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Influence of insecticides on cowpea aphid, *Aphis craccivora* (Hemiptera: Aphididae) and its host plant faba bean (*Vicia faba*) compared to natural oils

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Abstract
Using of insecticides to control different insects attacking bean plants causes great effects on the ecosystem. Also, glitches in environmental balance, in addition to pollution in air, soil, and water. In this study appears effects of neem oil, lemon oil, acetamiprid, and thiamethoxam on *Aphis craccivora* Koch**.** (Hemiptera: Aphididae) insects and on its host plant faba bean. Results indicated that lemon oil, acetamiprid, and thiamethoxam were more effective in toxicity on *A. craccivora* compared with neem oil was 42.63% after 72 hrs, while lemon oil was 70.00% after 72 hrs. The data in this work showed to neem oil and lemon oil were less phytotoxic than acetamiprid and thiamethoxam on bean leaves. The percentage of fertility results showed that values, in general, decreased as compared to the control with water (without any treatment) and there was a significant difference between control and all treatments, and there was a significant difference between all treatments, so the highest pollen fertility percentage (96.40%) was recorded in the control then the next treatment with lemon oil (94.41%). Also, the treatment with Neem oil (93.10%) respectively. but pesticide thiamethoxam and acetamiprid were recorded (75.3%) and (72.50%) The negative effects on pollen grains sterile were estimated and compared to control, also there was a significant difference between control and all treatments, and there was a significant difference between all treatments, the highest pollen sterile percentage were in pesticides. Acetamiprid was recorded in total sterile pollens (25.30%) and then thiamethoxam total pollens (22.20%). While the natural oils recorded less effect than insecticides where neem oil was recorded (6.20%). Then lemon oil its recorded (4.30%) and in the end, it was the control (3.60%). Also, the different shapes of cells that appeared during the examination with a light microscope, also there was a significant difference between control and all treatments, and there was a significant difference between all treatments, this type of cell register in the treatment with insecticides thiamethoxam **(**2.50%) and acetamiprid record (2.20%) then lemon oil (1.30%) and Neem oil (0.70%) not found in control.

Keywords: faba bean, *Aphis craccivora*, phytotoxicity, cytotoxicity and Egypt

Introduction
Faba bean (*Vicia faba* L.) is one of the most globally important legume crops. The faba bean decreased from 3.7 to 2.1 million ha between 1980 and 2014 and yields are highly different within specific countries (FAO, 2017) [10]. The global production of faba bean in 2014 was 4.1 million tons, which is approximately 21% greater than in 1994. The cultivated area of approximately 125 thousand Fadden in Egypt. The fresh and dry seeds of faba bean are highly nutritious because have a high protein content up to 35% in dry seeds and are sources of K, Ca, Mg, Fe and Zn, (Lizarazo *et al*.,2014; Longobardi *et al*.,2013) [19, 20]. Faba bean plants in the field attacking some different insects such as: *Aphis craccivora, A.gosspii, Bemisia tabaci*, *Liriomyza sativae* and *Thrips tabaci* are decreased production in this crop after harvest. Cowpea aphis, *A.craccivora* is one of the important sucking pests which attack many plants. *A. craccivora* infest faba bean plants causing damage directly by sucking the host plants and harm to leaves, stems, and fruits (Sharma and Joshi, 2010) [28]. In addition to indirectly harming by transmitting pathogens to many plants (Abbas *et al.,* 2009) [1], also, caused honeydew by this insect for the growth of black sooty mold which covers leaves resulting in disruption of photosynthesis (Cranshaw *et al.*,2000) [9]. Pesticides are used in agriculture to kill pests that many damage plants but the people are exposed to low levels of pesticides through food and water are not typically cause for concern. People at higher risk are those who work directly with pesticides.
Acetamiprid is an insecticide belonging to the chloropyridinyl neonicotinoid, it is a nicotinic agonist that reacts with nicotinc acetylcholine receptors (nACh-R). It is to control sucking insects (Mainly aphids, Thysanoptera, and Hemiptera). Also, it can be used combined with another pesticide with different modes of action. Thiamethoxam is systemic insecticide of the neonicotinoid class, it is absorbed quickly by plants and transported to all parts, including pollen. Mode of action this insecticide is the effect of nicotinic acetylcholine receptors in the control nervous system and causes paralyzes the muscles of insects. Residues pesticides are a risk to the health of humans, natural enemies, and the environment, so scientists search for alternatives pesticides such as

biopesticides and essential oils (Singh and Saratchandra, 2005) [29]. Biopesticides are natural materials from animals, plants, bacteria, fungi, and viruses that are used for different pests control.

Plant essential oils in general have been recognized as an important natural source of insecticides (Arun *et al.*, 2009) [5]. Natural products are not safer than synthetic insecticides, just because a plant has been used for centuries does not mean it is safe (Hinkle, 1995) [13]. Some plants contain some bioactive compounds that are biological actions against insects, including repellent, antifeedant, anti-ovipositional, and growth regulatory activities (Sertkaya *et al*., 2010 and Ahmed *et al., 1*984) [27, 3]. In this study, a comparison has been made between neem oil, lemon oil, acetamiprid, and thiamethoxam on *A.craccivora* insects and studying of effect on bean plants.

Some chemical or biological compounds and even some natural oils are often used to control fungal, bacterial, viral, and insect pests. Some of them may be harmful, so it is necessary to know the extent of their toxicity or the defect that may occur. The pollen viability test is one of the tests conducted to determine the extent of the toxicity of these compounds used on cells and their harmful effect, which leads to the lack of the natural sequence of cell division and its development until it reaches the maturity of the pollen, especially the plants that humans consume, as it may be an indicator of harm to human health and also, the amount of the crop.

Materials and methods

1. The compounds used in this study

1. Chemicals: Acetamiprid 70% SP used recommended concentration 0.25 gm/L. Thiamethoxam 25% WG, used 0.2 gmL/L.
2. Oils: Lemon is natural oil used 5ml/L. Neem oil is natural oil used recommended concentration 1.5 ml/L.

2. Greenhouse experiments

This experiment was carried out in the greenhouse belonging to the Central Lab of Organic Agriculture (CLOA), ARC, Giza, Egypt.

1. In all greenhouse experiments, unless otherwise indicated, Nubaria cultivars were used.
2. Three bean seeds were sown in each pot.
3. Five replicates were used for each treatment; all pots (25cm in diameter) containing three Kg of soil received the same treatment regarding irrigation.
4. Planted pots were kept under normal open greenhouse conditions.

3. Effect of natural and chemical compounds on *Aphis craccivora*:

For mass rearing of *A.craccivora* used in this study, cultures of insects by planted faba bean plants in pots under semi-field conditions infested them artificially by infesting leaves with aphis were collected from a farm in Giza Government, October 2020. After 10 days from the start, the experiment resulted in the colony of *Aphis* used. Prepared concentrations recommended for acetamiprid, thiamethoxam, lemon oil, and neem oil: 50gm/,0.2gm/L,5ml/L,1.5ml/L in the order. Sprayed these compounds on bean plants covered *A. craccevora*. Has been Calculated mortality of *A. craccevora* after 24, 48, and 72 hours according to Abbott (1925).

4. Phytotoxicity of natural and chemical compounds on faba bean plants:

Sprayed acetamiprid, thiamethoxam, lemon oil, and neem oil at recommended concentrations on bean plants under semi-field conditions except for control that was sprayed by water. All plants were rated foliar damage after 7 days. A numerical phytotoxicity rating scale from 0 to 3(0=no visible injury; 1=light injury,25% foliar injury;2=moderate injury,50% and3=complete foliar injury, >75% (Poe, 1970).

5. Cytotoxicity of treatments

Pollen grains analysis, flower buds were obtained from plants for all treatments and control for the following examinations

Specimens were randomly collected from different plants; unopened floral buds of suitable sizes were killed and fixed in a freshly prepared Carnoy's fixative (mixture of alcohol, chloroform, and glacial acetic acid in a volume ratio of 6:3:1). Anthers were stained with aceto carmine staining solution 1%; Pollen grains from each group were examined; their fertility was estimated through stain-ability tests. Pollen grains with stained were taken as apparently fertile, while shriveled and unstained pollen were counted as sterile¸ and bursting pollen grains were taken also **(**Haridy, 2016**).**

6. Photomicrographs

Photomicrographs from the freshly prepared desirable slides having clear pollen grains were taken with a digital imaging microscope eyepiece system of HiROCAM (High Resolution Optics Camera) according to Kumar and Singhal (2012) [15].

7. Statistical analysis

Statistical analysis was applied using SPSS 11.0 for Windows Statistical Program and the LSD analyses by ANOVA were applied to the values obtained from:

Results and Discussion

1. Toxicity of natural and chemical compounds on *Aphis craccivora* Koch and semi-field conditions:

The results in Table (1) signalize to the highest recorded effect were lemon oil, acetamiprid and thiamethoxam, also no significant between it. But Neem oil was the least impact on *A. craccivora* where recorded mortality of 42.63%. (Gaber *et al*., 2015) [11] said that thiamethoxam and acetamiprid were the highest effects in reducing populations of *A. gossypii* in cotton plants, causing an average reduction percentage ranging from 73.58 to 96.42%, these our data agreement with him. (Iramu *et al.,* 2011) [14] observed using essential oil formulations such as Fungatol-Neem spray was found to be most effective on the tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae). Addition to (Masatoshi, 1999) [21] reported that using high concentrations of essential oils can kill different species of aphis. (Al-Antary *et al.*, 2017) [4] showed the highest mortality which ranged between 94.25 to 97.75% on green peach aphid *Myzuz persicae* with treatment lemon oil, so lemon oil may be used in IPM to provide a safe environment.

Umme (2013) [32] determined comparative toxicity of neonicotinoid, imidacloprid, and neem oil against different types from aphis attacking vegetables showed that imidacloprid had the toxic effects even on low does, i.e. 30-100% for 0.00625-1069 g/ml. The neem oil had no such toxic effects on the higher dose, it caused the highest death rate due to sticking of aphis on the surface of leaves i-e,83% on 2.5 g/ml. (Raghuraman *et al*.,2008) said that using acetamiprid compound in three doses (20, 40, 80 g ai/ha) was effective in suppressing the populations of jassid and whitefly significantly up to nine days on cotton plants. Acetamiprid and thiamethoxam are an effect on nicotinc acetylcholine receptors in their nervous system causing hyperactivity and muscle spasms and death to insects after 24 and 48hr.

Table 1: Mortality of *Aphis craccivora* after treatment by neem oil, lemon oil acetamiprid and thiamethoxam under semi-field conditions.

Compounds	Con.	Mortality after treatments %		
		24 hr.	48 hr.	72 hr.
Neem oil	1.5ml/L.	49.13 C	45.10 b	42.63 b
Lemon oil	5ml/L.	62.16 b	68.23 a	70.00 a
Acetamiprid	0.25gm/L.	76.46 b	78.03 a	70.13 a
Thiamethoxam	0.2gm/L.	65.46 b	69.23 a	63.76 a
LSD 0.05	-	8.833	11.85	14.73

Values in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

2. Phytotoxicity of natural and chemical compounds on faba bean leaves:

Results in table (2) indicated that acetamiprid, thiamethoxam, neem, and lemon oil significantly more phytotoxicity than neem oil and the control, but neem and lemon oil were less phytotoxicity than acetamiprid and thiamethoxam. Observations recorded for the phytotoxicity evolution of Acetamiprid showed that there were no phytotoxic symptoms on rice plants (Bidisha *et al*., 2018) [6], this was not compatible in this study. *Beauveria basiana*, Azadirachtin, and paraffinic oil were not phytotoxic to any of the herb species tested (Cloyd and Cycholl, 2002) [8]. Also, Lawson and Wieres (1991) [18] said that oils such as paraffinic oil were safe for flowers and fruit. Kumar *et al.* (2020) [16] recorded no phytotoxicity in the chili plants due to the treatment of imidacloprid as seed treatment even at the highest dose. Kumawat and Kumar (2007) [17] found that some novel insecticides did not show any phytotoxic on soybean plants.

Teble 2: Phytotoxicity of neem oil, lemon oil, acetamiprid and thiamethoxam on bean leaves.

Compounds	Con.	Mean Phytotoxicity rating
Neem oil	1.5ml/L.	0.66 bc
Lemon oil	5ml/L.	0.99 b
Acetamiprid	0.25gm/L.	1.88 a
Thiamethoxam	0.2gm/L.	2.44 a
Control	-	0.00 c
LSD 0.05	-	0.74

Values in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

3. Effect of treatment on pollen grain viability (fertile n), sterile and bursting.

The effect of insecticides thiamethoxam and acetamiprid, in addition to some natural oils, lemon oil, neem oil, on PGs% fertile and sterile. The total C N refers to the number of all single cell counted. Results showed that pesticides reduced the percentage of pollen viability. Various sterile pollen types such as un-stained pollens and wrinkled pollens, pollens were encountered in pesticide treated (Figs 2b, 2c).

An evaluation of the percentage of fertility results showed that values, in general, decreased as compared to the control with water (without any treatment) and there was a significant difference between control and all

treatments, and there was a significant difference between all treatments, so the highest pollen fertility percentage (96.40%) was recorded in the control then the next treatment with lemon oil (94.40%). Also, the treatment with neem oil (93.10%) respectively. but pesticide Thiamethoxam and Acetamiprid were recorded (75.3%) and (72.50%) respectively (Table 3).

The negative effects on pollen grains sterile were estimated and compared to control, also there was a significant difference between control and all treatments, and there was a significant difference between all treatments, the highest pollen sterile percentage was in pesticides acetamiprid was recorded in total sterile pollens (25.30%) which is the sum of both unstained pollens (25%) and wrinkled pollens (10.30%) and then Thiamethoxam total pollens (22.20%) its recorded (21.2%) in unstained pollens and (1.00%) in wrinkled. While the natural oils recorded less effect than insecticides, where neem oil was recorded (6.20%) So the ratio of unstained pollens (4.80%) and wrinkled pollens (1.40%) from the total sterile pollens. Then lemon oil its recorded (4.30%) for total sterile pollens, it was distributed between unstained pollens (3.90%) and wrinkled pollens (0.40%). And in the end, it was the control (3.60%) where the lowest number of total sterile pollen was recorded, and also distributed between unstained pollens (2.80%) and wrinkled pollens (0.80%). And also, the different shapes of cells that appeared during the examination with a light microscope the bursting cell fig (3), also there was a significant difference between control and all treatments, and there was a significant difference between all treatments, this type of cell register in the treatment with insecticides thiamethoxam **(**2.50%) and acetamiprid record (2.20%) then lemon oil (1.30%) and neem oil (0.70%) not found in control The maternal cells divide by the first meiosis and then the second meiosis to produce the tetrad cells, and then these cells separate and move away from each other (fig.1). These resulting cells are immature pollen grains, called microspores, then develop and divide by mitosis to produce mature pollen grains through a series of biochemical reactions, which produce some of their components from gene expression. A defect may occur during the maturation process as a result of exposure to some mutagenic factors that prevent the formation of One of the components involved in these interactions prevents the completion of this process. And this agrees with Marina and Elkonin (2013) decided that meiotic irregularities usually result in microspores with unbalanced genome structures, which most frequently are non-viable. It is known that anomalies of chromosome segregation and cytokinesis are the result of the disturbances of the genetic system controlling assembling. and abnormalities in pollen development in Zh10-*asc*1 plants could be explained by mutation(s) in a gene encoding a transcription factor that regulates the expression of other genes involved in pollen development. Presently, a number of transcription factors required for pollen development have been found and their role in male-sterile phenotype was shown. Also, Souza *et al.* (2012) suggested that the sterility occurred during the development of the gametophyte, probably starting in the microspore stage and is aborted at the beginning of their development.

And Bursting pollen examples of damaged pollen grains. Treatment can induce structural damage to the grains. Modifications of PCG Release following Exposure to the treatments, PCG are naturally released from the pollen grain when the cytoplasm is expelled from the grain through the pore (fig. 3), However, we observed that the release could also occur through cracks of the exine when the grain was damaged (fig 3) arrowed. Pollen grains release PCG. PCG is expelled from the grain via the pore. Only a small proportion of the grains release their cytoplasm, and the remaining grains stay intact. However, in the fragile pollen, PCG release can also occur through breaks of the exine according to Motta *et al*. (2006). Cytoplasmic granules can be seen next to the pollen grains that came out of them. Our results showed that the interaction of pollen with (pesticide) pollutants increases the proportion of pollen grains releasing their PCG. These reasons also decreased the percentage of fertile pollen grains in the treated plants using pesticides compared with the control can be attributed to their toxic effects on pollens. In fact, this toxic effect becomes more evident for use of pesticides. This decreased ratio may produce a negative effect on fruit productivity and the quality of bean plants in the long run. These results agreed with Cali (2008) found that pesticides could give rise to mutation by changing the genetic structure of plants. Chromosomal changes resulted due to fungicides application had stimulated mutation similar to mutation occurred by mutagens chemical substance.

Fig 1: Meiocytes during meiosis I and II; (a) normal cell during meiosis I, (b) normal tetrad during meiosis II, (c) Immature pollen (microspore). Scale bar = 20μm.

a b c

Fig 2: PGs viability; (a) normal fertile PG (red) and the other is transparent without PCG; (b) unstained sterile pollen; (c) wrinkled pollen arrow. scale bar = 20µm.

Fig 3: Pollen grains release PCG. PCG is expelled from the grain via the pore. And the remaining grains stay intact, the fragile pollen, PCG released through breaks of the exine. Un mature pollen. Scale bar = 20µm PCG =Pollen cytoplasmic granules.

Table 3: Effect of different treatments on pollen grain fertility.

Treatment	Sterile %			Fertile %	Bursting %	Total N of PGs
	Unstained PGs	Wrinkled PGs	Total N of Sterile			
Lemon oil	3.90^{d}	0.40^{c}	4.30^{d}	94.40^{b}	1.30^{b}	1000
Neem oil	4.80^{c}	1.40^{a}	6.20^{c}	93.10^{c}	0.70^{c}	1000
Thiamethoxam	21.20^{b}	1.00^{ab}	22.20^{b}	75.30^{d}	2.50^{a}	1000
Acetamiprid	25.00^{a}	0.30^{c}	25.30^{a}	72.50^{e}	2.20^{a}	1000
Control	2.80^{e}	0.80^{b}	3.60^{e}	96.40^{a}	0.00^{d}	1000
LSD 0.05	0.126	0.136	0.085	0.033	0.098	

Values in each column followed by the same letter are not significantly different according to LSD test (P = 0.05). These variables were transformed according to this equation X + 1.

Conclusion

It is concluded that natural oils are considered promising safely control compounds without any effect on the host plant crops.

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