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# COMPATIBILITY AND SYNERGISTIC INTERACTION OF ENTOMOPATOGENIC FUNGI *BEAUVERIA BASSIANA* , *ISARIA FUMOSORSEA* AND OXYMATRINE AGAINST *LIRIOMYZA BRYONIAE*

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## Abstract
The individual and combined effect of oxymatrine insecticide and the entomopathogenic fungi, *Beauveria bassiana* and *Isaria fumosorsea* against the leafminer *Liriomyza bryoniae* (Kaltenbach,) [Diptera: Agromyzidae] was assessed. The oxymatrine was achieved 83.3% and 78.6% mortality of larvae and pupae at half field doses respectively. The mortality rate of larvae by *Beauveria bassiana* was 55.6 %, at a concentration of $10^7$ spores/ml, and the LC50 value was 8.4 x$10^4$ spores/ml. larvae mortality by *Isaria fumosorsea* was 52% at a concentration of $10^7$ spores/ml with LC50 value of 2.6 $10^5$ spores/ml. The mortality rate of pupae was 54.3% and 42.3%by by *Beauveria bassiana* and *Isaria fumosorsea* respectively at $10^7$ spores/ml concentration. There was an increase in the mortality rate due to the combined action of the *Beauveria bassiana* and the oxymatrine ( 88%) it does exceed synergistic effect ( chi square= 4.92), and71.3% by the combination of the oxymatrine and *Isaria fumosorosea* synergistic effect (chi square= 4.65). In the case of pupae treatment, an increase in the rate of mortality was achieved, but it did not express synergism.
***Keywords*:** Biocontrol**,** Leaf Miners, *Liriomyza* spp., *Liriomyza bryoniae* , oxymatrine, entomopatogenic fungi , *Beauveria bassiana*, *Isaria fumosorsea*

## introduction
The leafminer, *Liriomyza bryoniae*, is considered an exotic pest, as it was recorded in Iraq in 1996 and caused great economic losses to vegetable crops, whether in greenhouses or in open cultivation (Al-Mashhadani, 1998) andcaused losses in tomato production of up to 17% and losses in marketing value of up to 20% on tomatoes crop that cultivated in greenhouses (Walker , 2012). The leafminer (*Liriomyza* spp.; Diptera: Agromyzidae), is one of the most important insect pests that attack vegetable crop plants such as cucumbers, tomatoes, beans, fava beans, cabbage, lettuce, melons and zucchini. The insect attacks the leaves of plants, creating tunnels in them, where it feeds on the layer of cells between the two skins of the leaf. There are large numbers of leafminer species of the Diptera order, including large numbers of leafminer larvae of the family Agromyzidae, the tunnels of which are often filamentous, and the faeces on both sides of the tunnel are filamentous. These tunnels are also characterized by the presence of two symmetrical circular openings. ( Ravsaheb , 2013).

Synthetic and natural insecticides for *Liriomyza* species control have been extensively worked and are commonly used by farmers and producers regardless of production scale and crop (Ferguson, 2004). The effectiveness of these insecticides have been reduced because of their indiscriminate use, which has adversely impacted the natural enemies or non-target organisms and resulted in the development of resistance to several groups of insecticides (Rauf, et al. 2000).

To reduce the attack of *Liriomyza*, in addition to synthetic insecticides, farmers can use biological control agents such as predators (Pohl et al. 2012 ; Jat, et al. 2021 ), parasitoids (Foba et al. 2015) and entomopathogenic microorganisms (Migiro et al.
2011 ; Thabu, et al. 2022), as well as substances that induce plant resistance (Vieira et al. 2016). Entomopathogenic fungi (EPF), in particular Deuteromycetes, including *Beauveria bassiana* (Bals.-Criv.) Vuill. are alternative candidates as microbial control agents because of their rapid killing rate in laboratory assays relative to other entomopathogens. The possibility for commercial scale production on artificial media, and their contact route of infection that allows for pest targeting using standard spray applications should be investigated. (Rehner and Buckley 2005 ; Jat, et al. 2022).

*The entomopathogenic fungus Isaria fumosorosea* was known as *Paecilomyces fumosoroseus* for more than 30 years and was recently transferred to the genus *Isaria* (Zimmermann, 2008). Some of the more commonly known susceptible organisms include weevils, plant beetles, aphids, whiteflies, psyllids, wasps, termites, thrips, and a wide variety of butterflies and moths , it therefore has received significant attention as a possible biological control agent for several economically important insect pests of agricultural crops (Van ,2012.; Kim, et al., 2018 ).

The Oxymatrine pesticide of Quinolizidine alkaloids , a plant derived from the roots of wild plants belonging to the legume family is *Sophra* , it has been used to control different insect pests of various crops (Liu, et al, 2007). This study was conducted to evaluate the efficacy of this insecticide (Oxymatrim) , *Beauveria bassiana* , *Isaria fumosorosea* and their combinations on the larvae and pupae of the *Liriomyza bryoniae*.

## Materials and Method

The field experiment is completed during the autumn season in 2021. Cucumber seeds (Cucumis sativus ) BEITH ALPHA (Akgen, Green South) variety were sown, in greenhouse. Plants were left in the greenhouse to be infected with pests, including *Liriomyza bryoniae* without pesticide . (Haider & Wajih ,2016).

### Fungal isolates

The isolates of *Beauveria bassiana* and *Isaria fumosorosea* were obtained from the Agricultural Research Directorate / Ministry of Science and Technology . It was previously isolated from Iraqi gardens and farms soil using the technique of insect bait traps using larvae of *Galleria mellonellaa*).

### Pesticides

**Oxymatrine:** Active ingredient Oxymatrine 2.4% It is an organic insecticide the active substance of this pesticide is Oxymatrine 2.4%, a natural extract from the roots of the Sophra japonica plant Belonging to the family Leguminosaea . In the form of a bottle of 250 ml manufactured by Agrichem Australia the percentage of use is 150-180 ml / 100 liters of water.

**Abamectin:** is a naturally occurring acaricide / pesticide isolated from the fermentation of the soil microorganism Streptomyces avermitilis. (Also known as avermectin) In the form of a bottle of 100 ml manufactured by Al-kaseh the percentage of use is 35-50 ml / 100 liters of water.

**Bioassay**

A leaf dip bioassay of leaves containing young larvae and pupae spray were used. Mined leaves were collected from the unsprayed portion of the cucumber field. a total of 60 actively-mining larvae had been obtained for each treatment. Using a pair of forceps, leaves containing mining larvae were dipped briefly into the pesticide mix (Three concentrations, field, half field and quarter field) or plain water (Untreated control). After dipping, each leaf was briefly held over the rim of the beaker to allow excess liquid to drain off and then placed on a paper towel to drain off the remaining liquid. When the liquid had been drained off, each leaf was transferred to a clean and appropriately-labeled Petri dish. The Petri dishes were incubated at room temperature conditions and leaves were checked for larval mortality after two days. Percentage leaf miner larval mortality for each pesticide treatment was then calculated. Mortality data were corrected for control mortality (Abbott, 1925; WHO, 2009): $\%\ Corrected\ mortality = X - Y / 100 - Y \times 100$ Where X = percentage observed mortality in the treated sample, and Y = percentage mortality in the untreated control. After spraying the pupae, the same steps were followed to monitor the mortality rate.

**Effect of *Beauveria bassiana* and *Isaria fumosorosea* on leaf miner in laboratory condition**

The pathogenic activity of local isolates of *B. bassiana* , and *I. fumosorosea* was measured in vitro condition at concentrations, $10^5$, $10^7$and $10^9$ spores / ml + 0.05% Tween 20 on larvae and pupae of leafminers . Three replicates were used for each treatment. Two procedures were done: pupae spraying, a leaf dip bioassay of leaves containing young larvae,. The control treatment was treated with water +0.05% Tween 20. The mortality rate was counted after 4, 6 and 10 days of treatment with fungi and three days for chemical pesticides. Mortality percentages were corrected using Abbott's formula: Abbott’s corrected mortality= ((% mortality in treatment-% mortality in control)/(100-% mortality in control))×100. (Abbott 1925). The LC50 values were calculated according to the method of Finney (1971).

**Compatibility Test:** Compatibility assay is performed on potato-dextrose agar medium using poisoned food technology, and the effect of insecticide is evaluated in experiments related to vegetative growth of fungus *Beauveria bassiana* and *Isaria fumosorosea* .

This test was conducted to evaluated the compatibility between chemical pesticide (Oxymatrine) and bio agents (Entomopathogenic fungi EPF).The in vitro compatibility of this compound was determined using the recommended dose , half and quarter recommended dose by the poison food technique.( Nene and Thapliyal 1997; Mishra and Gupta, 2012).

To get the desired concentration of insecticide, the recommended field application rate/dose as well as its half dose and quarter dose was added to the PDA medium (100 mL) in a flask and mixed thoroughly before solidification (medium temperature 48°C) . About 20 mL of medium was then poured equally into 9 cm diameter sterile Petri plates and allowed to solidify in a laminar air flow chamber. The Petri plate containing the desired pesticide-poisoned medium was inoculated by transferring a 10 mm diameter culture disc into the center of each Petri plate. The culture disc was cut using a sterile cork borer from 10-day-old EPFs grown on PDA. After inoculation, Petri plates were sealed with Para film and incubated at 28±2°C and 80±5% relative humidity for a12-hr photoperiod. For each treatment, three replications were maintained. Pesticide-un amended PDA media served as the control treatment for comparison under the same conditions. The radial growth of each individual treatment, including each EPF, was measured at 14 DAI and compared with a standard check. The percent of growth inhibition of each EPF over the untreated check was estimated separately for the respective insecticides by using the following formula: $I=C-T/C\times100$

Where I=percent growth inhibition, C=colony diameter in the control, T=colony diameter with treatment. ( Rajanikanth et al. , 2010).

**Synergistic of *B.bassiana and I. fumosorosea* with insecticide**

The same procedure and doses used for evaluating each compound alone were used for the combination of *B.bassiana* and *I. fumosorosea* with oxymatrine. The data corrected by using Abbott (1925) equation. The expected mortality of the treatment of the combination of both agents was obtained using the formula:

$$E = O_a + O_b (1-O_a \backslash 100)$$

Where E represents the expected mortality and $O_a$ represents the observed mortality caused by the oxymatrine alone and $O_b$ is observed mortality caused by of each fungi alone ( Salama, 1984). Chi squared test $\chi^2$ was performed by calculating the $\chi^2$ value using the formula:

$$\chi^2 = (O_c - E)^2 / E$$

Where $O_c$ represents observed mortality for the treatment of combination, and then compared to the table value for df 1 (>3.84).if the calculated $\chi^2$ value exceeds the tabulated value, it indicates a non- additive effect (either synergistic or antagonistic) of the two control agents . A significant interaction of combination was determined through the difference of ($O_c - E$), where positive = synergistic and negative= antagonistic .if the tabulate value exceeds the calculated $\chi2$ value; it represents an additive effect at $P\leq0.05$.

**Statistical analysis**

The results were analyzed using the Spss version 20 program that includes the Duncan DMRT test to compare rates in all parameters and determine the significant differences at a probability level of 0.05. The T test and Probity analysis were also used to measure Lc50.

**Results and discussion**

**Effect of insecticides**

Results of the effect oxymetrin and abamectin pesticides on *Liriomyza bryoniae* larvae at field and half field doses that presented in Fig (1) indicated that the abamectin was superior (94.6 and 89.4% mortality), and its effect at the half- dose was not differ significantly from the effect of oxymetrine at field dose (83.3 and83.9 respectively), This is reflected by the results of the pupation and adults emergence. Where the percentage of adult emergence decreased, in the same manner, which resulted in the final results of mortality for abamectin and oxymetrin .

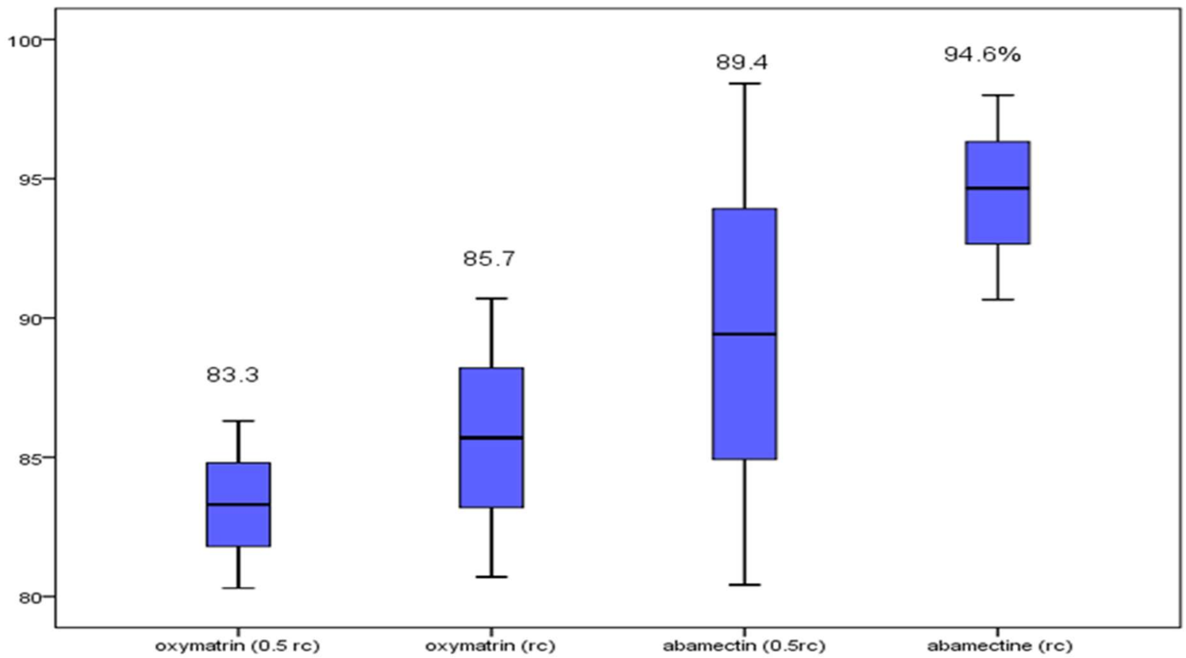



**Fig (1): Effect of oxymetrine and abamectin pesticides on larvae of *L. bryoniae***

Desai, et al.(2018) stated that the pesticide Abamectin was more effective in controlling *L. trifolii* in the concentration of 1.4 ml / L, with the highest kill rate of 58%. Kaspi et al.( 2005 ) pointed to the insecticide/miticide abamectin as an important chemical control method against *Liriomyza bryoniae* leafminers and the mite complex that attacks numerous greenhouse crops.

Oxymatrine as plant based insecticide was used to treat citrus plantlets in the nursery and the result showed that jasmine whitefly adult's mortality was 47.5% after three days of treatment (Tark and Mohammed, 2011). Oxymatrine has also been used to control different insect pests of various crops (Liu, et al, 2007). Oxymatrine is known to target insect acetylcholine receptors, which in turn affects acetylcholinesterase production (Cheng et al. 2018). And also Matrine has shown antifeedant activities against termites (*Coptotermes formosanus* Shiraki) and two spotted spider mites (*Tetranychus urticae Koch*) (Bakr et al. 2012 ; Sain et al. 2017).

Wu et al. (2019) showed that different matrine treatments caused a dose dependent increase in *Spodoptera litura* mortality at different time intervals. Hwang et al. (2009) described the efficacy of a chemical formulation (KNI3126) based on a mixture of matrine and neem oil against different sucking insect pests and phytophagous mites, thus confirming the toxic as well as biological action of matrine against phytophagous arthropods with different feeding habits. Matrine has the characteristics of specificity and naturalness (Zandari et al. 2015).

laboratory study of Baker and Hassan (2019). evaluated the effectiveness of the chemical pesticides Actara, Abamectin, Oxymatrim, and two bioformulation Varanestra (V. lecanii) and Almite (H. thompsonii) and their combination on adults and pupae of the *Liriomyza bryoniae* showed the treatment of Abamectin was superior in adult mortality 55.56% at the half-field concentration (FC-50%), followed by Actara was 46.67%, while the lowest mortality percentage of Oxymatrine treatment was 38.89%. The treatment of Varunestra was significantly higher in mortality percentage of adults was 55.60%, while the lowest mortality percentage for Almite treatment was 42.20%. There was no significant difference in adults mortality percentage for both the mixture (Actara + Varunestra) and the mixture (Actara + Almite), which reached 56.66% and 52.22%, respectively. The treatment of the mixture (Actara + Varunestra) was superior In reducing adults emergence percentage from pupae of 8.88%, which did not differ significantly from the treatment of the mixture (Actara + Almite), which reached to 12.22%.Results showed that Actara was compatibility with two bioformulation Varanestra (V.lecanii) and Almite (H. thompsonii) to control adults and pupae of the *Liriomyza bryoniae.*

**Effect of *Beauveria bassiana and Isaria fumosorosea* on larvae of *Liriomyza bryoniae***

The result of this study that presented in (figure 2) revealed that the impact of *B. bassiana* and *I. fumosorosea* on leaf miners larvae was related to isolate and concentration. The highest mortality rate after 5 days of treatment was recorded which was 62.4 % by *B.bassiana*, at a concentration of $10^9$ spores/ml, the lc50 value was 8.4 x$10^4$ spores/ml. The highest mortality rate of *I. fumosorosea* was 57.8% by the concentration of $10^9$ spores/ml with an lc50 value of 2.6 $10^5$ spores/ml.

So the result revealed that the effect was increased with the increasing of concentration and the fungus *B.bassiana* was more effective against larvae than the fungus *I. fumosorosea*.

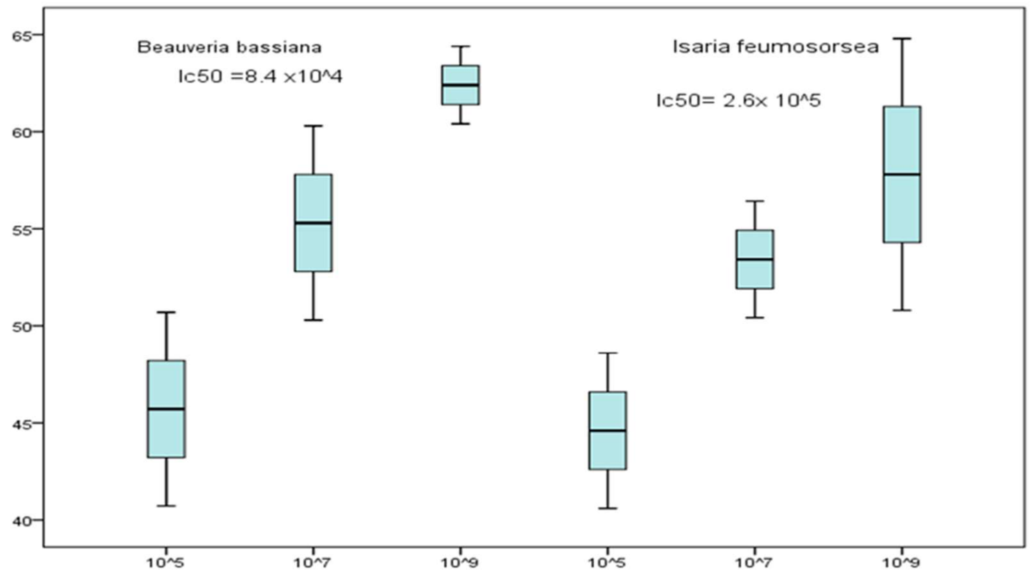



**Fig (2): Effect of *B. bassiana* and *I. fumosorsea* on leaf miner larvae**

The results of the study indicated that the biological resistance fungi had begun its effectiveness in causing infection and killing the fungus on the fifth day of treatment and continued to increase steadily with the passage of time. In order for the killing to occur, therefore, the post-treatment period has a very important role in biological control, as it is directly proportional to the rates of killing larvae when appropriate conditions are available (Luz et al., 1998). This agrees with many researchers who used insect pathogens, including fungi *Beauveria bassiana* and *Isaria fumosorosea*,

Ortiz-Urquiza and Keyhani (2013) *mentioned that prolonging the exposure of the insect to the fungus spores leads to an increase in the rates of insect killing, and the reason may be attributed to the increase in the number of fungal spores, which increases the chances of germination, penetration and infection events, thus, the insect's inability to repel the attack of the fungus on it, which increases the chances of its inevitable death.

In addition, increasing the exposure period increases the amount of enzymes that decompose the body wall of the insect secreted by the fungus, which facilitate the process of penetration and invading the body cavity of the insect, draining its contents and killing it (Shaker, 2015).

Different study referred to the activity of various strains of the hyphomycetous fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana* against dipteran pests (Watson et al. 1995, Renn et al. 1999).

The susceptibility of *Liriomyza trifolii* and *L. sativae* to 11 strains of entomogenous fungi was studied in the laboratory (Bordat et al. 1988). Pupae were placed in peat infected at a rate of about $10^8$ conidiospores/g with suspensions of *Beauveria bassiana* (4 strains), *Metarhizium anisopliae* (3 strains), *Paecilomyces farinosus* (1 strain) and *P. fumosoroseus* (3 strains). At 25°C, *L. trifolii* was susceptible to *P. farinosus* (23% adult emergence) and 2 strains of *P. fumosoroseus* (2.5 and 4% adult emergence). An ambient temperature of 20°C slowed metamorphosis and so contributed to mycosis development. *L. sativae* was generally less susceptible than *L. trifolii* to the tested strains. *M. anisopliae* 78 and *P. farinosus* 46 were highly efficient as adults emerged from only 23.5% and 27.5% of pupae.

The result of this study was in agreement with the result of Gandarilla, et al (2022) who found that the effect of *Beauveria bassiana* on the larvae of *Aedes aegypti* depends on the concentration and the type of isolate and the highest mortality was recorded in the case of the concentration 1.5 × $10^7$ conidia/mL which was 63%.

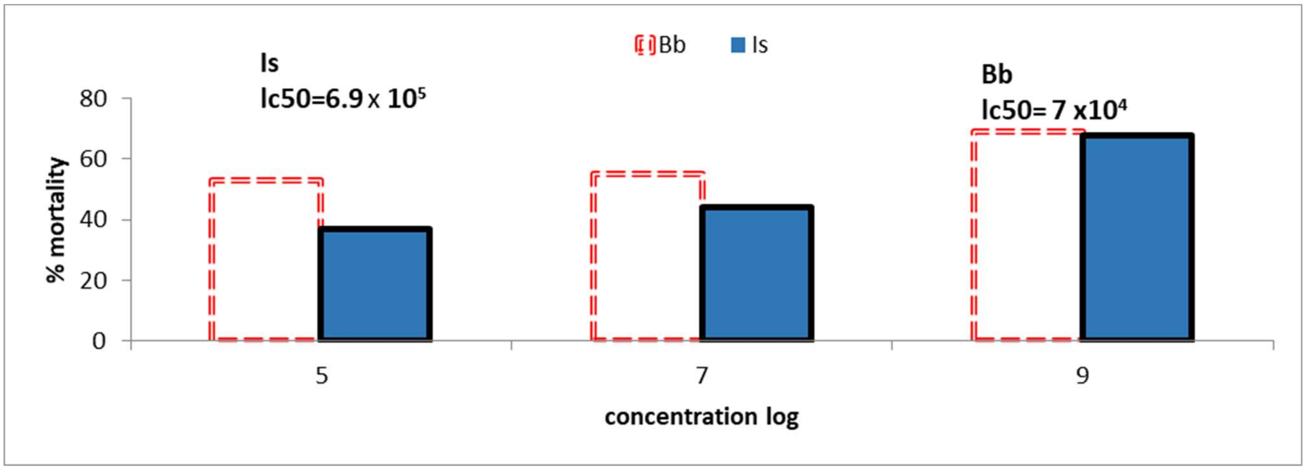
Seventeen isolates of *M. anisopliae* and three isolates of *B. bassiana* were evaluated for their pathogenicity to the adult pea leafminer, *Liriomyza huidobrensis*, in the laboratory. All the isolates were pathogenic to the pea leafminer, causing mortality between 40 and 100% at 5 d after exposure. The lethal time for 50% mortality (LT50) ranged from 2.6 to 5.4 d, whereas the LT90 values varied between 3.2 and 9.1 d depending on the isolate (Migiro, et al., 2011 ).

Another studies recorded the activity of *Beauveria bassiana* and *Isaria fumosorsea* as a biocontrol agent against *Liriomyza trifolii* (Migiro, et al., 2010 ; Wekesa, et al., 2011 ; Kisaakye, et al., 2021).

**Effect of *Beauveria bassiana* and *Isaria fumosorosea* on pupae of *Liriomyza bryoniae***

The result of this study that presented in Figure (3) revealed that the mortality rate of pupae differed according to the fungal isolates and conidial concentrations. The results also showed that the *Beauveria bassiana* caused 55% mortality in $1x10^5$ (conidia/ml) on the 10th day of incubation, and mortality reached 69% in $1x10^9$ (conidia/ml). At the same incubation time, *Isaria fumosorsea* caused 37% and 68% mortality in $1x10^5$ and $1x10^9$ (conidia/ml), respectively. The LC50 after 10 days of application with the *Beauveria bassiana* was 7 x $10^4$ (conidia/ml) , whereas it was 6.9 x $10^5$ (conidia/ml) , for *I. fumosorsea*.

The overall biocontrol potential of a fungal strain should be based also on its bioefficacy, including factors such as mycelial growth, sporulation, and mortality. As compared to the fungal strains, *Beauveria bassiana* showed faster and higher mycelial growth. However, based on overall biological efficacy, the EPFs that performed best were found to be *Beauveria bassiana* (62.1%) and *I. fumosorsea* (61.1%) . (Table 1). Evaluate the efficiency of two biological agents *Beauveria bassiana* Bals. and *Bacillus thuringiensis* Kurstaki and four chemical pesticides Levo 2.4 SL, Aster 20 SL, Diffuse 450 SC and Matrixine Plus EC on four important main pests that infect tomato plants, the pests studied were whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Meyrick Tuta absoluta* (Lepidoptera: Gelechiidae), *Sulzer Myzus persicae* (Homoptera: Aphididae) and *Tetranychus urticae* (Acari: Tetranychidae , the pesticide Matrixine Plus (2.4% Abamectine and Oxymatrin) recorded the highest average of mortality percentage of both larval and adult stages of all four pests( Abbas et al. 2020).




**Fig(3) Efficacy of *B. bassiana* and *I. fumosorsea* against leafminer pupae**

**Table (1) Biological index of *B. bassiana* and *I. fumosorsea* in controlling leafminer**

| Fungi | Growth (mm) | spores/ml ($10^9$ ) | Mortality | Biological efficacy index |
|---|---|---|---|---|
| ***Beauveria bassiana*** | 72 | 7.3 | 69 | 62.1 |
| ***Isaria fumosorsea*** | 71 | 6.4 | 68 | 61.1 |

Another study by Borisov and Ushchekov, (1997) revealed that the *P. lilacinus* and *M. anisopliae* were effective in reducing adult emergence from the soil by 70-94% and 60-88% respectively as compared with the untreated control .

Two biological control agents have been tested against *Liriomyza huidobrensis* pupae; the entomopathogenic fungus *Beauveria bassiana* and the entomopathogenic nematode *Heterorhabditis indica*,The results obtained when treatments with *B. bassiana* were able to kill 73-97% of the pupae and similarly treatments with *B. bassiana* and the surfactant Tween 80 were able to kill 73-93% of the pupae, Tween 80 demonstrated to increase the sporulation rate iduring the first 7 days following the application of the spores of *B. bassiana*.( Noujeim, et al.,2015).

Similar study showed the susceptibility of *Liriomyza huidobrensis* pupal stage to B. *bassiana* and *Metarhizium robertsii* depends on the type of isolate and bioagent and the fungus *M. robertsii* was the more effective one (Thabu, et al., 2022).

**Compatibility between the oxymatrine and fungal bio-agents**

The effect of the oxymatrine pesticide on *B. bassiana* growth was illustrated in Table (2 ). The results reflect the compatibility between the pesticide and the fungus. The study revealed that the reduction percentage of vegetative growth of the fungus was 0.0, -0.8, and 1.62 % at field, half, and quarter field doses respectively after 14 days. The reduction effect of the pesticides on *Isaria fumosorosea* vegetative growth at the recommended field dose was – 2.78, - 1.98, and 0.7 for field dose, half, and quarter dose respectively. (Table 3) .

**Table (2) Effect of oxymatrine on *Beauveria bassiana***

| Concentration | 3 days | % | Grade | 7days | % | Grade | 10days | % | Grade | 14 days | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Recommended dose 1.8 ml/L water | 3.67 | 5.98 | 1 | 5.47 | 7.34 | 1 | 6.83 | 5.53 | 1 | 8.23 | 0.00 |
| Half recommended dose 0.9 ml/L water | 4.03 | -3.42 | 1 | 5.8 | 1.69 | 1 | 7.30 | -0.92 | 1 | 8.30 | -0.81 |
| Quarter recommended dose 0.45 ml/ L water | 4.27 | -9.4 | 1 | 6.1 | - 3.39 | 1 | 7.47 | -3.23 | 1 | 8.10 | 1.62 |
| Control | 3.9 | | | 5.9 | | | 7.23 | | | 8.23 | |

**1=<50% reduction (harmless) 2= 50-79 slightly harmful 3= 80-90 moderately harmful 4= > 90% harmful e (Hassan, 1989).**

**Table (3) Effect of oxametrine on *Isaria fumosorosea***

| concentration | 3d. | % | grade | 7d. | % | grade | 10d. | % | grade | 14d. | % | grade |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| recommended dose 1.8 ml/L water | 2.4 | 2.7 | 1 | 6.07 | -5.2 | 1 | 7.57 | -5.58 | 1 | 8.63 | -2.78 | 1 |
| Half recommended dose 0.9 ml/L water | 2.53 | -2.7 | 1 | 6.03 | -4.62 | 1 | 7.5 | -4.65 | 1 | 8.57 | -1.98 | 1 |
| quarter recommended dose 0.45 ml/ L water | 3.20 | -29.73 | 1 | 6.0 | -4.05 | 1 | 7.2 | -0.47 | 1 | 8.33 | 0.79 | 1 |
| control | 2.47 | | | 5.77 | | | 7.17 | | | 8.4 | | |

**1=<50% reduction (harmless) 2= 50-79 slightly harmful 3= 80-90 moderately harmful 4= > 90% harmful e (Hassan, 1989).**

The reason for the compatibility, as these fungi may possess some enzymes that metabolize and decompose these pesticides into secondary metabolites, from which the fungi benefit as an important food source for their growth and reproduction by a process known as mycoremediation, as well as additives to modern pesticide preparations such as some the diffuse, binder and thickener materials that are polysaccharides (Xanthan gum and natural gum) and Propylene glycol containing carbohydrates, which is a ready food source for direct feeding by fungi, which increases their vegetative growth and production of conidia, many researchers have indicated the stimulating effect for some types of chemical pesticide preparations in the growth and reproduction of *B.bassiana* and *M. anisopliae*.( Anderson and Robert, 1983; Alves and Moino, 1998 ; Harbhajan, 2006).

Synergistic measurement

The synergistic measurement between pesticides and isolates of the pathogenic fungi achieved enhancing of the effectiveness at a significant level in controlling larvae. In the case of *B.bassiana* showed that there was an increase in the mortality rate due to the combined action of the fungus and the pesticide ( 88%) it does exceed synergistic status ( chi square= 4.92). In a similar case, the results of the combination of the pesticide and *Isaria fumosorosea* (chi square= 4.65) (Table 4). In the case of the treatment of pupae, the results showed an increase in the rate of mortality when the pesticide and the fungus were collected, but it did not express the state of synergism, but only the addition.

**Table (4) Synergistic bioassay of oxymatrine with *B. bassiana* and *I. fumosorosea* against larvae and pupae of *L. bryoniae***

| Host | oxymatrin | Beauveria. $10^7$ | expected | observed | Chi square |
|---|---|---|---|---|---|
| Larvae | 83.3 | 55.6 | 69.51 | 88.0 | 4.92 |
| Pupae | 78.6 | 54.3 | 90.22 | 79 | 1.4 |
| Host | oxymetrin | Issria. $10^7$ | expected | observed | Chi square |
| Larvae | 83.3 | 52 | 91.98 | 71.3 | 4.65 |
| Pupae | 78.6 | 42.3 | 87.65 | 81 | 0.5 |

- The value of the Chi square is greater than 3.83 at a degree of freedom 1, mean that the factors are synergistic

The synergistic action of mycoinsecticides with chemical insecticides can increase mortality and reduce the time until death in insects**.** The combined use of fungal pathogens and the full, or reduced, dose of chemical insecticides is a promising pest-control option. The application of synergists can effectively enhance the cost-effectiveness and eco-friendliness of insecticides by reducing the required quantity and extending the residual activity (Bitsadze, 2013 ; Sharififard, et al., 2011). Another study deliberates the possible synergistic action of oxymatrine and *Beauveria brongniartii* against *S. litura* in laboratory and half field dose conditions as both of these agents possibly affects their host through same site of action. Oxymatrine is known to target insect acetylcholine receptors, which in turn effects acetylcholinestrase production (Liu et al., 2007).

Also Asghari et al. (2009) and Baker and Hassan (2019) , _ referred to the activity of Oxymatrine against *Liriomyza* sp and its compatibility with entomopathogenic fungi to control this insect. Dayakar et al. (2000) have found that the combination of insecticides with *B. bassiana* and *M. anisopliae* showed 1.05–1.24 and 1.19–1.42 fold increase in virulence over the sole treatment, respectively. Quintela and McCoy (1997) have found that *B. bassiana* and *M. anisopliae* combined with sublethal doses of imidacloprid as a contact or oral treatment increased the mortality synergistically in Diaprepes abbreviates.

The combination of insecticide and entomogenous fungi was more deleterious to the insect than application of insecticides or entomogenous fungi alone. Evaluate the efficiency of combining *Beauveria bassiana* with certain insecticides (emamectin benzoate, methomyl and Bacillus thuringiensis) in tomato field revealed that mixing increases the efficiency of their insecticides, When treated larvae with mixture of fungi (*B. bassiana*) and insecticides percentages mortalities of second and fourth larval instar of emamectin benzoate + *B. bassiana* and methomyl + *B. bassiana* had the highest values after 24hrs. then decreasing gradually, but the highest values of percentage mortality of second and fourth larval instar when mixtures of *Bacillus thuringiensis* + *B. bassiana* were after fifth days at concentrate of insecticide 1/2RC and after third day at concentrate of insecticide 1/4RC ( Abdel Aziz et al., 2018) .

There was a significant increase (38%) with clothianidin in weevil mortality over clothianidin alone 1 d after treatment (Clavet et al., 2013). Joint application of *B. brongniartii* and matrine against *Spodoptera litura* caused a significant increase in mortality values under laboratory as well as field conditions and showing a strong synergistic interaction (Wu,et al., 2019 ).

Ali et al. (2017) observed similar increase in mortalities of *B. tabaci* treated with different combinations of oxymetrin and *Lecanicillium muscarium*. The possible synergistic action between both control agents was due to them both working on the same target site; oxymetrin and A. attenuatus both attack insect acetylcholine receptors (Bourne, et al., 2016).

The level of synergism observed under semi-field conditions was slightly lower than the laboratory conditions which can be explained by the variations in different biotic and abiotic factors or behavior of the target pest under semi field conditions (Xu, et al., 2011).

In order to increase the virulence of two commonly used entomopathogens namely *Beauveria bassiana* and *Metarhizium anisopliae* for effective mosquito control programs, we developed new combined formulation by the use of sublethal concentration of imidacloprid to second instar larvae C. quinquefasciatus for 92 h and the results revealed that the combination increased the mortality of second instar larvae C. *quinquefasciatus* and sublethal concentration was proved to be useful for effective larval control (Koodalingam, & Dayanidhi, 2021).

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