about six weeks from the day of infestation. So it was possible to count and record the following:

1. The number of eggs laid per female.
2. The number of hatched eggs.
3. The number of adult emerged.
4. The number of adult survival.
4. The percentage of adult mortality.

Evaluation of the biological efficiency of the neem kernel oil was based on the percentage mortality. The percentage mortality was calculated as follows:

$$\text{Percentage mortality} = \frac{\text{Number of adult emerged - number of adult survival}}{\text{Number of adult emerged}} \times 100$$

All the experiments were arranged in a randomized complete block design (RCBD) with four replications. The collected data were subjected to standard procedures of statistical analysis. The procedure described by Gomez and Gomez (1984) was used to estimate analysis of variance.

Results and Discussion

The lowest number of predators was found in Latty while the highest was found in EL Burgage. The natural enemies found were *Uscana semifumipennis*, Gir (egg- parasite), *Anisopteromalus calandrae*, How (larval-parasite), a mite and assassin bug *Xylocoris flavipes* (predators).

All concentrations of neem kernel oil reduced significantly number of eggs laid per female compared to the control (Table 1). The lowest mean number of eggs laid by *B. incarnatus* (13.00) on seeds treated with neem kernel oil was achieved by the rate of 5.0 ml/kg seeds. However, the highest mean number of eggs

laid by *B. incarnatus* (53.00) on seeds treated with neem kernel oil was achieved by the rate of 0.5 ml/kg seeds. Results indicated that the number of eggs laid per female decreased with increase of oil concentrations (Table 1). This result is in line with that obtained by Ivbijaro (1983) who reported that, a significant reduction in egg laying was found in a cowpea which mixed with Neem seed. The significant reduction in oviposition with high application of Neem kernel oil demonstrated the deterrence or avoidance of treated seed for oviposition. It is likely that the oil layer surrounding the seed deter the female from depositing their eggs on the oily surface. Also this result is comparable to that found by Siddig (1991). All concentrations of neem kernel oil reduced significantly percentages of eggs hatched compared to the control (Table 1). The lowest mean percentages of eggs hatched (00.00) on faba bean seeds treated with neem kernel oil was achieved by the rates of 3.00, 4.00 and 5.00 ml/kg seeds. However, the highest mean percentage of eggs hatched (9.50) on seeds treated with neem kernel oil was achieved by the rate of 0.5 ml/kg seeds. Results indicated that the percentage of eggs hatched decreased with increase of oil concentrations (Table 1). These results could be attribute to the physical mode of action of neem oil and it acts as ovicidal by blocking egg pore (micropyle) causing anoxia with a lethal critical efficacy, which increase in molecular weight and viscosity. Similar results were found by Siddig (1991); Schumtterer (1995); Mahdi (2002) and Abdel-Shafy and Zayed (2002).

Table (1) Mean number of eggs laid and percentage eggs hatched of *B. incarnatus* (Boh.) on faba bean seeds treated with different concentrations of neem kernel oil

Neem kernel oil concentration (ml/kg seeds)	Eggs laid per female	Percentage of eggs hatched
0.5	53.00 b	9.5 b
1.0	51.25 bc	1.75 c
2.0	46.25 c	1.00 c
3.0	35.50 d	0.00 d
4.00	24.31 e	0.00 d
5.0	13.00 f	0.00 d
Control	60.75 a	92.00 a
SE ±	1.93	1.54
CV%	8.93	6.22

Means with the same letters in the same column are not significantly different at 5% level of probability to Duncan's Multiple Range Test (DMRT).

All concentrations of neem kernel oil reduced significantly number of adult emergence compared to the control (Table 2). The lowest mean number of adult emergence (00.00) on seeds treated with neem kernel oil was achieved by the rates of 2.00, 3.00, 4.00 and 5.00 ml/kg seeds. However, the highest mean number of adult emergence (58.50) on seeds treated with neem kernel oil was achieved by the rate of 0.5 ml/kg. Results indicated that the number of adult survival decreased significantly with increase of neem kernel oil concentrations (Table 2). The lowest mean number of adults survival (00.00) on seeds treated with neem kernel oil was achieved by the rates of 2.00, 3.00, 4.00 and 5.00 ml/kg. However, the highest mean number of adult survival (9.1) on seeds treated with neem kernel oil was achieved by the rate of 0.5 ml/kg (Table 2). In addition to the above

mentioned points, Neem oil has insecticidal properties due to the active compound that has been isolated from the Neem tree and also there were other effects include repellent and growth distributing effect.

All concentrations of neem kernel oil increased significantly percentage of adult mortality compared to the control (Table 2). These findings are in line with those obtained by Abdel-Shafy and Zayed (2002). The highest mean percentage of adult mortality (100.00) on seeds treated with neem kernel oil was achieved by the rates of 2.00, 3.00, 4.00 and 5.00 ml/kg. However, the lowest mean percentage of adult mortality (84.44) on seeds treated with neem kernel oil was achieved by the rate of 0.5 ml/kg. (Table 2).

Table (2) percentages of adults emergence, adults survival and percentages mortality of *B. incarnatus* on faba bean seeds treated with different concentrations of Neem kernel oil

Neem kernel oil concentration (ml/kg seeds)	Number of adults emergence	Number of adults survival	Percentages of adults mortality
0.5	58.50 b	9.1 b	84.44 b
1.0	33.80 c	4.75 bc	85.95 b
2.0	0.00 d	0.00 d	100.00 a
3.00	0.00 d	0.00 d	100.00 a
4.00	0.00 d	0.00 d	100.00 a
5.0	0.00 d	0.00 d	100.00 a
Control	94 a	100.00 a	0.00 c
SE ±	1.90	1.97	1.59
CV%	7.20	6.60	5.86

Means with the same letters in the same column are not significantly different at 5% level of probability to Duncan's Multiple Range Test (DMRT).

Conclusions

1. All concentrations of neem kernel oil reduced significantly number of eggs laid per female, percentage of eggs hatched, number of adult emerged and number of adult survival and significantly increased percentage of adult mortality.
2. The highest mean percentage of adult mortality (100.00) on seeds treated with neem kernel oil was achieved by the rates of 2.00, 3.00, 4.00 and 5.00 ml/kg.

Recommendations

1. Use of neem kernel oil at the rate of 2.00 ml/kg seeds at least to control this insect.
2. Application of integrated control programs on store insect pests.

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الأعداء الطبيعية وتأثير زيت بذرة النيم علي خنفساء الفول الصغيرة(*Bruchidius incarnatus*) تحت ظروف الولاية الشمالية – السودان

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المستخلص

أقل عدد للمفترسات وجد في لتي بينما أعلاه وجد في البرقيق . المتطفلات التي وجدت كانت متطفلات بيضية ويرقية. المفترسات التي وجدت كانت العثة والبق السفاك. كل تراكيز زيت بذرة النيم قلل معنوياً عدد البيض لموضوع، النسبة المئوية للبيض الفاقس، النسبة المئوية للحشرات البالغة الخارجة و النسبة المئوية لبقاء الحشرات البالغة بينما زاد معنوياً النسبة المئوية لموت الحشرات البالغة. عدد البيض الموضوع، النسبة المئوية للبيض الفاقس، النسبة المئوية للحشرات البالغة خارجة و النسبة المئوية لبقاء الحشرات البالغة نقص معنوياً بزيادة تراكيز زيت بذرة النيم بينما زادت معنوياً النسبة المئوية لموت الحشرات البالغة.