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Research Article

Synergistic Effects of Combining Three Commercial Bioproducts Against *Tuta absoluta* (Meyrick) Larvae (Lepidoptera: Gelechiidae)

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Abstract

Background and Objective: The use of entomopathogenic agents for crop pest management is a viable alternative to synthetic chemical pesticides. *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) are fungi considered the most promising extensively widely applied bio-control agents in protecting a wide range of economic crops. Fungal toxins are thought to play a crucial part in the pathogenicity process during insect infestation. The bioinsecticides' synergy could help to control the invasive pest more safely and effectively. **Materials and Methods:** Suspensions of Beauveroz (*Beauveria bassiana*) and Metarhoz-P (*Metarhizium anisopliae*), were evaluated as to their virulence against *T. absoluta* larvae at 3 different doses. As a comparison, Abamectin was utilized as a positive control, while water was used as a negative control. **Results:** All the commercial compounds caused significant mortality among *T. absoluta* larvae, with approximately 52% mortality after 5 days of the treatment. Over 5 days, mortality of *T. absoluta* larvae when exposed to a combined treatment of *B. bassiana*, *M. anisopliae* and Abamectin reached 92%. The results under field conditions, showed significant differences ($p < 0.001$) among these products while adding the surfactants increased the mortality larvae. Combined treatments of these 3 commercial compounds showed a synergistic effect acceded the effect obtained using each compound alone. Bio-pesticides, *B. bassiana* and *M. anisopliae* formulations caused mortality rates among *T. absoluta* larvae similar to the Abamectin treatment. **Conclusion:** Observations indicated that both fungus candidates and Abamectin proved effective against *T. absoluta* larvae. The combined use showed a high potentiality indicating a positive synergistic effect.

Key words: Tomato leaf miner, *Tuta absoluta*, biological control, entomopathogenic fungi, commercial formulations, synergist effects

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), the tomato pinworm, is one of the most harmful invasive alien pests to tomatoes and certain cultivated family Solanaceae that have economic value including potato, tobacco Eggplant and others¹. Tomato leaf miner and/or the tomato pinworm *T. absoluta* is a destructive oligophagous insect that attacks a variety of plant species, with a strong affinity for Solanaceae species. It has a significant liking for the tomato (*Solanum lycopersicum* L.), which is the primary host². Under the ideal laboratory conditions (25°C, 65% RH), the insect has been proven to have one generation of around 24 days³.

T. absoluta as a highly invasive insect pest necessitates variable effective and long-term IPM strategies that reduce the insect population densities to be below the economic threshold level. The use of chemical pesticides, to combat *T. absoluta*, is one of the main strategies of the tomato farmers' since the insect acquired resistance against a wide range of insecticides. Therefore, an interest to use the eco-friendly control methods as well IPM approaches has been demanded and increased in the last decades. One of these approaches is biological control, which has eco-friendly sound management tactics¹.

Entomopathogenic fungi (EPF) are an environmentally acceptable alternative to chemical pesticides, with little toxicity to non-target organisms⁴ and a low lower risk of resistance in the target insect under field settings⁵. *B. bassiana* and *M. anisopliae* were reported to be effective control methods against all larval stages of *T. absoluta* in this study.

Against expanding and spreading of *T. absoluta* entomopathogens play important roles in pest management, which can be applied in different ways to protect crops from insect damage⁶. In the view of traditional biological control, spores of entomopathogens are released through spray application or infected cadavers to colonize and get the best permanent presence either under field and/or greenhouse conditions⁷. Naturally, this capacity represents an additional role and an alternative method of action of EPF against insects in its agroecosystem, given that the corresponding of these fungi still has an entomopathogenic activity when present in a suitable environment. *Beauveria bassiana* Vuill. (Ascomycota: Hypocreales) is a well-known microbial control agent against a wide range of arthropod species and its active ingredient in many commercially marketed fungal-based products⁸.

The study aimed to evaluate the efficiency of two commercial bio-pesticides as well as Abamectin in suppressing populations of *T. absoluta* and their interactions with larval mortality on tomato plants under laboratory

conditions. Moreover, improving mortality rates among the tomato leaf miner larvae by applying these compounds in combination.

MATERIALS AND METHODS

Insect colony: *Tuta absoluta* larvae were collected from tomato greenhouses at Al-Watania Agricultural Farms, Qassim region, Saudi Arabia, within the coordination of (26°34'31.0"N 43°41'23.2"E). Larvae were collected from inside their leaf tunnels and then raised on tomato plants in a growth chamber at 21-25°C, 60% RH and a 14:10 hrs photoperiod (L:D). Larvae were separated from their mines for the bioassays and individuals were categorized into distinct larval instars based on size and colour⁹.

Entomopathogenic agents sources: Before testing the EPF against *T. absoluta* larvae, a quantity of 5 kg of Beveroz 1.15% PW (*Beauveria bassiana*) (1×10^8 CFU g⁻¹) and Metarhoz-P (*Metarhizium anisopliae*) (1×10^8 CFU g⁻¹), were imported from India (Utkarsh Agrochem Pvt., Ltd., India). The fungal extracts were kept at a temperature of 18°C according to the instructions from the supplying company. The recommended rate for Beveroz 1.15% PW product was 10 g L⁻¹ of water, while the Metarhoz-P was 8 g L⁻¹ of water. The direct spray method was used in the transactions. The hand spray method was used in laboratory and growth chamber treatments using 2 L Handheld pressure sprayer (Inc., L. and G. Company), while automatic spraying in the greenhouse experiment through the Sandryloke sprinkler at a rate of 1.5 kg/200 L of water. Abamectin is a natural fermentation product derived from the soil bacterium *Streptomyces avermitilis* Rahman *et al.*¹⁰ was used as a counterpart in all experiments for comparison as a positive control. The Bio-insecticide Abamectin was imported by Al-Yaseen Agricultural Company in Qassim, Saudi Arabia, where the recommended rate of treatment in spraying was 50 mL/1 L of water. The surfactants (spreading agent) were also used to reduce the surface tension in the transactions and to speed up the process of adhesion to the surface, as the spreading material consisted of carbon 30-5% nitrogen and the material was imported from Al Deif Agricultural Company in Qassim Region, Saudi Arabia.

Lab bioassay (*in vitro*)

Assessment of entomopathogenic against *T. absoluta* larvae

Effect of using each compound alone: The laboratory experiment was carried out in May, 2021 at the Entomology lab, College of Agriculture at Qassim University, according to

a completely randomized block design. The targeted treatments included Beauveroz suspension, Metarhoz-P suspension and Abamectin in addition to the negative control (water only). Larvae counts were fixed to 50 individuals in each treatment and replicated 4 times. Using a sterile hand-held spraying device, larval instars of *T. absoluta* were sprayed with 20 mL of the commercial products as mentioned below:

- Beauveroz suspension at concentration of 2.5×10^8 conidia mL^{-1} suspension
- Metarhoz-P suspension at concentration of 2.5×10^8 conidia mL^{-1} suspension
- Abamectin 50 mL/1 L
- Control spraying with sterile water only

Spraying was carried out on sterile Petri-dishes (9 cm diameter) padded with 3 moistened filter paper discs. A filter paper was used to drain the excess of the applied volume¹¹. The number of dead and survival larvae was recorded at 1 day, 3 and 5 days intervals post-treatment. *T. absoluta* larvae were tested for survival after being exposed to doses of Beauveroz, Metarhoz-P and Abamectin suspensions as mentioned above. The studies were carried out at 25-27°C, with RH of 50-70% and a 12 L:12 D light. The application of treatments occurred on day 0.

Effect of combining the three compounds: The same procedure and doses used for evaluating each compound alone were used for the combination of the 3 compounds, the 2 bio-insecticides and Abamectin, to estimate their efficacy:

- Abamectin (50 mL/1 L) + *M. anisopliae* (2.5×10^8 conidia mL^{-1})
- Abamectin (50 mL/1 L) + *B. bassiana* (2.5×10^8 conidia mL^{-1})
- *M. anisopliae* (2.5×10^8 conidia mL^{-1}) + *B. bassiana* (2.5×10^8 conidia mL^{-1})
- Abamectin (50 mL/1 L) + *M. anisopliae* (2.5×10^8 conidia mL^{-1}) + *B. bassiana* (2.5×10^8 conidia mL^{-1})
- Control sprayed with sterile water only

As previously stated, data on insect survival rates were examined using the STATISTICA for Windows (Stat Soft 2005). Insect mortality data were recorded after 1, 3 and 5 days. The dead larvae were incubated in Petri dishes with potato dextrose agar (PDA) media to observe the appearance of fungal growths. The Abbott¹² formula was used to correct mortality percentages:

$$\text{CM} = \frac{\text{Nc} - \text{Nt}}{\text{Nt}} \times 100$$

Where:

CM = Corrected mortality (%)

Nc = Live individuals are in the control after the treatment

Nt = Live individuals in the treatment after the treatment

Calculation of the synergistic effects among the three commercial compounds used: Data on mortalities that were obtained as a result of each single compound effect (Table 1-3), were calculated over the 3 observations times by the Abbott¹² formula and Henderson and Tilton¹³ formula, as well as, our observations in the zero-time (called the Initial Kill).

The mortalities that resulted from the effectiveness of the combinations of the 3 compounds were obtained by subtracting the resulting values in Table 4 (the results of combinations) from the mortality values in Table 1 that resulted from the single effect of each compound separately. The same procedure was taken on the 3rd day (Table 3 and 4) observations and the 5th day (Table 5 and 6).

Greenhouse bioassay (*in vivo*)

Evaluation of the three compounds against *T. absoluta* on tomato under plastic house conditions: The experiment was conducted in the greenhouses of Al-Watania Agricultural Farms in Qassim, Saudi Arabia (26°34'31.0"N 43°41'23.2"E). The spraying rate according to the manufacturer's recommendation was 10 g L^{-1} of water for the Beauveroz suspension, 8 g L^{-1} of water for Metarhoz-P suspension and 50 mL/100 L of water for Abamectin. Automatic spraying in the field through the Sandryloke sprinkler at a rate of 50 L/1000 L of water was performed. The greenhouses used were highly infested during treatments, the temperature reached 36°C. The spraying was carried out by selecting 4 greenhouses, where each one was divided into 3 different replicates. Each of the 2 bio-insecticides and Abamectin were mixed with water, according to the rate recommended by the supplying company (UTKARSH Company, India) and a surfactant (spreading agent, containing 30% carbon and 5% nitrogen) was added at the rate of 50 mL/10 L of water. The purpose of using surfactants was to increase the effectiveness of the adhesion of those commercial compounds on the tomato leaf surface, where the *T. absoluta* larvae live inside tomato tunnels. Spraying began in the early morning hours. Larvae were examined regularly at intervals of 24, 72 hrs and

5 days post-treatment and dead larvae were recorded. A two-way analysis of variance was used to establish the significant differences between treatments of the main effects (ANOVA) ($p=0.01, 0.05$). Mortality percentages were corrected according to Henderson and Tilton (1955)¹³ formula:

$$\text{Corrected (\%)} = (1 - \frac{N \text{ in Co before treatment} \times N \text{ in T after treatment}}{N \text{ in Co after treatment} \times N \text{ in T before treatment}}) \times 100$$

Where:

N = *T. absoluta* larvae population

T = No. *T. absoluta* in treated treatments

Co = No. in check treatment (control)

Statistical analysis: Statistical significance of differences in *T. absoluta* larval mortality in the bioassays was determined using two-way ANOVA in STATISTICA for Windows (Stat Soft 2005). The data were corrected for control mortality using Abbott¹² formula for Lab bioassay and Henderson and Tilton¹³ formula for greenhouse treatments. Significant differences among treatment means were calculated based on Tukey's HSD at $p<0.05$. Cadavers were examined for the presence of fungal mycelia either during the final evaluation of the bioassays or after surface-sterilized cadavers were placed on a BSM medium and incubated in the dark at 24°C for 10 days. To measure the significant differences between treatments and doses, enable to make direct comparisons among treatments' means were done by calculating the LSD values. Any difference larger than the LSD was considered a significant result.

RESULTS

Efficiency of *B. bassiana*, *M. anisopliae* and abamectin against *T. absoluta* larvae: Bioassay results showed that the two Bio-insecticidal products of *B. bassiana*, *M. anisopliae*, as well, Abamectin caused significant mortality rates in *T. absoluta* larval stage in both lab and greenhouse treatments. The three commercial products had minor changes in larval stages mortality particularly among the effectiveness of the two fungal toxins (Table 1-3) when used each compound alone, and, however, they were non-significantly different. The $p = 0.001$ was found for all of the mortality statistics, indicating that the treated groups were significantly different from the controls (water treatment). There were non-statistical differences in mortality ($p = 0.05$) even though each product had a different (median mortality time in days). The mortality did not differ statistically ($p = 0.05$). In all laboratory bioassays on days 1, 3 and 5, the

general mortality based on *B. bassiana* (Beveroz 1.15% PW) formulation was significantly different from *M. anisopliae* (Metarhoz-P). In all situations, Abamectin outperformed *B. bassiana* and *M. anisopliae*, the lowest mortality (15.77%) was observed, with *M. anisopliae* of 1 day of treatment. Mortality rates increased over time (days) in all treatments (Table 1-3). However, the mortality percentage after 5 days was identical for all 3 products, with about 10% of the larvae still alive at the end of the trial (Table 3 and Fig. 1). In the controls, there was no evidence of conidiogenesis.

Effectiveness of *B. bassiana*, *M. anisopliae*, Abamectin and their combination against *T. absoluta* larvae:

Applications of the three commercial products separately or in combinations against *T. absoluta* larvae led to a mortality rate ranging from 25-39%. The effectiveness of Abamectin for *T. absoluta* larvae was higher when applied separately or in combination with one or both of the other bio-insecticide (Table 4 and 5). While overall mortality percentage of *T. absoluta* larvae was a noticeable increase when mixed with the suspension of *B. bassiana* and *M. anisopliae*. The potential for the combined use was higher, indicating a beneficial synergistic effect. While the general mortality rate showed a very significant increase when the 3 compounds were mixed and applied to the different larvae compared to the single treatment for each compound and the binary mixture for every two compounds ($p<0.01$). Concerning the rate of doses for each compound separately or in combination, the statistical analysis confirmed that the double dose of every single compound or in combination gave a higher mortality rate for larvae in their different stages, while the treatment of larvae with half recommended dose gave the lowest death rate ($p<0.01$) (Table 6). The comparison among the mortality rates of larvae in different stages between days for each insect pathogen showed a higher mortality rate on the 5th day of treatment and the highest death rate was on the 5th day when using the double dose of each bio-insecticide. While the mortality rate of the combined application was much higher than that of using *B. bassiana* *M. anisopliae* or Abamectin, separately.

Table 7, shows the participation of each bio-insecticide alone when mixed with other compounds in the mortality rate of *T. absoluta* larvae under laboratory conditions. The contribution of the abamectin in increasing the larval mortality rate when used in combination with the *M. anisopliae* compound in recommended dose was (5.12-6.79%), while the contribution of the *M. anisopliae* compound in the mixture when used in recommended dose was (18.93-25%). On the other side, the contribution of Abamectin to the

Table 1: Mortality (%) of *B. bassiana*, *M. anisopliae* and Abamectin used separately against *T. absoluta* larvae under laboratory conditions after 1 day of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin	24.88 $\pm$ 4.77	35.24 $\pm$ 5.55	44.00 $\pm$ 2.45	34.71	26.03	13.61*
<i>Metarhizium anisopliae</i>	15.36 $\pm$ 2.44	22.02 $\pm$ 3.85	25.71 $\pm$ 2.86	21.03	15.77	8.48*
<i>Beauveria bassiana</i>	19.24 $\pm$ 2.50	29.95 $\pm$ 3.90	33.33 $\pm$ 2.00	27.51	20.63	8.22*

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

Table 2: Mortality (%) of *B. bassiana*, *M. anisopliae* and Abamectin used separately against *T. absoluta* larvae under laboratory conditions after 3 days of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin	31.31 $\pm$ 4.86	46.07 $\pm$ 3.41	55.62 $\pm$ 5.83	44.33	33.25	17.25*
<i>Metarhizium anisopliae</i>	23.41 $\pm$ 3.06	27.86 $\pm$ 5.14	32.86 $\pm$ 4.29	28.04	21.03	9.27*
<i>Beauveria bassiana</i>	27.02 $\pm$ 4.00	35.33 $\pm$ 4.90	41.43 $\pm$ 5.08	34.60	25.95	12.23*

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

Table 3: Mortality (%) of *B. bassiana*, *M. anisopliae* and Abamectin used separately against *T. absoluta* larvae under laboratory conditions after 5 days of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin	40.40 $\pm$ 4.43	51.67 $\pm$ 7.04	64.29 $\pm$ 6.43	52.12	39.09	14.90*
<i>Metarhizium anisopliae</i>	30.00 $\pm$ 4.06	32.22 $\pm$ 5.02	40.36 $\pm$ 5.75	34.19	25.64	6.24*
<i>Beauveria bassiana</i>	33.41 $\pm$ 6.46	42.50 $\pm$ 6.98	46.19 $\pm$ 5.41	40.70	30.53	10.22*

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

Table 4: Effects of different combinations among the three commercial products against *T. absoluta* larvae under laboratory conditions after 1 day of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin+ <i>M. anisopliae</i>	27.50 $\pm$ 3.73	40.95 $\pm$ 4.35	49.43 $\pm$ 2.73	39.29	29.47	14.01*
Abamectin+ <i>B. bassiana</i>	36.43 $\pm$ 9.92	47.67 $\pm$ 9.77	56.29 $\pm$ 4.25	46.79	35.10	12.39*
<i>M. anisopliae</i> + <i>B. bassiana</i>	22.86 $\pm$ 2.14	31.43 $\pm$ 2.86	35.24 $\pm$ 5.60	29.84	22.38	7.73*
Abamectin+ <i>M. anisopliae</i> + <i>B. bassiana</i>	43.10 $\pm$ 5.98	57.33 $\pm$ 3.23	62.00 $\pm$ 3.27	54.14	40.61	9.49**

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

Table 5: Effects of different combinations among the three commercial products against *T. absoluta* larvae under laboratory conditions after 3 days of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin+ <i>M. anisopliae</i>	35.71 $\pm$ 6.43	52.86 $\pm$ 2.86	60.33 $\pm$ 4.84	49.63	37.23	8.68**
Abamectin+ <i>B. bassiana</i>	43.57 $\pm$ 8.39	66.29 $\pm$ 9.31	72.90 $\pm$ 2.21	60.92	45.69	18.21*
<i>M. anisopliae</i> + <i>B. bassiana</i>	27.38 $\pm$ 1.64	38.93 $\pm$ 2.79	43.33 $\pm$ 4.67	36.55	27.41	7.25**
Abamectin+ <i>M. anisopliae</i> + <i>B. bassiana</i>	59.05 $\pm$ 4.35	82.67 $\pm$ 9.15	90.00 $\pm$ 6.67	77.24	57.93	23.10*

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

Table 6: Effects of different combinations among the three commercial products against *T. absoluta* larvae under laboratory conditions after 5 days of application

Treatments	Mortality (% $\pm$ SE)			Residual effect (%)	General mortality (%)	Tukey's HSD at 0.05 level
	1/2 Recommended dose	Recommended dose	Double recommended dose			
Abamectin+ <i>M. anisopliae</i>	43.33 $\pm$ 7.01	56.79 $\pm$ 4.94	70.48 $\pm$ 6.50	56.87	42.65	8.06**
Abamectin+ <i>B. bassiana</i>	54.13 $\pm$ 7.25	70.57 $\pm$ 8.37	80.24 $\pm$ 2.95	68.31	51.23	19.43*
<i>M. anisopliae</i> + <i>B. bassiana</i>	36.39 $\pm$ 5.05	45.71 $\pm$ 1.75	51.07 $\pm$ 6.15	44.39	33.29	10.06*
Abamectin+ <i>M. anisopliae</i> + <i>B. bassiana</i>	66.43 $\pm$ 7.11	86.00 $\pm$ 9.80	93.33 $\pm$ 6.67	81.92	61.44	15.47*

Interaction between treatments and concentrations at 0.05 level was significant, *Significant at the 0.05 probability level, **Significant at the 0.01 probability level and $\pm$ Standard error of the mean

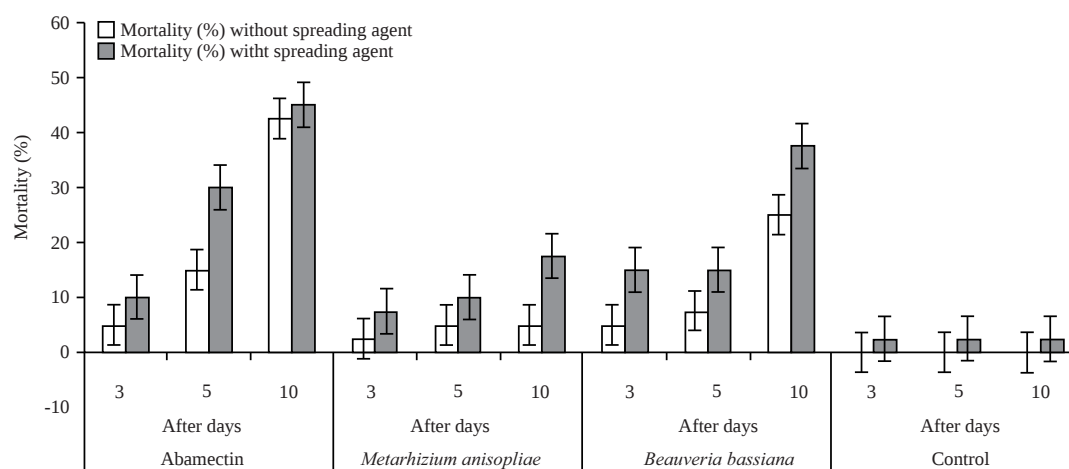


Fig. 1: Toxicity of three compounds against the tomato leaf miner, *T. absoluta* larvae on the tomato leaves under plastic house conditions



Fig. 2(a-d): Efficiency of application of the three compounds, (a) Abamectin, (b) *Beauveria bassiana*, (c) *Metarhizium anisopliae* compare to and (d) untreated control in controlling *T. absoluta* in tomato plants under greenhouse conditions

larval mortality rate of *T. absoluta* when combined with *B. bassiana* was (12.43-20.22%), while the contribution of *B. bassiana* was (25.67-30.96%). The synergistic effect was more pronounced when combined the three compounds at the double recommended dose and the highest percentage of larval mortality was recorded (57.14%) from the contribution of *M. anisopliae* product in this mixture, while the mixture of *M. anisopliae* and *B. bassiana* did not present a noticeable difference in increasing mortality rate compared to separate use of each compound.

Greenhouses bioassay (in vivo): The two bioinsecticides and Abamectin were applied on tomatoes infested with *T. absoluta* under the greenhouses. Except for *M. anisopliae*, the other formula *B. bassiana* and Abamectin significantly

reduced the tomato leaf infestation, in the Qassim region (Fig. 1). Abamectin was significantly higher, whereas *M. anisopliae* had a non-significant difference. The lowest mortality from the study compared to the untreated control was observed in *M. anisopliae*, (Fig. 1). In addition, mortality was observed in the Abamectin and *B. bassiana* treatments, with non-significant differences among them (Fig. 1). The efficacy of the 3 commercial compounds used and their results were shown in tomato plants' growth in the greenhouses, by reducing *T. absoluta* infestations as a result of larval stage mortalities, (Fig. 2a-c). However, the bio-insecticide *B. bassiana* and Abamectin significantly reduced the tomato leave infestation (Fig. 2a-b), since the interaction with the spreading agent was statistically significant. The untreated control rows showed the highest leaf infestation that differed significantly from the bioinsecticides (Fig. 2d).

DISCUSSION

Tuta absoluta overrun and quick outbreaks in the Qassim Region, Saudi Arabia in recent years have highlighted the pest's substantial economic implications in both greenhouses and open-field tomato production. The goal of this study was to see how effective three commercial products *Beauveria bassiana*, *Metarhizium anisopliae* and Abamectin and their combination for controlling *T. absoluta*, because as known the three biocontrol agents are important parts of biological control for pests with high economic value.

Several studies suggested that EPF, *B. bassiana* and *M. anisopliae* are promising biocontrol agents for use as bio-insecticide to control *T. absoluta* and other various pest targets¹⁴. *B. bassiana* has been the most commonly used species in such an approach¹⁵.

In bioassays under laboratory conditions, the EPF as well as, Abamectin, utilized separately against *T. absoluta* larvae, caused significant mortality rates after one day of application, were pathogenic to *T. absoluta* larvae. Mortality rates increased when applied different combinations among the three commercial products after one day of application, they were statistically different among the treatments ($p = 0.01$). Allegrucci *et al.*¹⁶ found that *T. absoluta* survival rates after being fed on tomato leaves colonized by *B. bassiana* were shown less effective at reducing *T. absoluta* survival rate when compared to the survival rates after being exposed to conidial spores of *B. bassiana* and *M. anisopliae* on tomato leaves directly.

The present study revealed that *B. bassiana* and *M. anisopliae* were successfully effective even when used separately or in combinations with other compounds based on conidial viability and concentrations of spores/mL¹⁴. Moreover, the obtained results showed that, although the initial mortality was lower (1 day post-treatment), the highest mortality rate occurred 5 days after treatment. *B. bassiana* was more infective at several concentrations than *M. anisopliae*. Their efficacy varied from minor effects (increase in mortalities by lower rates, to a mixture, compared with single effects. On the side, mixing these compounds led to an increase in their effectiveness causing very effectively mortality percentages. Also, it turned out that the combinations among these three compounds became more toxic against *T. absoluta* larvae, which led to an increase in the mortality percentages, especially on the 3rd and 5th day post-treatment. The study suggested that the cumulative effects of the following factors may be an increase in the pathogenic potential of *B. bassiana* and *M. anisopliae*: (a) Ability of the conidial spores to

Table 7: Combined effect of three commercial compounds to control *T. absoluta* larvae in the laboratory

Increasing mortality percentages as a result of the synergistic reaction of mixing												
Commercial compounds used	Observation periods	Abamectin			<i>Metarhizium anisopliae</i>			<i>Beauveria bassiana</i>			Abamectin+ <i>M. anisopliae</i> + <i>B. bassiana</i>	
		1/2	Recomm- ended	Double	1/2	Recomm- ended	Double	1/2	Recomm- ended	Double	1/2	Recomm- ended
Abamectin	1st day	-	-	-	2.62	5.71	5.43	11.55	12.43	12.29	18.22	22.00
	3rd day	-	-	-	4.41	6.79	4.71	12.26	20.22	17.28	27.28	27.74
	5th day	-	-	-	2.93	5.12	6.19	13.73	18.57	15.95	33.97	34.33
<i>Metarhizium anisopliae</i>	1st day	12.14	18.93	23.72	-	-	-	7.50	9.41	9.53	27.74	35.31
	3rd day	12.30	25.00	27.47	-	-	-	3.97	11.07	10.47	36.64	54.81
	5th day	13.33	24.57	30.12	-	-	-	6.39	13.49	10.71	36.43	53.78
<i>Beauveria bassiana</i>	1st day	17.19	25.67	30.68	3.62	1.48	1.91	-	-	-	23.86	27.78
	3rd day	16.55	30.96	31.47	0.36	3.60	1.91	-	-	-	32.02	46.34
	5th day	20.72	28.07	39.88	2.98	3.21	4.88	-	-	-	26.98	43.50
												47.14

adhesive and attach to insect cuticle surface of an insect¹⁶, (b) Toxic materials which secrets by these fungi such as chitinases, Pr1 and Pr2 proteases, etc.¹⁷, (c) The existence of collagenous protective film enables the fungal spores to penetrate the insect immunity system when the fungus grows and destroys the insect hemolymph. The results obtained from this research are in agreement with the findings of Freed *et al.*¹⁸, who reported that both *B. bassiana* and *M. anisopliae* were the most microbial pesticides used against *T. absoluta* life stages in greenhouses and field conditions. It is clear from the data that the use of different concentrations of both EPF and Abamectin resulted in different mortality based on the control agent type and dose of concentrations used. From these results, it could be concluded that both *B. bassiana* and *M. anisopliae*, as well as, Abamectin contained virulent characteristics to be more suitable for to use of ineffective bio-control programs against *T. absoluta* larvae. Besides, the efficiency and the characters of those fungi and Abamectin against other *T. absoluta* larvae in both greenhouses and Lab were addressed in the present study. Although fungal hyphae have been found in plant tissues¹⁹, hyphae are not known to cause infection, even after ingestion. Ingestion of dried mycelia by *Helicoverpa zea* (Lepidoptera: Noctuidae) (Boddie) larvae was shown to be poisonous²⁰. Even though this was an *in vitro* study, ingestion of living hyphae/mycelia could likely harm insect herbivores. Another idea is that EPF poisons have harmful impacts on insects. Abdel-Baky *et al.*¹⁴ suggested that the moderate dose (8×10^5 conidia mL⁻¹) and the higher one (10×10^5 conidia mL⁻¹) spore concentration mL⁻¹ of *B. bassiana* and *M. anisopliae* were more virulent and can develop as an effective and potent bio-control agent against *T. absoluta* eggs and other life stages in IPM programs.

Present findings indicate that using *B. bassiana* and *M. anisopliae* in controlling *T. absoluta*, within IPM programs, leads to (i) Decreasing pesticide usage to a minimal level, (ii) Minimizing exposure of beneficial organisms to chemical pesticides residuals, (iii) Enhancing natural enemies' role and activity in pest control and (iv) Minimize chemical pesticide residues amounts in foods. The concluding remarks that obtained from the present laboratory and greenhouse bioassay. Field and greenhouse experiments against tomato harmful pests are necessary to fully judge the potential of EPF effectiveness against *T. absoluta*. This context is in line with the organic agriculture or clean agriculture concept, which does not depend on any chemical inputs (either pesticides and/or fertilizers) in the production of tomatoes and other crops.

CONCLUSION

The present study characterizes the role of *B. bassiana* and *M. anisopliae* as EPF to protect tomatoes from *T. absoluta* infestation, a notoriously difficult to control tomato pests. Obtained data indicated the response of *T. absoluta* larvae (*in vitro* and *in vivo* studies) to those three commercial products. Moreover, spraying *B. bassiana* and *M. anisopliae* separately or in combination with each other and with Abamectin gave successful results, so the obtained results indicated the positive effects of the combined use, whether separately or in combination with others. This method had a promise for reducing the damage caused by insect pests, while also reducing the demand for highly harmful insecticides.

SIGNIFICANCE STATEMENT

Our findings showed that the fungi candidates in the commercial products *Beauveria bassiana*, *Metarhizium anisopliae* and Abamectin were effective against *Tuta absoluta* larvae. While the combined use of these three products showed high efficiency and positive synergistic effects. Moreover, the novelty of this research is that it combines the three biocidal components stated above to maximize their efficiency as a mortality factor, hence reducing the cost and hazards of utilizing synthetic pesticides on public health and the environment.

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