DETERMINATION OF THE TOXIC EFFECTS OF DIATOMACEOUS EARTH AND ENTOMOPATHOGENIC FUNGI AGAINST THE TOMATO LEAFMINER MOTH, *TUTA ABSOLUTA* (MEYRICK) [LEPIDOPTERA: GELECHIDAE]

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Abstract. This study was performed in the Department of Plant Protection, Bursa Uludağ University, the tomato varieties Albeni and UG 12406 740 were provided by United Genetics Turkey Seedling Company. This study sought to determine the toxic effects of diatomaceous earth and entomopathogenic fungi against the tomato leafminer moth, *Tuta absoluta*, as an alternative to insecticides. Diatomaceous Earth (DE) was combined with *Paecilomyces fumosoroseus* strain PFs-1 (Priority) and *Verticillium lecanii* strain Bb-1 (Nibortem) to manage *T. absoluta* larvae, alongside a positive control of Azadirachtin (Nimbecidine) and an untreated control group. Laboratory investigations revealed that Nibortem + DE and Priority+ DE exhibited the maximum efficacy, attaining a larval mortality rate of 96.6% and 93.3% within 96 hours. In field studies with the Albeni variety, optimal toxic effects (elevated mortality, minimal infestation) were seen with Nibortem + DE and Priority + DE combinations, which achieved high mortality rates of 93.3% and 92.3%, with infested fruit at 44.6% and 52.4%. In the UG 12406 740 variety, optimal toxic effects were seen with Nibortem + DE and Priority + DE combinations, which achieved high mortality rates of 88.8% and 77.7%, with infested fruit at 37.4% and 47.6%, respectively.

Keywords: white holo i, fossilized remains, synergistic interaction, Paecilomyces fumosoroseus, bioinsecticide

Introduction

The tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is widely cultivated as crop worldwide. It has attained a significant position due to its elevated nutritional properties and its application in the food industry (Keskin and Gül, 2004; Canpolat, 2016; Gölükçü et al., 2016). The primary food crops in the solanaceous family with worldwide spread are the potato and the tomato. It ranks second in both production and use among Solanaceae crops, following potato. Additional members include nightshades, eggplants, tamarillo, horse nettles, and various decorative flowers and fruit plants cultivated for their nutritional value (Naika et al., 2005). As a major agricultural product, it spans a cultivation area of 5.4 million hectares and boasts a global production volume exceeding 192 million tons (FAOSTAT, 2024).

The tomato leafminer moth, *Tuta absoluta* (Meyrick) [Lepidoptera: Gelechiidae], is presently regarded as a significant agricultural menace to European tomato cultivation, resulting in losses ranging from 80% to 100% in regions without control methods (Desneux et al., 2010, 2011; Huda et al., 2020; Biondi et al., 2024). It migrates and

reproduces in swarms, earning a reputation for rapidly devastating tomato crops in just over 48 hours, leading farmers to refer to it as tomato ebola. The moth and its larva consume the leaves of the tomato plant, depriving it of the nutrients necessary for flowering and fruit development (Larraín et al., 2014). It possesses a reproductive rate of 10 to 12 generations annually under greenhouse conditions, enabling rapid territorial invasion (Desneux et al., 2010; Abdel Farag El-Shafie, 2020). The larvae of this moth primarily develop on tomato plants (*Solanum lycopersicum* L.) (Desneux et al., 2010); however, both larvae and adults may also feed and reproduce on various solanaceous plants (Caparros Megido et al., 2013) and other species such as cape gooseberry (*Physalis peruviana* L.), bean (*Phaseolus vulgaris* L.), and *Malva sp.* (Tropea Garzia et al., 2012). Upon hatching, the larvae ingest the leaves and fruits, forming galleries within the mesophyll layer, so diminishing the plants' photosynthetic capacity and impeding their growth. These galleries permit the ingress of a varied assortment of diseases (Urbaneja et al., 2009; Desneux et al., 2010).

In its native distribution regions, extensive pesticide application resulted in *T. absoluta* developing resistance to numerous widely utilized insecticides, including abamectin and deltamethrin in Argentina (Lietti et al., 2005; Mawcha et al., 2025) and cartap, permethrin, and methamidophos in Brazil (Siqueira et al., 2000; Mawcha et al., 2025). Regrettably, *T. absoluta* has rapidly acquired pesticide resistance to conventional control products (Lietti et al., 2005; Domínguez et al., 2019). Therefore, the amalgamation of chemical pesticides with more ecologically sustainable control methods, such as cultural, mechanical, and biological strategies, is essential, as the persistent application of chemical pesticides may adversely affect beneficial insects, result in elevated levels of chemical residues in food, and contaminate soil and water resources (Aktar et al., 2009; Landgren et al., 2009). The formulation of control strategies that incorporate bioinsecticides as an environmentally sustainable alternative to chemical pesticides is a critical priority within an integrated pest management framework, owing to their minimal environmental persistence and biodegradability (Khater, 2012; El Hajj et al., 2017).

An alternative to diminish the reliance on synthetic pesticides is the use of entomopathogenic organisms, such as the fungus Paecilomyces fumosoroseus (Wraight et al., 2000; Chhipa et al., 2024). The predominant fungal species are *Metarhizium* spp., Beauveria spp., Nomuraea rileyi, Verticillium lecanii, and Hirsutella spp. V. lecanii are opportunistic, broadly dispersed ascomycete fungi belonging to the order Hypocreales. V. lecanii (Zimm.) is commonly referred to as "white holo" and induces mycosis in several insects belonging to the orders Homoptera, Coleoptera, and Lepidoptera. V. lecanii targets a broad spectrum of insects and is classified among the highly varied aggregation species. Concerns over food quality and environmental preservation have prompted the exploration of alternative and integrated insect control strategies. The application of chemical pesticides as a control technique is widely sought after and is the most effective method to diminish T. absoluta infestation levels. Nonetheless, the necessity for alternative management strategies is advocated, given that the pest has acquired resistance to numerous pesticides and the adverse consequences of pesticide overuse on the ecosystem and beneficial arthropods (Bawin et al., 2014). The principal mode of action for EPF entails the fungus infiltrating the insect's cuticle, the external protective layer. This is accomplished by enzymes including chitinases, proteinases, and lipases, which decompose the cuticle's structural elements, facilitating the fungus's invasion into the haemocoel, the body cavity that houses the insect's hemolymph (Mora et al., 2018; Paschapur et al., 2021). Upon entry, the fungi multiply and generate toxins

and secondary metabolites such as destruxins and beauvericins, which inhibit the insect's immune responses and exacerbate tissue degradation, ultimately resulting in the insect's demise (Paschapur et al., 2021; Mantzoukas et al., 2022).

Various families of entomopathogenic fungi (EPF), including Hypocreales and Entomophthorales, employ distinct infection methods that commence with spore attachment, germination, and penetration of the insect cuticle, subsequently leading to fungal proliferation within the host (Mora et al., 2018). The fungi can produce new infectious conidia on the host's body, promoting the dissemination of the fungus to more hosts (Shin et al., 2020). Entomopathogenic products constitute a comprehensive strategy for pest management, utilising both direct physical and biochemical mechanisms to incapacitate and eradicate pest populations, while ensuring safety for non-target organisms and promoting ecosystem health (Devi, 2024).

Diatomaceous earth is a naturally occurring substance composed of the fossilized remains of unicellular diatoms and algae. They exhibit a significant oil absorption capacity and have demonstrated efficacy as insecticides (Athanassiou et al., 2005, 2009; Reka et al., 2022). Click or tap here to enter text. Diatomaceous earth (DE) comprises unicellular or colonial silicified remains of algae (Bacillariophyceae). Inert dusts have been documented as effective for controlling numerous pests (Golob, 1997; Zeni et al., 2021). Attribute the effects of DE to desiccation resulting from adsorption and its abrasive characteristics, which disrupt the epicuticular wax layer, leading to dehydration and mortality within hours or days (Subramanyam and Roesli, 2000; Shah and Khan, 2014).

The efficacy of entomopathogenic fungal treatments appears to be contingent upon the larval instar phase as well as the concentration of the fungi (Abdel-Raheem et al., 2015). Moreover, numerous studies indicate that the combined application of parasitoids, predators, and microbials can improve the effectiveness against *T. absoluta* (Miranda et al., 1998; Mansour and Biondi, 2021), such as the simultaneous use of *Trichogramma achaeae*, *Bacillus thuringiensis*, and *Macrolophus caliginosus* (Mansour et al., 2018), or *Bacillus thuringiensis* and *Nesidiocoris tenuis* (Meza-Basso, 2006). The amalgamation of *Bacillus thuringiensis* with *Beauveria bassiana* demonstrated superior efficacy compared to the fungus in isolation (Younes et al., 2018). This work aims to determination of the toxic effects of the diatomaceous earth and entomopathogenic fungi against the tomato leafminer moth, *T. absoluta* (Meyrick) [Lepidoptera: Gelechiidae].

Materials and methods

Insects culture

The insects were obtained from a greenhouse infected with *T. absoluta* in the village of Bucak, Kozan/Adana, where the leaves were infested with larvae and adult insects. Later, the insects were released in the cages where tomato plants were planted. In this way, the seedlings were infected with tomato moths.

Bio pesticides

This study determines the toxic effects of the diatomaceous earth and entomopathogenic fungi against the tomato leafminer moth, *T. absoluta* (Meyrick) [Lepidoptera: Gelechiidae]. The fungi were obtained from AGROBEST GRUP. Diatomaceous Earth were obtained from Entoteam R&D Food Agriculture Industry Tic. Ltd. Şti.

Laboratory bioassays

The investigation was performed on April 27, 2024, in the Entomology Laboratory of the Faculty of Agriculture at Bursa Uludağ University, situated in Bursa province. The Tomato Moth was reared on indigenous tomato cultivars during the experiment. Cages are utilized for cultivating tomato seedlings. The experiment employs a completely randomized design, with each process replicated three times. (CRD). Twenty-one petri dishes were utilized in the tests, with ten third-instar larvae allocated to each dish. The study included seven applications, each employing a fully randomized design that was replicated three times. (CRD). Data collection was undertaken at 24, 48, 72, and 96 hours following the applications.

Field experments

Following the bioassay trials, the bio-pesticides were assessed in field conditions for their effectiveness against *T. absoluta*. This investigation was carried out at the Faculty of Agriculture, Bursa Uludağ University, in Bursa province, on June 8, during the 2024 growing season. The tomato cultivars Albeni and UG 12406 740 are supplied by United Genetics Turkey Seedling Company. Alternatively, 7-liter containers and potting soil are utilized for cultivating tomato seedlings. The experiment has a completely randomized design (CRD) with each treatment reproduced thrice. Two industrial tomato types were utilized, with seven plots allocated for each variety. Each plot contained three rows, each with nine plants. Nine plants were utilized for data collection, arranged in three rows. The dimensions of the plant area were 0.4×0.8 m in relation to the organic area. Bioinsecticide applications occur after 30 days.

Estimation of infested tomato fruits with T. absoluta

The infested tomato fruits containing *T. absoluta* larvae were evaluated three weeks after the initiation of the contract and the composition of the tomato fruits. Nine plants were cultivated in each plot, and the cross diagonals were meticulously examined; the quantities of healthy and diseased fruits were documented to compute the proportion of infected fruits. The infection rate of fruits per plot was evaluated weekly by counting the infested and total quantity of fruits directly on the plants. It is defined as the ratio of the number of fruits infested with *T. absoluta* to the total number of fruits per plant.

The toxic effects of the diatomaceous earth, priority, and nibortem, against larvae of the tomato leafminer T. absoluta after six applications

Field tomato plants were planted in Bursa. Six bio-insecticides from *Table 1* were applied, and the control (water spray) was applied six times at seven-day intervals throughout a two-month period. The experimental field was partitioned into 14 plots, with 7 plots designated for each tomato variety, Albeni and UG 12406 740. Each application was conducted thrice, with the applications randomly allocated utilising a complete randomised design (CRD). Seven leaves were randomly harvested from three plants before application, after spraying on the 1st, 3rd, 5th, and 7th days, placed in a pepper bag, and analysed in a laboratory setting. The adjusted mortality rate was computed using

the formula derived from Henderson and Tilton (1955). The results of laboratory bioassay for statistical analysis was showed based on the transformed (arcsine) values.

Table 1. List of bio-pesticides used against T. absoluta

Bio-Pesticides	Trade Name	Dosage
Paecilomyces fumosoreus strain PFs-1	Priority	250 ml/da
Verticillium lecani strain V1-1	Nibortem,	250 ml/da
Diatomaceous earth	Entoteam	200 ml/da
Azadiractin	Nembecidine	500 ml/da

Experiment design and data analysis

These experim ents were designed in a Completely Randomized Design. The obtained data were statistically analyzed according to the analysis of variance (ANOVA). The data were analyzed using GenStat (Computer Program) Version 18, and the means were separated using the Duncan Multiple Range Test (DMRT) at $P \le 0.05$ (Gomez and Gomez, 1984).

Results and discussion

Laboratory bioassays

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta after 24 hours

The best result was achieved by NIBORTEM+ DE, which caused 70% larval mortality, followed by PRIORITY+ DE and PRIORITY, which caused 60% mortality. NIBORTEM and DE death resulted in a 56.6% mortality rate. NEMBECIDINE is the positive control with 83.3%. All applications are not significantly different from each other, but they are significantly different from the untreated control (*Table 2*).

Table 2. The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 24 hours

Treatment	Dosage	±SD	Mortality (%)
PRIORITY	250 ml/da	0.0	60 (50.7)b
NIBROTEM	250ml/da	3.9	56.6(48)b
DE	200 ml/da	7.3	56.6 (48)b
PRIORITY+ DE	250 ml/da	6.2	60 (50.7)ab
NIBORTEM+ DE	250 ml/da	0.0	70 (56.7)ab
NEMBECIDINE	500 ml/da	13.1	83.3 (65.8)a
CONTROL	-	0.0	0.0 (0.0)c
C.V(%)	-	-	15.3
$SE\pm$	-	-	6.31

Means carrying the same letter(s) in the same column are not significantly different at $P \le 0.05$. The values in parentheses were converted using Arcsine

The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 48 hours

The best result was achieved by NIBORTEM+ DE, which provided 83.3% larval mortality, followed by NIBORTEM and PRIORITY, which gave a 70% mortality rate. PRIORITY+DE resulted in a death rate of 63.3%. The lowest mortality rate was recorded at 56.6% in Diatom. NEMBECIDINE is the positive control with 90%. NEMBECIDINE is the most effective bio-insecticide against *Tuta absoluta* larvae after 48 hours. DE alone was the least effective treatment, though it still reduced larval survival compared to the control, there is significante different between NEMBECIDINE and DE alone. Other applications are not significantly different from each other, but they are significantly different from the untreated control (Table 3). Literature suggests that numerous entomopathogenic fungi have been evaluated for the control of *T. absoluta*. For example, in a study by Shalaby et al. (2013), B. bassiana and M. anisopliae were tested for their efficacy on *T. absoluta* larvae (Neonate "newly hatched", 2nd and 3 rd instars) and eggs under laboratory conditions. The present results align with the findings of Abdel-Raheem et al. (2015), which indicated that increased concentrations of B. bassiana and M. anisopliae corresponded with elevated mortality rates in T. absoluta larvae. Raheem et al. (2015) reported that the LC50 values for B. bassiana, M. anisopliae, and V. lecanii isolates, administered at a concentration of 10⁷ spores per ml to the 1st, 2nd, and 3rd larval instars of *T. absoluta*, were $0.28 \times 10^{5} - 0.11 \times 10^{5} - 3.25 \times 10^{5}$, $0.45 \times 10^{5} 0.46 \times 10^5 - 5.47 \times 10^5$, and $0.32 \times 10^5 - 0.27 \times 10^5 - 3.28 \times 10^5$ conidia per ml, respectively.

Table 3. The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 48 hours

Treatment	Dosage	±SD	Mortality (%)
PRIORITY	250 ml/da	0.0	70 (56.7)bc
NIBORTEM	250 ml/da	0.0	70 (56.7)bc
DE	200 ml/da	7.3	56.6 (48)c
PRIORITY+ DE	250 ml/da	3.5	63.3 (52.7)bc
NIBORTEM+ DE	250 ml/da	17.6	83.3 (65.8)ab
NEMBECIDINE	500 ml/da	13.6	90 (71.5)a
CONTROL	-	0.0	0.0 (0.0)d
C.V(%)	-	-	17.3
SE±	-	-	10.69

Means carrying the same letter(s) in the same column are not significantly different at $P \le 0.05$. The values in parentheses were converted using Arcsine

The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 72 hours

The highest result achieved by NIBORTEM+ DE with 96.6% larval death, whereas PRIORITY+ DE produced an 83.3% mortality rate. PRIORITY yielded a mortality rate of 80%. The minimum mortality rate, 73.3%, was recorded with DE and NIBORTEM. NEMBECIDINE serves as a complete positive control. All applications exhibit minimal differences among themselves, however, they are markedly distinct from the untreated control (*Table 4*).

Table 4. The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 72 hours

Treatment	Dosage	±SD	Mortality (%)
PRIORITY	250 ml/da	7.4	80 (63.4)abc
NIBORTEM	250 ml/da	3.8	73.3 (58.8)bc
DE	200 ml/da	23.3	73.3 (58.8)bc
PRIORITY+ DE	250 ml/da	17.6	83.3 (65.8)abc
NIBORTEM+ DE	250 ml/da	10.6	96.6 (79.3)ab
NEMBECIDINE	500 ml/da	0.0	100 (90)a
CONTROL	-	0.0	0.0 (0.0)d
C.V(%)	-	-	16.8
${ m SE}\pm$	-	-	12.15

Means carrying the same letter(s) in the same column are not significantly different at $P \le 0.05$. The values in parentheses were converted using Arcsine

Under laboratory conditions according to Mohamed Mahmoud et al. (2021), report that the death rate of T. absoluta larvae increased in accordance with the duration of exposure post-infection. The greatest concentration (D1) of the two fungus effectively targeted and exterminated the larvae more efficiently than the lower concentration suspensions (D2 and D3). One-week post-infection, viable larvae emerged within the Petri dishes treated with the two fungal suspensions D2 and D3, subsequently maturing into adults. The results indicate that throughout time, particularly for D2 and D3, the fungus Clonostachys spp. demonstrated a progressively increasing efficacy, although 100% larval mortality was recorded after 3 days of infection at the maximum D1 concentration (Mohamed Mahmoud et al., 2021). Data in Table 4 show that NIBORTEM+ DE with 96.6% larval death, whereas PRIORITY+ DE produced an 83. The same results obtained by of Rodriguez et al. (2006), who reported over 90% larval mortality following treatment with both M. anisopliae and B. bassiana in a pot experiment. Laboratory studies demonstrated that B. bassiana could induce 68% larval mortality (Cabello et al., 2009). Under laboratory conditions Halder et al. (2019) reported that the entomopathogenic fungus B. bassiana IIVR strain had the highest efficacy against the second instar larvae of T. absoluta, resulting in larval mortalities of 33.35% and 53.36% after 48 and 72 hours post-application, respectively. Neem oil (1%) shown fatal efficacy against this oligophagous pest, with mortality rates of 46.69% and 60.03% recorded after 48 and 72 hours post-application, respectively.

The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 96 hours

The best result was achieved by NIBORTEM+ DE, which provided a 96.6% larval mortality rate, followed by PRIORITY, which gave a 93.3% mortality rate. PRIORITY+ DE resulted in a 90% mortality rate. NIBORTEM death was achieved with an 86.6% mortality rate. The lowest mortality rate was reported in DE at 76.6%. It is a 100% positive control with NEMBECIDINE. All applications are not significantly different from each other, but they are significantly different from the untreated control (*Table 5*). The entomopathogenic fungi and DE utilised in this investigation, at a recommended dosage, exhibited toxicity to *T. absoluta* larvae in all bioassays tested. In all treatments,

mortality rates increased with the increase in time (days). Numerous studies have indicated that control of *T. absoluta* can be attained through the application of entomopathogenic fungi, particularly *M. anisopliae* (Shalaby et al., 2013; Contreras et al., 2014; Shiberu and Getu, 2017) and *B. bassiana* (Qazzaz et al., 2015; Youssef, 2015; El-Kichaoui et al., 2016; Ndereyimana et al., 2019). Abdel-Raheem et al. (2015) reported that the LC50 values for *B. bassiana*, *M. anisopliae*, and *V. lecanii* isolates, administered at a concentration of 10^7 spores per ml to the 1st, 2nd, and 3rd larval instars of *T. absoluta*, were $0.28 \times 10^5-0.11 \times 10^5-3.25 \times 10^5$, $0.45 \times 10^5-0.46 \times 10^5-5.47 \times 10^5$, and $0.32 \times 10^5-0.27 \times 10^5-3.28 \times 10^5$ conidia per ml, respectively. In accordance with Jallow et al. (2019), it was discovered that second instar larvae exposed to tomato leaf discs treated with azadirachtin (3 g/L), *B. thuringiensis* (0.5 g/L), or *B. bassiana* (1.5 g/L) exhibited death rates of 70–86%, 55–65%, and 45.5–58.5%, respectively. The bioassay findings indicated that *T. absoluta* exhibited significant susceptibility to both tested commercial neem formulations. A comparable vulnerability to neem formulations has been previously documented (Hosseinzadeh et al., 2019).

Table 5. The toxic effects of the diatomaceous earth, Priority, and Nibortem, on mortality larvae of the tomato leafminer T. absoluta after 96 hours

Treatment	Dosage	±SD	Mortality (%)
PRIORITY	250 ml/da	10.6	93.3 (85.3)ab
NIBORTEM	250 ml/da	16.6	86.6 (68.5)ab
DE	200 ml/da	23.3	76.6 (61.1)b
PRIORITY+ DE	250 ml/da	13.6	90 (71.5)ab
NIBORTEM+ DE	250 ml/da	10.6	96.6 (79.3)a
NEMBECIDINE	500 ml/da	0.0	100 (90)a
CONTROL	-	0.0	0.0 (0.0)c
C.V (%)	-	-	15.7
$\mathrm{SE}\pm$	-	-	12.15

Means carrying the same letter(s) in the same column are not significantly different at $P \le 0.05$. The values in parentheses were converted using Arcsine

Field experiments

Percentage of infested tomato fruits in Albeni and UG 12406 740 varieties

The ANOVA followed by Duncan post-hoc tests revealed significant variation among treatments in reducing fruit infestation by *Tuta absoluta*. The lowest infestation was achieved with NEMBICIDINE (29.6%), the UG 12406 740 genotypes and 38.6% in Albeni genotypes, followed by DE+Nibrotem (40% and 44.6%), in UG 12406 740 and Albeni, respectively, while the untreated control showed the highest infestation (78.3 and 80.6%), for UG 12406 740 and Albeni genotypes. These findings suggest that combined applications, particularly NIBORTEM + DE, are more effective in suppressing fruit damage compared to single bio-pesticides or untreated controls. UG 12406 740 had a lower infestation percentage (29.6%) compared to Albeni (37.8%), which suggests it is numerically more resistant to *Tuta absoluta* (*Table 6, Figure 1*). However, the difference was not statistically significant (p = 0.121). baed on motioned results both varieties are susceptible, but UG 12406 740 appears slightly more tolerant. El Hajj et al. (2017) reported that in the initial trial of 2014, a virtually same pattern of treatment effects,

temporal factors, and their interaction on the examined variables was observed. Repeated analysis of variance indicated a very low effect size of time on the quantity of larvae per leaf and the percentage of damaged fruits. The treatments, duration, and their interaction significantly affected the quantity of mines and larvae per leaf, as well as the percentage of damaged fruits. A post hoc Tukey test revealed a significant effect of treatments relative to the control across the three examined parameters. No substantial differences were seen between the treatments concerning these characteristics.

Table 6. Percentage of	finfested tomato j	fruits in Albeni and	<i>UG 12406 740 varieties</i>

Treatment	Tomatoes genotypes	Mean	SE±	SD±
PRIORITY	Albeni	67.667	7.510	13.0
	UG124	59.333	7.510	9.3
NIBORTEM	Albeni	64.667	7.510	22.5
	UG124	52.000	7.510	18.0
DE	Albeni	67.667	7.510	4.9
	UG124	62.667	7.510	13.0
PRIORITY+ DE	Albeni	52.667	7.510	10.3
	UG124	49.333	7.510	13.2
NIBORTEM+ DE	Albeni	44.333	7.510	17.3
NIBORTEM# DE	UG124	40.000	7.510	11.8
NEMBECIDINE	Albeni	38.667	7.510	11.3
NEWBECIDINE	UG124	29.667	7.510	1.9
CONTROL	Albeni	80.667	7.510	6.0
CONTROL	UG124	78.333	7.510	13.0

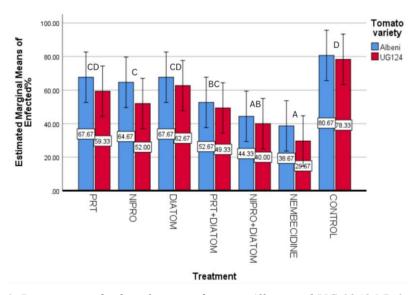


Figure 1. Percentage of infested tomato fruits in Albeni and UG 12406 740 varieties

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in Albeni variety after week 1

Table 7, illustrates that the toxic effects of diatomaceous earth, Priority, and Nibortem on the larvae of the tomato leafminer *T. absoluta* in the Albeni variety are not statistically different from one another, although they are significantly different from the untreated

control. Optimal Toxic Effects (Elevated Mortality, Minimal Infestation) NIBORTEM + DE: Mortality progressively escalated over time (46.6% \rightarrow 73.3% by day 7). Infested fruit: 44.6% — significantly lower than individual treatments. Proposes a synergistic interaction between Nibortem and Diatomaceous Earth. PRIORITY + DE: Mortality exhibited a progressive increase (26.6% \rightarrow 60%). Infested fruit: 52.4%, indicating a significant protective effect. Demonstrates that the combination of Priority and DE yields superior performance compared to either individually. NEMBECIDINE used as a positive control, Exhibited the greatest mortality by day 7 (80%). Infested fruit: 37.9% — the minimal infestation rate, indicating robust field efficacy. Although it has slower early mortality, it demonstrates exceptional long-term efficacy. In the absence of other factors, NIBORTEM (monotherapy) exhibits a moderate increase in mortality (33.3% to 73.3). Infestation: 61.6% — signifies satisfactory, although suboptimal, performance. PRIORITY (alone): Mortality rose from 40% to 63.3%. Infestation: 65.9%, suggesting diminished efficacy relative to its combination with DE. DE (alone): Commenced with a mortality rate of 16.6% and escalated to 46.6% by day 7. Infestation: 67.4%, indicating that diatomaceous earth alone offers relatively limited protection. Untreated Control exhibited consistently low mortality rates (3.3% across all days). Infested fruit: 80.9%, demonstrating the inherent effects of *T. absoluta* when not controlled.

Table 7. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in Albeni variety after week 1

Treatment	Before	The mean r	Infested			
	treatment	1day	3day	5day	7day	Fruit
PRIORITY	30	(40)abc	(43.3)b	(50) ab	(63.3) ab	65.9
NIBORTEM	30	(33.3) bcd	(33.3)bc	(53.3) ab	(73.3) ab	61.6
DE	30	(16.6) de	(23.3) c	(33.3) b	(46.6) c	67.4
PRIORITY+ DE	30	(26.6) cd	(30) bc	(53.3)ab	(60) bc	52.4
NIBORTEM+ DE	30	(46.6) ab	(46.6) b	(60) a	(73.3) ab	44.6
NEMBECIDINE	30	(53.3) a	(63.3) a	(66.6)a	(80) a	37.8
CONTROL	30	(3.3) e	(3.3) d	(3.3) c	0.3± d	80.8
C.V(%)	_	31.8	26.6	25.7	15.7	
SE±		1.0±	0.9±	1.1±	0.9±	

Means carrying the same letter(s) in the same column is not significantly different at P≤0.05

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety after week 1

As shown in *Table 8*, the toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer *T. absoluta* in UG 12406 740 variety, all applications are not significantly different from each other, but they are significantly different from the untreated control. Best toxic Effects (High Mortality, Low Infestation) NIBORTEM + DE: Mortality steadily increased over time (43.3% \rightarrow 80% by day 7). Infested fruit: 37.4% — much lower than single treatments. Suggests synergistic action of Nibortem with Diatomaceous Earth. PRIORITY + DE: Mortality also increased gradually (30% \rightarrow 73.3%). Infested fruit: 47.6%, showing a substantial protective effect. Indicates that Priority combined with DE performs better than either alone.

NEMBECIDINE as positive control, showed the highest mortality by day 7 (86.6%). Infested fruit: 29.6% — the lowest infestation, suggesting strong field performance. Despite being slower in early mortality, it has excellent long-term efficacy. Otherwise, NIBORTEM (alone): Moderate mortality increase ($26.6\% \rightarrow 70\%$). Infestation: 52.2% — indicates good, but not optimal, performance. PRIORITY (alone): Mortality increased from 26.6% to 63.3%. Infestation: 58.6%, indicating lower efficacy compared to its combination with DE. DE (alone): Started with relatively high mortality (23.3%) and reached 53.3% by day 7. Infestation: 60.5%, suggesting DE alone provides only partial protection. Untreated Control, consistently low mortality (6.6% across all days). Infested fruit: 72.6%, showing the natural impact of T. absoluta when left unmanaged.

Table 8. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety after week 1

Treatment	Before	The mean r	Infested			
	treatment	1day	3day	5day	7day	Fruit
PRIORITY	30	(26.6) ab	(36.6) a	(46.6) b	(63.3) bc	58.6
NIBORTEM	30	(26.6) ab	(40) a	(53.3) b	(70) abc	52.2
DE	30	(30) ab	(36.6) a	(50) b	(53.3) c	60.5
PRIORITY+ DE	30	(30) a	(40) a	(63.3) ab	(73.3) ab	47.6
NIBORTEM+ DE	30	(43.3) a	(50) a	(70) ab	(80) ab	37.4
NEMBECIDINE	30	(40) a	(53.3) a	(76.6) a	(86.6) a	29.6
CONTROL	30	(6.6) b	(6.6) b	(6.6) c	(6.6) d	72.6
C.V(%)	_	41.1	24.3	23.6	16.9	
$SE\pm$	_	1.1±	0.9±	1.2±	1.0±	

Means carrying the same letter(s) in the same column is not significantly different at $P \le 0.05$

Laboratory bioassays demonstrated that the entomopathogenic fungi and DE employed in this study, at the indicated dosage, exhibited toxicity to T. absoluta larvae across all investigated bioassays. In all therapies, fatality rates escalated with the passage of time (days). Multiple studies have demonstrated that the management of T. absoluta can be achieved by the utilisation of entomopathogenic fungi. In greenhouse conditions, 24 hours post-larvae deposition of T. absoluta on tomato leaves, symptoms were distinctly observed in young leaves that had been sprayed a week prior with suspensions at D1 concentration of Clonostachys spp. and B. bassiana, as well as with sterile distilled water; conversely, fewer symptoms were noted on plants treated with the insecticide. Twenty-four hours after the second application of the examined fungi or chemical formulations, there was an increase in the quantity of infected (mined) leaves, particularly for SDW and the Clonostachys spp. suspension. The mean number of diseased leaves was three. Forty-eight hours after the second application of fungal suspensions combined with the insecticide, the quantity of infected leaves remained unchanged, in contrast to the SDW, which exhibited a gradual rise to five infected leaves. Following a duration of 72 hours post the second application, all larvae were eradicated; the average quantity of leaves treated with SDW peaked at nine, while the number of leaves subjected to B. bassiana preparation and chemical insecticide remained unchanged. The average number of infected leaves for the fungal preparation of Clonostachys spp. rises to five (Mohamed Mahmoud et al., 2021). Our results of synergistic action of Nibortem with Diatomaceous

Earth. PRIORITY + DE: Mortality also increased gradually $(30\% \rightarrow 73.3\%)$. Infested fruit: 47.6%, showing a substantial protective effect, agree with the findings obtained by Mohammed (2002); Abbas (2011), and El Hajj et al. (2017). They reported the significant efficacy of combining BT with other biopesticides in their research. The use of BT alone shown a substantial difference when compared to the control for the same parameters evaluated.

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in Albeni variety after week 2

Table 9 indicates that the toxic effects of diatomaceous earth, Priority, and Nibortem on the larvae of the tomato leafminer T. absoluta in the UG 12406 740 variety are not statistically distinct from one another. At the same time, they are considerably different from the untreated control. Optimal Toxic Effects (Elevated Mortality, Minimal Infestation) NIBORTEM + DE: Mortality steadily climbed over time $(33.3\% \rightarrow 66.6\%)$ by day 7). Infested fruit: 44.6% — considerably lower than individual treatments. Proposes a synergistic interaction between Nibortem and Diatomaceous Earth. PRIORITY + DE: Mortality exhibited a progressive rise (26.6% \rightarrow 60%). Infested fruit: 52.4%, demonstrating a considerable protective effect. Demonstrates that the combination of Priority and DE delivers higher performance compared to either independently. NEMBECIDINE employed as a positive control, Exhibited the largest mortality by day 7 (83.3%). Infested fruit: 37.9% — the least infestation rate, showing significant field efficacy. Although it has decreased early mortality, it displays great longterm efficacy. In the absence of additional circumstances, NIBORTEM (monotherapy) displays a substantial increase in mortality (20% to 56.6%). Infestation: 61.6% — denotes satisfactory, yet suboptimal, performance. PRIORITY (alone): Mortality jumped from 13.3 to 53.3%. Infestation: 65.9%, indicating reduced efficacy in comparison to its combination with DE. DE (alone): Initiated with a mortality rate of 16.6%, which increased to 43.3% by day 7. Infestation rate: 67.4%, suggesting that diatomaceous earth provides only marginal protection. The untreated control demonstrated continuously low mortality rates of 6.6% during all days. Infestation of fruit: 80.9%, illustrating the intrinsic impact of T. absoluta in the absence of control measures.

Table 9. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in Albeni variety after week 2

Treatment	Before	The mean r	Infested			
	treatment	1day	3day	5day	7day	Fruit
PRIORITY	30	(13.3) ab	(30) c	(40) b	(53.3) bc	65.9
NIBORTEM	30	(20) ab	(33.3) bc	(46.6) b	(56.6) bc	61.6
DE	30	(16.6) ab	(26.6) c	(36.6) b	(43.3) c	67.4
PRIORITY+ DE	30	(26.6) ab	(40) bc	(46.6) b	(60) b	52.4
NIBORTEM+ DE	30	(33.3) ab	(46.6) ab	(56.6) ab	(66.6) b	44.6
NEMBECIDINE	30	(40) a	(60) a	(70) a	(83.3) a	37.8
CONTROL	30	(6.6) b	(6.6) d	(6.6) c	(6.6) d	80.8
C.V(%)	_	62.4	25.1	24.2	16.0	
SE±		1.3±	0.8±	1.0±	0.8±	

Means carrying the same letter(s) in the same column is not significantly different at P≤0.05

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety after week 2

As shown in Table 10, the toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety, all applications are not significantly different from each other, but they are significantly different from the untreated control. Best toxic Effects (High Mortality, Low Infestation) NIBORTEM + DE: Mortality steadily increased over time (36.6% \rightarrow 70% by day 7). Infested fruit: 37.4% — much lower than single treatments. Suggests synergistic action of Nibortem with Diatomaceous Earth. PRIORITY + DE: Mortality also increased gradually (33.3% \rightarrow 63.3%). Infested fruit: 47.6%, showing a substantial protective effect. Indicates that Priority combined with DE performs better than either alone. NEMBECIDINE used as positive control, showed the highest mortality by day 7 (86.6%). Infested fruit: 29.6% — the lowest infestation, suggesting strong field performance. Despite being slower in early mortality, it has excellent long-term efficacy. Otherwise, NIBORTEM (alone): Moderate mortality increase (23.3% \rightarrow 60%). Infestation: 52.2% — indicates good, but not optimal, performance. PRIORITY (alone): Mortality increased from 13.3% to 50%. Infestation: 58.6%, indicating lower efficacy compared to its combination with DE. Scovero et al. (2025) reported that there is no interaction between treatments and replicates in any species or stage was found, namely: i) 1st-2nd instar T. absoluta larvae, GLM, $\chi 2 = 12.758$, df = 8, P = 0.120; ii) 3rd-4th instar T. absoluta larvae, GLM, $\chi 2 = 13.196$, df = 8, P = 0.105; iii) 1st-2nd instar M. pygmaeus nymphs, GLM, $\chi 2 = 7.723$, df = 7, P = 0.358; iv) 3rd-4th instar M. pygmaeus nymphs, GLM, χ 2 = 6.163, df = 8, P = 0.629. Treatment significantly impacted the mortality of 1st-2nd instar T. absoluta larvae, with increased mortality observed in those feeding on leaflets treated with Naturalis® or Mycotal® compared to those on untreated leaflets.

Table 10. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety after week 2

Treatment	Before	The mean r	Infested			
	treatment	1day	3day	5day	7day	Fruit
PRIORITY	30	(13.3) d	(33.3) c	(43.3)cde	(50) c	58.6
NIBORTEM	30	(23.3) c	(40) bc	(53.3) bc	(60) bc	52.2
DE	30	(13.3) d	(33.3) c	(40) de	(46.6) c	60.5
PRIORITY+ DE	30	(33.3) b	(50) b	(53.3) bc	(63.3) bc	47.6
NIBORTEM+ DE	30	(36.6) b	(53.3) b	(63.3) b	(70) b	37.4
NEMBECIDINE	30	(46.6) a	(70) a	(76.6) a	(86.6) a	29.6
KONTROL	30	(10) d	(10) d	(10) f	(10) d	72.6
C.V(%)	_	21.2	18.2	14.9	16.8	
SE±		0.5±	0.7±	0.7±	0.9±	

Means carrying the same letter(s) in the same column is not significantly different at $P \le 0.05$

The therapy had no significant impact on the mortality of 3rd-4th instar *T. absoluta* larvae and *M. pygmaeus* nymphs of various instars. The four indigenous isolates exhibited moderate mortality responses (LT50 ranged from 4.1 to 4.3 days), comparable to or slightly exceeding that of the commercial product ECO-Bb (LT50 4.2 days). DE (alone):

Started with relatively high mortality (13.3) and reached 46.6% by day 7. Infestation: 60.5%, suggesting DE alone provides only partial protection. Untreated Control, consistently low mortality (10% across all days). Infested fruit: 72.6%, showing the natural impact of T. absoluta when left unmanaged. The results of synergistic action of Nibortem with Diatomaceous Earth. PRIORITY + DE: Mortality also increased gradually $(33.3\% \rightarrow 63.3\%)$. Infested fruit: 47.6%, showing a substantial protective effect, in consistency with the studies conducted by Bayram Y. (2019) who reported that both entomopathogenic fungi products individually and their combinations with SO shown highly significant efficacy in controlling the pest's eggs and larvae (P < 0.05). The simultaneous use of these two entomopathogenic fungi products proved more successful than the administration of each product individually in controlling both the egg and larval stages of the pest across both growing seasons. The individual application of the two entomopathogenic fungi products, B. bassiana and M. anisopliae, resulted in mean mortalities of 79.8% and 70.2% against the eggs during the initial growing season, whereas their combinations with SO achieved mean mortalities of 86.4% and 74.8% against the same developmental stage of the pest, respectively. M. anisopliae + SO combination was more effective against larvae of the pest, causing 83.2% and 81.4% mortalities in the highest dose in 2014 and 2015, respectively (Bayram, 2019). Likewise, in findings similar to the obtained data, Tadele and Emana (2017) evaluated the effect of three different concentrations of a local isolate of both B. bassiana and M. anisopliae against larvae of T. absoluta using the concentrations of 2.5×107 , 2.5×108 , and 2.5×108 109 conidia ml-1 under laboratory and greenhouse conditions and reported that mortalities caused by B. bassiana isolate at the different concentrations ranged from 79.17% to 95.83% in the laboratory and 73.0% to 84.04% in the greenhouse, and the highest mortality rate was observed at 2.5×109 conidia ml⁻¹. The same as B. bassiana isolate, the M. anisopliae isolate also caused the highest mortality at the highest concentration.

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer Tuta absoluta in Albeni variety after week 3

Table 11 indicates that the toxic effects of diatomaceous earth, Priority, and Nibortem on the larvae of the tomato leafminer *T. absoluta* in the Albeni variety are not statistically distinct from one another. At the same time, they are considerably different from the untreated control. Among the treatments, NEMBECIDINE used as positive control, exhibited the highest overall larval mortality over time, with values steadily increasing and reaching peak effectiveness 90% by day 7. This is consistent with its known rapid mode of action. NIBORTEM + DE and PRIORITY + DE combinations also achieved high mortality rates of 76.6% and 63.3%, respectively, indicating potential synergistic or additive effects when diatomaceous earth is combined with synthetic or biological insecticides.

The standalone use of DE resulted in moderate mortality levels of 50%, while PRIORITY and NIBORTEM individually showed slightly lower effectiveness rates of 53.3% and 63.3%, respectively, than their respective combinations with DE. Notably, the CONTROL (untreated) treatment showed consistently low mortality rates 13.3% by day 7, as expected.

Table 11. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in Albeni variety after week 3

Treatment	Before	The mean r	number morta leav	Infested		
treatment	treatment	1day	3day	5day	7day	Fruit
PRIORITY	30	(20)cd	(36.6) d	(43.3)c	(53.3)c	65.9
NIBORTEM	30	(16.6)cde	(43.3) bc	(50)bc	(63.3)bc	61.6
DE	30	(20)cd	(30) dc	(43.3)c	(50)c	67.4
PRIORITY+ DE	30	(33.3)abc	(46.6) bc	(56.6)bc	(63.3)bc	52.4
NIBORTEM+ DE	30	(36.6) ab	(53.3) ab	(63.3) b	(76.6)ab	44.6
NEMBECIDINE	30	(43.3)a	(60) a	(80) a	(90)a	37.8
KONTROL	30	(3.3)ef	(3.3) e	(3.3) d	(13.3)d	80.8
C.V(%)	_	36.3	18.5	16.8	16.7	
$SE\pm$	_	0.9±	0.7±	0.8±	0.9±	

Means carrying the same letter(s) in the same column is not significantly different at P≤0.05

The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer Tuta absoluta in UG 12406 740 variety after week 3

As shown in Table 12, the toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety, all applications are not significantly different from each other, but they are significantly different from the untreated control. Among the tested treatments, NEMBECIDINE used as positive control, consistently resulted in the highest larval mortality, reaching 90% larvae dead by day 7 and corresponded with the lowest fruit infestation rate (29.67%), confirming its strong toxic action. The combinations NIBORTEM + DE and PRIORITY + DE also showed high levels of larval mortality, particularly from day 3 onward, with 7day mortalities of 76.6% and 70% larvae, and fruit infestation rates of 37.48% and 47.63%, respectively. These results suggest a potential synergistic or additive effect when diatomaceous earth (DE) is combined with chemical or biological insecticides. Tsoulnara and Gordon (2016) evaluated the effectiveness of B. bassiana on third instar larvae, documenting a 90% death rate at elevated concentrations. Similarly, the outcome concurred with the findings of Rodriguez et al. (2006), who reported over 90% larval mortality following treatment with both M. anisopliae and B. bassiana in a pot experiment. In accordance with the study by Ismail (2024), it was discovered that death rates in response to various treatments, whether administered individually or in combination, significantly changed at different time intervals when treated to 2nd instar Fall Army Worm larvae (FAW) (Table 1). The mortality rate associated with the combined treatment of B. bassiana, DE, and chlorantraniliprole was substantially greater than that of the individual therapies. Furthermore, the combination of all therapies exhibited a strong synergistic interaction at various time intervals. The maximum mortality rate (100%) was attained with the synergistic application of B. bassiana (1 x 10⁸ conidia/mL), diatomaceous earth (DE), and chlorantraniliprole after 8 and 10 days. Conversely, the lowest mortality rates (3.33% and 5.06%) were recorded with individual doses of low-concentration DE and B. bassiana, respectively, after two days.

Table 12. The toxic effects of the diatomaceous earth, Priority, and Nibortem, against larvae of the tomato leafminer T. absoluta in UG 12406 740 variety after week 3

Treatment	Before treatment	The mean number mortality % of Larva after/7 leaves				Infested
		1day	3day	5day	7day	Fruit
PRIORITY	30	(23.3) b	(40) c	(50) bc	(60) bc	58.6
NIBORTEM	30	(23.3) b	(53.3) b	(60) bc	(70) bc	52.2
DE	30	(23.3) b	(36.6) c	(43.3) c	(53.3) c	60.5
PRIORITY+ DE	30	(30) ab	(46.6) bc	(56.6) bc	(70) bc	47.6
NIBORTEM+ DE	30	(46.6) a	(56.6) b	(66.6) ab	(76.6) b	37.4
NEMBECIDINE	30	(50) a	(70) a	(83.3) a	(93.3) a	29.6
KONTROL	30	(13.3) bc	(13.3) d	(20) d	(20) d	72.6
C.V(%)	_	24.1	16.0	18.9	14.6	
SE±	_	0.7±	0.7±	1.0±	0.9±	

Means carrying the same letter(s) in the same column is not significantly different at P≤0.05

In contrast, the single applications of PRIORITY, NIBORTEM, and DE resulted in moderate mortality levels, ranging between 60%,70% and 53.3%, larval mortality rates by day 7, and their corresponding fruit infestation rates were higher than the combination treatments, indicating reduced efficacy. This results is consisty with Silva et al. (2020) reported that the S50 (survival50) period of 2nd and 3rd instar T. absoluta larvae feeding on the leaves of tomato plants inoculated with B. bassiana LPP139 isolate 30 days ago against tomato moth was 4 days and all larvae died on the 7th day of the study. A similar situation was found for I. farinosa, and although the highest mortality rate (58%) was obtained from the seed inoculation method, there was no statistical difference between the methods (F = 1.833; df = 2; p = 0.179) (Taşçı and Muştu, 2023).

The control treatment showed the lowest larval mortality throughout the experiment, with 13.3% to 20% larvae dead by day 7, and exhibited the highest fruit infestation rate (72.65%), underscoring the damage potential of *T. absoluta* in the absence of control measures.

Conclusion and recommendations

- Based on the aforementioned results, a combination of DE and Entomopathogenic fungi can be regarded as a promising control agent for *T. absoluta*.
- PRIORITY + DE and NIBORTEM + DE combinations also achieved high mortality rates, indicating potential synergistic or additive effects when diatomaceous earth is combined with synthetic or biological insecticides.
- PRIORITY+ DE resulted in a 90% mortality rate, after 96 hours.
- The optimal outcome achieved by NIBORTEM+ DE, which provided a 96.6% larval mortality rate, after 96 hours.
- Field assays: Both tomato varieties were susceptible, but UG12406-740 was slightly more tolerant.
- Further comparison research should be undertaken to assess the effects of these combinations of DE and Entomopathogenic fungi on different insect pests.

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