

Effectiveness of chemical insecticides and entomopathogenic fungi *Lecanicillium muscarium* against *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) infesting geothermal crops in southern Tunisia

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Abstract

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The whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), is one of the most serious pests of protected and geothermal crops. To improve control of *B. tabaci*, we studied the efficacy of a group of chemical insecticides and a biological product based on an entomopathogenic fungus, offering a diversity of modes of action and innovative activity pathways. The results showed that these treatments were effective in reducing the development stages of *B. tabaci*. *L. muscarium* caused a significant reduction of the different developmental stages of *B. tabaci* with a percentage varying between 57.87 and 79.69 %. Egg and larvae reduction percentages were above 60% for Imidacloprid, Pyriproxyfen, Pyrethrinoids, Sulfoxaflor, and Imidacloprid + lambda-cyhalothrin. These treatments can be used as part of an integrated control program against whiteflies associated with crops in geothermal greenhouses in southern Tunisia.

1. INTRODUCTION

The whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), is widely distributed throughout the world (Byrne, 1999). In southern Tunisia, *B. tabaci* constitutes a real menace for crops in heated greenhouses, through the transmission of viruses (Bel Kadhi, 2004). It is one of the most common and economically important invasive pest species in at least 54 countries worldwide (Oliveira et al., 2001). The presence of *B. tabaci* in the Mediterranean has long been known and was reported in Tunisia in 1997 (Chermiti, 1997). *B. tabaci* is extremely polyphagous, and can feed on more than 900 wild and cultivated host plants in tropical, subtropical, and Mediterranean areas (Perring, 2001). It grows on many crops, including vegetables, ornamentals, and field crops (Nauen et al., 2014). According to Perring, (2001), *B.*

tabaci contains at least 19 species, the two most widespread being “B” and “Q” (Erdogan et al., 2008). In Tunisia, two species have been reported: the “B” biotype named *B. argentifolii* and the “Q” biotype with a predominance of the first biotype (Chermiti, 1997). It is of economic importance as it is an effective vector of viruses to many families (Brown and Czosnek, 2002; Fernández et al., 2009), including TYLCV virus (Tomato yellow leaf curl virus) (Jones, 2003; Roditakis et al., 2017). The most important control strategy against *B. tabaci* is the use of insecticides, as even a single individual can cause severe damage through viral transmission (Wang et al., 2017). Neonicotinoids have been considered an important group of insecticides used against *B. tabaci* for several years (Wang et al., 2010; Basit et al., 2013; Wang et al., 2017). Imidacloprid, the first neonicotinoid introduced

in 1991, offers excellent contact and systemic activity and has therefore been largely responsible for the management of *B. tabaci* in horticultural and agronomic production systems worldwide (Nauen and Denholm, 2005; Jeschke and Nauen, 2008). In recent years, new insecticidal chemicals have been introduced that offer a diversity of new modes of action and pathways of activity to control whiteflies effectively. It has been proven that Flupyradifurone is a new chemical agent that simultaneously fights whitefly and significantly prevents viral infection due to its rapid inhibitory effect (Roditakis et al., 2017). The new insecticide sulfoxaflor shows high efficacy against a wide range of species of sap-feeding insects, including those resistant to neonicotinoids (Babcock et al., 2011; Zhu et al., 2011; Wang et al., 2017). Sulfoxaflor acts on nicotinic acetylcholine receptors (nAChR) in the insect nervous system (Babcock et al., 2011). Several substances are effective against whiteflies such as diafenthiuron, buprofezin, imidacloprid, and pyroproxifen (Palumbo et al., 2001). Effective chemical compositions or the use of entomopathogenic fungi must be studied to demonstrate potential ways to overcome contamination and resistance issues (Ali et al., 2017). Fungi of the genus *Lecanicillium* are important insect pathogens that have been marketed since 1980 to control aphids and whiteflies (Wang et al., 2004; Lazreg et al., 2009). A strategy for managing insecticide resistance is recommended, emphasizing the rotation of more effective insecticides from different chemical classes, as well as the use of new chemicals and other integrated pest management strategies (Ahmad et al., 2002).

The current work aims to test the effectiveness of 10 active ingredients; imidacloprid + lambda-cyhalothrin, sulfadiazine, abamectin + thiamethoxam, pyrethroids, pyriproxifen, imidacloprid, fluroxypyr, Flufenamide, Thiphenazine and EPF *Lecanicillium muscarium* against *Bemisia tabaci* eggs, larvae and adults in heated greenhouses of tomatoes and peppers in southern Tunisia.

2. MATERIAL AND METHODS

2.1. Experimental design

The experiment was conducted in three greenhouses naturally infested with *B. tabaci* in Ben Ghilouf El Hamma Gabes Tunisie (33°88'73'N, 9°59'98'E, 78m), two greenhouses

of *Solanum lycopersicum* of the Maria variety and one greenhouse of sweet pepper *Capsicum annuum* of the variety Chargui. These are precocious crops planted in October in geothermal tunnel greenhouses with an area of 500 m², conducted in conventional mode. The selected greenhouses are in full production condition, during the experiment the farmers did not carry out any treatment with insecticides. For each greenhouse, the plants were grown in four twin rows and each row is considered a block. The tests were conducted according to the device of completely random blocks. Each block was divided into elementary plots separated by buffer zones (untreated). Within the block, each elementary plot was treated with a product while avoiding borders.

2.2. Insecticides

Insecticides used in biological tests were given with doses and precautions for use by the specialized service of the General Directorate for the Protection and Quality Control of Agricultural Products of the Ministry of Agriculture, Water Resources and Fishing in Tunisia as part of field trials for approval (Table 1).

2.3. Sampling procedures

On 18 April 2016, treatments were carried out for all three greenhouses. Only the *L. muscarium* treatment was repeated every 7 days. The first samples were taken the same day immediately before the treatment, and then samples are taken every week. For each elementary plot, twelve different plant leaves are randomly taken. Forty-eight sheets were collected for each treatment. These leaves were examined under a binocular magnifying glass (Leica® Model MS5) to count the number of living individuals of each developmental stage of *B. tabaci*. The number of eggs, first instar larvae (L1), second instar larvae (L2), third instar larvae (L3), fourth instar larvae (L4), pupae and adults were counted. The pupae were counted together with the larval stages. This allows monitoring of the population structure of *B. tabaci* after treatment and evaluation of the effectiveness of each treatment.

2.4. Statistical analyzes

The efficacy of these insecticides against the different stages of development of *B. tabaci* was established according to the reduction formula. Reduction percentages were calculated using

Table 1. Characteristics of tested insecticides

Active ingredient	Chemical group (IRAC MoA No.)	Conc. a.i., Formulation type	Doses	Mode of action
Imidacloprid + lambda-cyhalothrine	Neonicotinoids (4A) /Pyrethroids (3A)	150 g/l + 50 g/l ; SC	30 cc/hl	nAChR competitive modulators
Sulfoxaflor	Sulfoxaflor (4C)	240 g/l ; SC	250 ml/ha	nAChR competitive modulators
Abamectin + Thiamethoxam	Avermectines (6)/ Neonicotinoids (4A)	33.2g/L + 152.4 g/L ; SC	0.5 L/ha	tamate-gated chloride channel (GluCl) allosteric modulators + nAChR competitive modulators
Pyrethrins	Pyrethroids (3A)	18.61 g/l ; EC	160 cc/hl	Sodium channel modulators
Pyriproxyfene	Pyriproxyfene (7C)	100 g/l ; EC	50 cc/hl	Juvenile hormone mimics
Pyriproxyfene	Pyriproxyfene (7C)	100 g/l ; EC	75 cc/hl	
Spores de <i>Iecanicillum muscarium</i> souche 1979	Entomopathogenic fungi	48 g/kg (1.1*1010 spores/g) ;WG	1 g/l de produit + 2.5 ml/l d'adjuvant	Fungal agents
Imidacloprid	Neonicotinoids (4A)	200 g/L ; OD	50 ml/hl	nAChR competitive modulators
Flupyradifurone	Butenolide (4D)	200 g/l ; SL	60 cc/hl	
Teflubenzuron	Benzoylurées (15)	150 g/L ; SC	25 cc/hl	Inhibitors of chitin biosynthesis affecting CHS1
Buprofezin	Buprofezin (16)	25 g/l ; EC		Inhibitors of chitin biosynthesis, type 1

Conc. a.i.: Concentration of active ingredient

Abbott's formula (Abbott, 1925) : $R\% = (1 - \frac{\text{no. in T after-treatment}}{\text{no.in Co. post-treatment}}) \times 100$

Where; no = insect population, T = Treated elementary plot, Co = Control elementary plot

For the analysis of variance (ANOVA), the number of eggs, larvae and adults of *B. tabaci* as well as the rate of reduction after treatments were subjected to a unidirectional analysis associated with a Duncan test ($P < 0.05$) using XLSTAT 2019, Excel® 2013.

3. RESULTS AND DISCUSSION

3.1. Toxicity on eggs of *B. tabaci*

The results showed that all treatments were effective in reducing the *B. tabaci* population. According to the pre-treatment sampling recorded before spraying, the average number of

eggs ranged from 18.27 to 21.87 eggs/leaflet in tomato greenhouse 1, from 16.05 to 18.95 eggs/leaflet in tomato greenhouse 2 and from 18.82 to 20.75 eggs/leaf in pepper greenhouse, no significant difference between treatments (Table 2). However, after application of the tested treatments, the number of whitefly eggs decreased significantly in the first week after treatment in all three greenhouses compared to untreated controls. Statistical analysis showed that the treatments were effective against *B. tabaci* eggs throughout the trial period compared to the untreated control, which equaled 19.07 eggs/leaflet in tomato greenhouse 1, eggs/leaflet in tomato greenhouse 2 and 26.12 eggs/leaf in pepper greenhouse at the last sampling date. On the other hand, on treated plants, the number of eggs was reduced between 1.85 and 9.80 eggs/leaflet in tomato greenhouse 1, between 5.67 and 13.25 eggs/leaflet in tomato

Table 2. Effect of different insecticides against *B. tabaci* eggs on tomato and pepper under heated greenhouse conditions

	0 DAT	7 DAT	14 DAT	21 DAT	28 DAT
Number of eggs <i>B. tabaci</i>/Tomato leaflet in greenhouse 1					
Untreated-control	18,17 a	17.82 a	17.27 ab	23.25 a	19.07 a
Teflubenzuron	21,87 a	8,02 b	19,80 a	13.00 b	9.80 b
Imidacloprid	22,80 a	7,45 b	7,65 c	7,77 bc	2,30 c
<i>lecanicillum muscarium</i>	22,20 a	8,42 b	10,77 abc	4,25 c	2,80 c
Pyriproxyfene 50cc /hl	20,30 a	3,80 bc	5,10 c	8,50 bc	2,27 c
Pyriproxyfene 75cc /hl	21,80 a	1,775 c	10,32 bc	8,32 bc	3,60 c
Pyrethrinoïdes	18,27 a	4,20 bc	10,95 abc	3,72 c	1,85 c
<i>F</i>	0,811	16.892	5.626	24.276	69.048
<i>P</i>	0,562	0,001	< 0,0001	< 0,0001	< 0,0001
Number of eggs <i>B. tabaci</i>/Tomato leaflet in greenhouse 2					
Untreated-control	17,00 a	22,05 a	22,75 a	20,50 a	25,57 a
Imidacloprid	16,05 a	11,12 cd	8,25 c	10,75 b	7,67 b
Abamectin + Thiamethoxam	18,95 a	9,55 d	8,02 c	10,42 b	11,57 b
Flupyradifurone	17,85 a	10,82 cd	5,85 c	8,10 b	7,37 b
Buprofezin	17,52 a	15,97 b	15,70 b	15,05 ab	10,35 b
Pyrethrins	17,30 a	13,60 bcd	9,75 c	11,77 b	9,37 b
Sulfoxaflor	18,67 a	14,42 bc	10,50 c	14,52 ab	13,25 b
<i>F</i>	0,388	16,375	22,848	3,864	15,040
<i>P</i>	0,887	< 0,0001	< 0,0001	0,001	< 0,0001
Number of eggs <i>B. tabaci</i>/Pepper leaf					
Untreated-control	20,75 a	24,07 a	28,07 a	32,10 a	26,12 a
Imidacloprid	18,82 a	7,95 b	4,75 c	4,60 b	13,45 b
Imidacloprid + lambda-cyhalothrine	19,25 a	4,32 bc	9,12 bc	7,17 b	11,52 b
<i>lecanicillum muscarium</i>	19,22 a	2,42 c	11,82 b	7,72 b	9,60 b
<i>F</i>	0,366	58,817	33,012	7,235	8,514
<i>P</i>	0,778	< 0,0001	< 0,0001	0,000	< 0,0001

DAT : Day After Treatment ;

Different letters denote means are significantly different from one another, as determined by Duncan

greenhouse 2, and between 9.60 and 13.45 eggs/leaf in pepper greenhouse. Egg reduction percentages were above 70% for Imidacloprid, *L. muscarium*, Pyriproxyfene, Pyrethrinoïdes, Sulfoxaflor, and Imidacloprid + lambda-cyhalothrin (Fig. 1).

3.2. Toxicity on *B. tabaci* larvae

The study concerning the effect of the different treatments against *B. tabaci* larvae shows a significant efficiency during the completely sampling period (Table 3). At the beginning, the average number of larvae varied between 37.45 and 46.97 larvae/leaflet in tomato greenhouse 1, between 13.47 and 16.12 larvae/leaflet in tomato greenhouse 2 and between 21.35 and

24.07 larvae/leaf in pepper greenhouse. From the first week after treatment, there was a significant decrease in the numbers of larvae for the different treatments in the three greenhouses as compared to the untreated control. The treatments reduced the number of larvae between 0.50 and 8.20 larvae/ leaflet in tomato greenhouse 1, between 5.67 and 12.30 larvae/leaflet in tomato greenhouse 2, and between 7.52 and 15.15 larvae/leaf in the pepper greenhouse. On the other hand, the number of *B. tabaci* larvae remained high following the Buprofezin treatment with 20.05 larvae/tomato leaflet at the last sampling date. The percentages of larvae reduction were above 60% for Imidacloprid, *L. muscarium*, Pyriproxyfene, Pyrethrinoïdes, Sulfoxaflor,

Table 3. Effect of different insecticides against *B. tabaci* larvae on tomato and pepper under heated greenhouse conditions

	0 DAT	7 DAT	14 DAT	21 DAT	28 DAT
Number of larvae <i>B. tabaci</i>/Tomato leaflet in greenhouse 1					
Untreated-control	40,17 a	39,62 a	47,80 a	33,47a	28,22 a
Teflubenzuron	38,27 a	27,42 ab	33,07 b	19,02 b a	8,20 b
Imidacloprid	46,40 a	20,60 bc	16,02 c	8,05 c	1,72 c
<i>Iecanicillum muscarium</i>	41,95 a	12,45 c	22,17 bc	5,55 c	1,45 c
Pyriproxyfene 50cc /hl	46,97 a	21,37 bc	17,40 c	7,75 c	2,50 c
Pyriproxyfene 75cc /hl	39,95 a	25,45 bc	15,42 c	5,05 c	0,50 c
Pyrethrinoides	37,45 a	20,55 bc	19,72 bc	3,00 c	1,30 c
<i>F</i>	0,887	6,273	13,031	34,482	73,982
<i>P</i>	0,505	< 0,0001	< 0,0001	< 0,0001	< 0,0001
Number of larvae <i>B. tabaci</i>/Tomato leaflet in greenhouse 2					
Untreated-control	14,07 a	16,50 a	19,22 a	18,10 a	22,15 a
imidacloprid	15,90 a	8,32 b	9,025 b	6,30 c	8,15 c
Avermectines	15,17 a	8,00 b	9,250 b	10,25 abc	12,30 bc
Flupyradifurone	16,12 a	9,12 b	7,125 b	8,27 bc	5,67 c
Buprofezin	15,52 a	12,05 ab	14,60 ab	15,95 ab	20,05 ab
Pyréthrines	15,45 a	8,52 b	9,07 b	7,02 c	10,22 c
Sulfoximines	13,47 a	12,70 ab	11,25 ab	12,70 abc	9,30 c
<i>F</i>	0,191	4,756	3,233	5,234	9,245
<i>P</i>	0,979	0,0001	0,004	< 0,0001	< 0,0001
Number of eggs <i>B. tabaci</i>/Pepper leaf					
Untreated-control	21,47 a	28,92 a	26,70 a	20,92 a	19,15 a
Imidacloprid	21,35 a	2,75 c	5,95 b	9,30 ab	15,15 ab
Imidacloprid + lambda-cyhalothrine	23,37 a	11,17 b	15,55 ab	4,57 b	9,75 b
<i>Iecanicillum muscarium</i>	24,07 a	3,45 c	13,65 b	5,30 b	7,52 b
<i>F</i>	0,584	34,400	6,009	4,817	6,224
<i>P</i>	0,626	< 0,0001	0,001	0,003	0,001

DAT : Day After Treatment ;

Different letters denote means are significantly different from one another, as determined by Duncan

Flupyradifurone, Abamectin + Thiamethoxam, Pyrethrins and Imidacloprid + lambda-cyhalothrin.

3.3. Toxicité sur les adultes de *B. tabaci*

Treatments applied against *B. tabaci* adults on tomato and pepper crops showed significant efficacy. The treatment with *L. muscarium* was effective against *B. tabaci* adults from the first week of treatment compared to the untreated control. Similarly, the other chemical treatments were effective in reducing adult numbers. The number of adults in tomato greenhouse 2 increased over the sampling period on both treated and untreated plants (Table 4), but there

was a significant difference when compared with the untreated control. The ANOVA analysis shows a significant effect of the treatments which are found to be effective during the whole follow-up period on the adults even compared to the reference treatment Imidacloprid with a reduction of more than 40% (Fig. 1). The Teflubenzuron treatment did not result in a significant reduction in adults during the follow-up period.

The different active substances used in this study showed significant efficacy against eggs, larvae and adults of *B. tabaci* over time compared to an untreated control in heated

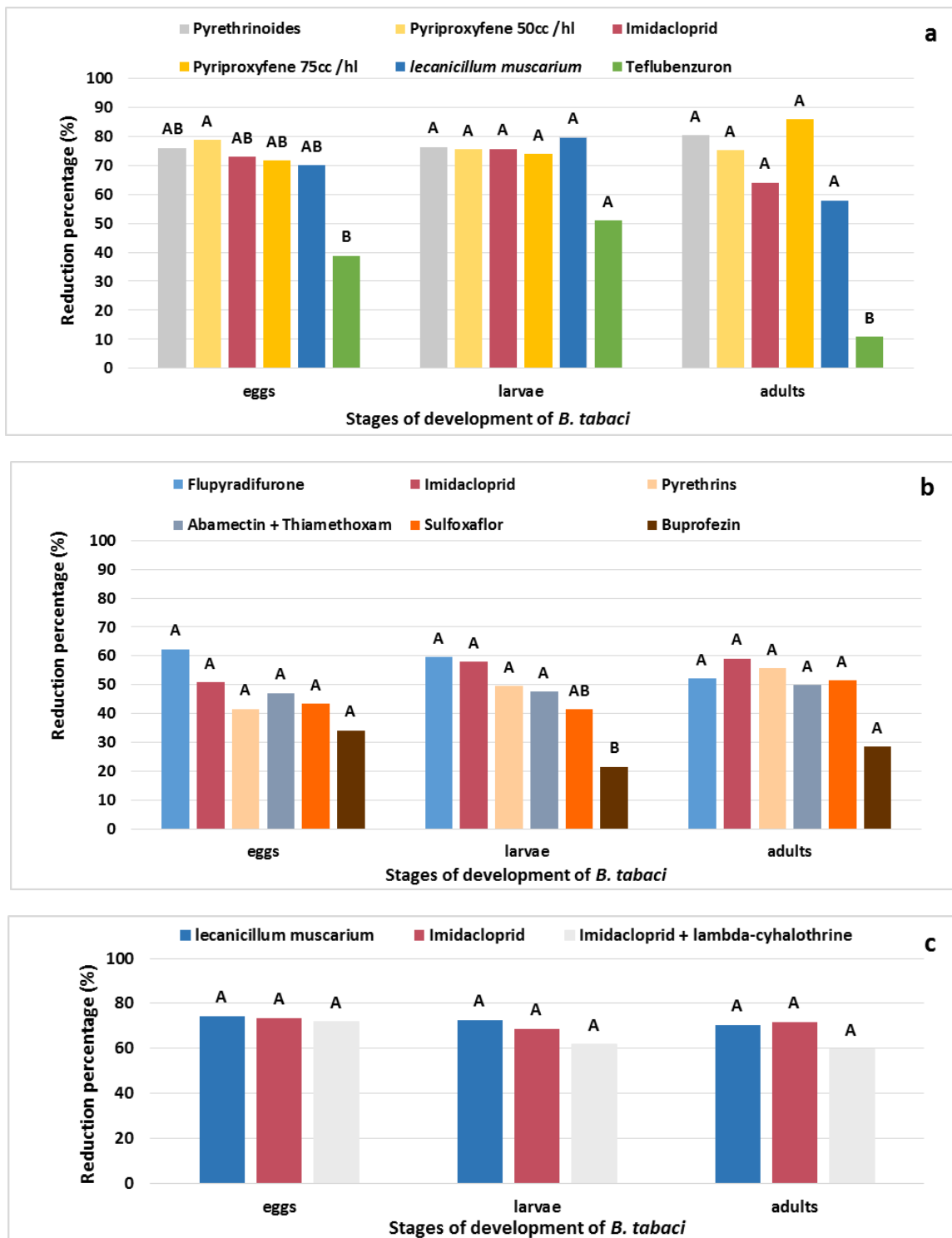


Fig. 1. Reduction in the number of eggs, larvae and adults of *B. tabaci* under: (a) tomato greenhouse 1, (b) tomato greenhouse 2 and (c) pepper greenhouse.

greenhouses in southern Tunisia. Imidacloprid was tested in the three greenhouses to compare its effect on the *B. tabaci* population with other active substances as neonicotinoid insecticides played a major role in the chemical control of *B. tabaci* (Bass et al., 2015). Imidacloprid has been shown to be particularly effective against a wide range of Hemiptera pests including *B. tabaci*

(Palumbo et al., 2001). In our study, this substance showed efficacy against the different stages of development of *B. tabaci*, the same as the entomopathogenic fungus *L. muscarium* for tomato and pepper crops. This fungus causes significant reductions in eggs, larvae and adults of *B. tabaci*. This is confirmed by several studies showing that this fungus has potential for

suppressing *B. tabaci* larvae in greenhouses on a range of plants (Down *et al.*, 2009) and has high pathogenicity to all developmental stages of *B. argentifolii* (Gindin *et al.*, 2000). Other laboratory studies showed that second instar *B. tabaci* larvae were the most susceptible to infection by *L. muscarium* after 3 and 7 days of incubation (Cuthbertson *et al.*, 2005). Similarly, the chemicals showed high efficacy against developmental stages of whiteflies with different reduction rates which can be explained by the different modes of action of these substances. For the Imidacloprid + lambda-cyhalothrin based treatment tested in a pepper greenhouse, the percentages of *B. tabaci* were reduced over time. This means that the combination of two chemical families can improve efficacy against this pest. A study showed that alteration and association between pyrethroids and another active substance against sucking gnawing showed better control of *B. tabaci* (Sarr *et al.*, 2016). Thus, a mixture of insecticides with different modes of action can delay the emergence of resistance (Basit *et al.*, 2013).

Pyrethrins, Pyriproxyfen, and Teflubenzuron showed moderate efficacy against different stages of *B. tabaci* development with reduced proportions of *B. tabaci* eggs not exceeding 50% during the entire follow-up period. For pyridine derivatives, increasing the application dose of this treatment has no effect on mortality at different stages of development. In relation to benzoylureas, they are insect growth regulators that act by inhibiting chitin synthesis when ingested by insects and preventing molting of larvae. In our study, the effect of benzoylurea was greater against caterpillars.

In the second tomato greenhouse, the five substances tested Avermectins, Flupyradifurone, Pyrethrins, Buprofezin and Sulfoxaflor showed a significant effect on the different stages of development of *B. tabaci*. sulfoxaflor constitutes a new chemical family of neonicotinoid insecticides shows broad-spectrum efficacy against many biting-sucking insects (Longhurst *et al.*, 2013; Zhu *et al.*, 2011) and exhibits good insecticidal activity against *Myzus persicae* (Yu *et al.*, 2008). Similarly, Flupyradifurone has a significant effect against the different stages. It is the first systemic insecticide and competitive modulator at the orthosteric binding site of insect nAChRs of the new class of butenolide (Nauen *et al.*, 2014). It acts reversibly as an antagonist at insect nicotinic acetylcholine

receptors. This is why this substance leads to a significant decline in the population of *B. tabaci*. Laboratory tests confirm the results found, such as flupyradifurone showing adult mortality of *B. tabaci* and *T. vaporariorum* after 72 hours (Nauen *et al.*, 2015).

In our study, Buprofezin does not have a significant effect against the larval stages of *B. tabaci*, however (Palumbo *et al.*, 2001) hand mentioned that Buprofezin is effective against the larvae of *B. tabaci* on cotton. It inhibits the synthesis of chitin and acts specifically on the disruption of the moult of immature stages of whiteflies (Toscano *et al.*, 2001). This discrepancy may be due to climatic conditions or a difference in the host plant or in the dose applied. But another study shows Buprofezin had no significant effect on adults and eggs (Sohrabi *et al.*, 2011). Thus, pyrethrins act weakly on *B. tabaci* larvae. Field experiments showed moderate efficacy of red after 7 days of treatment against *B. tabaci* larvae (Golmohammadi *et al.*, 2014).

According to our study, chemical control shows significant effectiveness against the population of *B. tabaci* in heated greenhouses in southern Tunisia. For a long time, chemical control has been used as the main method of controlling whitefly (Wang *et al.*, 2017; Wang *et al.*, 2010). But, chemical control is widely used to manage *B. tabaci* which leads to reduced susceptibility of *B. tabaci* which has developed resistance to a number of insecticides worldwide (Palumbo *et al.*, 2001) even to the new substances such as insect growth regulators (IGR) and neonicotinoids (Horowitz *et al.*, 2005; Nauen and Denholm, 2005). In addition, the *B. tabaci* population was highly susceptible to infection by the entomopathogenic fungus *L. muscarium*. Given its importance, this entomopathogenic fungus *L. muscarium* has shown potential for incorporation into an integrated management program (IPM) for the biological control of *B. tabaci* on tomato and pepper plants.

4. CONCLUSION

The entomopathogenic fungus *L. muscarium* has shown significant efficacy on the different developmental stages of *B. tabaci* on tomato and pepper crops. These results encourage us to use this fungus in the control of whitefly from the egg stage, which requires the monitoring of the life cycle of this insect to determine the

intervention time. In addition, the determination of the conditions of introduction of this fungus in geothermal greenhouses requires further studies. Similarly, chemical treatments result in high mortality rates on the different developmental stages of *B. tabaci* but there is still a risk of the emergence of resistant whitefly biotypes. This requires the combination of different control methods to control this pest and the development of biological control methods.

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