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The Aphicidal and Repellent Activities of Oxymatrine, *Bacillus amyloliquefaciens*, Nimbecidine and Spinosad against Adults of Oleander Aphids (*Aphis nerii* Boyer De Fonscolombe) under Laboratory Conditions

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ABSTRACT

Background: The study aimed to determine the effect of biological and chemical control against the oleander insect, *Aphis nerii*, under laboratory conditions.

Methods: The current study was conducted in the Plant Protection Laboratory of Diyala Agriculture, Diyala Province, Baqubah district, Iraq, During May 2023 to evaluate commercial products, which included oxymatrine, spinosad, the bacteria *Bacillus amyloliquefaciens* and nimbecidine, in addition to alphacypermethrin pesticide with three concentrations of 10, 20 and 30% against adults of Oleander aphids, *Aphis nerii*, the study included two experiments, to evaluate the mortality rate and the second to evaluate the repellency rate under laboratory conditions.

Result: The alphacypermethrin pesticide with a concentration of 30% recorded the highest mortality (93.3%) after 24 hours, while the bacteria *Bacillus amyloliquefaciens* with a concentration of 10% and the oil neem (nimbecidine) with a concentration of 30% recorded the highest mortality (63.3% and 63.3%, respectively), after 48 hours. The oxymatrine 30% and 20% were superior in recording the highest repellency (66.6% and 66.6%) after 5 and 15 minutes, respectivelyand the bacteria 20% recorded the highest repellency (60.0%) after 10 minutes. The results of this study indicate the possibility of replacing chemical pesticides with biological control agents as eco-friendly alternatives.

Key words: *Aphis nerii*, *Bacillus amyloliquefaciens,* Nimbecidine, Oxymatrine, Spinosad.

INTRODUCTION

Oleander aphids (*Aphis nerii* Boyer de Fonscolombe) belong to the order Hemiptera and the family Aphididae. The adult and nymph insects feed on the Nerium plant by sucking the sap, which leads to leaf curling. These insects excretion honeydew, which collects dust particles and on which black sooty molds develop, reducing the respiration, transpirationand photosynthesis of the plant. Also, the insects acts as vector and transmit several plant viruses, which weaken the plant (Raccah and Fereres, 2009; Brault *et al.,* 2011). Environmentally friendly methods must be employed for managing insect pest menace and to increase crop production instead of chemical pesticides to promote plant growth and prevent environmental damage; thus, botanical extracts and microorganisms are the best alternatives to these harmful compounds (Salim and Hassan, 2022; Ali, 2023; Kouache *et al.,* 2024; Al-Hayali and Qader 2024a,b). In this context, biological management of pests utilizing strains of antagonistic bacterial and fungal organisms is becoming more important; besides, the soil has untapped potential and contains a number of possible bio-control agents (Salim and Abed, 2015; Al-Hayali *et al.* 2025).

A bacteria called *Bacillus amyloliquefaciens* is known for producing a wide range of bioactive chemicals and compounds having fungicidal and insecticidal properties (Alvarez *et al.,* 2012; Zouari *et al.,* 2016; Torres *et al.,* 2017). Yun *et al*. (2013) found that *B. amyloliquefaciens* has an aphicidal impact against *Myzus persicae* and Beris *et al.*

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(2018) found that *B. amyloliquefaciens* has a mosquitocidal effect against mosquitoes. Spinosad is a mixture of spinosyn A and spinosyn D and is a secondary metabolite produced by fermentation from the bacterium *Saccharopolyspora spinosa*. According to Elzen *et al*. (1998), Spinosad works well against some borers, Colorado potato beetles, leaf miners, trips and caterpillars. The use of botanical products and their derivatives as insect pest management tools is one of the most important alternatives to synthetic insecticides for managing insect pests (Salim *et al.,* 2016; Al-Hayali and AL-Zuhairi, 2024; Saha and Modi, 2024).

It has been demonstrated that neem products have a variety of chemical components and that extracts from its seeds and kernels negatively affect the biology and life cycles of several insects (Schmutterer, 1997; Das *et al.,* 2010; Salim *et al.,* 2020; Ahmed *et al.,* 2024). Oxymatrine is one of the main compounds extracted from the ancient Chinese herb *Sophora flavescens* Aiton, which belongs to the Leguminosae family (Mao and Henderson 2007). Oxymatrine has been demonstrated to have a potent anti-feedant impact on a variety of insects, including termites (Verma *et al*. 2009). The objective of the current study was to assess the effectiveness of biological ,as well as chemical control and certain plant extracts, against only adults of oleander aphids under lab conditions.

MATERIALS AND METHODS

The current study was conducted during May 2023 at a plant protection laboratory in the Directorate of Diyala Agriculture, Diyala Province, Baqubah district, Iraq.

Insect breeding

100 whole insects were collected, each whole was placed in Petri plates of 9 cm × 2 cm on two fresh oleander leaves, In controlled laboratory conditions at a temperature of 33-35°C and a relative humidity of 55-60% with the neck of the leaf covered with a piece of cotton moistened with water, which was moistened daily to ensure that it did not dry out, in addition to replacing the paper when needed and left for observation. When these females began giving birth to nymphs, A number of nymphs were taken in one day to follow their life cycle until they turned into winged adults for use in the current study experiments.

The experiment treatments

All the experimental material were collected from the local market in Diyala Province, Baqubah district that included (OXYMATRINE 2.4 SL), a natural extract from the Sophora plant with a dilution of 2-3 ml per liter of water from Agrichem Company, Australia;. Alphacypermethrin 10%, trade name (FLASH 10% EC) product by: Tagros Chemicals India LTD, with a dilution of 1.25 ml per liter. (NIMBECIDINE 1% EC); the composition is Azadirachtin 1% W/W, with a dilution of 2-3 ml per liter of water; T. Stanes Company, Indian origin. The bacteria *Bacillus amyloliquefaciens* (QST 713) trade name (AMYLOLAND), which had a number of bacteria colonies of 2.2 billion per gram with a dilution of 1 kg per 300 liters of water, are of Chinese origin. Spinosad 0.024% W/V, trade name (SUCCESS 0,02 CB) produced by Dow Agro Sciences, British, with a dilution of 1 ml per 4 liters of water. Each commercial product was prepared as suspension stock, then diluted by distilled water to 10, 20 and 30%.

The oleander aphids

The adults of *Aphis nerii* were collected from Nerium plants in the general gardens in Diyala Province, Baqubah district**and** the insects were fed on oleander leaves.

Mortality testing

Petri plates of 9 cm × 2 cm were utilizedand were swabbed with cotton that had previously been treated with 1 ml of each concentration separately for all treatments, In addition, dip oleander leaves in each concentrate and leave them to air dry for 5 minutes, then put one leaf in each Petri plate as food during the experiment, while water was used as a control, Then, each Petri plate was filled with ten adult *Aphis nerii*and each treatment was repeated three times (Gafoor and Qadr, 2011). For three days, the number of dead aphids in each replicate was noted every day. By the following formula the adult mortality rate was calculated:

$$\text{Mortality \%} = \frac{\text{Number of dead aphide}}{\text{Total number of a phids}} \times 100$$

Repellency testing

Divided into two equal halves, Petri plates of 9 cm × 2 cm were utilized were marked with a circle with a diameter of 2 cm in the center. One half was treated by wiping one milliliter (ml) of each concentration with a cotton piece, while the other half was treated with just water and allowed to air dry. *Aphis nerii* adults by 10 insects were introduced into each petri plate in the circle. Three replications of each treatment were madeand the percentage of adults in the untreated group was noted after five, tenand fifteen minutes (Talukder and Howse, 1993). The following formula was used to calculate percentage repellency (PR) values:

$$PR = 2(C-50\ \%)$$

Where:
C = % adults in the untreated part.

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the data from the factorial experiment(Fisher and Yates, 1968).

RESULTS AND DISCUSSION

The results in Table (1) illustrated that the pesticide and the bacteria recorded the highest increase in the mean mortality percentages of adults, which amounted to 78.8 and 57.7%, respectively, after 24 and 48 hours as compared

Table 1: Effect of different concentrations of treatments on mortality percentage in adults of oleander aphids after 24, 48 and 72 hour.

Treatments		(B)	24 hour				48 hour				72 hour		
(A)		10%	20%	30%	Mean	10%	20%	30%	Mean	10%	20%	30%	Mean
Control		6.66	6.66	6.66	6.6	16.6	16.6	16.6	16.6	30.0	30.0	30.0	30.0
Pesticide		66.6	76.6	93.3	78.8	30.0	23.3	6.6	20.0	3.3	0.0	0.0	1.1
Bacteria		33.3	36.6	43.3	37.7	63.3	56.6	53.3	57.7	3.3	6.6	3.3	4.4
Spinosad		23.3	36.6	30.0	30.0	36.6	36.6	30.0	34.4	6.6	3.3	20.0	10.0
Neem		40.0	20.0	20.0	26.6	33.3	40.0	63.3	45.5	16.6	23.3	16.6	18.8
Oxymatrine		16.6	36.6	43.3	32.2	46.6	53.3	50.0	50.0	16.6	10.0	6.6	11.1
Mean		31.1	35.5	39.4		37.7	37.7	36.6		12.7	12.2	12.7	
	A	13.9				15.5				6.8			
CD 5%	B	9.8				11.0				4.8			
	A×B	24.2				26.9				11.9			

Table 2: Effect of different concentrations of treatments on repellency percentage in adults of oleander aphids after 5,10 and 15 minute.

Treatments		(B)	5 minute				10 minute				15 minute		
(A)		10%	20%	30%	Mean	10%	20%	30%	Mean	10%	20%	30%	Mean
Pesticide		26.6	60.0	80.0	55.5	60.0	73.3	86.6	73.3	53.3	80.0	80.0	71.1
Bacteria		33.3	-20.0	46.6	20.0	46.6	60.0	26.6	44.4	20.0	40.0	40.0	33.3
Spinosad		-6.6	-13.3	40.0	6.6	0.0	13.3	20.0	11.1	-6.6	-20.0	40.0	4.4
Neem		33.3	26.6	0.0	20.0	6.6	53.3	13.3	24.4	20.0	53.3	26.6	33.3
Oxymatrine		20.0	46.6	66.6	44.4	-13.3	33.3	46.6	22.2	13.3	66.6	13.3	31.1
Mean		21.3	20.0	46.6		20.0	46.6	38.6		20.0	44.0	40.0	
	A	36.0				33.5				24.4			
CD 5%	B	27.9				26.0				18.9			
	A×B	62.3				58.1				42.3			

to the control treatment's 6.6 and 16.6%, while the mortality percentages increased in the control treatment to 30% after 72 hours over the other treatments, whereas no significant differences in mortality percentages were noted between the concentrations after 24, 48 and 72 hours. The pesticide with a concentration of 30% was superior in recording the highest mortality (93.3 %) after 24 hours, also the bacteria with a concentration of 10% and the neem with a concentration of 30%, which amounted to 63.3% and 63.3%, respectively, after 48 hours.

The results in Table (2) showed that all treatments recorded increases in repellency percentages in adults after 5, 10and 15 minutes. In addition to the superiority of the pesticide treatment, the other treatments such as oxymatrine, bacteria recorded the highest increase in the mean repellency percentages of adults, which amounted to 44.4% and 44.4% after 5 and 10 minutes respectively, also each of bacteria and neem were recorded the highest increase amounted to 33.3% after 15 minutes, no significant differences in repellency percentages were noted between the concentrations after 5 minutes, whereas the concentration 20% recorded the highest increase (46.6%) compared to concentration 10% (20.0%) after 10 minutes, also the concentrations 20% and 30% recorded the highest increase reaching (44.0 and 40.0%) after 20 and 30 minutes respectively compared to concentration 10% (20.0%). The oxymatrine 30% and 20% recorded the highest repellency, reaching 66.6% and 66.6% after 5 and 15 minutes, respectively. After 10 minutes, the bacteria 20% recorded the highest repellency, reaching 60.0%.

Our findings revealed that all treatments have aphicidal and repellent activity against the oleander aphid, *Aphis nerii*. Aqueous extract of the Neem plant to reduced the larval population of *Vanessa cardui* to 83.3%, 100% and 100% after 14, 21 and 28 days, respectively (Salim *et al.* 2016). *Azadirachta indica* led to a reduction in the larval population of *Spilosoma oblique* on cabbage (Abbas *et al.* 2015). The mortality rate of *Ocnogyna loewii* caterpillars treated with *Azadirachta indica* increased to 74.0% in comparison to the control (Salim *et al.* 2020). Savi *et al.* (2021) reported that oxymatrine and azadirachtin provide effective control against *Tetranychus evansi* on tomato; oxymatrine treatments caused reduced fertility (73.1-90.7%) and higher mortality (60-88%), where oxymatrine controlled the immature individuals and adult females of *T. evansi* faster than azadirachtin; also, Oxymatrine-based treatments had a significant effect on newly hatched *T. evansi* larvae. oxymatrine has the ability to stop mitosis at any point in the cell cycleand this refers to the cytotoxic effects of oxymatrine; it has no distinctive genotoxic effects, also, oxymatrine can be recommended as a safe bio-pesticide for use (Akdeniz and Özmen, 2011).

Spinosad is a broad-spectrum insecticide that provides effective control of many orders of insects, such as Orthoptera, Coleopteraand Lepidoptera, Diptera and Thysanoptera (Cloyd and Sadof, 2000; Thompson *et al.,* 2000). Spinosad led to high mortality in numbers of nymphs and adults of potato leafhopper *Empoasca fabae* on cowpea plants, which reached 3.3 and 5.6, respectivelyand also showed superiority in corrected mortality percentage of nymphs and adults, which amounted to 44.7% and 39.45%, respectively (Khamas *et al*. 2018). Fang *et al*. (2002) reported that the spinosad could be a replacement for protecting the grains against *Rhyzopertha dominica* and *Plodia interpunctella* in stored wheat. According to López *et al*. (2019), there is insecticidal activity against *Myzus persicae* by all strains of *B. amyloliquefaciens*and the bacteria also have an inhibitory effect on the ability of insects to reproduce as a result of their presence inside the aphid. This suggests that the bacteria's live cells and active enzymes are essential to the bacteria's ability to kill *M. persicae* aphids. Torres *et al*. (2022) reported that CBMDLO3, one of the *B. amyloliquefaciens* strains, showed a decrease in the fecundity of female adults and the survival of pupae and larvae of the housefly *Musca domestica*, therefore, the *B. amyloliquefaciens* strain could be a new alternative for biological control. Al-Hayali *et al*. (2024) reported that *Bacillus amyloliquefaciens*, oil neem, spinosadand oxymatrine possess strong impacts against aphids on apricot.

CONCLUSION

The results showed that oxymatrine, the microorganism *Bacillus amyloliquefaciens*, nimbecidineand spinosad possess active effects as toxic and repellent factors versus oleander aphids, *Aphis nerii*, under laboratory conditions.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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