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Abstract. In Iraq, *Bemisia tabaci* is a major Tomato pest that causes significant crop losses, which lowers farmers' revenue. The purpose of this study was to detect entomopathogenic fungus from dead whiteflies and to assess their relative frequency and different structural characteristics. Only two fungal species, *Trichodrema virede* and *Beauveria bassiana*, with relative frequencies of 15.07 and 10.94%, respectively, were validated microscopically as recognised entomopathogenic species out of the 11 fungal species isolated from the whitefly cadavers. The 7th day following inoculation was when T. virede and B. bassiana had the largest mortality impact on *B. tabaci* nymphs and adults, with an average mortality of more than 60% (with a concentration of 106 conidia/ml). In comparison to nymphs, suspension *T.virede* and *B.bassiana* . were significantly twice as pathogenic.

Keywords. Bemisia tabaci, Tomata, Entomopathogenic fungi, Trichoderma.

1. Introduction

Recent studies have illuminated the defence mechanisms of many fungal species against insect pests. It has been discovered that these entomopathognic fungi , which include both specialised and non-specialized kinds including Beauveria and Trichoderma, significantly contribute to protecting plants [1]. Furthermore, they utilise a variety of processes to support plant development. These bacteria control plant infections through mycoparasitism, competition, antibiotic synthesis, and direct encouragement of plant development once they have colonised the plant [1].

Fascinatingly,entomopathognic fungi spend a significant amount of their life cycle inside the tissues of plants without showing any symptoms in the host [1,2]. Important agricultural crops including wheat, maize, and tomatoes are among the many plants in which this endophytic relationship with fungus has been noted [3,4]. One such fungus, Beauveria bassiana, is mainly found in soil and has a wide spectrum of insecticidal action against insects at various developmental stages.. *B. bassiana* has also demonstrated the capacity to defend plants against particular plant diseases and can even function as a biofertilizer [5,6]. Previous studies have looked closely at the insecticidal and antibacterial activities of endophytic *B. bassiana* [7]. The tomato fruitworm (Helicoverpa armigera) and tomato leafminer (Tuta absoluta), two chewing-tunneling lepidopteran pests, have been successfully introduced into tomato

plants using a variety of application techniques, and *B. bassiana* effectiveness as an insecticide has been confirmed [8]. Our study's objective was to assess several techniques for introducing *B. bassiana* as an endophyte in tomato plants [8]. Furthermore, in greenhouse circumstances, we looked at the effectiveness of this fungal endophyte against the sap-sucking insect known as the greenhouse whitefly (Bemisia tabaci) [9]. Our findings have important ramifications for investigating alternate pest control strategies given the widespread dependence on chemical pesticides for managing whiteflies in many nations. Microorganisms known as mutualistic endophytes, including bacteria and fungus, live in plant tissues throughout the entirety of their life cycle or at particular phases without manifesting any symptoms of illness. These mutualistic endophytes have occasionally shown that they can lessen phytopathogen infections and insect herbivory on colonised plants [10].

2. Materials and Method

2.1. Insect-Harming Fungus White Fly Fungus Isolation and Identification

In February 2022, insect cadavers with exterior fungal development were gathered from tomato farms in Basra-Al-Zubayr, a governorate in southern Iraq, and kept in ptri-dishes with potato dextrose agar (PDA) medium. Up until the fungus started to develop further, the infected ptri-dishes were incubated in an incubator at 25 °C and 755% R. H. The use of a compound microscope was used to examine the spores of pure cultures.Insects that were expected to be infected were surface-sterilized in a 1% solution of sodium hydrochlorite for 30 seconds before being cleaned with distilled water. The insects were then raised in ptri plates (15 insects per dish) using PDA medium and maintained in an incubator with the same temperature and R.H. conditions. Recognising isolated fungus *B. tabaci* nymphs and adults' bioassays using conidia suspension method . After the growth of the fungi, they were purified and isolated again on PDA medium, and the fungi were identified according to the taxonomic keys. [11].

2.2. Bioassays on B.tabaci Nymphs and Adults Utilising the Conidia Suspension Technique

Three concentrations of the fungus suspension *T.virede* and B.bassiana ($1x10^4$, $1x10^5$ and $1 x 10^6$ spores/ml). Each replication of the treatments and controls contained 10 adult *B. tabaci*, and there were three replicates of each kind. In tiny plastic cages, 1 cc of the fungal slurry was sprayed over each copy before being moved to a 9 cm Petri plate. Water was used as the control. The *B. tabaci* nymphs underwent the same experiment. Every two days—three, five, and seven days after inoculation—researchers looked at the mortality rates of *B. tabaci* adults and nymphs [12].

2.3. Biocontrol of Bemisia tabaci

Biocontrol Using lab bioassays, *T.virede* and *B. bassiana* were assessed against adult *Bemisia tabaci.* The capacity of *T.virede* and *B.bassiana* to systemically colonise the whole plant was tested using spraying techniques. Using a hand sprayer, a spore suspension with a density of $1x\ 10^6$ spores/mL was applied to the whole plant. Plants in the control group received the same amount of distilled water treatment. Twenty adult *B. tabaci* were released onto each of the treated plants, which were housed in cages protected by insect netting. A translucent synthetic canvas entomological cage with dimensions of 60 cm by 60 cm by 80 cm held four plants. After that, the plants were housed in a controlled environment in a greenhouse, and the mortality of *B. tabaci* at various phases of life was noted. By comparing the number of pupae that emerged to the total number of pupae, the percentage of adult mortality was calculated. The dead whiteflies were kept in an incubator at room temperature while mycosis was looked for. Four replicates were used in each bioassay. The whiteflies were tracked for 14 days after being raised on the test plants for their whole existence. After one day, three days, seven days, and fourteen days, the number of adult living plants on each plant was counted.

2.4. Statistical Analysis

To ascertain if there were any statistically significant differences between the groups, the obtained data were subjected to a one-way analysis of variance (ANOVA) study. The least significant difference (LSD) test was used for post hoc multiple comparisons of the means, with a significance

threshold of P 0.05. To compare similar samples, paired t-tests were also used. All data analysis used SPSS, version 21.0, a statistical programme.

3. Results

3.1. Fungal Community Isolated from Whitefly Adults

The results obtained revealed that all fungi were recovered from all whitefly samples. The antagonistic fungi percent isolated from whitefly ranged from 15.07% . Statistical analysis revealed a significant difference of fungal densities ($P < 0.05$). Highest fungal frequency was recorded for *Beauveria bassiana* (15.07%), followed by *Aspergillus niger* (11.87%) and *Trichoderma virede* (10.94%). However, the lowest concentration was recorded for *Cladosporium cladosporioides* and *C.oxysporum* with a percent of 4 and 8% (Table 1).

Table 1. Relative frequency (Rf), of the fungal specie isolated from the whitefly adults.

Fungal strain	Fungal families	Relative frequency (%)
Aspergillus niger	*Trichocomaceae*	11.87
A.carponrius	*Trichocomaceae*	9 .32
A.flavus	*Trichocomaceae*	9.64
A. oryzae	*Trichocomaceae*	10.41
Alternaria chlamydospora	*Pleosporaceae*	8.43
Beauveria bassiana	*Cordycipitaceae*	13.07
Cladosporium oxysporum	*Cladosporiaceae*	8.11
C.cladosporioides	*Cladosporiaceae*	4.60
Fusarium moniliformae	*Nectriaceae*	7.10
Penicillium oxalicum	*Trichocomaceae*	6.51
Trichoderma virede	*Trichocomaceae*	10.94
L.S.D.0.05		2.83

3.2. Bemisia Tabaci Adults and Nymphs Bioassays using Conidia Suspension Method

The mortality was higher when the highest rate of conidia suspension was sprayed onto the infested leaves with B. tabaci adults and nymphs. Infected adults and nymphs began to die 3 days after exposure to conidia (Tab. 2). The average mortalities of the insects sprayed with T.virede, and B. bassiana . varied from 23.14 (104 conidia/ ml) to 37.45 % (106 conidia/ml) and from 44.16 (104 conidia/ml) to 71.19 % (106 conidia/ml) after 7 days of inoculation, respectively. After 7 days of the exposure of B. tabaci nymphs to conidia of B. bassiana, the mortality 28.76–67.14 % were recorded as in the lowest (104 conidia/ml) to the highest (106 conidia/ml) treatment T.virede showed some pathogenicity; the mortality value was estimated 55.46 % after 7 days of inoculation (106 conidia/ml) (Tab. 2).

Table 2. Comparative effect of T.virede and b.bassiana on mortality rate (%) of Bemisia tabaci adults using conidia filtration method.

Fungi concentration (conidia ml$^{)1}$)	***B.tabaci* adult % mortality after**		
B. bassiana	**3 days**	**4 days**	**7 days**
0	2.06 ± 1.67c	2.34 ± 0.97c	6.13 ± 1.81c
104	23.14 ± 2.98b	42.25 ± 3.80b	44.16 ± 5.41b
105	35.76 ± 5.18 a	55.34 ± 5.67a	65.11 ± 4.94a
106	37.45 ± 5.84 a	53.49 ± 4.58a	71.19 ± 4.08a
T.virede	3 days	4 days	7 days
104	16.76 ± 2.76 b	16.78 ± 1.15b	27.44 ± 6.91b
105	22.87 ± 3.65 b	39.88 ± 2.25a	55.11 ± 1.60a
106	33.98 ± 5.62 a	44.09 ± 2.69a	59.19 ± 5.33a

Means inside a column with the same letter do not differ substantially (LSD-test after one way ANOVA: ($P > 0.05$) .

Table 3. Comparative effect of *T.virede* and *B.bassiana* on mortality rate (%) of Bemisia tabaci adults using conidia filtration method.

Fungi concentration (conidia ml^{-1})	*B. tabaci* nymphs % mortality after		
B. bassiana	**3 days**	**4 days**	**7 days**
0	2.06 ± 1.67c	2.34 ± 0.97c	6.13 ± 1.81c
104	17.17 ± 5.76b	31.85 ± 1.23b	46.32 ± 3.90b
105	29.41 ± 3.10 a	42.77 ± 3.24a	61.52 ± 4.05a
106	28.76 ±2.84 a	47.92 ± 2.50a	67.14 ± 4.23a
T.virede	3 days	4 days	7 days
104	9.70 ± 4.01 b	15.22 ± 2.14b	29.65 ± 2.61b
105	25.14 ± 2.66 a	33.17 ± 4.30a	51.73 ± 4.41a
106	25.77 ± 3.02 a	40.83 ± 5.04a	55.46 ± 3.31a

Means inside a column with the same letter do not differ substantially (LSD-test after one-way ANOVA : (P>0.05).

3.3. Bemisia Tabaci Biocontrol in Plants

We examined the survival of adult *B. tabaci* on tomatoes treated with *T.virede* and *B. bassiana* to control tomatoes in order to ascertain if these treatments had any impact on B. tabaci performance. After 14 days of feeding, we discovered variations in the adult B.tabaci's survival rate on tomatoes treated with *T.virede* and *B.bassiana*.When compared to control tomatoes, B.bassiana had the greatest mortality rate (= 55.69) (Tab.4). However, there were significant differences between the three treatments and fewer B. tabaci feeding on the *T.virede* with tomatoes as compared to *B. tabaci* and control tomatoes. Then the fungus *T.virede* with a mortality rate (=47.82). It is noted too that the death rate increased after days of treatment with biological control fungi.

Table 4. Effect of biological control fungi on adult white bear using concentration of 1x 10^6 spores/ml.

Treatment	Survival rate, 3 days after	Survival rate, 4 days after	Survival rate, 7 days after	Survival rate, 14 days after
Control (water only)	3.65 ± 1.83c	4.76 ± 3.73c	6.74 ±13b	24.87± 4.11c
T. virede	5.74 ± 3.40b	14.83±4.45b	26 .87 ± 23a	47.82 ± 5.39 b
B.bassiana	10.85 ± 5.76a	18.63 ± 2.80a	31.73 ± 10 a	55.69 ± 2.43 a
Mean	6.74	12.74	21.78	42.79

Means inside a column with the same letter do not differ substantially (LSD-test after one-way ANOVA : (P>0.05).

4. Discussion

It is crucial to understand the diversity of *B.tabaci* natural enemies in the agroecosystem. Due to the lack of general concepts and trustworthy methods to define microbial species, studies on the soil microbial and insect communities tend to focus more on genetic diversity than species diversity, making it challenging to assess the entomopathogenic fungal biodiversities of the adult whitefly populations in Iraq. Two significant entomopathogenic fungi, *T.virede* frequency was 10.94% and *B.bassiana* frequency was 15.07%, were commonly isolated from the whitefly adults examined. Whitefly adults contained several non-entomopathogenic fungus, including *Cladosporium* Spp, *Aspergillus* spp., and *Penicillium* spp.. The relationship between the Prays oleae life cycle and the climate has a deleterious impact on the variety of fungi [13]. The kind of crop or vegetation where the insect is prevalent is one of the crucial elements that might influence the richness and spread of the entomopathogenic fungus species [14,15]. It was shown that the presence of naturally occurring entomopathogenic fungus is positively connected with plant variety.Our findings corroborated those of and showed that *T.virede* and *B.bassiana* induce death in *B. tabaci* populations and have promise as biological control agents. said that *B.bassiana* has shown great promise as a greenhouse whitefly

biocontrol agent[16,17]. [18], reported that the most Paecilomyces spp. [19], noted that the entomopathogenic fungus produces endochitinases, involved in the degradation of B. tabaci chitin to facilitate the infection process. Thus, the fungal chitinases played a vital role in plant defense against B. tabaci which resulted in the mortality of both fourth instar nymphal and adult B. tabaci [19]. Chitinases from whitefly-associated fungi can be used to develop transgenic plants and increase fungus virulence against this pest insect [20]. Bing & Zhong (2008) pointed out that Aspergillus spp., *Penicillium* spp are opportunistic fungi and can infect insects. [21], mentioned that the mortality rate induced by opportunistic fungal species may be due to the characteristic of their fast growing, which may infect, injured or weakened the insect. Those opportunistic fungi are known for producing a vast array of secondary metabolites that are gaining importance for their biotechnological applications [22]. Fungal secondary metabolites have high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor, repellent etc. [23]. The mortality rates differences between nymphs and adults of *B.tabaci* may be due to the discrepancy in producing abundant amounts of cuticle lipids (such as long-chain wax esters) [24,25]. demonstrated that the nymphs of whitefly are covered with long-chain wax esters, which could potentially act as a barrier preventing the spores from coming into contact with nutrients or other cues that trigger germination, but it is unknown whether they play a significant role in affecting spore germination by fungilytic or fungistatic toxicity, thus protecting nymphal stages more efficiently than adults. Moreover,[26], indicated that the germination and penetration of entomopathogenic fungal spores on *B. tabaci* cuticle represent an important step in the process of fungal infection using penetration pegs from appressoria or occasionally by direct hyphal penetration [27].

Conclusions

Given chemicals' direct and indirect impacts on people, animals, and the environment in general, there is a growing interest in finding safe and beneficial substitutes. In this situation, researchers are focusing on entomopathogenic fungi as a secure and productive substitute. One of these options is entomopathogenic fungi, which help to strengthen plant defences and fend off pathogens and insects like *B. tabaci*. entomopathogenic fungi can help to enhancing plant immunity and triggering natural defence systems because of their intimate interactions with host plants. Consequently, using entomopathogenic fungus may lessen the need for dangerous chemical pesticides and lessen adverse effects on the environment and public health..Therefore, the development and utilization of Entomopathognic fungi fungi may offer an optimal solution in the future to combat pest problems and maintain plant health through natural and environmentally-friendly means. It is important to continue scientific studies in this field to gain a better understanding of the capabilities of entomopathognic fungi fungi in interacting with plants and combating pests, as well as to develop effective strategies**.**

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