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Some microbial treatments against the tomato leaf miner; *Tuta absoluta* (Merick) under natural field conditions

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Abstract: In an attempt to find more effective methods and safety to control the tomato leaf miner, *Tuta absoluta*, this research was conducted to study the efficacy of the four bio-treatments ; two bacterial isolates (*Bacillus subtilis*. and *Pseudomonas Fluorescence*), one fungal isolate (*Trichoderma viride*) and Spinosad as a microbial insecticide compared with the most common insecticides used against the dipterous and lepidopterous leaf miners in Egypt; Profenofos and Aphox. In the two successive seasons; 2011 and 2012, the fungal isolate *Trichoderma viride* achieved the lowest infestation(numbers of infested leaflets, mines, infestation percentages) which were 167, 195, and 21.9% resp.in season 2011 and 198, 222 and 26.2% resp. in season 2012. While the highest infestation was occurred in case of using the insecticides, Aphox (423, 559 and 55.9% resp.& 384, 839 and 72.2%. and Prohpenofos (436, 562 and 57.7% &391, 534 and 51.7% resp.).during 2011 and 2012 respectively. The other treatments occupied the intermediate levels.
On the other hand, the yield of tomato fruits was increased by 75% over the check in case of plants treated with *Trichoderma viride* isolate, but the pesticide treatment given weak or no impact in increasing tomato crop.
In season 2012, the infested tomato fruits percentages with *T. absoluta* larvae and Rhizoctonia soil rot disease(that Frequently observed incidentally) were recorded the minimum levels obtained by spraying *Trichoderma viride* isolate(21.38 and 10.32% resp.). In contrast, the maximum levels were recorded in Prophenofos (44.87 and22.78% resp.) and Aphox treatments(47.22 and 27.78% resp.).

Keywords: *tomato*

Introduction

Chemical control has been the main method of control used against *T. absoluta* in all tomato production regions in Argentina since 1970. Horticultural growers have tried to decrease its injure applying insecticides two times a week during a single cultivation period. Effective chemical control was difficult to achieve because of the mine-feeding behavior of larvae, lack of a threshold action, and deficient spraying technology (Marcela *et. al*., 2005).In addition to destruction of natural enemy populations (Campbell *et al*.,1991), build up of insecticide residues on tomato fruits (Walgen bach *et al*.,1991) and in the environment and fundamentally the rapid development of insecticide resistance (Siquira *et al*.,2000a and Siquira *et al*.,2000b).

Common insect pathogens used against insectpests according to Weinzierl, 1989 are *Bacillus thuringiensis, B.popilliae, B.lentimorbus* (bacterial pathogens) and *Baeuvaria bassiana* (fungal pathogen).

This investigation was carried out in the experimental farm at the faculty of Agricultural, Kafr El-Sheikh University during the two successive seasons 2011 and 2012 to study the following aims:

1. The effect of some uncommon bio- treatments used to control the tomato leaf miner*; Tuta absoluta* (Meyrick) infesting tomato plants.

2. Evaluating the percentages of infested tomato fruits by *T. absoluta* larvae in the tested treatments.

Materials and methods

1. Feild studies

Field experiments were carried out at the experimental farm of the Faculty of Agriculture, Kafr El-Sheikh University during two successive seasons ; 2011 and 2012. One tomato variety namely Rosa was planted in the green house on April, 21st and transplanted to the experimental field on May, 11 and 15th of 2010 and 2011, respectively. The experimental area was 1/6 Feddan divided into seven plots. Each plote (80 m²)was divided into three replicates. The normal agricultural practices of growing were done usual.

Six control treatments were used in the two tested seasons (four bio-treatments and two chemical insecticides), added to one untreated plot. Spraying treatments was started after one month from transplanting in the experimental field using cp_3 Knapsack. Spraying was applied twice a week until the tomato leaflets dryness.

Leaflet samples were taken randomly after twenty four hours from applying treatments.A sample was consisted of

108 leaflets/3 replicates for each treatment. Samples were transferred to the laboratory to examine numbers of infested leaflets, mines and estimating the infestation percentages.

Fruit samples were collected weekly for one month.Three plants were selected randomly to pick up its fruits for each treatment. Mean tomato fruit yield, infested tomato fruits by *Tuta absoluta* larvae and infested tomato fruits by the tomato leafminer larvae& fungi were evaluated.

2. Tested materials.

2.1. Bio-treatments:

2.1.1. Microbial isolations:

2.1.1. (a). Bacillus subtilis.

2.1.1. (b). Pseudomonas Fluorescence

2.1.1. (c). Trichoderma viride

2.1.2.Bacterial insecticide.

2.1.2. (a). Spinosad:

Spinosad dust (0.125%WP) Dow AgroSciences product is a mixture of the two most active naturally occurring metabolites of the soil bacterium *Saccharopolyspora spinosad*. Spinosad is a mixture of about 85% Spinosyn A and 15% Spinosyn D.,structurally, these compounds are macrolides and contain an unique tetracyclic ring system to which two different sugars are attached(Abdel-Mageed,2008).

Spinosyn A, Empirical Formula C41H65NO10;MW 731.98.

Spinosyn D, Empirical Formola C42H67NO10; MW 746.00.

2.3. Chemical insecticides:

2.3.1. Profenofose:

Common name is Selecron (72% EC).It is an organophosphorus compound applied at rate of 100cm/100L water.

2.3.2. Aphox:

The common name is Pirimicarb(50%DG). It is a carbamate compound applied at rate of 250 gm/100L water.

The insecticides used against all leaf miners in Egypt are also used to control the white flies and aphids which have systemic effect.

3. Laboratory studies:

3.1. Preparation of microbial inocula:

The microorganisms were recultured on suitable liquid media, potato dextrose broth (PDB) for fungi or nutrient broth (NB) for bacteria in flasks of 500 ml capacity containing 200 ml medium. In case of fungus, cultures were incubated at 28^{o} C for 10 days. Spore suspension of *Trichoderma* isolate was sieved through double cheesecloth and hand homogenized and counts of spores were adjusted to 10^{6}spore/ml using hemocytometer. In cases of bacterial isolates, cultures were incubated at 28^{o} C for 5 days in shaking incubator (140 r/min.) and cell suspension was adjusted to 10^{8} cell/ml. *Bacillus subtilis*, *Pseudomonas fluorescence* and *Trichoderma viride* isolated by El-Khateeb (2004) were kindly obtained.

3.2. Procedure of application:

Cultures were amended with calculated aliquots of an adhesive surfactant (New-Film, 1265 registered by ministry of Agric., Egypt) as recommended (30 ml/100 L) and hand homogenized before fine spraying onto the upper and the lower leaf surfaces of plant cotyledons until runoff. Plants, sprayed with sterilized tap water (likely amended with the adhesive surfactant), served as check treatment (control).

3.2. Identify the pathogens.

During the examination of tomato leaflets samples picked from plots treated by *Trichoderma viride*, some larvae were found showing symptoms of microbial disease. Larvae had soft bodies, static, cuticle color was close of brown. The pathogen was isolated from the diseased larvae to be defined.

Results and discussion

1. The effect of some bio- treatments used to control the tomato leaf miner; Tuta absoluta (Meyrick) infesting tomato plants.

Table (1) and figure (1) showed the number of infested tomato leaflets, the number of mines and the infestation percentages in the two successive seasons; 2011and 2012 according to the four bio- treatments tree isolations and one bioinsecticide) and two chemical insecticides. In the first season, the *Tricoderma* sp. isolate gave the least infestation (167, 195, and 21.9% resp.) followed by Spinosad (233, 301 and30.8%), the *Bacillus subtilis* isolate (246, 338 and 32.9% resp.), the *Pseudomonas* Fluorescence isolate (334, 475 and 44.2% resp.). In the latest ranking, Aphox (423, 559 and 55.9% resp.) and Prohpenofos(436, 562 and 57.7%). The nearby trend was recorded in the second season 2012. Since the lowest infestation level related with *Tricoderma viride* treatment (198, 222 and 26.2% resp.).The middle infestation levels related with *Bacillus subtilis,*Spinosad.and *Pseudomonas Fluorescence* treatments (261, 351 and 34.5% & 270, 383 and 35.7% & 321, 466 and 42.4% resp.).While Aphox and Prophenofos recorded the highest infestation levels (384, 839 and 72.2% &391, 534 and 51.7% resp.).

Generally, the chemical insecticides Aphox and prophenofos recorded greater infestation than microbial treatments *(Tricoderma viride,Bacillus subtilis,Pseudomonas Fluorescence*) and Spinosad. While the greatest values were obtained in check during the two seasons 2011 and 2012 (614, 889 and 81.2% & 546, 839 and 72.2% resp.) Statically analysis showed significant differences among the tested treatments in their efficiency of *T. absoluta* control.

Figure (1) showed one of the diseased larvae that was obtained during the examination of samples. Leaflet samples taken from plants treated with *Trichoderma viride* isolate**.**

Table (1): The effect of the four bio- treatments compared to chemical insecticides against T. absoluta during 2011 and 2012 seasons.

Seasons	**2011[A]**			**2012[B]**		
Treatments	**Inf. leaflets**	**mins. No.**	**Infestation%**	**Inf. leaflets**	**mins. No.**	**Infestation%**
Bacillus subtilis	246[d]	338[d]	32.9[e]	261[d]	351[d]	34.5[d]
Spinosad	233[d]	301[d]	30.8[e]	270[d]	383[d]	35.7[d]
Pseudomonas Fluorescence	334[c]	475[c]	44.2[d]	321[c]	466[c]	42.2[c]
Tricoderma viride	167[e]	195[e]	21.9[f]	198[e]	222[e]	26.2[e]
Prophenofos	436[b]	534[b]	51.7[c]	391[b]	562[b]	51.7[b]
Aphox	423[b]	559[b]	55.9[b]	384[b]	516[b]	50.8[b]
Control	614[a]	889[a]	81.2[a]	546[a]	839[a]	72.2[a]

The values were an average of three determinations.
Values followed by the same letter in column are not significantly different at $p < 0.01$.
a, b, c, d, e, f and g: comparison of means by treatments.

Table (2): The percentages of yield and infested tomato fruits under tested treatments in 2012 season.

Treatments.	**yield/12 plants**	**Average yield/plant**	**%yield increasing**	**Infested fruits by**			
				Larvae		**Larvae + fungi**	
				Total	**%**	**Total**	**%**
Bacillus subtilis	100[b]	8.33[b]	38.89[b]	37[b]	37.00[e]	18[d]	18[f]
Spinosad	97[b]	8.08b[c]	34.72[c]	37[b]	38.14[d]	21[b]	21.65[de]
Pseudomonas Fluorescence	91[c]	7.58[c]	26.39[d]	34[c]	37.36[de]	19[cd]	20.88[e]
Tricoderma viride	126[a]	10.50[a]	75.00[a]	31[d]	21.38[f]	13[e]	10.32[g]
Prophenofos	78[d]	6.5[d]	8.33[e]	35[c]	44.87[c]	18[d]	22.78[c]
Aphox	72[e]	6[e]	0.00[f]	34[c]	47.22[b]	20[bc]	27.78[b]
Control	72[e]	6[e]	0.00[f]	40[a]	55.56[a]	24[a]	33.33[a]

The values were an average of three determinations.
Values followed by the same letter in column are not significantly different at $p < 0.01$.
a, b, c, d, e, f and g: comparison of means by treatments.

The pathogen was isolated and defined by Microbiology Laboratory,Botany Department in Faculty of agriculture at Kafer elsheikh University. The pathogen was defined as *Trichoderma viride.*

These findings agreed with Lietti *et. al.*, 2005 they reported that some populations of *T. absoluta* have developed resistance to organophosphate and pyrethroid pesticides. Gonzalez-Cabrera *et. al.*, 2011 mentioned that newer compounds such as spinosad and *Bacillus thuringiensis* have demonstrated some efficacy in controlling European outbreaks of this moth.

Fig.(1): Infected larva after *Trichoderma viride* treatment.

2. Evaluating the percentages of infested tomato fruits by *T. absoluta* larvae in the tested treatments.

Data presented in Table (2) indicated that the maximum yield of tomato fruits during four weeks was took place in *Tricoderma viride* treatment (126 fruits/12 plants.) which increased by 75% over the check. While the minimum yield was occurred in Aphox treatment (72 fruits/12 plants)that equaled with check. The tomato fruits yield resulted from *Bacillus subtilis*, Spinosad and *Pseudomonas Fluorescence* treatments were occupied intermediate values (100, 97,and 91 fruits/12 plants resp.).

Concerning the infested tomato fruits percentages with *T. absoluta* larvae in season 2011 were arranged in ascending order as follows; 21.38, 37.00, 37.36, 38.14, 44.87, 47.22 and 55.56% in *Tricoderma viride*, *Bacillus sutilis*, *Pseudomonas Fluorescence.* Spinosad Prophenofos, Aphox treatments and finally check respectively.

During the examination of infested fruits by T. absoluta larvae, Found that a large number of them infected with *Rhizoctonia solani* fungus that causes Rhizoctonia soil rot disease. Symptoms are alternating light-and dark-colored zonate bands on fruit surfaces (figs.3 and 4). The infected tomato fruits must had a hole of tomato leaf miner larva (fig.2).The infected tomato fruits with fungus ranged between 41.94 and 60.00% from the total of infested tomato fruits with *T. absoluta* larvae according to the different treatments. The highest infestation percentage was obtained by spraying

Aphox(58.82%) then the control (60.00%), while the lowest infestation percentage was achieved by using *Tricoderma viride* isolation which was 41.94% (Table 2 and Fig.2).

The previously data can be interpreted that the tomato leaf miner larvae speed up the entry of *Rhizoctonia solani* fungus. So, the real danger of Tuta absoluta lies in its impact on quantity and quality of the tomato crop.

Fungi of the genus *Trichoderma* are important biocontrol agents (BCAs) of several soil borne phytopathogens (Benítez et al. 2004). The biocontrol by fungal species of the *Trichoderma* genre, of root and crown rot caused by *R. solani*, are being used as an alternative to chemical fungicides (Papavizas, 1985; Limon et al. 1999; Rey et al. 2001).

Statistical analysis for yield increasing percentages showed significant differences among all experimented treatments. While the infested tomato fruits by *T. absoluta* larva and by *T. absoluta* larva with fungi revealed that *Trichoderma viride* differ highly significant than the others.

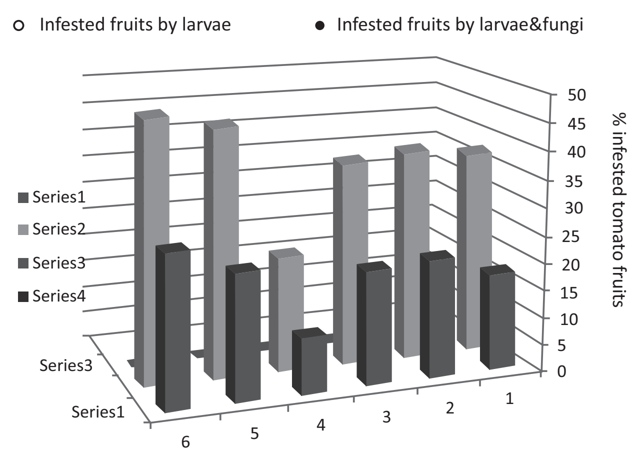

Fig. (2): The percentages of infested tomato fruits by *T. absoluta* larvae and *T. absoluta* larvae and fungi in the tested treatments

Fig. (3 and 4): Tomato fruits infected with Rhizoctonia soil rot disease.

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