

1 **Effectiveness of *Bacillus thuringiensis* (Shigetane) Commercial Products**
2 **against the tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:**
3 **Gelechiidae)**

4
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6
7 **ABSTRACT**

8 The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is one of
9 the most important pests causing significant economic losses in plant species belonging the
10 Solanaceae family. The preferred management method for *T. absoluta* currently involves
11 insecticide application. However, beside the undesired effects of insecticides, chemical
12 treatments can also negatively impact the efficiency of integrated pest management programs
13 (IPM). *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (Bt) is a pathogen with
14 formulations used as host-specific bioinsecticides. These formulations decompose quickly in
15 the environment, thereby reducing non-target effects and residue problems compared to
16 chemical pesticides. In this study, the effectiveness of six commercial *Bt* products, belonging
17 to *aizawai* and *kurstaki* strains, against *T. absoluta* was assessed under laboratory conditions,
18 using manufacturer-recommended doses. The efficacy of the *Bt* products varied between 70
19 and 97.5%. The lowest and highest mortalities were recorded in *B. thuringiensis* var. *aizawai*
20 and *B. thuringiensis* var. *kurstaki* products, respectively. Mortality reached 100% within three
21 days following insecticide treatments, whereas peak mortality in *Bt* applications was noted after
22 a post-treatment period of fifteen days. These findings highlight the potential of certain *Bt*
23 products as effective components of IPM programs for *T. absoluta*, suggesting the need for
24 further field studies to optimize their use in agricultural practices.

25 **Keywords:** *Bacillus thuringiensis*, development time, mortality, tomato, *Tuta absoluta*

26
27 **INTRODUCTION**

28 The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae),
29 originating from South America, stands as one of the most economically detrimental pests
30 affecting a range of plant species within the Solanaceae family (Miranda et al., 1998; Garzia,
31 2009). Initially reported in Spain in 2006, the pest subsequently spread throughout Europe and
32 the Mediterranean countries (Urbaneja et al., 2007; Arno et al., 2009). In Turkey, following its

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33 first appearance in 2009, it rapidly proliferated and emerged as a prominent pest in both
34 greenhouse and field tomato cultivation (Kılıç, 2010; Karut et al., 2011). *T. absoluta* larvae feed
35 between the two epidermal layers of tomato leaves, creating irregular transparent galleries that
36 eventually turn brown, causing complete leaf desiccation. Furthermore, the larvae also feed on
37 tomato fruits, and their excrement fosters an environment conducive to decay and the
38 development of secondary microorganisms. Collectively, these damages result in significant
39 losses in fruit quality and yield (Korycinska and Moran 2009; Desneux et al., 2010).

40 The predominant method for controlling *T. absoluta* involves insecticide application among
41 existing practices (Tropea et al., 2012; Roditakis et al., 2018). However, due to the limited
42 penetration of insecticides into plant tissues and the rapid development of resistance attributed
43 to *T. absoluta*'s high reproductive capacity, chemical control alone often fails to yield the
44 desired results (Biondi et al., 2018; Buragohain et al., 2021). Moreover, the indiscriminate and
45 intensive use of insecticides poses adverse effects on human and environmental health.
46 Consequently, alternative control methods, such as biological and biotechnical control, have
47 gained preference for the better management of the pest (Lietti et al., 2005; Gonzales-Cabrera
48 et al., 2011; Desneux et al., 2022).

49 Numerous natural enemies of *T. absoluta* from Hymenoptera and Hemiptera group of insects
50 have been identified (Miranda et al., 1998; Marchiori et al., 2004; Luna et al., 2007; Bajonero
51 2008; Cabello et al., 2009; Kabiri et al., 2010; Doğanlar and Yiğit 2011). In addition to predators
52 and parasitoids, microorganisms are also employed for pest control (Buragohain et al., 2021).
53 *Bacillus thuringiensis* (Shigetane 1902) (Bacillales: Bacillaceae) (*Bt*) is a unique soil-dwelling
54 bacterium utilized in the biological control of *T. absoluta* (Palma et al., 2014; Dammak et al.,
55 2016; Biondi et al., 2018). Commercial products derived from various subspecies of *Bt* are
56 deployed in managing insect species across different families. While *B. t* var. *kurstaki* is
57 effective against lepidopteran larvae, *B. t* var. *israelensis* and *B. t* var. *tenebrionis* are used to
58 control mosquitoes and coleopteran pest species, respectively (Gelernter, 2004; Palma et al.,
59 2014; Dammak et al., 2016).

60 Studies investigating the efficacy of *Bt* products against on *T. absoluta* commenced with *B.*
61 *t* var. *kurstaki* (*Btk*), sourced from South America in the early 2000s. Giustolin et al. (2001)
62 demonstrated *Btk* induced mortality across all developmental stages of *T. absoluta* larvae.
63 Subsequently, there has been a notable increase in research assessing the efficacy of *Bt* products
64 in managing the pest (Niedmann and Meza-Basso, 2006; Gonzalez-Cabrera, 2011; Sarr et al.,
65 2021). Niedmann and Meza-Basso (2006) revealed that two indigenous strains of *Bt* exhibited

66 lethal effects against *T. absoluta* in Chile. Gonzalez-Cabrera (2011) reported that the impact of
67 *T. absoluta* could be significantly diminished by exclusively applying *B. t*-based formulations,
68 obviating the need for chemical insecticides. Sarr et al. (2021) demonstrated a reduction in the
69 proportion of damaged fruits and an improvement in tomato yield, particularly with the
70 application of *Bt* products. Furthermore, it has been revealed that more favorable outcomes in
71 pest management could be achieved by combining *Bt* with various biocontrol agents (Gonzalez-
72 Cabrera et al., 2011; Alsaedi et al., 2017; Jamshidnia et al., 2018; Asma et al., 2018).

73 Environmentally friendly agents such as *Bt* strains are essential for a sustainable Integrated
74 Pest Management (IPM) program against tomato pests. Therefore, this study aims to evaluate
75 the effects of specific *Bt* commercial products with the potential to be used in biological control
76 programs against *T. absoluta*.

77

78 MATERIAL AND METHODS

79 Host Plant Rearing

80 Tomato (*Lycopersicon esculentum* L.) cultivar Soray was used as a host plant in this study.
81 The production of tomato plants was carried out in the a specialized rearing room adjusted at
82 25±2 °C temperature and 70±5% humidity with long day lighting (16 Light: 8 Dark)h. The
83 plants were grown in pots (15x15 cm) containing potting soil.

84

85 Tomato Leaf Miner Rearing

86 The initial population of *T. absoluta* was obtained from tomato fields of Adana, and bioassay
87 studies were completed at Cukurova University, Faculty of Agriculture, Department of Plant
88 Protection, Laboratory of Insect Molecular Genetics and Biotechnology. The production was
89 carried out in three fully grown tomato plants in net cages. The cages, each measuring
90 70x70x150 cm, were placed in the rearing room adjusted to 25±2 °C temperature of and 70±5%
91 humidity, with long-day lighting (16 Light: 8 Dark)h. To maintain the *T. absoluta* production,
92 dead tomato plants were replaced with new healthy plants during mass rearing period.

93

94 *Bacillus thuringiensis* Products

95 In this study, six registered *Bt* products in Turkey were tested. In addition to those products,
96 two commercial insecticides, spinetoram 120 g L⁻¹ (Radiant™, Dow AgroSciences, Istanbul,
97 Turkey), and spinosad 480 g L⁻¹ (Laser™, Dow AgroSciences, Istanbul, Turkey), widely
98 preferred in pest control by growers, were used as positive controls. The features and

99 recommended doses of the products are given in Table 1. Except for Dacron, all products were
100 registered against *T. absoluta*.

101

102 **Bioassay Experiment**

103 Leaves obtained from the upper half of 40 cm tall tomato plants (40 cm in height) were used
104 in the experiments. The recommended doses of the products, given in Table 1, were prepared
105 using distilled water, and were applied to the tomato leaves by leaf dipping method. In the
106 process, the leaves were dipped in the prepared solution for three seconds and then, allowed to
107 dry on a paper for 30 minutes under laboratory conditions. The petiole of the tomato leaves
108 were wrapped in wet cotton to provide moisture and keep the leaves alive during the
109 experiments. The leaves were placed in rectangular transparent plastic containers of 12x6x6
110 cm, where the lids were covered with nets for ventilation. One newly hatched first instar of *T.*
111 *absoluta* larvae was transferred to each leaf with the help of a fine-tipped paint brush. The first
112 instar larvae were obtained from *T. absoluta* eggs kept in cabinet adjusted to 25±1°C
113 temperature and 70±5% humidity. The larvae released on leaves treated with distilled water
114 were considered as controls. The prepared units were placed in a cabinet adjusted to 25±1°C
115 temperature, 70±5% humidity, and long-day lighting (16 hour Dark:8 hour Light).
116 Experimental units were checked daily, and the number of live/dead larvae and the development
117 of the larvae that remained alive were recorded. The stages of the larvae were determined
118 depending on the head capsules they left after each molting. A total of ten individuals were used
119 per replicate and each treatment was set up with 10 replicates (100 individuals) in bioassay
120 experiments. The mean development time of larval instars was determined from individuals
121 that remained alive and completed the immature development (Kandil et al., 2020). To
122 determine adult longevity, individuals reaching the adult stage were carefully transferred to
123 separate containers, and provided with honey as a regular consistent food source. These
124 containers were kept under controlled environmental conditions, including a temperature of
125 25±1°C, relative humidity of 70±5%, and a photoperiod of 16 hours light/8 hours dark. Each
126 adult was observed daily, and their survival was recorded until death.

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Table 1. Characteristics of *Bacillus thuringiensis* products and insecticides used in the experiments.

| <i>Bt</i> products | | | | | |
|--------------------|-------------|--|-----------------------------|------------------|------------------|
| Name | Formulation | Strain | Isolate | Bacteria density | Recommended rate |
| Agree 50 | WG | <i>B. thuringiensis</i> spp. <i>aizawai</i> + | GC-91 | %50 | 100 g /100 L |
| Dacron | WP | <i>B. thuringiensis</i> berliner var <i>kurstaki</i> | Serotype 3a 3b, SA- 11 5300 | 32000 IU/mg | 100 g /100 L |
| Delfin | WG | <i>B. thuringiensis</i> berliner var <i>kurstaki</i> | Serotype 3a 3b, SA- 11 | 32000 IU/mg | 100 g /100 L |
| Dipel DF | WG | <i>B. thuringiensis</i> subsp <i>kurstaki</i> | ABTS-351 | 32000 CLU/mg | 100 g /100 L |
| Florbac | WG | <i>B. thuringiensis</i> var. <i>aizawai</i> | ABTS-1857 | 35000 DBM/mg | 150 g /100 L |
| Rebound | WP | <i>B. thuringiensis</i> var. <i>kurstaki</i> | - | 16000 IU/mg | 200 g /100 L |
| Insecticides | | | | | |
| Active ingredient | | | | | |
| Laser | SC | 480 g/l Spinosad | | 25 ml/100 l | |
| Radiant | SC | 120 g/l Spinetoram | | 50 ml/ da | |

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Statistical Analyses

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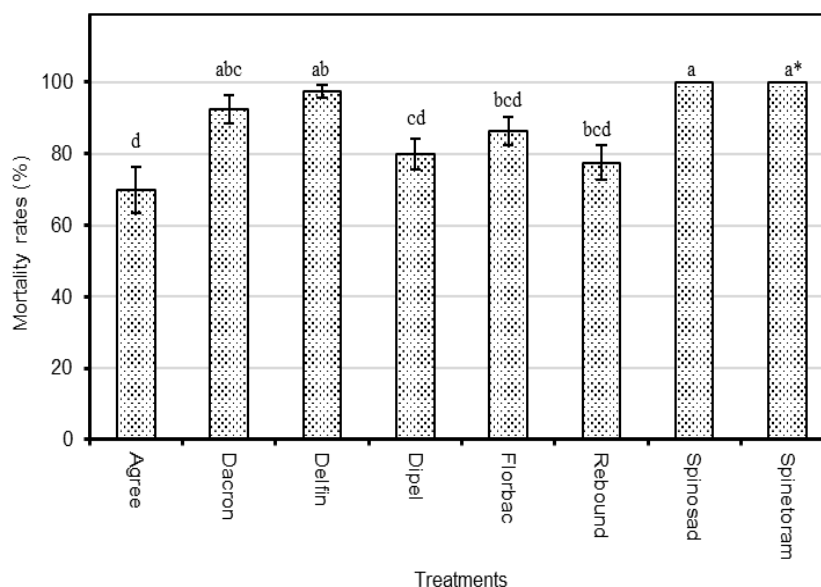
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Corrected mortality rates of 3, 7, 10, and 15 days after application and cumulative mortality were calculated using the Abbott formula (Abbott, 1925). Before conducting the analysis, we assessed the normality using the Shapiro–Wilk test and checked for homogeneity of variances using Levene’s test. In case of violation of assumptions, the data were transformed using $\text{Log}_{10}(X+1)$ and arcsin for homogeneity of variances. The original data were presented in the results. Data were analyzed using the One-Way ANOVA, followed by separation of means using the Tukey test. All analyses were conducted using SPSS 25.0 (Chicago, IL, USA).

138 RESULTS

139 Effects of *B. thuringiensis* Products on Mortality of *Tuta absoluta*

140 The insecticides, spinosad and spinetoram, exhibited the highest cumulative mortality rates,
 141 both reaching 100%, indicative of their potent lethal effects. Delfin and Dacron, belonging to
 142 *Bt* category, followed with mortality rates of 97.5 and 92.5%, respectively, and were
 143 statistically fell within the same group [F(7,79)=9.74, P=0.0001]. The remaining *Bt* products
 144 demonstrated mortality rates ranging from 86.2 to 70.0%, showcasing variability in their
 145 effectiveness (Figure 1).



146 **Fig. 1.** Cumulative mortality rates (\pm SE) of *Tuta absoluta* caused by different *Bacillus thuringiensis* products and two insecticides (Spinosad and Spinetoram). * Values with different letters denote statistically significant difference (Tukey; $p < 0.05$).

147 On the third day after application, a 100% mortality rate was observed for spinosad and
 148 spinetoram, while Dipel exhibited a low rate of 1%. Mortality rates increased for all products
 149 by day 7, ranging from 9 for Agree to 50% for Delfin, signifying varied responses to the
 150 treatments. On day 10, except for Delfin (67%), all products exhibited mortality rates below
 151 50%, indicating sustained but varied efficacy across treatments. On the 15th day, the highest
 152 and lowest mortality rates were observed in Delfin (94.2) and Agree (55.2%) treatment units,
 153 respectively, highlighting the durability and variability of the treatments (Table 2).

154 In the first instar larvae, the highest mean number of dead individuals was detected for Delfin
 155 (3.6), followed by Rebound (1.9) and Dipel (1.6). The three products differed statistically from
 156 the control experimental unit [F(6, 69)=10.1, P=0.0001]. No mortality was observed in Agree,
 157 which belonged to the same category as the control group. In the second instar larvae, the
 158

159 highest average numbers of dead individuals were found in Dacron (4.6), followed by Florbac
 160 (4.5). Other products showed varied mean mortality values between 0 and 2.8; the differences
 161 were statistically significant [F(6, 69)=16.08, P=0.0001]. In the third instar larvae, the mean
 162 numbers of dead individuals were close to each other, with the highest in Agree and Dacron
 163 (2.8). All the products were statistically different from the control, but showed no difference
 164 between each other [F(6, 69)=8.27, P=0.0001]. In the fourth instar larvae, the highest mean
 165 mortality values was determined as two for both Agree and Florbac. The values varied between
 166 0.6 and 1.5 for the other products. In the pupal stage, the highest and lowest mean numbers of
 167 dead individuals were recorded respectively for Rebound (0.8), and Delfin (0.1); the statistical
 168 difference, however was not significant [F(6,69)=1.40, P=0.226]. In the sum of the first and
 169 second instars, the mean number of dead individuals exceeded approximately 50% for the three
 170 products (Dacron, Delphin and Florbac) (Table 3).

171 **Table 2.** Corrected mortality rates (\pm SE) of commercial *Bacillus thuringiensis* (Bt)-based
 172 products and two insecticides (Spinetoram and Spinosad) after post-treatment period of
 173 3, 7, 10 and 15 days.

| Products | Days | | | |
|------------|-------------------------------|---------------------------------|--------------------------------|---------------------------------|
| | 3 | 7 | 10 | 15 |
| Agree | 0.00 \pm 0.00 ^{b*} | 9.00 \pm 3.48 ^c | 13.00 \pm 3.34 ^b | 55.25 \pm 4.38 ^c |
| Dacron | 0.00 \pm 0.00 ^b | 16.00 \pm 4.00 ^{bc} | 44.00 \pm 6.86 ^a | 64.50 \pm 7.00 ^{bc} |
| Delfin | 0.00 \pm 0.00 ^b | 50.00 \pm 7.60 ^a | 67.00 \pm 9.07 ^a | 94.25 \pm 3.07 ^a |
| Dipel | 1.00 \pm 1.00 ^b | 32.00 \pm 4.16 ^{ab} | 46.00 \pm 7.18 ^a | 78.50 \pm 4.47 ^{abc} |
| Florbac | 0.00 \pm 0.00 ^b | 21.00 \pm 4.33 ^{bc} | 45.00 \pm 7.49 ^a | 76.25 \pm 6.57 ^{abc} |
| Rebound | 0.00 \pm 0.00 ^b | 26.00 \pm 6.15 ^{abc} | 37.00 \pm 6.15 ^{ab} | 82.25 \pm 6.17 ^{ab} |
| Spinetoram | 100 \pm 0.00 ^a | - | - | - |
| Spinosad | 100 \pm 0.00 ^a | - | - | - |

174 * Means within the same column with different letters denote statistically significant difference (Tukey; p
 175 < 0.05).

176 **Table 3.** Mean (\pm SE) numbers of mortality *Tuta absoluta* individuals at different
 177 larval instars treated with commercial *Bacillus thuringiensis* (Bt)-based products

| Products | Larval instars and pupa | | | | |
|----------|------------------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|
| | I | II | III | IV | Pupa |
| Agree | 0.0 \pm 0.00 ^{c*} | 2.0 \pm 0.36 ^{bc} | 2.8 \pm 0.44 ^a | 2.0 \pm 0.61 ^a | 0.7 \pm 0.30 ^a |
| Dacron | 0.5 \pm 0.30 ^{bc} | 4.6 \pm 0.76 ^{ab} | 2.8 \pm 0.46 ^a | 1.2 \pm 0.38 ^a | 0.3 \pm 0.30 ^a |
| Delphin | 3.6 \pm 0.61 ^a | 2.8 \pm 0.44 ^{abc} | 2.0 \pm 0.55 ^a | 1.4 \pm 0.26 ^a | 0.1 \pm 0.10 ^a |
| Dipel | 1.6 \pm 0.54 ^{ab} | 2.5 \pm 0.54 ^{abc} | 2.6 \pm 0.30 ^a | 1.5 \pm 0.37 ^a | 0.2 \pm 0.13 ^a |
| Florbac | 1.0 \pm 0.29 ^{bc} | 4.5 \pm 0.63 ^a | 2.4 \pm 0.26 ^a | 0.6 \pm 0.26 ^a | 0.3 \pm 0.15 ^a |
| Rebound | 1.9 \pm 0.62 ^{ab} | 1.5 \pm 0.16 ^c | 2.0 \pm 0.59 ^a | 2.0 \pm 0.53 ^a | 0.8 \pm 0.29 ^a |
| Control | 0.0 \pm 0.00 ^c | 0.0 \pm 0.00 ^d | 0.1 \pm 0.10 ^b | 0.6 \pm 0.22 ^a | 0.4 \pm 0.22 ^a |

178 * Means within the same column with different letters denote statistically significant difference
 179 (Tukey; p < 0.05).

180 **Effects of *B. thuringiensis* Products on Development and Longevity of *Tuta absoluta***

181 In the first instar, statistically significant differences were observed in mean development
 182 times. The longest and shortest times were recorded for Dipel (4.37 days) and the control (3.33
 183 days), respectively [F(5, 161)=2.72, P=0.02] (Table 4). In the second instar, the mean
 184 development times varied between 2.77 and 5.00 days. In the third instar, a statistically
 185 significant difference in mean development times was observed, exceeding those of the control
 186 [F(5, 161)=9.62, P=0.0001]. In the pupal stage, mean development times were close to each
 187 other and did not show statistically significant differences [F(5, 161)=1.83, P=0.10]. Total mean
 188 development times ranged between 24.5 and 20.27 days, with statistically significant
 189 differences observed [F(5, 161)=12.7, P=0.0001]. Except for Florbac, adult longevities in all
 190 treatments were longer and statistically different from control [F(5, 161)=6.42, P=0.0001]
 191 (Table 4).

192 **Table 4.** Mean (\pm SE) development time (day) of different larval stages, and adult longevity of
 193 *Tuta absoluta* calculated from the larvae do not dead and completed development after *Bacillus*
 194 *thuringiensis* treatment.

| Products | Larval instars and pupa | | | | | | | Longevity |
|----------|-------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|---------------------------------|--------------------------------|
| | n | I | II | III | IV | Pupa | Total | |
| Agree | 24 | 3.45 \pm 0.20 ^{ab*} | 4.16 \pm 0.48 ^{ab} | 4.54 \pm 0.37 ^a | 3.54 \pm 0.24 ^{ab} | 8.12 \pm 0.06 ^a | 23.83 \pm 0.63 ^{ab} | 12.33 \pm 1.13 ^{ab} |
| Dacron | 6 | 4.33 \pm 0.42 ^{ab} | 4.83 \pm 0.30 ^a | 3.50 \pm 0.22 ^{ab} | 3.66 \pm 0.33 ^{ab} | 8.16 \pm 0.18 ^a | 24.50 \pm 0.42 ^a | 15.16 \pm 0.60 ^a |
| Dipel | 16 | 4.37 \pm 0.32 ^a | 3.62 \pm 0.32 ^{ab} | 3.37 \pm 0.28 ^{ab} | 3.56 \pm 0.47 ^{ab} | 8.12 \pm 0.17 ^a | 23.06 \pm 0.50 ^{ab} | 13.68 \pm 0.76 ^a |
| Florbac | 12 | 3.91 \pm 0.28 ^{ab} | 5.00 \pm 0.68 ^a | 3.33 \pm 0.28 ^{ab} | 2.83 \pm 0.40 ^{ab} | 7.58 \pm 0.19 ^a | 22.66 \pm 0.93 ^{abc} | 8.66 \pm 1.00 ^b |
| Rebound | 18 | 3.66 \pm 0.19 ^{ab} | 2.77 \pm 0.26 ^c | 2.88 \pm 0.25 ^b | 4.22 \pm 0.40 ^a | 7.94 \pm 0.17 ^a | 21.50 \pm 0.49 ^{bc} | 14.66 \pm 0.94 ^a |
| Control | 86 | 3.33 \pm 0.13 ^b | 3.50 \pm 0.14 ^{bc} | 2.74 \pm 0.09 ^b | 2.72 \pm 0.13 ^b | 7.88 \pm 0.07 ^a | 20.27 \pm 0.26 ^c | 11.04 \pm 0.32 ^{ab} |

195 * Means within the same column with different letters denote statistically significant difference (Tukey; $p <$
 196 0.05).

197 **DISCUSSION**

198 Although there were statistical differences, the effectiveness of *B. thuringiensis* (*Bt*)
 199 products, manifest by mortality rates exceeding 70.0%, was confirmed in this study. Similarly
 200 the effectiveness of *Bt* products on larval mortality of *T. absoluta* was confirmed under
 201 laboratory and greenhouse conditions (Hafsi et al., 2012; Birgücü et al., 2014; Jallow et al.,
 202 2019; Kandil et al., 2020; Sandeep Kumar et al., 2020a, b; Buragohain et al., 2021; Sarr et al.,
 203 2021). Although the application method is different, Hafsi et al. (2012) also found an average
 204 of 72.5% larval mortality at seven days after the treatment of the *Bt* product (Bt 32000) under
 205 laboratory conditions. Jallow et al. (2019), reported 55%–65% mortality when second- instar
 206 *T. absoluta* larvae were exposed to tomato leaves treated with *Bt* (Dipel).
 207

208 It can be suggested that the high mortality rate in the first two larval stages, with over 50%
209 mortality in three *Bt* products (Delfin, Florbac, and Dacron), could increase the success in the
210 biological control of tomato leaf miner. Similar results were reported in different studies, and
211 mortality in the first and second larval stages was found to be higher than other larval stages in
212 comparison (Giustolin et al., 2001; Gonzalez-Cabrera et al., 2011; Hashemitassuji et al., 2014).
213 Coelho and França (1987) argued that this was because the new larva that emerged from the
214 egg was feeding by chewing the leaf surface to reach the mesophyll layer. This behavior
215 increases the chance of getting bacterial toxins into the digestive system of the larvae.

216 *B. thuringiensis* products prolonged the larval development period in infected individuals
217 that survived and completed their development. These results were aligned with other
218 researchers who demonstrated the effect of *Bt* products on *T. absoluta* larvae, reporting a
219 significant increase in larval and pupal development periods (Kandil et al., 2020). Similar
220 results were also reported for other lepidopteran pests. Yang et al. (2008) determined that the
221 *Bt* YL17 isolate disrupted the development of the 3rd larval stage of *Spodoptera exigua*
222 (Hübner, 1808) (Lepidoptera: Noctuidae), and prolonged the total immature development. Erb
223 et al. (2001) reported that *Bt* had a sub-lethal effect on *Lymantria dispar* (Linnaeus, 1758)
224 (Lepidoptera: Lymantridae) fourth instar larvae and prolonged the development period. Barker
225 (1998) reported 12.4 days longer total immature development time for *Bt* treated *Cochylis*
226 *hospes* (Walsingham, 1884) (Lepidoptera: Tortricidae) larvae compared to the control group.
227 Similarly, Huarong et al. (2005) found that after *Bt* applications, larval development of *Ostrinia*
228 *nubilalis* (Hübner, 1796) (Lepidoptera: Pyralidae) was prolonged when compared to the
229 control.

230 The mortality rates of the *Bt* products were found to be close to the that of the insecticides.
231 However, the highest mortality rate (100%) was reached on the 3rd day in insecticide
232 treatments, while it was reached on the 15th day in *Bt* treatments. This could be due to the
233 different modes of action of the insecticide and *Bt*. While insecticides lead to immediate death
234 after application, mortality in *Bt* applications may occur after a few hours or weeks (Perez et
235 al., 2015). *B. thuringiensis* strains generate toxins during both the initial sporulation phase and
236 the growth stage, resulting in the formation of parasporal crystalline inclusions. Upon ingestion
237 by insects, these toxins dissolve within the midgut. Subsequently, midgut proteases trigger the
238 toxins through proteolysis, binding them to precise receptors on the insect cell membrane. This
239 binding results in cell disruption, ultimately leading to the death of the insect (Schnepf et al.,
240 1998; Palma et al., 2014).

241 In this laboratory study, we demonstrated that *Bt* products are at least as effective as
242 insecticides but require more time to achieve the maximum mortality rate. Therefore, for a
243 successful IPM program in greenhouses, these products should be applied repeatedly at specific
244 time window (one week) supported with supplemental application of other natural enemies,
245 such as predators or parasitoids.

246

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251

252 REFERENCES

- 253 1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ.*
254 *Entomol.*, 18: 265–267.
- 255 2. Alsaedi, G., Ashouri A. and Talaei-Hassanloui, R. 2017. Evaluation of *Bacillus*
256 *thuringiensis* to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under
257 laboratory conditions. *Agric. Sci.*, 8: 91–599.
- 258 3. Arno, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodriguez, D., Garreta, A., Gomez,
259 A. and Gabarra, R. 2009. *Tuta absoluta*, a new pest in IPM tomatoes in the northeast of
260 Spain. *IOBC/WPRS Bull.*, 49: 203–208.
- 261 4. Asma, C., Ons, I., Sabrine, B. and Kaouthar, L. 2018. Life-stage-dependent side effects
262 of selected insecticides on *Trichogramma cacoeciae* (Marchal) (Hymenoptera:
263 Trichogrammatidae) under laboratory conditions. *Phytoparasitica*, 46: 105–113.
- 264 5. Aynalem, B. 2022. Empirical review of *Tuta absoluta* Meyrick effect on the tomato
265 production and their protection attempts. *Adv. Agric.*, 2595470.
266 <https://doi.org/10.1155/2022/2595470>.
- 267 6. Bajonero, J. 2008. Biology and life cycle of *Apanteles gelechiidivoris* (Hymenoptera:
268 Braconidae) parasitoid of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agron. Colomb.*,
269 26(3): 417–426.
- 270 7. Barker, J. F. 1998. Effect of *Bacillus thuringiensis* subsp. *kurstaki* toxin on the mortality
271 and development of the larval stages of the banded sunflower moth (Lepidoptera:
272 Cochylidae). *J. Econ. Entomol.*, 91 (5): 1084–1088.

- 273 8. Birgüçü, A. K., Çelikpençe, Y., Karaca, İ. and Bayındır A. 2014. Growth inhibitory
274 effects of bio- and synthetic insecticides on *Tuta absoluta* (Meyrick, 1917)
275 (Lepidoptera: Gelechiidae). *Turk. J. Entomol.*, 38 (4): 389–400.
- 276 9. Biondi, A., Guedes R. N. C., Wan F. H. and Desneux N. 2018. Ecology, worldwide
277 spread, and management of the invasive South American Tomato Pinworm, *Tuta*
278 *absoluta*: Past, present, and future. *Annu. Rev. Entomol.*, 63: 239-258.
- 279 10. Buragohain P., Kumar Saikia, D., Sotelo-Cardona, P. and Srinivasan, R. 2021.
280 Evaluation of Bio-Pesticides against the South American Tomato Leaf Miner, *Tuta*
281 *absoluta* Meyrick (Lepidoptera: Gelechiidae) in India. *Horticulturae*, 7(9): 325.
- 282 11. Cabello, T., Gallego, J. R., Vila, E., Soler, A., Del Pino, M., Carnero, A., Hernandez, E.
283 and Polaszek, A. 2009. Biological control of the South American Tomato Pinkworm,
284 *Tuta absoluta* with releases of *Trichogramma achaeae* in tomato greenhouses of Spain.
285 *IOBC/ WPRS Bull.*, 49: 225–230.
- 286 12. Coelho, M. C. F. and França, F. H. 1987. Biologia e quemotaxia da larva e descrição da
287 pupa e adulto da traça-do-tomateiro. *Pesqui Agropecu Brasil*, 22: 129–135.
- 288 13. Dammak, M., Ben Khedher, S., Boukedi, H., Ikbel, C., Laarif, A. and Tounsi, S. 2016.
289 Involvement of the processing step in the susceptibility/tolerance of two lepidopteran
290 larvae to *Bacillus thuringiensis* Cry1Aa toxin. *Pestic. Biochem. Physiol.*, 127: 46–50.
- 291 14. Desneux, N., Han, P., Mansour, R., Arno, J., Brévault, T., Campos, M. R., Chailleux,
292 A., Guedes, R. N. C., Karimi, J., Konan, K. A. J., Lavoit, A. V., Luna, M. G.,
293 Perez- Hedro, M., Urbaneja, A., Verheggen, F. J., Zappala, L., Abbes, K., Ali, A.,
294 Bayram, Y., Cantor, F., Andrew, G. S. Cuthbertson, A. G. S., Vis, R. D., Erler, F.,
295 Firake, D. M., Haddi, K., Hajjar, M. J., Ismoilov, K., Jaworski, C. C., Kenis, M., Liu,
296 H. T., Madadi, H., Martin, T., Mazih, A., Messelink, G. J., Mohamed, S. A., Nofemela,
297 R. S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P. R., Wan, F. H.,
298 Wang, M. H., Wang, S., Zhang, Y. B. and Biondi, A. 2022. Integrated pest management
299 of *Tuta absoluta*: practical implementations across different world regions. *J. Pest Sci.*,
300 95:17–39.
- 301 15. Desneux, N., Wajnberg, E., Burgio G., Arpaia, S., Wyckhuys-Kris A. G., Narvaez-
302 Vasquez, C.A., Gonzalez-Cabrera, J., Tabone, E., Frandon J., Pizzol, J., Poncet C. and
303 Urbaneja A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*:
304 ecology, geographic expansion and prospects for biological control. *J. Pest Sci.*, 83:
305 197–215.

- 306 16. Doğanlar, M. and Yiğit, A. 2011. Parasitoid complex of the Tomato Leaf Miner, *Tuta*
307 *absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Hatay, Turkey. *KSU J. Nat.*
308 *Sci.*, 14(4): 28–37.
- 309 17. Erb S., Bouchier, R., van Frankenhuyzen, K. and Smith, S. 2001. Sublethal effects of
310 *Bacillus thuringiensis* Berliner subsp. *kurstaki* on *Lymantria dispar* (Lepidoptera:
311 Lymantriidae) and the Tachinid parasitoid *Compsilura concinnata* (Diptera:
312 Tachinidae). *Environ. Entomol.*, 30(6): 1174–1181.
- 313 18. Garzia, G.T. 2009. *Physalis peruviana* L. (Solanaceae), a host plant of *Tuta absoluta* in
314 Italy. *IOBC/WPRS Bull.*, 49: 231–232.
- 315 19. Gelernter W. 2004. The rise and fall of *Bacillus thuringiensis* tenebrionis.
316 *Phytoparasitica*, 32: 321–324.
- 317 20. Giustolin, T. A., Vendramim, J. D., Alves, S. B., Vieira S. A. and Pereira, R.M. 2001.
318 Susceptibility of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae) reared on two species of
319 lycopersicon to *Bacillus thuringiensis* var. *kurstaki*. *J. Appl. Entomol.*, 125: 551–556.
- 320 21. Gonzalez-Cabrera, J., Molla, O., Monton H. and Urbaneja, A. 2011. Efficacy of *Bacillus*
321 *thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick)
322 (Lepidoptera: Gelechiidae). *BioControl*, 56: 71–80.
- 323 22. Hafsi, K. A., Chermiti, B. and Nasraoui, B. 2012. Response of the tomato miner *Tuta*
324 *absoluta* (Lepidoptera: Gelechiidae) to thirteen insecticides in semi-natural conditions
325 in Tunisia. *Bull. OEPP/EPPO Bull.*, 42 (2): 312–316
- 326 23. Hashemitassuji, A., Safaralizadeh, M. H., Aramideh, S. and Hashemitassuji, Z. 2014.
327 Effects of *Bacillus thuringiensis* var. *kurstaki* and spinosad on three larval stages 1st,
328 2nd and 3rd of tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in
329 laboratory conditions. *Arch. Phytopathol. Plant Prot.*, 48(5): 377–384.
- 330 24. Huarong, L., Oppert, B., Higgins, R. A., Huang, F., Buschman, L. L. and Zhu, K. Y.
331 2005. Susceptibility of Dipel resistant and susceptible *Ostrinia nubilalis* (Lepidoptera:
332 Crambidae) to individual *Bacillus thuringiensis* protoxins. *J. Econ. Entomol.*, 98(4):
333 1333–1340.
- 334 25. Jallow, M. F. A., Dahab A. A., Albaho, M. S. and Devi, V. Y. 2019. Efficacy of some
335 biorational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)
336 under laboratory and greenhouse conditions in Kuwait. *J. Appl. Entomol.*, 143: 187–
337 195.

- 338 26. Jamshidnia, A., Abdoli, S., Farrokhi, S. and Sadeghi, R. 2018. Efficiency of spinosad,
339 *Bacillus thuringiensis* and *Trichogramma brassicae* against the Tomato Leafminer in
340 greenhouse. *BioControl*, 63: 619–627.
- 341 27. Kabiri, F., Vila, E. and Cabello T. 2010. *Trichogramma achaeae*: An excellent
342 biocontrol agent against *Tuta absoluta*. *Sting Newsletter Biol. Control*, 33: 5–6.
- 343 28. Kandil, M. A., Abdel-Kerim R. N. and Moustafa M. A. 2020. Lethal and sub-lethal
344 effects of bio-and chemical insecticides on the tomato leaf miner, *Tuta absoluta*
345 (Meyrick) (Lepidoptera: Gelechiidae). *Egyptian J. Biol. Pest Control*, 30:1–7.
- 346 29. Karut, K., Kazak C., Döker I. and Ulusoy M. R. 2011. Mersin ili domates seralarında
347 Domates yaprak galeri güvesi *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:
348 Gelechiidae)'nın yaygınlığı ve zarar durumu. *Turk. Entomoloji Derg.*, 35 (2): 339–347.
349 (in Turkish with abstract in English)
- 350 30. Kılıç, T. 2010. First record of *Tuta absoluta* in Turkey. *Phytoparasitica*, 38: 243–244.
- 351 31. Korycinska, A. and Moran, H. 2009. Plant Pest Notice: South American tomato moth,
352 *Tuta absoluta*. *Dep. Environ. Food Rural Aff. Food Environ. Res. Agency (Fera)*, 56: 1–
353 4.
- 354 32. Lietti, M. M. M., Botto, E. and Alzogaray, R.A. 2005. Insecticide resistance in
355 Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotrop.*
356 *Entomol.*, 34 (1): 113–119.
- 357 33. Luna, M. A. G., Sanchez, N. and Pereyra, P.C. 2007. Parasitism of *Tuta absoluta*
358 (Lepidoptera: Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae)
359 under laboratory conditions. *Environ. Entomol.*, 36(4): 887–893.
- 360 34. Marchiori, C. H., Silva, C. G. and Lobo, A. P. 2004. Parasitóides de *Tuta absoluta*
361 (Meyrick, 1917) (Lepidoptera: Gelechiidae) coletados em plantas de tomate em lavras,
362 estado de Minas gerais, Brasil. *Braz. Arch. Biol. Technol.*, 50(6): 434–437.
- 363 35. Miranda, M. M. M., Picanço, M., Zanuncio, J. C. and Guedes, R.N.C. 1998. Ecological
364 life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol Sci.*
365 *Technol.*, 8: 597–606.
- 366 36. Niedmann L. L. and Meza-Basso, L. 2006. Evaluation of native strains of *Bacillus*
367 *thuringiensis* as an alternative of integrated management of the tomato leaf miner (*Tuta*
368 *absoluta* Meyrick; Lepidoptera: Gelechiidae) in Chile. *Agricultura Técnica*, 66(3): 235–
369 246.

- 370 37. Palma L., Muñoz, D., Berry, C. Murillo, J. and Caballero, P. 2014. *Bacillus*
371 *thuringiensis* toxins: An overview of their biocidal activity. *Toxins*, 6(12): 3296–3325.
- 372 38. Perez, J., Bond, C., Buhl, K. and Stone, D. 2015. *Bacillus thuringiensis* (Bt) General
373 Fact Sheet; National Pesticide Information Center, Oregon State University Extension
374 Services. <http://npic.orst.edu/factsheets/btgen.html>.
- 375 39. Roidakis, E., Vasakis, E., Garcia-Vidal, L., Del Rosario, M. A. M., Rison, J. L.,
376 Haxaire-Lutun, M. O., Nauen, R., Tsagkarakou, A. and Bielza, P. (2018). A four-year
377 survey on insecticide resistance and likelihood of chemical control failure for tomato
378 leaf miner *Tuta absoluta* in the European/Asian region. *J Pest Sci.*, 91(1), 421–435.
- 379 40. Sandeep, Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Venkatasamy, B.,
380 Irulandi, S. and Prabhu, S. 2020 (a). Potential of Cry1Ac from *Bacillus thuringiensis*
381 against the tomato pinworm, *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera).
382 *Egyptian J. Biol. Pest Control*, 30: 81
- 383 41. Sandeep Kumar, J., Jayaraj, J., Shanthi, M., Theradimani, M., Venkatasamy, B.,
384 Irulandi, S. and Prabhu, S. 2020 (b). Potential of standard strains of *Bacillus*
385 *thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera:
386 Gelechiidae). *Egyptian J. Biol. Pest Control*, 30: 123.
- 387 42. Sarr, O. M., Bal, A. B., Fossati-Gaschnard O. and Gauthier, N. 2021. Effectiveness of
388 two biopesticides against the invasive tomato pest *Tuta absoluta*. *Entomologia Exp. et*
389 *Appl.*, 169: 674–685.
- 390 43. Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.
391 R. and Dean, D. H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins.
392 *Microbiol. Mol. Biol. Rev.*, 62: 775–806.
- 393 44. Tropea Garzia, G. G., Siscaro, G., Biondi, A. and Zappalà, L. (2012). *Tuta absoluta*, a
394 South American pest of tomato now in the EPPO region: Biology, distribution, and
395 damage. *EPPO Bulletin*, 42(2): 205-210.
- 396 45. Urbaneja, A., Vercher, R., Navarro, V., García Marí, F. and Porcuna, J. 2007. La polilla
397 del tomate, *Tuta absoluta*. *Phytoma España*, 194: 16–23.
- 398 46. Yang, X., Oluwafemi, A. R. and Zhang, H. 2008. Screening of highly toxic *Bacillus*
399 *thuringiensis* and its effects on the growth and development of *Spodoptera exigua*
400 (Lepidoptera: Noctuidae). *Entomol. Gen.*, 31(1): 95–104.