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Field Persistence and Application Timing of *Beauveria bassiana* and Two Surfactants to Suppress Tarnished Plant Bug Population in Cotton

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Abstract

A commercial and native isolates of *Beauveria bassiana* (Bb) and two emulsifiers, Tween-80 and starched-based sprayable bioplastic, applied at night and morning were evaluated in the field to measure the indices of damage caused by the tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) on cotton. Effects of solar radiation on TPB suppression, infectivity, and Bb sporulation were also examined. The TPB population was significantly reduced 7 days after spray (DAS) for all treated plots, suppressing population from 5-6 adults/10 sweep nets (SN) to 1 adult/10-SN 3-DAS and 0-1 adults/10-SN 7-DAS for all treatments. Little to no variation was observed in TPB nymph populations between treated and untreated plots. No significant differences in adult suppression were observed between morning and night application (MA, NA). The highest mortality rates and sporulation were found in insects exposed to cotton terminals sprayed with NI8+Tween-80-NA, 0-DAS. Although, no significant differences were observed between MA and NA, mortality and sporulation were higher in plots sprayed at night. Mortality and sporulation significantly decreased by the first day of application for all treatments regardless of NA or MA corresponding to the high susceptibility of Bb to sunlight. The highest percentage retention of all first position fruiting structures was observed in plots treated with NI8 + Tween-80-MA (88.30 ± 1.41), NI8+BioPlastic-MA (90.77 ± 1.28), and NI8 + Tween-80-NA (87.60 ± 1.15). Which were significantly higher than the controls. Overall, the seasonal plant mapping provided clear evidence of damage to cotton caused by TPB and how Bb regardless of the isolate, surfactant, or timing prevents in-

dices of cotton damage.

Keywords

Surfactants, UV Light, Microbial Control, *B. bassiana*, *Lygus*

1. Introduction

The tarnished plant bug (TPB), *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae), is a common pest occurring in many commercially grown crops across North America, but is most notably significant in cotton production *Gossypium hirsutum* L. (Malvales: Malvaceae) in Mississippi, and across the southern US [1], feeding at any growth stage of the cotton plant, but most of the damage occurs from feeding the onset of squaring through the blooming period [2], causing abscission of squares and bolls, leading to loss in yield [3]. Snodgrass [4] and Snodgrass *et al.* [5] [6] reported the difficulty of managing TPB due to its ability to become resistant to insecticides. Despite that, today, foliar-applied insecticides are the only method used to suppress TPB population, but to be effective, several applications are required to adequately control this pest [7]. Among biological control options, the entomopathogenic filamentous fungus *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), serves as an important natural agent to control TPB adults and nymphs [7]-[9]. However, its efficacy is shaped by multiple biotic and abiotic factors that affect or influence infectivity and sporulation. Of the various factors, temperature, humidity, and solar radiation are the most important environmental conditions affecting spores' survival [10].

In the nature ecosystem of Mississippi Delta, despite extreme temperatures, such as heat and cold waves, the *B. bassiana* strain NI8, originally reported in 2005 [8], can be found to live as epiphytes and as endophytes in plant tissues. Although *B. bassiana* is well known to be a cosmopolitan and naturally soil-inhabiting fungus [11], the native strain NI8 in the absence of host insects, is likely to live with plants rather than the soil [7]. This strain has been studied in the field to manage TPB in cotton, with few published non-target effects [12].

Many of the entomopathogenic pesticides that use *B. bassiana* as active ingredients such as GHA (BotaniGard 22WP) are utilized in a variety of agricultural situations to manage a diversity of pests. Its pathogenicity, which is the qualitative ability of a pathogen to cause disease is determined by the interaction of factors such as the physiology of the host, physiology of the fungus, and the environmental conditions [13]. Its pathogenicity, therefore, same as any other commercial or native strains can be improved by using formulating agents such as emulsifiable adjuvants [14]. This biological pesticide is usually applied in the form of spores, containing wettable powders conidia. Polar *et al.* [14] noted that water is a useful formulating agent because it is non-toxic, readily available, cheap, and can be dis-

persed using simple hydraulic sprayers. Thus, the use of wettable and sprayable surfactants is important to facilitate application, stability, and enhancement of spore activity. Tween-80 is one of the most useful wettable surfactants that have been shown to enhance the production of enzymes, making it indispensable for laboratory bioassays and field trials [15]. Accinelli and Abbas [16], and Portilla *et al.* [17], reported the feasibility of using an alternative bioplastic-based formulation as a carrier of microbiological agents, including atoxigenic isolates of *Aspergillus flavus*, *Trichoderma spp.*, and *B. bassiana*.

Besides the use of sprayable surfactant, the timing of application is an important factor that influences the dynamics of pest-pathogen interaction [9] [10] [18]. Conidia, hyphal bodies, and hyphae of all taxa of hyphomycetes fungi are highly susceptible to damage by solar radiation, particularly the UV portion of the solar spectrum (Inglis, 2001). However, significant differences in susceptibility to irradiation among strains within species have been observed. For example, Leland *et al.* [9] observed that GHA and NI8 conidia that were isolated from *Lygus hesperus* Knight (Hemiptera: Miridae) were generally more resistant to artificial sunlight than GHA and NI8 isolated from *L. lineolaris*. Therefore, this study examined whether morning or night application affects the effectiveness of *B. bassiana*-based bioinsecticide spores (commercial strain GHA and native strain NI8) against TPB in cotton fields, using Tween-80 or BioPlastic water-based emulsions.

2. Materials and Methods

2.1. Insect Colony

A laboratory-reared colony of TPB has been kept at the United States Department of Agriculture - Agricultural Research Service (USDA-ARS), Southern Insect Management Research Unit (SIMRU) in Stoneville, MS, following the protocol described by Portilla *et al.* [7]. The rearing system was developed to efficiently produce individuals of the same age in large numbers. Insects were kept in environmental chambers set to a 12-hour light and 12-hour dark cycle, at 27˚C and 60% relative humidity. Mixed-sex TPB adult aged 1 - 2 days were selected for both mortality and sporulation bioassays.

2.2. Fungus Cultures

The native *B. bassiana* strain NI8 used in the present study was attained from the collection of USDA-ARS-SIMRU, which has been produced on regular basis for research purposes. The sprayable *B. bassiana*-based commercial strain GHA and native strain NI8 were tested in two formulations using either Tween-80, or a Bioplastic-based adjuvant, and prepared according to the procedures described by Portilla *et al.* [17] and Portilla *et al.* [7]. 250 g from both strains, GHA (BotaniGard 22WP; LAM International Corporation, Butte, Montana) and NI8, both with a spore viability of $\geq$ 97% were combined in a gallon of water + Tween-80 (Sigma-Aldrich P8074, Darmstadt, Germany) or BioPlastic (1% solution) + Mepiquat

chloride-Plant growth regulator (MC-PGR), giving the following concentration for morning and night application: 1) NI8 2.5×10^{11} + 60 ml Tween-80 + 354 ml MC-PGR/ha; 2) GHA 2.0×10^{13} + 60 ml Tween-80 + 354 ml MC-PGR/ha; 3) NI8 2.5×10^{11} + 1% concentration of Bio-Plastic + 354 ml MC-PGR (75 L/ha; 4) GHA 2×10^{13} + 1% concentration of Bio-Plastic + 354 ml MC-PGR (75 L/ha); 3 as follow: 2.0 101 water containing 0.4 mL of Tween-80 or a 1% (wt/vol) BioPlastic solution (75 l/ha).

2.3. Tarnished Plant Bug Population Suppression on Night and Morning Treated Plots

The experiment was conducted at a cotton farm operated by the Southern Insect Management Research Unit, USDA, located near Leland, Mississippi. The study employed a factorial experiment within a randomized complete block design, ensuring consistency across both nocturnal and morning applications. Therefore, two sets of 25 plots (0.028 ha each plot) were planted with 8 rows of cotton, *Gossypium hirsutum* L. (DP1321B2RF Bollgard II ™, Delta and Pine Land Company ™, Scott, MS) in late May. Between cotton plots, four rows of corn, *Zea mays* L. (VT2Pro® corn, DKC66-97®, DeKalb Genetics Corp., DeKalb, IL) were planted in March, which served as a barrier to evade cross infection among plots and treatment. Each set of 25 plots were used for morning and night applications. The TPB population was monitored by sampling the treated plots on biweekly basis. Applications were initiated once 50% of the cotton plots or higher exhibited a TPB population to approximately 5-10 adults/10 sweep net samples. All cotton plots applications were made using a multi-boom sprayer tractor (TecjetTM-Conejet® TXVS12 nozzle). In July, all assigned morning and night plots were sprayed with commercial or native *B. bassiana* strains both surfactants' formulations with either Tween-80 or Bio-Plastic, and water alone as a control (five plots/treatments). The first population sample was collected from all plots at 0 day (0-D) before the morning and night applications. To evaluate suppression of TPB population, two more samples were done, 3-D and 7-D after spray. The number of TPB adults and nymphs collected in the set of 10 sweep net samples per plot each day (morning and night sprays) determined the population suppression. To better understand the TPB population suppression and the differences between before and after treatments during night at morning applications. Each treatment (GHA+Tween-80, GHA + BioPlastic, NI8+Tween-80, NI8+BioPlastic, and water control) were analyzed independently and the sub-treatments were: a) 0 days before spray morning application (0-DBSMA), b) 0 days before spray night application (0-DBSNA), c) 3 days after spray morning application (3-DASMA), d) 3 days after spray night application (3-DASNA), e) 7 days after spray morning application (7-DASMA), f) 7 days after spray night application (7-DASNA).

2.4. Bioassay Procedure to Evaluate the Effect of the Sunlight on *Beauveria bassiana* Spores

This experiment was conducted using the same plots for the population suppression

study with a factorial experiment (10 × 5 × 3 × 3) (treatments, replications, sub-replication top terminal, days of evaluation) within a randomized complete block design. Treatments for this experiment were as follow: 1) water control morning spray, 2) native strain NI8 + Tween-80 morning spray, 3) native strain NI8 + Bio-Plastic morning spray, 4) commercial strain GHA + Tween-80 morning spray, 5) commercial strain GHA + Bio-Plastic morning spray, 6) water control night spray, 7) native strain NI8 + Tween-80 night spray, 8) native strain NI8 + Bio-Plastic night spray, 9) commercial strain GHA + Tween-80 night spray, and 10) commercial strain GHA + Bio-Plastic night spray. Thus, on 0 day (0-D) one hour after treatment applications and 1-D and 2-D, thereafter, three top nodes terminals of cotton plant/plot (morning and night) were randomly cut and placed under laboratory conditions individually into a pop-up insect rearing mesh cage 30 cm^3 (http://amazon.com/). A total of four hundred and fifty cages and terminals were used for both sets of 25 plots study (three cages/plot, 15 terminal/treatment/per morning and night applications per each day). Thirty TPB mix-sexed adults 2 d old from SIMRU laboratory colony were released in each cage (13,500 TPB adults total). Cages containing sprayed cotton terminals and newly released TPB adults were gently agitated to ensure that the insects were exposed to the treated cotton foliage. The cages were then maintained at ambient room temperature for 24 hours. Then, TPB adults were removed from the cages and placed individually into a solo cup with solid diet (12). Daily observations were conducted for 10 days to determine mortality. Dead insects were kept in the same cup and were checked daily for fungal sporulation for 15 days. Adults TPBs were held in an environmental room at 27˚C, 65% RH, and 12:12 (L:D) photoperiod.

2.5. Effect of Native and Commercial *Beauveria bassiana* on TPB Population and Cotton Fruit Damages under Field Conditions

This experiment was conducted at the cotton farm at Leland, MS for both sets of the previously described cotton plots. Plant mapping was done on ten plants within each plot replication of both morning and night applications (500 plants in total). Data recorded on selected plant mapping date included total nodes, total fruiting nodes, first position squares, first position flower or boll, missing first position fruit, first position flowers and number flowers/boll nodes, percentage retention on square nodes, percentage retention top 3 fruiting position, percentage retention top 5 fruiting position, percentage retention nodes above white flowers, and percentage retention of all first position site including all undamaged squares, flowers and bolls.

2.6. Statistical Analysis

The study designs featured factorials in a randomized complete block configuration. Population suppression (1 × 6 × 5 × 3) (treatment, sub-treatments, replications, insect stages), effect of sunlight on spore germinations (10 × 5 × 3 × 3) (treatments, replications, sub-sample, days, times), and morning and night appli-

cation effects on the plant mapping (10 × 5) (treatments, replications) variables were analyzed using SAS 15.3 [19]. All variables for each experiment were analyzed by using a PROC GLM (ANOVA) followed by Tukey's HSD ($P = 0.05$) to detect differences between treatments.

3. Results

3.1. Tarnished Plant Bug Population Suppression

Commercial and native *B. bassiana* strains reduced TPB populations, regardless of surfactant type or application timing (morning or night). No statistically significant differences were observed in TPB density within the water control plots for nymphs (F = 2.37; df = 9, 20; $P = 0.0521$), adults (F = 0.46; df = 9, 20; $P = 0.7624$), or the total population (F = 0.72; df = 9, 20; $P = 0.6846$) when comparing pre- and post-spray periods conducted at night and in the morning. Population levels ranged between 4 and 5 TPB per 10 sweeps per plot during both sampling intervals (see **Figure 1(A)**). There were statistical differences in TPB adults, nymphs, and total population in plots before and after sprayed with NI8 + Tween80: (F = 12.35; df = 9, 20; $P = 0.0001$), (F = 3.46; df = 9, 20; $P = 0.0100$) and (F = 2.82; df = 9, 20; $P = 0.0435$) (**Figure 1(B)**); NI8 + BioPlastic: (F = 10.61; df = 9, 20; $P = 0.0001$), (F = 4.38; df = 9, 20; $P = 0.0029$) and (F = 4.11; df = 9, 20; $P = 0.0099$) (**Figure 1(D)**); and GHA + BioPlastic (F = 20.33; df = 9, 20; $P = 0.0001$), (F = 2.89; df = 9, 20; $P = 0.0230$) and (F = 3.91; df = 9, 20; $P = 0.0053$) (**Figure 1(E)**), respectively. There were no statistical differences in TPB nymph population (F = 4.53; df = 9, 20; $P = 0.0024$) in plots sprayed with GHA + Tween80, but highly significant differences were observed in adults (F = 12.83; df = 9, 20; $P = 0.0001$) and total population (F = 2.33; df = 9, 20; $P = 0.0024$) (**Figure 1(C)**). No significant differences were found between plots morning and night applications at any day of evaluation for any surfactant combined with commercial or native strain, except for GHA + BioPlastic where the population before spray in night plots was lower than morning plots and was the only treatment that reduced the population to 0 adults/10 sweep nets 7-D after spray (**Figure 1(E)**). **Figures 1(A)-(D)** shows that the adult TPB reduced significantly its population by ~4-fold 3-D after spray and >4-fold 7-D after all *B. bassiana* treatments regardless of night or morning applications or adding the surfactants Tween-80 or BioPlastic. No reduction in TPB nymph population was observed for any treatment. On the contrary, a slight increment in population was observed after spray in all treatments including water control (**Figures 1(A)-(E)**).

3.2. Effects of Sunlight on *Beauveria bassiana* Spore Germination

Figure 2(A)-(C) indicates that sunlight seems to interfere with TPB infection, affecting the propagule persistence of *B. bassiana* sporulation regardless of morning and night applications or surfactant's protection.

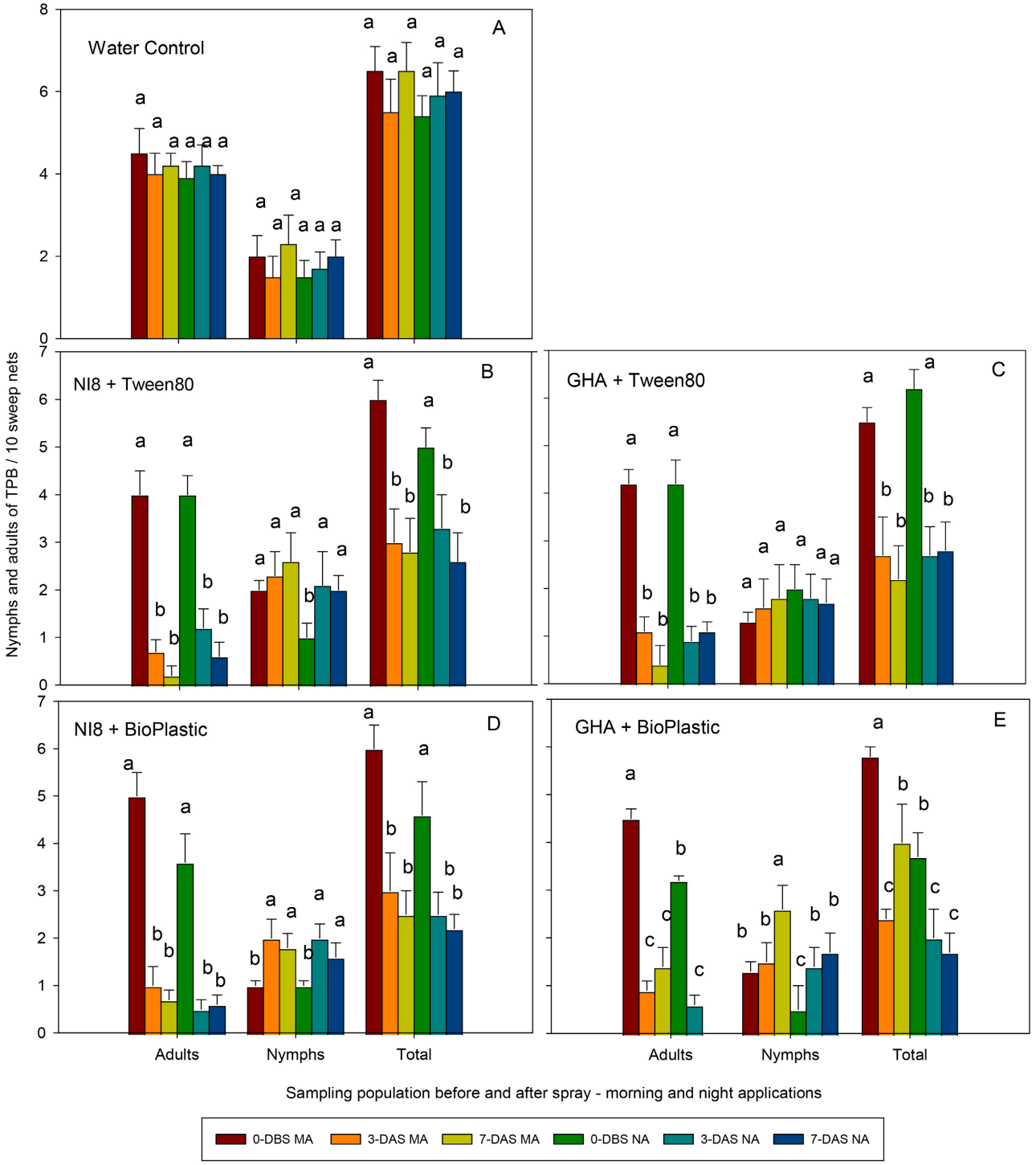

Figure 1. Mean ± SD number of tarnished plant bug nymphs, adults, and total population /10 sweeps/plot 0 days before spray (0-DBSA) and 3 and 7 days after spray (3-DAS, 7-DAS) morning and night applications (MA, NA, respectively). (A) Population of TPB adults sampled in control cotton plots before and after spraying water. (B) Populations of TPB sampled plots in cotton plots before and after spraying the native strain NI8 + Tween-80. (C) Populations of TPB adults sampled in cotton plots before and after spraying the commercial strain GHA + Tween-80. (D) Populations of TPB adults sampled in cotton plots before and after spraying the native strain NI8 + BioPlastic. € Populations of TPB adults sampled in cotton plots before and after spraying the commercial GHA strain + BioPlastic. Means separated by a common letter are not significant different according to Tukey Test LSD ($p = 0.05$).

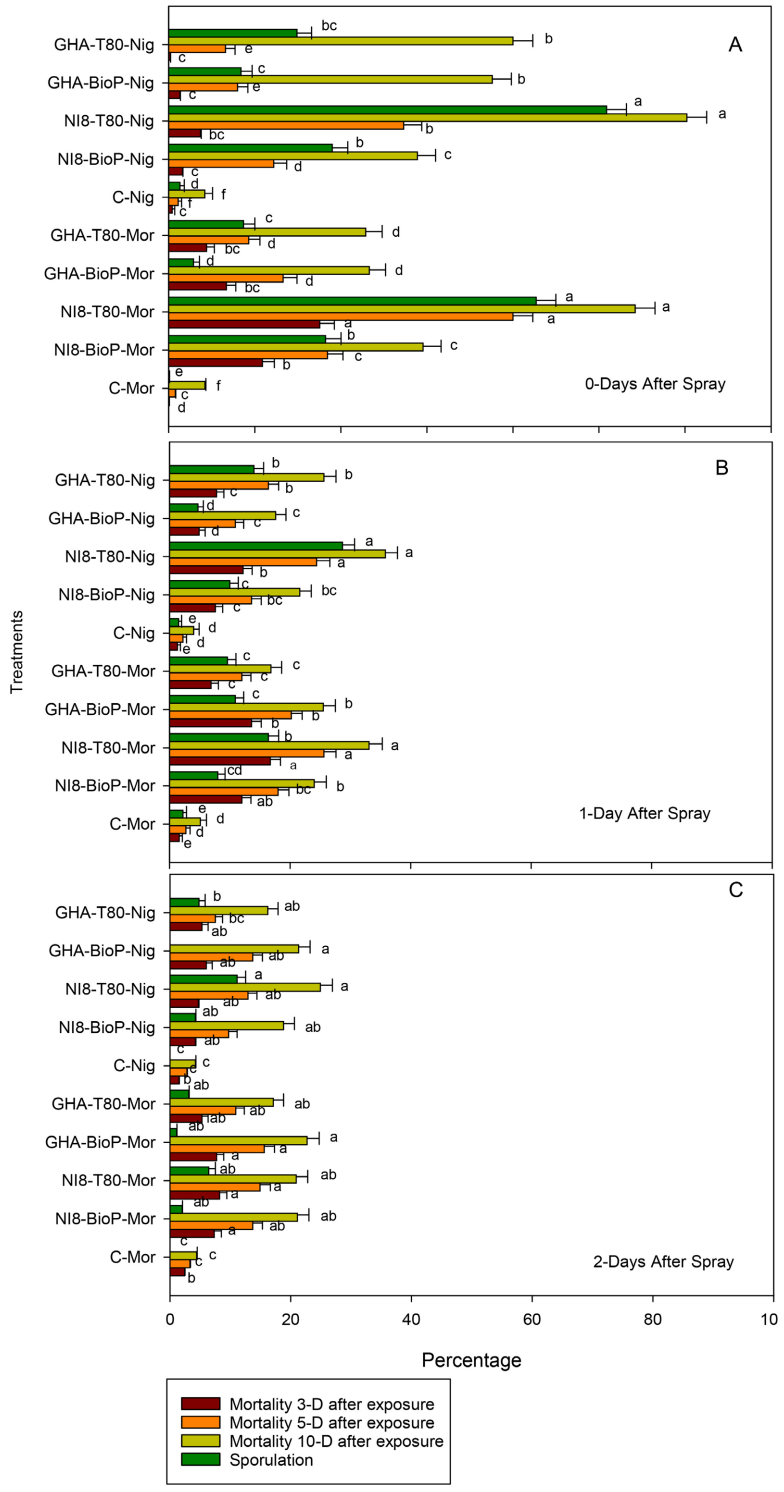

Figure 2. Mean ± SD cumulative mortality and sporulation percentage of tarnished plant bug adults exposed to sprayed cotton leaves with commercial GHA and native NI8 strains of *Beauveria bassiana* in combination with two surfactants Tween-80 and BioPlastic. (A) TPB adults exposed to sprayed cotton terminals 0 days after spray. (B) TPB adults exposed to cotton terminals 1 day after spray. (C) TPB adults exposed to cotton terminals 2 days after spray. Means separated by a common letter among colored bars are not significant different according to Tukey Test LSD ($p = 0.05$).

Virulence factors of both commercial and native strain were highly susceptible to damage by solar radiation where their inactivity was clearly noticeable across the experiment (Figure 2(A)-(C)). Although native Delta strain NI8 + Tween-80 applied during morning time showed highly significant differences with a greater performance compared to the other treatments 3-D after exposure on plots 0-D, 1-D, and 2-D after treatment; its viability 5-D after exposure increased the same as its application during nighttime with no significant differences among timing, which continue with similar enhance throughout the 10-D after exposure. The Delta native strain NI8, when used with Tween-80 during nighttime applications, demonstrated greater efficacy, resulting in higher mortality and sporulation (Figures 2(A)-(C)). However, no statistical differences were observed for either mortality or sporulation when the same treatment was administered in the morning. Water controls consistently showed less than 5% mortality and under 2% sporulation 10 days post-exposure compared to the experimental groups subjected to *B. bassiana* infection stress due to UV exposure. Cross contamination may have caused mortality and sporulation in the control group. Figure 1(A) shows highly significant differences among treatments in 0-D after spray at 3, 5, and 10 days after exposure for cumulative morality 3-D: ($F = 8.76$; $df = 44, 4455$; $P = 0.0001$), 5-D: ($F = 13.98$; $df = 44, 4455$; $P=0.0001$) and 10-D: ($F = 18.27$; $df = 44, 4455$; $P = 0.0001$) and for sporulation ($F = 25.22$; $df = 44, 4455$; $P = 0.0001$). Similarly, Figures 2(B)-(C) reveal highly significant differences among treatments in 1-D after spray at 3, 5, and 10 days after exposure for cumulative morality 3-D: ($F = 4.20$; $df = 44, 4455$; $P = 0.0001$), 5-D: ($F = 6.04$; $df = 44, 4455$; $P = 0.0001$) and 10-D: ($F = 7.84$; $df = 44, 4455$; $P = 0.0001$) and sporulation ($F = 6.93$; $df = 44, 4455$; $P = 0.0001$) and 2-D after spray at 3, 5, and 10 days after exposure for cumulative morality 3-D: ($F = 2.32$; $df = 44, 4455$; $P = 0.0001$), 5-D: ($F = 3.84$; $df = 44, 4455$; $P = 0.0001$) and 10-D: ($F = 5.57$; $df = 44, 4455$; $P = 0.0001$) and sporulation ($F = 4.54$; $df = 44, 4455$; $P = 0.0001$). Figures 2(A)-(C) shows that adult mortality and *B. bassiana* sporulation declined with increasing sunlight exposure across all treatments; yet degradation patterns differed significantly among strains, surfactants, and application time. Across all treatment groups, adult mortality due to *B. bassiana* infection and the viability of spores are strongly correlated with exposure to ultraviolet light.

3.3. Effect of Native and Commercial *Beauveria bassiana* on TPB Population and Fruit Damage Based on Yield Cotton Plant Mapping

As previously noted, both commercial and native *B. bassiana* strains were effective in reducing TPB populations, irrespective of surfactant type or timing of application (morning or night). Population density is closely linked to crop injury, which was evident in control groups when compared to treated plots. Water control had the highest injury with the lowest parameters values, and the highest rate of missing first position (Table 1). There were highly significant differences among treatments for total nodes ($F = 2.68$; $df = 32, 967$; $P =$

0.0001), total fruiting nodes (F = 2.69; df = 32, 967; $P = 0.0004$), first position square (F = 8.34; df = 32, 967; $P = 0.0001$), first position flowers or bolls (F = 3.26; df = 32, 967; $P = 0.0001$), missing first position (F = 6.73; df = 32, 967; $P = 0.0001$), and number of flowers/boll node (F = 2.57; df = 32, 967; $P = 0.0001$) (Table 1).

Table 1. Effect of morning (Mor) and night (Nig) application of *Beauveria bassiana* with sprayable bioplastic and Tween-80 formulations under field conditions on *L. lineolaris* population and the damage of fruits based on within-season yield cotton plant mapping.

Treatments	Indices of *L. lineolaris* damage (means ± SE)					
	Total Nodes	Total Fruiting Nodes	First Position Square	First Position Flowers or Bolls	Missing First Position	Number of Flowers/boll node
Control-Mor	13.90 ± 0.32 c	9.26 ± 0.33 c	3.45 ± 0.20 d	2.76 ± 0.18 bc	3.05± 0.25 ab	3.37 ± 0.24 c
NI8 [1]-BioPlastic-Mor	15.31 ± 0.26 abc	10.80 ± 0.35 ab	5.29 ± 0.14 ab	4.14 ± 0.23 a	1.37 ± 0.16 de	4.96 ± 0.30 a
NI8-Tween80-Mor	15.02 ± 0.27 abc	9.96 ± 0.29 abc	5.18 ± 0.15 ab	3.81 ± 0.25 a	0.97 ± 0.13 d	4.21 ± 0.27 abc
GHA[2]-BioPlastic-Mor	14.39 ± 0.28 bc	10.34 ± 0.32 abc	4.63 ± 0.18 bc	3.48 ± 0.21 abc	2.23 ± 0.21 bcd	3.48 ± 0.29 bc
GHA-Tween80-Mor	15.48 ± 0.24 ab	9.37 ± 0.38 bc	3.88 ± 0.19 cd	2.73 ± 0.21 c	2.76 ± 0.24 b	4.55 ± 0.27 abc
Control-Night	14.61 ± 0.28 bc	10.06 ± 0.36 abc	4.56 ± 0.22 bc	3.24 ± 0.22 abc	2.26 ± 0.20 bc	3.94 ± 0.28 abc
NI8-BioPlastic-Nig	15.44 ± 0.29 abc	10.54 ± 0.34 abc	5.44 ± 0.16 a	3.69 ± 0.21 ab	1.41 ± 0.13 cde	4.50 ± 0.26 abc
NI8-Tween80-Nig	15.96 ± 0.22 a	10.93 ± 0.31 a	5.11 ± 0.18 ab	3.98 ± 0.19 a	1.84 ± 0.21 cde	4.92 ± 0.25 a
GHA-BioPlastic-Nig	15.32 ± 0.26 abc	10.68 ± 0.40 abc	5.22 ± 0.16 ab	3.90 ± 0.20 a	1.56 ± 0.18 cde	4.67 ± 0.27 ab
GHA-Tween80-Nig	15.15 ± 0.25 abc	10.26 ± 0.30 abc	3.18 ± 0.18 d	3.38 ± 0.18 abc	3.70 ± 0.21 a	4.41 ± 0.25 abc

Note: [1] Native Strain; [2] Commercial Strain; Means ± SE followed by the same letter in each column are not significantly different ($p < 0.05$ Tukey test).

Applying native strain NI8 + Tween80 at night outperforms other treatments, yielding the most total nodes, fruiting nodes, and flowers per boll node (Table 1). No significant differences were detected in the number of first position flowers or bolls relative to the commercial GHA + BioPlastic strain applied during nighttime. Likewise, there were no significant variations in the number of flowers per boll node compared to the same treatment administered in the morning. Table 2 demonstrates that the application of NI8 combined with Tween80 during morning and nighttime maintained superior performance, exhibiting highly significant differences among the other treatments. Those combinations resulted in a higher retention rate at square nodes (F = 8.34; df = 32, 967; $P = 0.0001$), top 3 fruit position (F = 10.15; df = 32, 967; $P = 0.0001$), top 5 fruit position (F = 8.15; df = 32, 967; $P = 0.0001$), nodes above white flowers (F = 9.29; df = 32, 967; $P = 0.0001$), and all first positions (F = 8.86; df = 32, 967; $P = 0.0001$). No significant differences were observed in the retention (%) on flowers per boll node (F = 2.20; df = 32, 967; $P = 0.2204$) (Table 2).

Table 2. Effect of morning (Mor) and night (Nig) application of *Beauveria bassiana* with sprayable bioplastic and Tween-80 formulations under field conditions on *L. lineolaris* population and the retention of fruits based on within-season yield cotton plant mapping.

Treatments	Indices of *L. lineolaris* on cotton retention (%) (means ± SE)					
	Retention on squares nodes	Retention Top 3 Fruit Position[1]	Retention Top 5 Fruit Position[1]	Retention Nodes above white flowers	Retention All first position[2]	Retention on flowers/boll node
Control-Morning	54.19 ± 2.74 e	73.67 ± 2.74 cde	64.60 ± 2.77 e	53.69 ± 2.94 e	67.94 ± 2.30 d	79.93 ± 3.12 a
NI8[3]-BioPlastic-Mor	86.27 ± 2.22 a	96.02 ± 1.24 a	93.00 ± 1.54 a	86.28 ± 2.22 a	88.30 ± 1.41 a	78.21 ± 3.07 a
NI8-Tween80-Mor	83.90 ± 2.41ab	96.34 ± 1.24 a	92.00 ± 1.73 ab	83.91 ± 2.41 ab	90.77 ± 1.28 a	79.68 ± 3.44 a
GHA[4]-BioPlastic-Mor	57.37 ± 2.93 e	75.38 ± 2.45 cde	68.20 ± 2.62 de	56.74 ± 2.99 de	71.76 ± 2.32 de	69.36 ± 3.81 a
GHA-Tween80-Mor	73.24 ± 2.83 bc	85.69 ± 2.33 bc	82.40 ± 2.40 abc	73.24 ± 2.82 bc	78.57 ± 1.86 bcd	68.96 ± 3.20 a
Control-Nig	68.24 ± 3.01 cd	81.00 ± 2.60 cd	75.40 ± 2.89 cd	68.24 ± 3.00 cd	66.39 ± 2.09 d	78.94 ± 3.09 a
NI8-BioPlastic-Nig	81.77 ± 2.36 ab	90.67 ± 1.96 ab	87.60 ± 1.88 ab	81.77 ± 2.36 ab	84.37 ± 1.69 abc	80.28 ± 2.58 a
NI8-Tween80-Nig	85.47 ± 2.12 a	96.00 ± 1.27 a	91.00 ± 1.67 ab	85.47 ± 2.12 a	87.60 ± 1.15 a	76.35 ± 2.99 a
GHA-BioPlastic-Nig	83.32 ± 2.46 a	93.00 ± 1.79 ab	88.60 ± 2.11 ab	82.47 ± 2.57 ab	85.99 ± 1.59 ab	81.91 ± 2.74 a
GHA-Tween80-Nig	62.11 ± 2.61 de	77.33 ± 2.55 cde	70.00 ± 2.58 de	72.11 ± 2.61 bc	74.47 ± 1.95 de	78.54 ± 2.36 a

Note: [1] With undamaged squares; [2] Include all undamaged squares, flowers and bolls; [3] Native Strain; [4] Commercial Strain; Means ± SE followed by the same letter in each column are not significantly different ($p < 0.05$ Tukey test).

4. Discussion

Our results demonstrated that regardless of the time of the day when *B. bassiana* is applied, spores are rapidly deactivated by solar radiation. However, application during nighttime period could maximize spore survival. These results corroborated with previous studies [20] [21] where was demonstrated that spores released during the day tend to be transported higher up in the atmosphere and would die before returning to the ground. The authors suggest that, for short-life fungal conidia of entomopathogenic fungi such as *B. bassiana*, spore dispersal should typically occur during nighttime or early morning to enhance survival rates. Extensive research has been dedicated to improving protection against ultraviolet radiation for entomopathogenic fungi, specifically *B. bassiana* and *Metarhizium anisopliae* var. *acridum* [22]-[27]. Yet, this factor is seen as a significant obstacle to the effective commercialization and acceptance of entomopathogens by farmers for controlling insect pests in field crops [13].

Our field study demonstrated that under direct sunlight and outdoor conditions, the half-life of *B. bassiana* spores sprayed on cotton leaves could range from 1 - 2 days (**Figure 2**). These results are comparable to the findings by Portilla *et al.* [18] in cotton leaves and Daoust and Pereira [28] in wheatgrass and alfalfa but differed from Inglis *et al.* [29] who found that conidia life span sprayed on strawberries leaves last up to 4 days. Thus, regardless of field conditions or crop type, *B. bassiana* experiences irreversible damage after UV exposure [29]. Interestingly, although sunlight reduces the survival of these fungus spores, the combination of the native entomopathogenic NI8 + Tween80 applied at night kept its germination

and virulence ability against TPB adults (>70%) and (> 80%), respectively. However, due to its rapid autooxidation at high temperatures the sporulation and mortality rates decreased significantly the first day after spray, ending with ~ 20% mortality and less than 10% sporulation by the second day after spray, both morning and night applications. Similar results were found by Portilla *et al.* [30] with mortality of TPB no greater than 60% both by direct spray and contact with no significant difference between morning at night but, decreasing significantly less its mortality to < 20% and sporulation to ~5% by the second day after spray regardless of the time of the application. Tobar *et al.* [31] and Jia *et al.* [27] demonstrated that the survival of the conidia was relative to the exposure time under UV and after irradiation the spores can change its nutritional form but keep its germination abilities against *Hypothenemus hampei* Ferrari. (Coleoptera: Curculionidae: Scolytidae) and *Locusta migratoria* L. (Orthoptera: Acrididae), respectively.

On the other hand, the present study indicated that efficacy is not only measured based on the number of propagules landing on the plant leaves, but also the number of propagules that eventually contact the host cuticle. **Figure 1** showed high mortality on TPB adult population, suppressing to less than 1 adult per 10 sweep nets 7 days after spray regardless of the strain and surfactant, with no significant differences between morning and night applications but highly significant differences were found among control. Therefore, it seems that spores on insect cuticles are more sunlight-resistant than those on leaf surfaces. Consistent with previous research [32], our findings show that spores on the upper leaf surface contribute to *B. bassiana*'s limited persistence in the environment, as demonstrated in **Figures 2(A)-(C)**. It's important to mention that Tween80 and Bioplastic are not sunlight protectants; instead, they act as emulsifiers to assist with spore dispersion in water, helping spore solution spread evenly across leaf and insect host surfaces. This function can affect spore survival positively, particularly for spores that make landfall on underside leaves or successfully infect the intended organism. Mwambury *et al.* [33] noted that Tween80 aids spore protection against environmental stresses like UV, freezing, and soil chemistry by coating individually each spore. Yet, as mentioned by Portilla *et al.* [17] both emulsifiers Tween80 and Bioplastic act primarily as dispersing agents and offer only negligible protection against UV degradation. They also noted that the compatibility of Bioplastic does not differ from Tween-80 and could be considered attractive for use with native or commercial strains of *B. bassiana* to control TPB in cotton due to its glue-sticky action when wet and hard when dry. Also, these authors observed that the formulation NI8 + Bioplastic was more effective when applied direct to the insect than applying it to cotton [17]. Accinelly and Abbas [16] explained why application of atoxigenic aspergillus works when applied directly to corn. It's glued to the host and can dissolve through respiration to release spores.

A significant aspect of this research was the assessment of retention indices, including all undamaged squares, flowers and bolls, which were evaluated throughout plant mapping [34]. Our study demonstrated an evident relation between the sea-

son plant mapping (**Tables 1-2**) and the within-season insect scouting (**Figure 1**). Population density demonstrates a significant association with crop injury, as observed in the control groups (morning and night) relative to treated plots. Portilla *et al.* [17] reported that TPB moved into cotton after mustard was mowed, negatively affected fruit survival, especially in plots sprayed with GHA + BioPlastic; the absence of first position fruit was similar to the water control, which differed from our study where water control exhibited the most severe injury, alongside the lowest parameter values and the greatest frequency of missing first positions and the lowest indices of cotton percentage retentions. It is worth noting that some strains of *B. bassiana* are beneficial in promoting plant growth or having the potential to translocate nitrogen [11], which significantly increase root biomass and number of leaves [35]. In our study, this positive behavior should not be discarded if we compare the greater percentage rate of damage observed in plots treated with only water. Hence, *B. bassiana,* both native and commercial strains, might exert a dual effect by controlling insects and enhancing plant growth. Additional research is required before this can be considered as a possibility.

5. Conclusions and Recommendations

Overall, our results indicated that *B. bassiana,* both native and commercial, suppressed TPB adult population in the field regardless of the surfactants and time of applications (**Figure 1**). Plots sprayed with native NI8 plus either Tween-80 or Bioplastic had a better impact in preventing damage from TPB than the commercial GHA applied in the morning or at night (**Tables 1-2**). It was evident that NI8 + Tween80 applied during night had grater rates of mortality and sporulation at 0-d after application when compared with the rest of the treatments (**Figure 2(A)**). However, its viability did not last more than 2 days after sunlight exposure regardless of combination of strain, surfactant, or time of application (**Figure 2(C)**). Finally, neither the native strain nor the commercial strain control population of TPB nymphs (**Figure 1**). These results are in confirmation with other studies [17] [36] who noted that TPB nymphs' population could be located on unreachable places or could be the product of a new generation, suggesting that population were eggs at the time of application. But, differed from Portilla *et al.* [37] who observed greater control of nymph population on cotton plots treated with *B. bassiana* than water control. Portilla *et al.* [37] and Little *et al.* [38] demonstrated that *B. bassiana* alone or in combination with insect growth regulators (IGR) can suppress TPB adults and nymphs' population as effective as synthetic insecticides; however, the performance of *B. bassiana* alone was not enough to prevent cotton damage and affect lint cotton yields. Therefore, the combination of the entomopathogenic fungi *B. bassiana* combined either with Tween80 or Bioplastic could be implemented with other integrated program management (IPM). Additionally, strategic applications will be necessary such early morning, evenings, or shaded areas to avoid exposure to sunlight. Portilla *et al.* [30] noted that while solar radiation quickly inactivates *B. bassiana* spores, which may hinder TPB control, the NI8 strain offers a valuable

option for IPM and can help reduce chemical pesticide use.

Consent for Publication

All authors read and approved the manuscript for publication.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author Contributions

Conceptualization, M.P.; data curation, M.P.; formal analysis, M.P.; investigation, M.P., H.A., and C.A.; methodology, M.P.; H.A., and C.A.; supervision, M.P. And H.A.; validation, M.P.; visualization, M.P.; writing - original draft, M.P.; writing - review & editing, M.P., H.A., and C.A. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors do not have conflicts of interest to declare that are relevant to the content of this article.

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