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Biocidal Effect of Ethanolic and Aqueous Extracts of Some Plants on adult worker of Honey Bee *Apis mellifera L.* (Hymenoptera: Apidae)

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Abstract

Laboratory experiments were conducted at the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology (SUST). This study aimed to evaluate the effects of ethanolic extracts of (neem seeds and cafur leaves) and aqueous extract of clove buds, on non-target beneficial insect, the adult workers of honeybee *(Apis mellifera sudanensis)*. Three different concentrations of each plant extracts were used (2.5% 5%and 7.5%). They mixed with honey syrup and then used as food bait for honeybee workers. The mortality was recorded after 24hr, 48hr, 72hr, and 7 days of feeding.

The results demonstrated that the high concentration of neem seed extract (NSE) 7.5% caused the highest mortality percentages (80% and 100%) to *A. m. sudanensis* workers, after 72 hours and 7 days respectively. During the four assessment periods the high concentrations of each tested plant caused highest mortality percentage to the workers than the low concentrations.

The result also showed that, the workers which fed on honey syrup that containing high concentrations of neem seeds and cafur leaves extracts, obtained a mortality percentages increased within the elapse of time. While in the case of clove buds extract, the activity rate of workers decreased according to the decrease of toxicity after 72 and 7 days of feeding.

The results concluded that, the highest doses of neem seed extract could be considered as toxic to honey bee workers and should be avoid to be sprayed on field crops for pest control in area that containing honey bee hives. In addition, the low concentration (2.5%) of clove buds extract could be considered as safe and less toxic after 7days of feeding, accordingly it could be sprayed for pest control on field crops (before flowering by 7 days) in area that containing honey bee hives.

Key wards: ***Apiss mellifera, Biocidal effect, Honey bee worker, Natural extracts.***

1. Introduction

Honeybee is vital for pollination and ecological services, boosting crops productivity in terms of quality and quantity and production of colony products: wax, royal jelly, bee venom, honey, pollen and propolis. In addition, honeybees are most important plant pollinators and almost one third of diet depends on bee's pollination, worth billions of dollars (Ullah *et al*., 2021). Estimated the value of increased yield and quality of crops, due to pollination by honeybees, in the United States of America, for the year 2000, at US$14.6 billion. (Anonymous, 2013).Western honeybees, *A. mellifera* are important pollinators of a wide variety of agricultural crops , increasing both the quality and yield of crop plants. In addition, it is the most widely used insect for crop pollination (Klein *et al*., 2007, Garibaldi, *et al*., 2013, and Hung *et al*., 2018).

In Sudan, bee products can enter the national economy by 100% percentage, but there are difficulties facing the entry of the hproduct in the economic circle, the most important one is the marketing methods, which constitute 44.8% (ELhassan, 2019).

The bee pollination services yield substantial economic benefits for the agricultural production. However, in recent years, the bee population has decreased throughout the world (Lebuhn, *et al*., 2013). Several factors have been considered as the potential causes of this decline, for instance, the use of organic synthetic pesticides (Van der Sluijs ,*et al*., 2013). The natural products, such as botanical insecticides, might have repellent effects, inhibit oviposition, and change the feeding and hormonal systems of several arthropod pests These characteristics made the use of these bio pesticides attractive for pest management (Copping and Menn., 2000; Isman, 2006; Seiber, *et al*., 2014). The use of synthetic and botanical pesticides has detrimental effects to both natural enemies and pollinators in agricultural fields (Ndakidemi, *et al*., 2016). Due to the increasing use of botanical insecticide, the main objective of this study was to evaluate the effects of three botanical extracts on honey bee workers during their used as a control agents against pests of blooming trees and field crops that contains honey bee hives.

Objectives

Evaluate the biocidal effect of neem (*Azadirachta indica A. juss*) seeds ethanolic extract, cafure (*Eucalyptus camaldulensis*) leaves ethanolic extract, and clove (*Syzygium aromaticum*) buds aqueous extract, on honey bee workers (*Apis mellifera sudanensis*) as beneficial insect.

2. Materials and methods

2.1 Experiment site

The study was conducted at Laboratory of Entomology, College of Agricultural Studies, Sudan University of Science and Technology (SUST), Khartoum North, Shambat, during January to March 2023. The study included an investigation and a comparative studies of the effect of three botanicals, neem seed ethanolic extract, cafur leave ethanolic extract, and clove buds aqueous extract, on adult workers of honey bee.

2.2 Preparation of plant materials

Drier seeds of neem were obtained from the Department of Alternatives of Pesticides and Fertilization at the Environmental and Natural Resources and Desertification Research Institute (ENRDRI), National Center for Research (NCR).The neem seeds were crushed and powdered by using an electric blender (brand Panasonic).

In addition, healthy leaves of cafur plant were collected from experimental farm, College of Agricultural Studies, (Khartoum North). The leaves were washed and dried under shade for 5-7 days under room condition, and then they were grounded by using an electrical blender. While, buds of clove were brought from a local market in Omdurman, Sudan. Thereafter the buds were grounded using an electrical blender.

The prepared powder of each tested plants then were stored in plastic bag at room temperature until extraction.

2.3 Extraction of plant materials

To prepare ethanolic extract of neem seeds and cafur leaves, 100 gm powder of each material was placed in a separated conical flask (500ml). Then 100ml of ethanol 96% were added to each flask and they were placed in a shaker settled at 120-150 round per minute (for agitation of mixture) for 16 hrs at 25-35 ℃. After that, the solution was filtered using filter paper in a conical flask. The solution was then stored in a refrigerator until the experiment setup.

One hundred grams of prepared powder of clove buds were placed in a conical flask. and dissolved in amount of 100 ml of distilled water were added to the flask and then the flask was placed in a shaker settled at 120 to 150 rounds per minute (for agitation of mixture) for 16 hours at 25-35℃. Then the mixture was filtered by filter paper in a conical flask and stored in refrigerator until experiment setup.

2.4 Preparation of the concentrations

The extracts concentrations were prepared from the previous above mentioned stock solutions. To prepare 100% concentration for each botanical, amount of 100gm of each botanical extract (neem seeds and cafur leaves) were added to 100ml ethanol in a separated flask. While, 100gm of clove buds extract were added to 100ml distilled water.
From the above concentrated extracts, 20% concentration was prepared by dissolving 20ml of each of the stock solution in 80ml distilled water, thereafter, the three sub concentrations were prepared (2.5, 5 and 7.5%) by diluting the stock.

2.5 The insect feeding and treatments

Adults of honeybee workers *Apis mellifera sudanensis* (360 workers) were collected from a hive that obtained from Bahri, (Khartoum North) Sudan. These healthy workers, were tested and treated (feeded) in 36 plastic plates at the rate of 10 adults in each plate. The first nine plates containing syrup of honey bee mixed with neem seed ethanolic extracts, the second nine plates containing powder of cafure leaves, the third nine plates containing water extract of clove buds. In addition, the last nine plates containing botanical extracts without honeybee syrup as three plates for each plant as a control. Three different concentrations (2.5, 5 and 7.5%) were used for each treatment. All the plates then were covered, with muslin cloth for ventilation, and thereafter they kept under laboratory condition. The experiment were conducted using three replications. The mortality of feeded workers, was recorded after 24, 48 and 72 hours, and also after 7 days of feeding.

2.6 Data analysis

The experiments were designed in a Completely Randomized Design (CRD). The obtained data was statistically analyzed according to Analysis of Variance (ANOVA). The data were analyzed by Statistic 8.0 program software, and means were separated using Duncan Multiple Range Test (DMRT) at $P<0.05$. Data were transformed using arcsine.

3. Results

3.1 Effect of plant extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 24 hours of feeding.

The relative quantity of main effects and related interactions after 24h were significant at $P < 0.05$ as demonstrated in (Table 1). The high toxicity to adult honeybee worker was caused by concentration 7.5 % of neem seeds ethanolic extract, which gave 23.3% adult mortality, followed by the same concentration 7.5% of clove buds ethanolic extract which achieved 20% of adult mortality. However, extract of cafure leaves at highest concentration (7.5%) achieved only 16.6% of adult mortality which is similar to the percentage of adult mortality that achieved by concentration 5% of clove water extract where significantly not different with the effect of other treatments. Same adult mortality percentage (13.3 %) was caused by, water clove extract at concentration 2.5%, neem seeds extract at concentration 5%, and cafur leaves extract at concentration 5%. The lowest percentage of adult mortality (10%) after 24h of feeding was given by the same concentration (2.5%) of neem seeds extract and cafur leaves extract. At the low concentrations, 2.5 and 5 %, clove extract showed high

toxicity to adult honeybee workers compared to the same low concentrations for the other two botanical extracts (neem and cafur).

Table (1). Effect of some botanical ethanolic extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 24 hours.

Concentrations (%)		**Mortality (%)**	
	Neem	**Clove**	**Cafure**
2.5	10^{ab}	13.3^{ab}	10^{ab}
5.0	13.3^{ab}	16.6^{ab}	13.3^{ab}
7.5	23.3^{a}	20.0^{a}	16.6^{ab}
Control	0.7^{b}	0.7^{b}	0.7^{b}
C.V: 3.8 S.E± : 5.1 LSD :18.2			

Means followed by the same letter are not significantly different at (P < 0.05).

3.2 Effect of plant extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 48 hours of feeding.

The majority of main effects after 48h were also significant at $P < 0.05$. The results stated that, the highest mortality percentage (60%) was achieved by neem seed ethanolic extract at the highest concentration 7.5%, which was significantly different with the effect of the other two concentrations and the control. While the lowest percentage of mortality was caused by cafur ethanolic extract at the lowest concentration 2.5%. In addition, clove water extract achieved mortality percentages of (53.3% and 40%) at the two concentrations 7.5% and 5.0% respectively (Table 2). These results were also differ significantly with the control. The lowest adult mortality percentages (13.3%) was obtained by cafur ethanolic extract at the lowest concentration 2.5%, this result was not significantly different with the adult mortality that achieved by control. The results stated that, after 48 hours of feeding, neem seed extract was more toxic compared to other two botanical extracts.

Table (2): Effect of some botanical ethanolic extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 48 hours.

Concentrations (%)		**Mortality (%)**	
	Neem	**Clove**	**Cafure**
2.5	20.6^{cd}	30.0^{bcd}	13.3^{de}
5.0	30.0^{bcd}	40.0^{abc}	33.3^{bcd}
7.5	60^{a}	$53.3.^{ab}$	36.6^{abcd}
Control	0.7^{b}	0.7^{b}	0.7^{b}
C.V: 25.2 S.E± : 6.6 LSD : 23.6			

Means followed by the same letter are not significantly different at (P < 0.05).

3.3 Effect of plant extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 72 hours of feeding.

Results after 72h of workers feeding, obtained means of mortality significantly different as represented in (Table 3). The highest percentages (80% and 70%) of honeybee adult mortality were achieved by neem seed ethanolic extract at the two upper concentrations (7.5% and 5.0%) respectively. However, the lowest adult mortality (36.6%) was obtained by clove water extract at the lowest concentration 2.5%, even so, it was significantly different with the result that obtained by control.

The ethanolic extract of cafur leaves obtained adult mortality percentages (66.6% and 60.0%) at the two highest concentrations (7.5% and 5%) respectively after 72 h of feeding. No significant differences were noticed between these results and the results that achieved by clove water extract at the same higher concentrations.

All the results that obtained after 72 hours of adult honeybee feeding, by the different botanical extracts were significantly different with the results obtained by control. All treatments gave higher mortality compared to control.

In addition, it is clear that, higher concentrations gave higher toxicity and mortality. The toxicity and mortality percentage of adult worker *A. m. sudanensis* increased with the increase of concentrations of neem seed extract within the time pass.

Table (3): Effect of some botanical ethanolic extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 72 hours.

Concentrations (%)		**Mortality (%)**	
	Neem	**Clove**	**Cafure**
2.5	46.6^{cde}	36.6^{e}	40.0^{de}
5.0	70.0^{ab}	56.6^{bcd}	60.0^{bc}
7.5	80.0^{a}	$60.0.^{bc}$	66.6^{ab}
Control	3.8^{f}	3.8^{f}	3.8^{f}
C.V: 25.2 S.E± : 6.6 LSD : 23.6			

Means followed by the same letter are not significantly different at ($P < 0.05$).

3.4 Effect of plant extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 7days of feeding

Results after 7days of feeding indicated that, complete mortality (100%) was achieved by neem seed ethanolic extract at the highest concentration 7.5%, which was significantly different compared with the other all treatments and control. The highest concentration (7.5%) of cafur leaves extract obtained adult mortality (73.6 %), while the toxicity and mortality achieved by water clove extract, was decreased within the time elapse and obtained 53.3% adult mortality of honeybee worker *A. m. sudanensis* at the same concentration. In addition, the lowest concentration of water clove extract achieved low mortality percentage (26.6%) (Table 4).

The above mentioned results stated that, mortality percentage of honeybee worker due to the concentration of neem seed extract and cafur leave extract, was increased with the increase of the time pass compared to the clove buds extract where the mortality percentage decreased within the time elapse.

Significant differences were observed between all treatments where al lof them gave higher mortality than the control.

Table (4).Effect of some botanical ethanolic extracts on mortality of honey bee (*A. mellifera. sudanensis*) adult workers after 7 days.

Concentrations (%)		**Mortality (%)**	
	Neem	**Clove**	**Cafure**
2.5	56.6^{cd}	26.6^{e}	53.3^{cd}
5.0	76.6^{b}	46.6^{d}	63.3^{bc}
7.5	100.0^{a}	53.3^{cd}	73.3^{b}
Control	3.8^{f}	3.8^{f}	3.8^{f}
C.V: 9.8 S.E± : 4.4 LSD : 15.7			

Means followed by the same letter are not significantly different at (P<0.05).

4. Discussion

Honey bee *(Apis mellifera L.*) is the principal pollinator of cultivated plants. With the increased importance on organic agriculture, the use of botanical insecticides has also enlarged. However, the effects of these products on bees remain to be observed (Xavier *et al*., 2015).

The use of botanical insecticides has recently been promoted as an alternative pest control method, especially in crop systems where the conventional synthetic insecticides have limited use, such as in agro-ecological farming and organic agricultural systems (Isman, 2006; Seiber *et al*., 2014). However, botanical insecticides might cause adverse effects to non-target organisms such as bees. In addition, the use of synthetic and botanical pesticides has damaging effects to both natural enemies and pollinators in agricultural fields (Xavier *et al*., 2015, Ndakidemi *et al*., 2016). Whereas, bees can be contaminated with botanical insecticides during their repeated foraging visits to flowers in sprayed fields (Koch and Weiber, 1997).

This study aimed to investigate and compare the effect of two botanicals ethanolic extracts, neem seed and cafur leaves and water extract of clove buds powder on non-target beneficial insects honey bee adult workers. The present study showed that highest

Concentration of neem seed extract (NSE) 7.5% caused highest mortality percentage of 80%, 100% to adult workers, after 72 hours and 7 days respectively.

The present results are in agreement with Rembold *et al*., (1980) who found that neem oil extracts were acutely toxic to immature honeybees. In addition, Efrom *et al*., (2012) found a significant increase in the mortality of adult workers with an increased exposure time of the bees to different concentrations of neem oil. In addition, Telles, *et al*., (2020), studied the efficiency of four natural products for greater wax moth control, among them

was neem oil. They also evaluated their effects on adult bees and on the population growth of colonies. The results showed that, the neem oil caused wax moth mortality at low concentrations, but did not affect colony population growth. However, they did have a deadly result on adult bees.

In addition, neem seeds extract more toxic than cafur leaves extract and clove buds extract, and caused highest mortality percentage to adult honeybee workers. While cafur leave extract decreased the activity rate of adult worker. Moreover, the percentage of mortality was found increasing with the increase of the time elapse.

The result of this study indicated that, the adult workers feed on syrup of honey that containing higher doses (7.5%) of neem seed extract and cafur leave extract gave high percentages of mortality than the clove buds extract after 72hrs of feeding for neem and 7 days of feeding for cafur.

Several botanical insecticides widely used for crop protection against pests (e.g., Neem) and as other less used for this purpose caused lethal and sub-lethal effects on larvae and adult honey bees *A. mellifera*. Xavier *et al*., (2015) assessed the acute toxicity and subl-ethal behavioral effects of some botanical insecticides such as neem oil on honey bees, *A. mellifera*. They stated that, neem oil induced toxicity to both larvae and adult worker bees. Moreover, it was repellent to adult workers, and it also reduced the rate of walking activity in adult workers.

Potrich, *et al*., (2020) find out the effects of the aqueous extracts of some plants on Africanized *A. mellifera* workers. Their results showed that, through all the bioassays, all plant extracts decreased the life of the workers. In addition some of the tested plant caused morphometric modification in the cells of the midgut.
In a contrast, Pereira, *et al*., (2020) reported that, africanized is sensitive to synthetic insecticides, but may not be sensitive to botanical extracts. They determined the toxicity of botanical extracts and their essential on the bees *Apis mellifera*. Moreover, they explained that, although some extracts were toxic to bees, survival was always higher compared to the results get with the insecticide, which was fatal to 100% of bees. Their results also indicated that, there was no difference in intake of food including plant extracts or food comprising water only. In addition, they did not observe any harmful effects of the botanical extracts on bee respiration rates or flight. They concluded that, botanical extracts were selective to *A. mellifera*, and hence, have the potential for eco-friendly pest control. Moreover, some natural plant products have been studied by Ebert, *et al*., (2007) for potential use as in-hive fumigants for suppression of parasitic mites and other pests. They tested ten different products among them was clove oil. Their results indicated that, the tested products could all be used safely for treating bees orally if the product dosage is cautiously managed in the bee hive. Naumann, *et al*, (2012) tested standard, oil-free neem (*A. indica* A. Juss) seed extract (NSE) for repellency to honey bees. Their results concluded that, honey bees may be with success used in blooming crops that have been treated with doses of NSE adequate to manage herbivorous insect pests.
The difference in the results may be due to the differences in environment, or due to the difference in the type of extracts used, they used oil extracts while in this research ethanolic and aqueous extracts were used.
Based on the present result, the high concentrations of neem seed extract can be consider as toxic and not safe for adult honeybee worker. However, clove buds water extract at low doses after 72 hour and 7 days, could be consider as less toxic and safe for honeybee worker, when it used before flowering by 7 days.

These founding indicated that, plant extracts supposed to be utilized in beekeeping, should be well-tried on all improvement phases of honey bees. However, there must be an equilibrium between their toxicity to hive pests and their safety to the bees. Nevertheless, more experiments have to be done, in order to confirm these results.

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