Research Article

Field Efficacy of Entomopathogenic Fungus Metarhizium anisopliae and Botanical Insecticide Oxymatrine and Mineral Oil Nautilus against Oriental Yellow Scale, *Aonidiella orientalis* (Newstead) (Homoptera: Diaspididae)

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Abstract | Citrus is an important horticultural crop of Iraq. Its production is hampered by the incidence of many insect pests. The oriental yellow scale, *Aonidiella orientalis* (Newstead) (Homoptera: Diaspididae), is one of the most destructive insect pests of citrus, causing considerable economic loss to citrus farmers. Extensive applications of traditional synthetic insecticides against these insect pests often result in many ecological consequences. This field work was performed to have a comparative evaluation of the insecticidal efficacy of the entomopathogenic fungus *Metarhizium anisopliae* and the botanical pesticide oxymatrine 2.4% SL, and to determine if the effectiveness of these insecticides can be enhanced by their combined application with the mineral oil Nautilus 70% EW in controlling the oriental yellow scale, *Aonidiella orientalis*, on citrus trees. Results showed that both insecticides, *M. anisopliae* and oxymatrine, exhibited significant mortality of adults (56–69% and 52–67%, respectively) and crawlers (72–82%) of *A. orientalis*, the latter being more susceptible. Moreover, the combined application of these pesticides with mineral oil (nautilus) synergized their efficacy, exhibiting up to 95 and 87% population reduction of scale nymphs and adults, respectively. These findings suggest the incorporation of these biological or botanical insecticides and mineral oils in the integrated management of scale insect pests, including oriental yellow scale (*A. orientalis*). Moreover, optimizing practical implications and assessing the long-term impacts of these pesticidal treatments constitute the future perspectives of this study.

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Introduction

Citrus is considered one the most important genera of fruit trees, containing many varieties and cultivars, including sweet oranges, sour oranges, mandarins, grapefruits, lemons, etc. Citrus fruits are admired globally for their nutrition-rich composition, as these fruits contain vitamins, particularly vitamin C, fibers, minerals, and antioxidants such as carotenoids and flavonoids, all of which contributing to disease prevention and a healthy life (Adenaike and Abakpa, 2021). Oranges are produced worldwide, including Iraq, where the production of oranges reaches up to 70–80 thousand metric tons per annum (Redhewy *et al*., 2022).

However, the production of citrus in Iraq is hampered by a wide range of insect pests and diseases. The oriental yellow scale, *Aonidiella orientalis* (Newstead) (Homoptera: Diaspididae), is one of the most destructive insect pests of citrus all over the world including Iraq (Alkhalidy, 2000; Al-Saffar and Augul, 2020) which infest all cultivars of citrus in Iraq and cause damage to leaves and fruits just like mealybugs and cause considerable economic loss to citrus production. *A. orientalis* is a polyphagous pest that infests different host plants belonging to 36 genera among 25 plant families worldwide (Bragard *et al*., 2022). The adults and crawling nymphs of *A. orientalis* cause damage by sucking plant sap from all plant tissues causing discoloring and malformations of fruits and leaves and also release certain toxins into plant tissues by their saliva (Kondo *et al*., 2022).

Farmers in Iraq rely primarily on the application of highly persistent synthetic insecticides to control *A. orientalis* infestations in their orchards. Due to extensive applications, most of the insect pests have attained resistance to these insecticides (Awad and Ali, 2024). Moreover, these insecticides are harmful to humans, domestic animals, and wildlife and, contaminate the environment as well (Ali *et al*., 2021). This situation necessitates looking for alternative bio-ration and environment-friendly options to control insect pests, including *A. orientalis*. For instance, entomopathogenic fungus *Metarhizium anisopliae* and botanical insecticide oxymatrine would be promising insecticidal compounds that can be employed against mealybugs and scale insect pests (Sayed and Dunlap, 2019; Brilinger *et al*., 2025). Both of these insecticides are more target-specific and bear low mammalian toxicity compared to conventional and traditional synthetic insecticides (Zimmermann, 2007; Lim *et al*., 2014). Moreover, as the scale insects develop an impermeable shell around their bodies, mineral oils would be an effective synergist to dissolve this shell, rendering scale insect pests more susceptible to the applied insecticide (Bateman and Alves, 2000; Helmy *et al*., 2012; Johnson, 2020).

To this end, this study aimed to have a comparative evaluation of the insecticidal efficacy of the entomopathogenic fungus *Metarhizium anisopliae* and the botanical pesticide oxymatrine 2.4% SL, and to determine if the effectiveness of these insecticides can be enhanced by their combined application with the mineral oil Nautilus 70% EW in controlling the oriental yellow scale, *Aonidiella orientalis*, on citrus trees.

Materials and Methods

Insecticidal treatments

Indigenous isolates of the entomopathogenic fungus *Metarhizium anisopliae* were acquired from the fungal infected individuals of oriental yellow scale *Aonidiella orientalis* on citrus trees located in Baghdad, Iraq. Fungal culture was propagated in the lab on potato dextrose medium and conidia were harvested to make conidial suspension (@ 1.3×10^9 conidia/cfu mL^{-1}) to be used against target insect pest (*A. orientalis*). Botanical pesticidal formulation, Oxymatrine 2.4% Soluble concentrate (SL), was diluted @ 0.25 mL^{-1} L^{-1} of water, and the mineral oil formulation, Nautilus 70% EW, was applied @ 2 mL L^{-1} of water. These three insecticidal treatments were applied in all field trials using the same above-mentioned concentrations that were found effective in the literature.

Experimental protocol

For field trial, 18 trees of citrus (sweet orange; *Citrus sinensis*) were randomly selected in a citrus orchard located in Baghdad, Iraq. These trees were infested with oriental yellow scale *A. orientalis* and three trees were randomly selected for each treatment. Treatments were applied using a knapsack sprayer (SEMCO 14 PM, Japan) using 15 liters of water. For control treatment, only water was applied. For data recording, about 25 randomly selected leaves from each treated citrus tree were collected and observed in the lab under an optic microscope. Data regarding the population of citrus scale adults or crawlers were recorded at 3, 7, 14, and 21 days after treatment/ insecticidal spray. Mortality data was corrected according to Henderson and Tilton (1955) formula.

$$\textit{Corrected insect population reduction } (\%) = \frac{\textit{population in C before treatment} \times \textit{population in T after treatment}}{\textit{population in C after treatment} \times \textit{population in T before treatment}} \times 100$$

Where, C = control citrus tree, T = treated citrus tree

Statically analysis

Randomized complete block design (RCBD) was followed during this field trial. Data regarding the population reduction of citrus scale insects were statistically analyzed using one-factor analysis of variance (ANOVA) followed by the least significant different (L.S.D.) post-hoc test to compare the treatments. The standard level of significance used during the analysis was $\alpha = 0.05$. Statistical program Static Analysis Symposium (SAS, 2012) was used for the analysis of data.

Results and Discussion

All treatments caused a significant effect on the population of the crawler and adult stages of the pest and caused a considerable reduction at all observation times. There was a continuous increase in the population of scale crawlers and adults on the citrus branches in the control treatments during the study period.

The percent population reduction of crawler or nymphal stage of the scales in response to the application of *M. anisopliae* and nautilus applied alone or in combination has been shown in Table 1. Results showed a significant ($P \leq 0.05$) effect of the insecticidal treatments on the crawler insects. *M. anisopliae* caused a 72% population reduction at 3 days after treatment to 82% population reduction recorded at 21 days after treatment, and remained statistically higher than mineral oil nautilus application (Table 1). However, the combined application of fungus and mineral oil exhibited maximum and significant mortality of crawlers of oriental yellow scale as compared to other applications, causing 74 to 94% population reduction (Table 1).

A different trend of insecticidal potential of *M. anisopliae* and nautilus mineral oil was recorded in the case of adult individuals of oriental yellow scale. Mineral oil application caused 64% population reduction at 3 days after treatment to 82% population reduction recorded at 21 days after treatment, and it was significantly higher than *M. anisopliae* alone, which caused 56–69 % cumulative population reduction of adult scales (Table 2). However, the highest percent reduction in adult scales was recorded for the treatment of the fungus *M. anisopliae* and nautilus oil, *i.e.,* 66 – 87% (Table 2).

Table 3 indicates the effect of oxymatrine application on the adult stage of oriental yellow scale *A. orientalis* alone or in the combination with nautilus mineral oil.

Table 1: *Percent population reduction of the crawling stage (nymphs) of the oriental yellow scale A. orientalis by entomopathogenic fungus M. anisopliae, mineral oil nautilus, and by their combination on citrus trees under field conditions.*

Insect mortality (%)				Concentrations used	Insecticidal treatments
21 DAT	**14 DAT**	**7 DAT**	**3 DAT**		
82.3^{b}	80.2^{a}	78.7^{a}	71.8^{a}	@ 1.3×10^{9} conidia/cfu mL^{-1}	*Metarhizium anisopliae*
94.5^{a}	65.7^{b}	82.5^{a}	73.7^{a}	@ 1.3×10^{9} conidia/cfu ml^{-1} + @ 2 mL L^{-1}	*Metarhizium anisopliae* + nautilus mineral oil
70.3^{c}	62.2b	50.2^{c}	30^{b}	@ 2 mL L^{-1}	Nautilus mineral oil

cfcfu = colony forming units (spores or conidia), DAT = days after treatment; Letters beside mortality values indicate statistically significant differences among the treatments within each column (one-way ANOVA followed by LSD post-hoc test at α = 0.05).

Table 2: *Percent population reduction of the adult stage of the oriental yellow scale A. orientalis by entomopathogenic fungus M. anisopliae, mineral oil nautilus, and by their combination on citrus trees under field conditions.*

Insect mortality (%)				Concentrations used	Insecticidal treatments
21 DAT	**14 DAT**	**7 DAT**	**3 DAT**		
68.5^{b}	63.5^{c}	69.1^{b}	55.5^{b}	@ 1.3×10^{9} conidia/cfu mL^{-1}	*Metarhizium anisopliae*
87.2^{a}	81.4^{a}	76.2^{a}	66.4^{a}	@ 1.3×10^{9} conidia/cfu ml^{-1} + @ 2 mL L^{-1}	*Metarhizium anisopliae* + nautilus mineral oil
81.6^{a}	70.2^{b}	65.7^{b}	64.4^{a}	@ 2 mL L^{-1}	Nautilus mineral oil

cfu = colony forming units (spores or conidia), DAT = days after treatment; Letters beside mortality values indicate statistically significant differences among the treatments within each column (one-way ANOVA followed by LSD post-hoc test at α = 0.05).

Table 3: *Percent population reduction of the adult stage of the oriental yellow scale A. orientalis by the botanical insecticide oxymatrine, mineral oil nautilus, and by their combination on citrus trees under field conditions.*

Insect mortality (%)				Concentrations used	Insecticidal treatments
21 DAT	14 DAT	7 DAT	3 DAT		
52.2[b]	67.1[b]	57.3[c]	52.2[b]	@ 0.25 mL^{-1} L^{-1}	Oxymatrine
85.1[a]	67.0[b]	65.1[b]	56.6[b]	@ 0.25 mL^{-1} L^{-1} + @ 2 mL L^{-1}	Oxymatrine + nautilus mineral oil
81.1[a]	76.4[a]	72.5[a]	66.2[a]	@ 2 mL L^{-1}	Nautilus mineral oil

cfu = colony forming units (spores or conidia), DAT = days after treatment; Letters beside mortality values indicate statistically significant differences among the treatments within each column (one-way ANOVA followed by LSD post-hoc test at α = 0.05).

In this case, oxymatrine was found least effective, causing about 52–67% population reduction up to 14 days after treatment. For oxymatrine application, the highest population reduction was recorded as 67.1% after 14 days of treatment and then the population built up when observed at 21 days after treatment (Table 3). The highest population reduction (85.1%) was exhibited by the combined application of oxymatrine and nautilus oil recorded at 21 days after treatment. Nautilus mineral oil alone caused 66–81% population reduction of adult scales at 3 days to 21 days after treatment, respectively (Table 3).

This field study evaluated the effectiveness of different types of insecticidal treatments including an entomopathogenic fungus *M. anisopliae*, a botanical insecticidal formulation Oxymatrine and mineral oil Nautilus against the nymphs (crawlers) and adults of oriental yellow scale *A. orientalis* which is one the destructive pests of citrus orchards in Iraq (Bragard *et al*., 2022). Overall study results showed a considerable suppression of scale insects, both crawlers and adults, by the application of entomopathogenic fungus and botanical pesticides. Among both treatments, entomopathogenic fungus (*M. anisopliae*) showed more effectiveness and caused a higher population reduction as compared to the botanical insecticide Oxymatrine. Crawling stage individuals were more susceptible and more affected by the insecticidal treatments as compared to the adult individuals. Moreover, the combined application of these insecticidal compounds along with mineral oil (nautilus) significantly synergized the population reduction effect of both insecticidal treatments.

These results are in agreement with the findings of Flayieh (2007) and Amran and Mohammed (2020) demonstrating the effectiveness of another entomopathogenic fungi, *Beauveria bassiana* and *M. anisopliae,* against the crawling stage of oriental yellow scale (*A. orientalis*), causing the mortality of the crawling stage from 60 to 86%, and both entomopathogenic fungi were more effective against crawling stage than adults. *M. anisopliae* has been demonstrated as a very effective microbial insecticide against a wide range of insect pests, including lepidopterous, homopterous, mealybugs, psyllids, and scale insects (Ezz, 2012; Batool *et al*., 2022; Peng *et al*., 2022; Ullah *et al*., 2022).

Regarding the implication of mineral oil against scale insects, Grafton-Gardweel and Reagan (1995) demonstrated a mineral oil formulation (Sun Spray 6 EC) effective against the crawling stage of *A. orientalis* on citrus trees. Similarly, Salman *et al*. (2014) also showed that mineral oils can be effective against all life stages, including crawling and adult ones, of oriental yellow scale *A. orientalis.* Similarly, Al-Jassania *et al*. (2005) used a summer oil (Semerol) to control oriental scale insect *A. orientalis* on citrus trees; the mortality of adults was 66.19% after 7 days of treatment, and the better time to control it is in the spring. One of the reasons for the mortality of scale insects by mineral oils is that these oils get adsorbed inside the scale insect shells and kill the insects by their physical clogging of spiracles.

Similarly, the results of this study corroborate the findings of some previous studies demonstrating the insecticidal potential of the botanical insecticide oxymatrine. Oxymatrine is indeed a bioinsecticide derived from the plant Sophora flavescens, and it kills the target insect by ingestion or contact by paralyzing the peripheral and central nervous systems of insect pests (Bloomquist *et al*., 2018). For instance, oxymatrine and matrine have been promising botanical formulations being used to control different sucking and chewing insect pests on fruits and vegetables such as against fruit flies (Brilinger *et al*., 2025) and phytophagous mites (Aljboori and Dahwi, 2020; de Andrade *et al*., 2020; Zanardi *et al*., 2025).

Conclusions and Recommendations

The overall results of this field study demonstrated the insecticidal control potential of entomopathogenic fungus (*Metarhizium anisopliae*) and botanical insecticide (Oxymatrine 2.4% SL) against citrus yellow scale (*Aonidiella orientalis*) infesting citrus trees. More interestingly, this study proved the compatibility and synergistic effect of the application of these pesticides along with mineral oil (Nautilus 70% EW), both against adult and crawling (nymphal) stages of scale insects. These findings suggest the incorporation of these biological or botanical insecticides and mineral oils in the integrated management of scale insect pests, including oriental yellow scale (*A. orientalis*). Moreover, optimizing practical implications and assessing the long-term impacts of these pesticidal treatments constitute the future perspectives of this study.

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Novelty Statement

Field Efficacy of Entomopathogenic Fungus Metarhizium anisopliae and Botanical Insecticide Oxymatrine and Mineral Oil Nautilus to Prevent and Control Oriental Yellow Scale. With the focus on Entomopathogenic Fungus as a biocontrol as the future basis of botanical pesticide against Aonidiella orientalis (Newstead).

Authors' Contributions

All authors were contributed equally for all processes of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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