

The Controlling Of Ash Whiteflies *Siphoninus Phillyreae* (Haliday) (Hemiptera : Aleurodidae) By *Metarhizium Anisopliae* In The Field

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Abstract

This experiment was conducted in the Agriculture College fields for the Autumn season 2019 with the aim of examining the *M. anisopliae* effect on the mortality ratios of the whitefly *S. phillyreae* stages (eggs, instar^{2nd} and pupae), where the mortality rates for the tested fungal isolates varied (4.8×10^1 , 4.8×10^2 and 4.8×10^4) which also varied according to their used concentrations and the time periods following the treatment. As the mortality rates gradually increased, the concentration increased or the lengthy period after its use increased. The fungus *M. anisopliae* achieved mortality rates for eggs, instar^{2nd} and the pupae, respectively, and the highest mortality rate was at concentration 4.8×10^4 spor / ml of 3.66, 5.66 and 4.66% after a week of treatment, respectively, and then the mortality rates continued, and the mortality rates reached (33.3 and 1.33), (2.33 and 1.33) and (2.66 and 1.66)% after 14 and 21 days of treatment, respectively, and achieving 1.66, 0.66 and 0.33 %mortality rate after four weeks of treatment, to infect the fungus with all tested insect stages, respectively.

Key words: Concentration, Mortality, *Metarhizium anisopliae*, Pupae *Siphoninus phillyreae*, whitefly

Introduction

Whiteflies are the most important an insect pest on more an important economic plants , The Ash whiteflies *Siphoninus phillyreae* (Haliday) Which is recorded in the broad ,recently they are detected in Iraq ,they have more host plants especially the Citrus ,causing severe injury on other trees such as Buckthorn and Olive [1,2,3].Whiteflies are tiny insect with white powder covered pairs wings the type of mouth parts are piercing sucking , gradual life cycle from hatched eggs to crawling nymph stage with white waxy bristles on the outer edge ,the active nymphs remain mobile for a short time ,then find a suitable place for feeding and settle down to become adults [4]The nymphs and adult suck plant sap and secrete honeydew on the tree leaves that leads to fungi growth and collects dust ,thus prevent the plant physiological processes, especially the photosynthesis process[5] .Some natural enemies (parasite and predator) of *S. phillyreae* are *Encarsia inaron* , *Eretmocerus corni* [6].The seriousness of whiteflies and

widely distribution on many economic plants [7]. Pathogens such as fungi, Bacteria and Viruses are natural enemies to control whiteflies, additionally the predators and parasite importance in a biological control strategic programs that it used with the insecticide, [8] confirmed that it is a save more specific methods and need an excite environment conditions for success, without reminds, doesn't make a dangers for Human and his animals, it can applied before plant harvest, sometimes it presence in natural environment [9]. Fungi is the most important agent of biological control for effect of whiteflies species according Aleuridadae. [10] confirmed the hard ability insect infect [11] by cuticle penetration ability and growth in insect body [12]. The study aims to determine the most important of biological control by Entomopathogenic agent to control by biotic factor *Metarhizium anisoplae* to whiteflies *S. phillyreae* stages

Materials and methods

Fungus colony was imported to Iraq from Algeria in 2008. Was cultivated in sterilized petri dishes contains Potato Dextrose Agar (PDA) media with 1 gm of pure chitin for each media Wight 250gm. Add 0.05gm of streptomycin to bacterial inhabitation. After inoculation, dishes were put in an incubator at temperature $25 \pm 5\%$ for a period of 7 to 10 days. Fungal Spore Suspension was prepared using a petri dish contains the weeks old grown fungal colony, added 5ml sterile distilled water then spores were harvested using harvester (glass L shape loop), were filtered with sterile gauze installed on a glass funnel fixed on sterilized 100ml. To ensure the filtration of all spores added 5ml of water on the gauzes sides. Drained solution represents the stock solution [13]

Calculation of the fungal spores suspension *M. anisopliae* spores numbers.

The number of spores for the fungal spores suspension was calculated according to [14,15] using the Neubauer improved cell count slice by placing a drop on this slice of the base solution with the slide cover applied and calculating the number of blackboards in each square at a strength Zoom 40 × according to the following formula: -

$$\frac{N}{80} \times 6 \times 10 \times 10 = \text{Number of spore}$$

As:

N = the number of blackboards in the squares, = 80 the sum of the five squares, 610 = the dilution correction coefficient, 10 the correction coefficient of volume.

After the count, it was found that the concentration of the basic solution of *M. anisopliae* was 4.8×10^4 spor / ml. The dilution of the fungus represented the aforementioned numbers multiplied by the three dilutions of the fungus, which are 4.8×10^1 , 4.8×10^2 , 4.8×10^4 spor / ml means the first and second dilution. And the third, respectively. Concentrations of spores of *M. anisopliae* isolates were prepared 4.8×10^1 , 4.8×10^2 , 4.8×10^4 spor spore / ml respectively for the purpose of studying their effect on all of the insect's stages. Add to each concentration a few drops of Tween 80 solution at a concentration of 0.10% as a moisture preservative And diffuser. Took 4 sterile test tubes marked from 1-4. Each tube contains 9 ml of sterile distilled water. 1 ml of the base sputum Stock was withdrawn by a sterile pipette Pipit and added to tube 1 so the concentration became 10^1 , then withdraw 1 ml from tube 1 and add to tube No.

2 The dilution became 10^2 , and so on to concentration 10^4 for the *M. anisopliae*. The tubes containing the required concentrations were kept in the refrigerator at 4 ° C until the experiment [16].

Biological Effects of Mushroom Spores Suspension on the Life Roles of Black Fly Species and Genres on Citrus trees in the Field.

To find out the efficacy of the aforementioned isolates against the insect stages in field conditions, the experiment was conducted in the fields of the Faculty of Agriculture and selected six small citrus seedlings with different the black fly insect stages. Seedlings sprinkled spore the fungal isolates of the fungi *M. anisopliae* with concentrations of 4.8×10^1 , 4.8×10^2 , 4.8×10^4 spore / ml only, respectively. By three replicates per concentration, one refined included on one seedlings, while three other seedlings were sprayed with sterilized distilled water for comparison. After the spraying process, all the seedlings were covered with bags of the organza cloth, which was tied at its base. The readings were taken after 14 and 21,28 treatment days. The relative effectiveness (%) of fungi concentrations was calculated according to the Henderson and Tiltion equation defined by [17] as the ratio of the odds ratio or the gross product ratio as follows:

$$X 100 (P = (1 - ((CB) \times (TA)) / ((CA) \times (TB)))$$

As:

P = the relative effectiveness (%) of a fungus.

TA = average number of live pest individuals after treatment.

TB = average number of live pest individuals prior to treatment.

CA = average number of members of a live lesion in comparison after treatment.

CB = average number of members of a live pest in the comparison before treatment.

Results and Dissection

From tables (1-3), it is observed that the mortality rates for the tested fungal isolates varied according to their used concentrations and the time periods that followed the treatment. As the mortality rates increased gradually, the concentration increased or the period after its use increased. The fungus *M. anisopliae* achieved very modest eggs mortality rates, and the highest mortality rate was at concentration 4.8×10^4 spore / ml reached 3.66% after a week of treatment (Table 1). Then the mortality rates continued to reach (33.3 and 1.33)% after 14 and 21 treatment Days , respectively, it is noted from the table mentioned that a mortality rate reached 1.66% was achieved after four weeks of treatment so that the fungus infects all the insect tested eggs. The results of (Table 2) also show that the highest mortality rate of the aforementioned fungi concentrations against instar^{2nd} was at a concentration 4.8×10^4 spore / ml reached 5.66% after a week of treatment, and then followed in varying proportions, reaching (2.33 and 1.33)% After 14 and 21 treatment days, it is also noticed from this table a 0.66% mortality rate was achieved 0.66% after four treatment weeks so that the fungus infects all tested insect instars^{2nd}. Finally, it is observed from the Table results (3) that the highest rate of pupae mortality rate at concentration 4.8×10^4 spore / ml, which is 4.66% after a week of treatment, and then followed in

varying proportions, reaching, (2.66 and 1.66)% after 14 and 21 days, respectively, and notes from this table also achieved a 33% mortality rate. after four treatment weeks, infected all fungus of the tested insect pupae. These results are consistent with what was mentioned.[18] It showed that the black fly stages *Acaudalerodes rachipora* mortality rates increase with fungi concentrations and exposure duration increasing. The effect of sunlight, especially ultraviolet radiation, has a known effect on the pathogen's DNA [19], which results in a decrease in the pest mortality rates and its slow effect as biological factors in curbing the pest population in field application. Sometimes we notice their no utility and the need to re-apply them because the insect returns its population to the critical limits to the point where chemical interference is required [20]. These facts encouraged many researchers in this field to think about using other environmentally friendly substances and mix them with pathogenic fungi to increase their effectiveness and field application [21], recently used the mixing vegetable oils technique such as neem oil with these fungi to increase their efficiency and speed in killing when This oil has an effect on suffocating the insect and thus weakening it early so that the fungus can play the complementary role in its penetration and be a guaranteed source of fungal infection later. [22] explained that using oil with fungus boards in a dry environment helps conidia maintain its effectiveness for longer periods inside the oil droplets[23]. Growth regulators have been used in other applications as catalysts to accelerate fungal infection and increase mortality rates at mixing it with these fungi, as it works pesticide action in the double concentrations. Either in its recommended concentrations, its effect is slow in the pest growth and development by kaitin synthesis inhibiting process [24]. We note an increase in the mortality rate with the use of double doses of the fungus and the growth regulator Dimilin and Alsystin. This effect was observed in the treatment of the Desert Locust [25] as the fungus and the growth regulator combination caused the failure of the molting processes to prevent its development as well as increasing the effectiveness of the fungus in invading the insect body [26] . [27] used a Dimilin growth regulator mixture with *M. anisoplae* on the Desert Locust *Schistocera gregaria* nymphs and gave a 100% mortality rate.

Table (1): Relative Effectiveness of *M. anisoplae* against the white fly, *S. phillyreae* eggs in the field

Concentration spores/ml	daily Relative efficacy %			
	7days	14days	21days	28days
4.8x10 ¹	1.33	2.66	3.33	4.33
4.8x10 ²	2.33	3.66	3.66	0.33
4.8x10 ⁴	3.66	3.33	1.33	1.66
mean	2.44	3.22	2.77	2.11

Table (2): Relative Effectiveness of *M. anisoplae* against the white fly, *S. phillyreae* instar^{2nd} in the field

Concentration spores/ml	daily Relative efficacy %			
	7days	14days	21days	28days
4.8x10 ¹	2.66	3.33	3.33	1.33
4.8x10 ²	4.33	3.66	1.33	0.66

4.8x10 ⁴	5.66	2.33	1.33	0.66
mean	4.22	3.11	2.01	1.01

Table (3): Relative Effectiveness of *M.anisopliae* against the white fly, *S.phillyreae* pupae in the field

Concentration spores/ml	daily Relative efficacy %			
	7days	14days	21days	28days
4.8x10 ¹	3.33	3.66	1.66	1.33
4.8x10 ²	4.33	3.66	1.66	0.33
4.8x10 ⁴	4.66	2.66	1.66	0.33
mean	4.11	3.33	1.66	1.01

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