Research Article

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Efficacy of Betaproteobacteria-based insecticides for managing whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), on cucumber plants

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Abstract: The whitefly, *B. tabaci*, poses a significant threat to cucumber cultivation. While traditional insecticides are commonly used to manage this pest, they frequently raise ecological and health concerns. In contrast, naturally derived biopesticides offer a promising and environmentally friendly alternative for controlling whitefly populations, thereby reducing negative impacts on non-target organisms. This study evaluates the efficacy of two novel Betaproteobacteria-based insecticides: *Burkholderia* spp. strain A396 (Venerate XC) and *Chromobacterium subtsugae* strain PRAA4-1 (Grandevo WDG) against *B. tabaci*. Leaf-dipping bioassays were performed to compare the effectiveness of these biopesticides with spirotetramat (Movento® 240 SC) as a standard control. Both biopesticides significantly reduced egg hatching rates and decreased survival rates in nymphs and adults by 74.5, 94.8, and 76.5%, respectively, indicating concentration-dependent effects. Direct exposure to Venerate and Grandevo exhibited toxicity levels comparable to spirotetramat across all tested concentrations. However, spirotetramat residues were found to be more toxic to adult whiteflies than the other products, while the mortality associated with Venerate residues was relatively low. Both Venerate and spirotetramat produced significant sublethal effects on the duration of nymph development, although these effects were not consistently concentration-dependent; Grandevo did not affect nymph development. These findings suggest that the novel insecticides may effectively manage *B. tabaci* populations in Jordan, warranting further investigation under field conditions.

Keywords: biopesticide, mortality, survival, toxicity, whitefly

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1 Introduction

Agriculture is fundamental to global economic and social development, significantly contributing to biodiversity conservation and the preservation of ecological balance, which are crucial for sustainable resource use for future generations [1]. Contemporary agricultural practices emphasize not only economic advancement but also the social and environmental aspects of agricultural activities [2].

In Jordan, agricultural production has significantly increased over the past four decades, with the plant sector accounting for approximately 45% of national agricultural output. For instance, vegetable production has tripled since 1976, driven by advancements in irrigation technology, the use of plastic greenhouses, and the introduction of high-yield hybrid varieties, alongside an increasing demand for fresh produce [3]. However, agricultural yields face various abiotic and biotic stresses, with the whitefly, *B. tabaci* (Hemiptera: Aleyrodidae), posing a considerable threat to many vegetable and ornamental crops in both field and greenhouse environments [4,5]. This polyphagous pest can infest around 600 host plants [6,7], causing significant damage by feeding on plant sap [8] and injecting toxic saliva, which leads to physiological alterations in plant tissues [9]. Moreover, whitefly infestations result in indirect damage, including the transmission of several viral diseases [10] and the contamination of fresh market crops with honeydew and sooty mold, negatively impacting plant physiology and making crops unsalable [7].

Traditionally, crop protection against whiteflies has depended significantly on conventional insecticides, which have offered substantial advantages to agricultural practices. However, this dependence is increasingly viewed as insufficient due to the ecological and health-related concerns it raises [11,12]. The widespread use of pesticides has resulted in their accumulation within the food chain and the environment, leading to bioaccumulation and biomagnification with unforeseen toxicological consequences [13]. Consequently, there is an increasing focus on investigating environmentally sustainable pest management alternatives.

There are numerous opportunities to harness the chemical properties of plants and other organisms to reduce insect damage and improve agricultural productivity. The effectiveness of various naturally derived biopesticides has garnered international recognition and support within the context of sustainable agriculture [14]. For example, over 200 natural-origin pesticides are registered in the United States, with ongoing development efforts aimed at addressing the growing consumer demand for sustainable food alternatives [15,16].

Recently, biopesticides derived from *Burkholderia subtsugae* strain PRAA4-1 (marketed as Grandevo WDG) and *Chromobacterium* spp. strain A396 (marketed as Venerate™ XC) have been commercialized by Marrone Bio Innovations, located in Davis, CA, USA. These Betaproteobacteria-based biopesticides exhibit significant insecticidal activity against a wide range of arthropod pests in both field and greenhouse environments, attributed to their production of various bioactive metabolites [17,18]. Grandevo WDG primarily acts as a stomach poison, effectively reducing fecundity and deterring feeding behavior [19]. In contrast, Venerate™ XC demonstrates multiple modes of action, including the enzymatic degradation of exoskeletal structures upon contact or ingestion [20].

In Europe, Movento (active ingredient spirotetramat, a tetramic acid derivative) has been developed by Bayer Crop Science AG since 2003 as a systemic and persistent insecticide with ambimobile properties [5,21]. It demonstrates high efficacy against a range of pests, including whiteflies, aphids, scales, mealybugs, psyllids, certain thrips species, and spider mites. This effectiveness is achieved through acute toxicity, which significantly reduces the fecundity and fertility of adult females, in addition to disrupting lipid biosynthesis [21–23].

Research has demonstrated that *Burkholderia* and *Chromobacterium subtsugae* exhibit significant insecticidal effects against various pests, including *B. tabaci*. These biopesticides can induce mortality and sublethal effects on feeding, growth, and reproduction, thereby contributing to pest population management [24,25]. Specifically, *Burkholderia* has been shown to disrupt insect physiology through the production of metabolites that impair vital processes [26]. In comparison, spirotetramat, a synthetic insecticide, also demonstrates high efficacy against *B. tabaci* by affecting lipid biosynthesis, leading to decreased survival and reproduction [27]. While spirotetramat has a strong immediate impact, its residual activity has been noted to surpass that of the biopesticides [28]. However, the combination of *Burkholderia* and *Chromobacterium subtsugae* presents a promising alternative for integrated pest management (IPM), particularly as they may exert less harmful effects on non-target organisms compared to traditional insecticides [29].

This study aims to evaluate the efficacy of newly developed insecticides based on *Chromobacterium subtsugae* and *Burkholderia* against various life stages of *B. tabaci*. This assessment is particularly important due to the limited information available on these biopesticides and the presence of genetically diverse *B. tabaci* strains that exhibit differing responses to insecticides. The adoption of these innovative management strategies has the potential to improve reliability and sustainability in vegetable pest management, especially in contexts where the use of traditional pesticides is restricted or limited. Such approaches may play a significant role in IPM practices and organic farming, addressing economic, environmental, and health concerns associated with conventional insecticide applications. In this study, spirotetramat is utilized as a reference (standard) insecticide.

2 Materials and methods

2.1 Insect colonies and plant culture

Adult *B. tabaci* specimens were collected from infested cucumber plants in a field in Irbid, Jordan, located at approximately 35.85°N latitude and 35.85°E longitude. These specimens were subsequently maintained for several generations on young cucumber plants cultivated in plastic pots (15 cm in diameter) filled with potting soil. The plants were kept in a controlled growth chamber at a temperature of 25 ± 3°C, with a photoperiod of 16 h of light and 8 h of darkness, at the Department of Plant Production, Jordan University of Science and Technology, Irbid, Jordan. The whitefly colony was housed in a cage measuring 60 cm × 60 cm × 100 cm, which was covered with fine mesh on all sides. To ensure colony sustainability, a continuous supply of newly grown greenhouse plants was provided to replace older plants that deteriorated due to the high feeding pressure exerted by the whiteflies.

Cucumber seeds were sown in 50-cavity plastic seedling trays (50 cm × 30 cm × 0.6 cm) filled with commercial potting soil. After several days of growth under greenhouse conditions, seedlings at the primary leaf stage were individually transplanted into 15 cm diameter plastic pots containing the same potting soil. The experimental plants were maintained under greenhouse conditions at 25 ± 3°C with a photoperiod of 16 h of light and 8 h of darkness. They were fertilized weekly with a 20-9-20 water-soluble fertilizer (N:P:K) and irrigated as needed until they were utilized in the experiments.

2.2 Insecticides

This study employed two commercially formulated microbe-based insecticides: Grandevo WDG (30% *Chromobacterium subtsugae* strain PRAA4-1 and spent fermentation media) and Venerate XC (94.46% heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media), both obtained from Marrone Bio Innovations Inc., Davis, California, USA. Well-established Movento 240 SC (22.4% spirotetramat: cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4-yl-ethyl carbonate) was used as standard due to its effectiveness against a wide range of pests, its unique mode of action, and its systemic properties. The efficacy of these products was evaluated across three concentrations for their effects on different developmental stages of *B. tabaci* feeding on cucumber plants under laboratory conditions. The concentrations tested were 0.4, 1.2, and 2.4 g/L for Grandevo; 0.5, 1.0, and 2.0 mL/L for Venerate; and 0.02, 0.04, and 0.08 mL/L for Movento, representing the lower, middle, and upper limits of the recommended field rates for each product.

2.3 Effects of insecticides on whitefly

To assess the impact of the novel insecticides on the survival of adult whiteflies, as well as young and old nymphs and egg hatch rates, leaf disc bioassays were conducted using plastic Petri dishes (5.5 cm diameter) equipped with gauze-covered ventilation holes. The efficacy of direct application was evaluated using cucumber foliage, which was cut into discs (5 cm diameter) with a sharp-edged plastic tube. For each treatment, six plants at the third true-leaf stage were utilized.

2.4 Ovicidal effect of biopesticides

To evaluate egg hatch rates, approximately 30 adult whiteflies were confined to the undersurface of a fully expanded second leaf on each plant using a clip-on cage made from a plastic Petri dish (6 cm diameter). Adults were anesthetized with CO_2 for a few seconds before being transferred with a fine, moist camel-hair brush. These cages were constructed from a plastic Petri dish (6 cm × 1.5 cm) with two side holes covered with fine mesh for ventilation. After 12 h, the cages were removed, the infested leaves were excised, and the ovipositing sites were cut into discs (5 cm diameter) using a sharp-edged plastic tube. The number of eggs laid on each disc was counted using a hand lens at 20× magnification and recorded.

Subsequently, six leaf discs were immersed in each dilution of the test products for 10 s with gentle agitation. The discs were then placed on paper towels, abaxial side up, to air dry before being positioned with the abaxial surface facing upward on a 4 mm layer of 1% agar in a plastic Petri dish (5.5 cm diameter) that had been prepared 1 day prior to testing. The Petri dishes were maintained in an inverted position at 23 ± 3°C under a 16:8 (L) photoperiod. Six replicates were conducted for each treatment, and the proportion of egg hatching was recorded at 24-h intervals over a period of 2 weeks.

2.5 Effect of biopesticides on young and old nymphs

Similar procedures were employed to assess the direct effects of the test insecticides on young nymphs (3 days old) and older nymphs (10 days old). Adult whiteflies were introduced to the plants as described previously for egg hatch assessment. After a 12-h oviposition period, the infested leaves were left intact on the plants for 7 or 14 days to facilitate egg hatching and nymph development. Following these periods, leaves containing the respective immature stages were excised, and the infested areas were cut into 5 cm diameter discs for nymph counting prior to insecticide exposure.

The discs were treated with the appropriate dilution of the insecticides and maintained on agar as previously described. Live and dead nymphs were counted and recorded at 24-h intervals until adult emergence, and the total developmental time for treated young nymphs was documented. Each treatment was replicated six times.

2.6 Effect of biopesticides on survival of adults

The direct and residual effects of the formulated insecticides on adult whiteflies were evaluated using untreated cucumber foliage cut into discs, as previously described. For the assessment of direct application efficacy, leaf discs from each treatment were placed in plastic Petri dishes lined with a layer of agar, abaxial side up. Fifty adult whiteflies were transferred onto each leaf disc using a fine camel-hair brush. The leaf discs containing the insects were then treated with each insecticide at three different concentrations using 500 mL plastic spray bottles until thoroughly saturated. The Petri dishes were immediately inverted to remove excess spray solution and maintained in this inverted position under the aforementioned conditions.

Similar procedures were employed to quantify the residual effects of Grandevo, Venerate, and Movento on adult whiteflies, utilizing three concentrations for each insecticide. In this assessment, six leaf discs (5 cm diameter) were first dipped in each test product dilution for 10 s and then air-dried before being placed in agar in a plastic Petri dish. The leaf discs were subsequently infested with whiteflies at a density of 50 adults per dish, with each treatment replicated six times. Mortality was monitored in both tests at 24-h intervals until no further increase in mortality was observed. Control treatments for all bioassay tests consisted of leaf discs treated with tap water.

2.7 Statistical analysis

Mortality data were adjusted for control mortality using Abbott's formula [30]. Data were analyzed using analysis of variance (ANOVA) with SAS software version 9.2 [31], and mean values were compared using the least significant differences test at $P \leq 0.05$.

3 Results

3.1 Effect of novel insecticides on egg mortality

The results indicate that different life stages of *B. tabaci* exhibit varying susceptibility to the tested Betaproteobacteria-based insecticides, influenced by the specific product, concentration, and target life stage. In leaf-dip bioassays, these novel insecticides significantly reduced eclosion rates in a concentration-dependent manner ($F = 68.90$, df = 8, 45, $P < 0.0001$) (Table 1). Among the products evaluated, Grandevo demonstrated superior efficacy against eggs across all concentrations, achieving up to 74.5% egg mortality at the highest concentration. Venerate exhibited moderate ovicidal activity, resulting in egg hatching rate reductions of 40.2–63.3%, which correlated significantly with increasing concentration. In contrast, the acute contact toxicity of spirotetramat (Movento) to *B. tabaci* eggs was minimal, with only approximately 42.9% mortality observed at the highest labelled rate (0.8 mL/L) (Table 1).

3.2 Effect of novel insecticides on nymph mortality

A similar pattern in the efficacy of the novel insecticides against whitefly nymphs was observed, though with varying magnitudes of effect (Table 1). The insecticides exhibited significant toxicity toward both young (3 days old) and older (14 days old) nymphs in a concentration-dependent manner ($F = 46.44$, df = 8, 45, $P < 0.0001$ for young nymphs; $F = 61.38$, df = 8, 45, $P < 0.0001$ for older nymphs). All products demonstrated comparable efficacy against young nymphs, resulting in survival reductions of 47.7–72.2% when applied at the lowest and highest labelled rates, respectively. However, no significant differences in efficacy were observed between the highest and lowest concentrations for young nymphs. Grandevo at an intermediate concentration induced a marked decrease in nymph survival compared to the corresponding concentrations of other products (Table 1).

Table 1: Mean percent mortality of eggs and young and late instar nymphs of *B.* following the application of different concentrations of *Chromobacterium subtsugae* (Grandevo®), *Burkholderia* (Venerate®), and Spirotetramat (Movento®) to plant foliage targeting specific life stages of the whitefly

Insecticide	Concentration	Eggs	Young instar nymphs	Late instar nymphs
Chromobacterium subtsugae (Grandevo®)	4 mg per L	57.4 ± 3.03bc	40.3 ± 2.24c	31.4 ± 1.41ed
	12 mg per L	66.8 ± 3.36ab	44.6 ± 0.65c	65.9 ± 4.71b
	24 mg per L	74.5 ± 2.47a	71.2 ± 1.53a	94.8 ± 3.28a
Burkholderia spp. (Venerate®)	5 mL per L	40.2 ± 2.03e	42.1 ± 1.58c	19.9 ± 3.11e
	10 mL per L	52.0 ± 0.43cd	56.2 ± 2.09b	43.5 ± 2.84cd
	20 mL per L	63.3 ± 1.33b	72.7 ± 3.18a	89.7 ± 4.95a
Spirotetramat (Movento®)	0.2 mL per L	20.9 ± 1.09f	47.7 ± 1.52c	28.6 ± 3.15de
	0.4 mL per L	25.9 ± 1.80f	56.5 ± 1.02b	52.8 ± 2.21bc
	0.8 mL per L	42.9 ± 2.61de	65.6 ± 1.63a	89.3 ± 5.49a

Mean values ± SE within columns followed by the same letter are not significantly different ($P > 0.05$).

For late instar nymphs, the response to the application of insecticides mirrored that of young nymphs but with differing degrees of effectiveness (Table 1). The acute contact toxicity of the novel insecticides against older nymphs, when applied at the highest labelled rates, was notably pronounced, achieving up to 94.8% mortality depending on the specific insecticide. In contrast, older nymphs exhibited less vulnerability to the lowest labelled rates, with mortality not exceeding 31.4%, compared to the 47.7% mortality observed in young nymphs exposed to the corresponding concentrations.

3.3 Effect of novel insecticides on adult mortality

In terms of direct toxicity, the novel insecticides elicited comparable mortality levels in adult whiteflies when applied directly at relevant concentrations (Table 2). A significant increase in adult mortality was observed as a function of concentration ($F = 43.32$, df = 8, 45, $P < 0.0001$). All test insecticides applied at the lowest recommended field rates resulted in a maximum of 15.2% mortality. Increasing the concentration to the highest recommended field dosage produced a substantial suppressive effect, with maximum adult mortality reaching 76.5%.

The residual toxicity of the insecticides on adult whiteflies feeding on leaves previously treated with an aqueous solution is presented in Table 2. The adults responded to the residual activity similar to that observed for direct sprays, though with differing magnitudes. Exposure to treated leaves resulted in a significant reduction in adult survival in a concentration-dependent manner ($F = 76.63$, df = 8, 45, $P < 0.0001$). Generally, the residual effect of Venerate was less effective in suppressing pest populations compared to the other insecticides. Even at the highest recommended field dosage, Venerate achieved a maximum mortality of only 21%, indicating lower susceptibility of adult whiteflies to its residual activity. In contrast, Grandevo effectively reduced adult survival by up to 67.4% when applied to foliage at the highest labelled rate. However, spirotetramat demonstrated the highest residual efficacy, suppressing adult populations by up to 93.7% compared to the corresponding concentrations of other insecticides.

Overall, the residual activities of Grandevo and spirotetramat demonstrated greater effectiveness against adult whiteflies than their acute contact toxicity. In contrast, Venerate exhibited a reduced capacity to decrease adult survival compared to direct application.

3.4 Effect of novel insecticides on development and adult emergence

The sublethal effects of the insecticides on the development time of 3-day-old nymphs that survived insecticide application are presented in Table 3. Grandevo did not significantly impact the development period of nymphs at any concentration compared to the control group. In contrast, both Venerate and spirotetramat resulted in significant increases in development time ($F = 16.01$, df = 9, 40, $P < 0.0001$). Specifically, spirotetramat extended the development period by 4.1–4.9 days relative to the control, while Venerate prolonged nymph development by 2.1–3.1 days. However, the effects of spirotetramat and Venerate were not significantly correlated with the concentrations of the insecticides.

Table 2: Mean percent mortality of adult whiteflies following the application of *Chromobacterium subtsugae* (Grandevo®), *Burkholderia* (Venerate®), and Spirotetramat (Movento®) at different concentrations, with direct application to adults on infested host foliage (direct effect) and residual application to plant foliage for later feeding

Insecticide	Concentration	Direct effect	Residual effect
Chromobacterium subtsugae (Grandevo®)	4 mg per L	13.1 ± 2.86d	42.0 ± 4.19c
	12 mg per L	47.2 ± 4.64b	50.6 ± 3.09c
	24 mg per L	74.1 ± 3.73a	67.4 ± 3.90b
Burkholderia spp. (Venerate®)	5 mL per L	15.2 ± 2.92cd	8.1 ± 2.00ed
	10 mL per L	33.4 ± 1.65 bc	14.4 ± 2.29ed
	20 mL per L	76.5 ± 4.13a	20.2 ± 2.16d
Spirotetramat (Movento®)	0.2 mL per L	11.4 ± 2.10d	49.5 ± 3.91c
	0.4 mL per L	32.3 ± 3.21bc	76.9 ± 4.03b
	0.8 mL per L	67.7 ± 7.59a	93.7 ± 2.85a

Mean values ± SE within columns followed by the same letter are not significantly different ($P > 0.05$).

As indicated in Table 3, the proportion of adult emergence was significantly decreased following the exposure of young nymphs to insecticides, with both product type and concentration exerting an influence (F = 15.32, df = 9, 40, P < 0.0001). Adult emergence rates were measured at 51.8% and 30.4% for the lowest and highest recommended field doses, respectively, compared to 86.7% in the control.

4 Discussion

This study assessed the efficacy of two novel Betaproteobacteria-based insecticides on various life stages of *Bemisia tabaci*, comparing their effects to the widely used standard, spirotetramat (Movento). All developmental stages of whiteflies responded significantly to treatment with the novel insecticides, with effectiveness varying based on the specific product, concentration, and targeted life stage. Increased concentration was associated with reductions in egg eclosion as well as the survival rates of juveniles and adults. *Chromobacterium subtsugae* (Grandevo) exhibited high efficacy, achieving up to 74.5% mortality of whitefly eggs in leaf-dip bioassays, followed by *Burkholderia* (Venerate), and spirotetramat.

The Betaproteobacteria-based products demonstrated similar efficacy against both young and late instar nymphs, reflecting the outcomes observed with relevant concentrations of spirotetramat. Young nymphs experienced mortality rates of up to 72.7% when the insecticides were applied at the highest labelled rate. Importantly, older nymphs displayed increased susceptibility to direct applications of these products, resulting in survival reductions of up to 94.8% at the highest concentration.

The direct toxicities of the Betaproteobacteria-based insecticides were comparable to the insecticidal effects of spirotetramat across all tested concentrations. However, the residual activity of spirotetramat on adult whiteflies was more effective than its direct activity, achieving lower population levels than Venerate and Grandevo, with a maximum adult mortality of 93.7%. Grandevo exhibited moderate residual toxicity when adults came in contact with the treated leaves, while Venerate's residual effect resulted in a maximum adult mortality of only 20.2%, even at high concentrations.

In a previous research by Shannag and Capinera [32], spirotetramat was found to be more effective in suppressing green peach aphids (*Myzus persicae*) and Madeira mealybugs (*Phenacoccus madeirensis*) under laboratory conditions, although Venerate achieved competitive mortality levels similar to those of spirotetramat. Notably, young nymphs of Madeira mealybugs showed lower susceptibility to spirotetramat residues compared to green peach aphids. In contrast, the present study indicates that the novel insecticides exhibit comparable efficacy against whiteflies, suggesting that the response may be species-specific.

As noted by Nauen et al. [22], spirotetramat effectively eliminated nymphs of various aphid species in leaf-dip bioassays, achieving total mortality through ingestion by disrupting lipid biosynthesis, which subsequently affected growth and reproduction [25]. However, its contact activity was observed to reduce aphid populations by less than 50% even at high concentrations, consistent with our findings. The efficacy of spirotetramat is attributed to its systemic and translaminar properties, which facilitate substantial residual concentrations in plants that effectively target sap-sucking insects, although its contact efficacy is

Table 3: Mean developmental time of young instar nymphs of *B. tabaci* treated directly with insecticides and the proportion of adults emerging from young instar nymphs following direct application to nymphs on infested host foliage as affected by different concentrations of *Chromobacterium subtsugae* (Grandevo®), *Burkholderia* (Venerate®), and Spirotetramat (Movento®)

Insecticide	Concentration	Development time of young larvae	% adult emergence
Control	0	11.5 ± 0.23ed	86.7 ± 1.54a
Chromobacterium subtsugae (Grandevo®)	4 mg per L	12.3 ± 0.36d	51.8 ± 1.60b
	12 mg per L	11.1 ± 0.16e	48.0 ± 0.88bc
	24 mg per L	10.6 ± 0.21e	24.8 ± 1.75f
Burkholderia spp. (Venerate®)	5 mL per L	13.6 ± 0.28c	49.4 ± 2.66b
	10 mL per L	14.6 ± 0.24bc	38.9 ± 2.44ced
	20 mL per L	13.8 ± 0.17c	23.8 ± 3.07f
Spirotetramat (Movento®)	0.2 mL per L	16.4 ± 0.23a	45.3 ± 0.58bcd
	0.4 mL per L	15.7 ± 0.38ab	37.2 ± 0.90ed
	0.8 mL per L	15.6 ± 0.19ab	30.4 ± 1.73ef

Mean values ± SE within columns followed by the same letter are not significantly different (P > 0.05).

somewhat limited [9]. These characteristics of spirotetramat may be beneficial in IPM strategies [27,29].

Betaproteobacteria-based products have been reported to induce mortality in insects from various orders, including Lepidoptera, Hemiptera, Thysanoptera, and Coleoptera, as well as in mites. These products also have significant sublethal effects on feeding activity, fecundity, and oviposition [16,24,26,29,33].

Recent studies indicate that *Burkholderia* can effectively reduce populations of various insect pests, including aphids and whiteflies, by inducing both mortality and sublethal effects. These bacteria produce metabolites that disrupt insect physiology, leading to impaired feeding, growth, and reproduction [24–26]. Both *Burkholderia* and *Chromobacterium subtsugae* (Grandevo) negatively affect the reproduction of several insect pests, resulting in decreased egg-laying rates and changes in oviposition behavior, likely due to disruptions in hormonal balances or essential physiological processes [24,26,27]. Although *Chromobacterium subtsugae* may not cause immediate mortality, its effects on reproduction contribute to the overall reduction in pest populations, making it a valuable component of IPM strategies [26–29]. However, other research works have suggested that the reproduction of aphids exposed to *Burkholderia* was only minimally affected, while *Chromobacterium subtsugae* (Grandevo) had no impact on their reproduction [27].

In the present study, significant sublethal effects of *Burkholderia* and spirotetramat on the developmental period of nymphs were observed, although these effects were not concentration-dependent; *Chromobacterium subtsugae* did not affect development. Similar findings were noted when aphids were exposed to *Burkholderia* and spirotetramat treatments, which influenced the duration of time required for aphids to reach maturity, likely due to interference with hormonal regulation and metabolic processes [24,25,27].

Furthermore, the proportion of nymphs reaching adulthood was significantly decreased in plants treated with these novel insecticides in a dose-dependent manner. This inability of nymphs to mature may be attributed to the toxic residues of the insecticides present on plant tissues.

In agreement with our findings, the direct application of both Betaproteobacteria-based products (Venerate) and spirotetramat (Movento) has been demonstrated to negatively impact the emergence of adults from treated nymphs. This effect is likely attributed to their influence on feeding and growth, resulting in reduced survival rates to the adult stage, which indicates their potential effectiveness in decreasing future populations [24,25,28].

Overall, the newly formulated biopesticides based on *Burkholderia* and *C. subtsugae* exhibited efficacy against *B. tabaci* comparable to that of spirotetramat and may serve as viable alternatives to synthetic insecticides for managing the *B. tabaci* strains found in the region, although responses may differ among various insect species. Further research is needed to evaluate their effectiveness against whiteflies under field conditions and to assess their impact on beneficial natural enemies.

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