

Comparative Study Between ATOMES Novels, NOVOSECT SC21[®], ATO BED BUG[®] and NEO-BOOST[®] as a Bio-organic Solution in Managing Tomato Open Field Plantation in Hrajel Area in Lebanon

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Abstract

The increasing use of chemical insecticides has adversely effected the environment and increased insect resistance. Biopesticides have been noticed the potential to be an excellent alternative to chemicals to reduce the negative impacts to human health and the environment. Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop worldwide due to its nutritional importance. The effect of NOVOSECT SC21[®] (0.5 L/200 L), ATO BED BUGS[®] (1 L/200 L) and NEO-BOOST[®] (1 kg/200 L) against *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* was studied. A complete randomized block design (CRBD) was used with three replications, three treatments and one control in Hrajel area in Lebanon in the summer of 2020. We evaluated the level of infestation and larval mortality level of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* infection 24, 48 and 72 hr after the application. Results showed that NOVOSECT SC21[®] (Mix of metabolites of the Bacillus F.D. 777) was the most efficient in decreasing the infestation and inducing larval mortality level of *T. absoluta* and *L. trifolii* and the fungal infection induced by *A. solani* with significant difference with time, followed respectively by ATO BED BUGS[®] and finally NEO-BOOST[®].

Keywords: *Alternaria solani*, ATO BED BUGS[®], *Liriomyza trifolii*, NOVOSECT SC21[®], NEO-BOOST[®], Tomato, *Tuta absoluta*

1. Introduction

Vegetables are one of the most important crops in agriculture and tomato (*Lycopersicon esculentum*) is considered as one of the most cultivated and consumed vegetable crops in Lebanon, with a production of 300,157 tons in 2018 and a cultivated area of 3700 ha according to IDAL (Investment Development Authority in Lebanon) (IDAL, 2020). Over the years, the increased demand for vegetables has resulted in the increase in land cultivation and the adoption of intensive farming in greenhouses. However, the increasing pressure on the soil and the excessive use of chemical fertilizers weakens the soil and disturbs its microbiological balance leading with time to weaken seedlings, which makes them susceptible to diseases and pests.

The intensive use of chemical insecticides have serious drawbacks, including reduced profits from high insecticide costs, destruction of insect pest's natural enemy populations, the build up of insecticide residues on tomato fruit. In addition, the high use of chemical insecticides can adversely affect the environment and increase the insecticide resistance to many insects, including *Tuta absoluta* and *Liriomyza trifolii*, which are among the most important tomato pests in Mount Lebanon, causing severe problems to tomato crops and significant losses in production that could reach 100% if not controlled (Devine et al., 2007; Giorgini et al., 2018; MOA, 2016). The need to adopt new technologies and good agricultural practices should be taken into consideration in order to lower the production costs, lower the insecticide residues in the product, protecting the environment, and obtaining effective insect control (IDAL, 2020). Natural products are an excellent alternative to synthetic pesticides as they help reducing their negative impacts on human health, while protecting the environment and

beneficial pest population. Natural products (biopesticides) are safe and eco-friendly and more compatible with the environmental components than synthetic pesticides (Isman, 2000; Isman & Machial, 2006). Biopesticides include substances such as plant extracts, hormones, pheromones, entomophagous control, plant derived pesticide, etc. (Koul, 2008). Therefore, biopesticides have the potential to replace synthetic pesticides as they have generally short persistency on plants and high selectivity when used as extracts (Bakkali et al., 2008). The purpose of our study was to evaluate the bioactivity of NOVOSECT SC21[®] (Mix of metabolites of the *Bacillus* F.D. 777), ATO BED BUGS[®] and NEO-BOOST[®] (Powdered Peracetic Acid & Bio Silicate) on the infestation and mortality level of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* infection on tomatoes in an open field in Hrajel Lebanon.

2. Material and Methods

2.1 Experimental Site

The experiment was carried out during the 2019-2020 cropping season from June to September at a private tomato field in Hrajel, Mount Lebanon (33°01'41"N; 35°79'49"E), 1400 m above sea level. Water was available from two sources: artesian well and a lake which accumulates spring water. The irrigation system was a drip system.

2.2 Plant Source and Supply

Tomato seeds, var. Maysa (Antagro s.a.l.), with a germination rate 92-96% and a 99.9% purity were used in the research. This variety has a dense to medium foliage and is resistant to tomato spotted wilt virus (TSWV) caused by thrips. The seeds were planted in the nursery of the Faculty of Agriculture (April 27, 2020), and the seedlings were transplanted into the field in the mid of June (June 15, 2020) having a height between 12 and 15 cm. Two hundred and fifty-two tomato plants were evaluated for the efficiency of the biopesticides ATO BED BUGS[®], NOVOSECT SC21[®], and NEO-BOOST[®] (Powdered Peracetic Acid & Bio Silicate) in controlling two major pests encountered in Mount Lebanon: *Tuta absoluta* and *Liriomyza trifolii*, and the fungal disease *Alternaria solani*. In the field, the experimental design was randomized complete block design RCBD) with three replications, three treatments and the untreated control. The biopesticide treatments include (A) ATO BED BUGS[®] 1 L/200 L, (B) bacteria NOVOSECT SC21[®] 0.5 L/200 L, and (D) NEO-BOOST[®] (Powdered Peracetic Acid & Bio Silicate) 1 kg/200 L. The fourth treatment was the untreated control (C). The products used originated from Atomes F.D. Inc., Canada. Two hundred fifty-two tomato plants were planted in 3 blocks. Each block of 5.8 m length, was consisted of 12 plots distributed into two rows. Each plot contained 7 plants distanced 0.5 m. The distance between rows and between blocks was equal to 1 m. There were 2 m unplanted from each side of the field to prevent the existence of any infected plant or contamination from the neighboring area (Figure 1).



Figure1. Experimental field (Hrajel, 2020)

2.4 Field Trials

The experiment included two major field trials. The first set of field trials was conducted to assess the abundance and diversity of two pests: *Tuta absoluta*, *Liriomyza trifolii* and fungal presence: *Alternaria solani*. The second

set of field trials was conducted in order to evaluate the biopesticides effects on larval toxicity and fungal infestation level.

2.5 Collected Data

The pattern of infestation and resultant damage varied not only from species to species but within species, depending on life cycle stage: egg, larvae, pupa and adult. Signs of insect infestation were:

- ✓ Live insects mostly found inside and on plant parts, within leaves;
- ✓ Insect remains including whole carcasses body parts and cast stains;
- ✓ Frass, droppings and tunnels.

Direct observations in the field have advantages can help identify the pest, possible discovery of predators or any other predator previously not believed to be a predator, and the number of insects per unit of area and time. The number of individuals found and the population abundance were collected and compared.

2.5.1 Effect of Biopesticides on the Insect Population Infestation and Fungal Infection

One day prior to the first pulverizations of the ATO BED BUG[®], NOVOSECT SC21[®], NEO-BOOST[®], and the control), a first lecture was done in order to indicate *T. absoluta*, *L. trifolii* and *A. solani* symptoms on the whole plant (Figures 2-4). Two plants were chosen from each plot. The first treatment was applied on August 24, 2020, using a knapsack sprayer with a capacity of 20 L. A second, third and a fourth observation were completed after 24, 48, and 72 hr. The purpose of the observations was to determine if the level of infestation by these pests increased, what new symptoms appeared, the number of living larvae, the amount of frass, etc. In the case of *A. solani*, the level of infection was measured by observing the emergence of new symptoms (dark concentric rings) on the whole plant other than that marked before in the previous observation.



Figure 2. Symptoms of *Tuta absoluta* infestation (live larvae, frass) (Hrajel, 2020)



Figure 3. Symptoms of *Liriomyza trifolii* infestation (live larvae, frass) (Hrajel, 2020)



Figure 4. Symptoms of *Alternaria solani* infection (Hrajel, 2020)

2.5.2 Effect of Biopesticides on the Larval Mortality of *Tuta absoluta* and *Liriomyza trifolii*

During the second pulverization on the 13 September 2020, three plants per plot were chosen, and from each plant, three leaves presenting *Tuta* and/or *Liriomyza* symptoms were indicated one day before the treatment. 24, 48 and 72 hr after the pulverization, the symptoms were observed to evaluate the mortality of those two insects (Figure 5). The mortality of the *T. absoluta* larva was identified through the observation of the larvae mine. If the larvae color changes pale from green color, then from brown or black. This means that the larvae were dead. The dead larvae and insects became extremely withered because of dehydration (Kaoud, 2014). The mortality of *Liriomyza trifolii* was identified by observing the color and the size of the larvae tunnels on the leaf. If the tunnels remain small and their color changed from green to brown, this was an indication that the larvae were dead. The mortality level of *Tuta absoluta* and *Liriomyza trifolii* larvae was calculated using the Abbot's formula $[(Ca - Ta)/Ca] \times 100$, where Ca represents the number of live control larvae after treatment and Ta the number of live test larvae after the treatment.



Figure 5. Different symptoms of *Tuta absoluta* and *Liriomyza trifolii* on the leaf and the fruit (Hrajel, 2020)

2.6 Statistical Analysis

The statistical analysis was computed using IBM SPSS statistics 16.0, for the Anova analysis, Duncan test and T-test. The design of the experiment was based on RCBD design (Randomly Complete Block Design).

3. Results

3.1 Effect of Biopesticides on *Alternaria solani* Infection

The results obtained showed that there is no significant difference between the level of infection between 24 and 48 hr ($F = 9.761$, $P = 0.794 > 0.05$). Besides, using the paired sample statistics between the level of infestation of *A. solani* at 48 and 72 hr after the treatment shows that the means are 1.041 ± 1.96 and 0.90 ± 0.22 , respectively at 48 and 72 hr, with a very strong correlation between these times (correlation = 0.906) which indicates that there is no significant difference between the level of infection at 48 and 72 hr (Table 1).

Since there is no significant difference between the infection levels of *A. solani* among time, we will be based later on the results obtained at 24 hr after the treatment to indicate the efficiency of each treatment.

In addition, ANOVA test indicates that there is a significant difference among the 4 different treatments at 24 hr.

Table 1. Average infestation level of *Alternaria solani*

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	2.22	0.77	0.833	0.5	1.08075	0.669
NOVOSECT SC21 [®]	2.167	0.77	0.611	0.611	1.03975	0.654
NEO-BOOST	2.389	0.611	0.666	0.389	1.01375	0.80
Control	2.5	1.888	2.05	2.11	2.137	0.224

Table 1 shows that the three products used had approximately similar effects (ATO BED BUG[®]: 1.08±0.66; NOVOSECT SC21[®]: 1.03±0.65 and NEO-BOOST[®]: 1.01±0.8) on reducing the emergence of new *Alternaria* symptoms on the whole plant, similar results were obtained by the Duncan test.

3.2 Effect of Biopesticides on *Tuta absoluta* Infestation

The results obtained using T-test between 24 and 48 hr, indicate a reduction in *Tuta absoluta* level of infestation/plant: the mean after 24 hours is 1.36±0.49, while the mean at 48 hr is 0.91±0.22. The paired samples correlation indicates that there is a moderate correlation between 24 and 48 hr ($r = 0.547 < 0.8$), which means that there is a moderate relationship between the reduction of *Tuta absoluta* level of infestation at 24 and 48 hr with a significant difference ($P = 0.037 < 0.05$). The decrease of the level of infestation by *Tuta absoluta* is greater at 48 hr than its decrease at 24 hr after the treatment.

In addition, there is no significant difference between the level of infestation by *Tuta absoluta* at 48 and 72 hr after the treatment, with a mean of infestation equal to 0.91±0.22 and 1.08±0.23, respectively, and a very strong correlation and statistical relation between these two times ($r = 0.880$) (Table2).

ANOVA test showed that there is a highly significant difference between the efficiency of the tested groups at 48 hr and 72 hr after the treatment, $F = 28.68$, $P = 0.000$; $F = 42.50$, $P = 0.000$ respectively.

Table 2. Average infestation level of *Tuta absoluta*

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	3.389	1.5	0.666	0.999	1.6385	1.053
NOVOSECT SC21 [®]	3.278	0.944	0.277	0.055	1.1385	1.277
NEO-BOOST	3.278	0.778	0.611	1.055	1.4305	1.078
Control	2.555	1.777	2.111	2.222	2.16625	0.277

Table 2 shows that NOVOSECT SC21[®] was the best in controlling the emergence of new *Tuta absoluta* symptoms on the whole plant at 72 hours after the treatment (1.138±1.27) followed by ATO BED BUG[®] and NEO-BOOST[®] respectively (1.63±1.05; 1.43±1.07). The Duncan test showed similar results.

3.3 Effect of Biopesticides on the Larval Mortality of *Tuta absoluta*

The T-test between 24 and 48 hr after the treatment shows that the mean of the level of *Tuta absoluta* mortality/leaf are 0.71±0.15 and 0.7±0.22 respectively, with a very strong correlation value (0.949), and no significant difference (Table 3).

The ANOVA test showed that there is a highly significant difference between groups among time: at 24 hr $F = 30.59$, $P = 0.000$; at 48 hr $F = 41.1$, $P = 0.000$; at 72 hr $F = 563.35$, $P = 0.000$). NOVOSECT SC21[®] showed to be the most effective with a mortality level reaching 100% at 72 hr after the treatment. While ATO BED BUG[®] has been more effective than NEO-BOOST[®] among time. Similar results were also obtained using the Duncan test.

Table 3. Mortality percentage of *Tuta absoluta* larvae after the treatment

	24 hr	48 hr	72 hr
ATO BED BUG	12.75%	82.77%	79.36%
NS SC21	83.11%	92.40%	100%
NEO-BOOST	24.08%	79.10%	87.80%

3.4 Effect of Biopesticides on *Liriomyza trifolii* Infestation

The T-test between the level of infestation by *Liriomyza trifolii* after 24 and 48 hr of the treatment, showed that the mean of the level of infestation by *Liriomyza*/plant is equal to 0.65 ± 0.065 , and 0.93 ± 0.13 at 24 and 48 hr after the treatment, respectively. The correlation between the level of infestation/plant at 24 and 48 hr after the treatment is weak ($0.369 < 0.8$), which mean that there is a significant difference between the level of infestation by *Liriomyza trifolii* after 24 and 48 hr from the treatment. While the T-test between the level of infestation of *Liriomyza trifolii* 48 and 72 hr after the treatment is respectively 0.93 ± 0.132 and 0.58 ± 0.11 , with a highly significant difference between the level of infestation by *Liriomyza trifolii* after 48 and 72 hr (Table 4).

The ANOVA test showed that there is no significant difference among groups at 24 and 48 hours after the infestation: at 24 hr $F = 1.149$, $P = 0.387$; while at 48 hr $F = 3.479$, $P = 0.07$.

Table 4. Average infestation level of *Liriomyza trifolii* after the treatment

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	2	0.583	0.722	1.167	1.118	0.553
NOVOSECT SC21 [®]	1.666	0.5	0.778	0.333	0.81925	0.514
NEO-BOOST	1.833	0.791	0.722	0.5	0.9615	0.514
Control	1.499	0.75	1.5	1.111	1.215	0.311

Table 4 shows that the products used had approximately the same effect on controlling the emergence of new *Liriomyza trifolii* symptoms on the whole plant (ATO BED BUG[®] 1.118 ± 0.55 ; NOVOSECT SC21[®] 0.81 ± 0.51 ; NEO-BOOST[®] 0.96 ± 0.51). Similar results were obtained using the Duncan test.

3.5 Effect of Biopesticides on *Liriomyza trifolii* Larval Mortality

The T-test between 24 and 48 hr after the treatment showed that the mean of *Liriomyza trifolii* dead larvae/leaf has decreased 0.84 ± 0.14 and 0.71 ± 0.23 respectively with a high correlation value (0.889) indicating a strong relation between the level of mortality after 24 and 48 hr from the treatment, with no significant difference among time ($0.32 > 0.05$) (Table 5).

ANOVA test showed that there is a highly significant difference between groups at 24 and 48 hr after the treatment: at 24 hr $F = 1.149 \pm 0.387$; at 48 hr $F = 3.479 \pm 0.070$.

At 48 hr after the treatment, NOVOSECT SC21[®] and ATO BED BUG[®] induced the highest mortality, 90.83% and 88.97%, respectively (Table 5). The same results were observed at 72 hr, where the mortality for NOVOSECT SC21[®], ATO BED BUG[®], and NEO-BOOST, were 98.88%, 98.60%, and 88.68%, respectively. Similar results were observed using the Duncan test.

Table 5. Mortality percentage of *Liriomyza trifolii* larvae after the treatment

	24 hr	48 hr	72 hr
Ato BedBug	78.35%	88.97%	98.60%
NOVOSECT SC21 [®]	73.59%	90.83%	98.88%
NEO-BOOST	32.01%	76.10%	88.68%

4. Discussion

Biopesticides such as clove oil (eugenol), BT F.D. 777 are excellent alternatives to synthetic insecticides as a means to reduce residues and protect the environment (Isman & Machal, 2006). The objective of our research was to evaluate the effect of ATO BED BUG[®], NOVOSECT SC21[®] and NEO-BOOST[®] on the level of infestation and mortality of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani*.

4.1 Effect of Biopesticides on the Level of *Tuta absoluta* Infestation

The results showed that the effect of the treatments was completely opposite from the control: the resistance level of *Tuta absoluta* population decreased with time showing the best results of efficiency at 48 hr after the treatment. At 24 hr, biopesticides and the control showed same effect, while at 48 hr after the treatment, the three biopesticides (ATO BED BUG[®], NOVOSECT SC21[®] and NEO-BOOST) had the same opposite effect as the control. While at 72 hr after the treatment, NOVOSECT SC21[®] was the most efficient on reducing the level of

infestation by *Tuta absoluta*, followed ATO BED BUG[®] and NEO-BOOST[®]. Through time, ATO BED BUG[®] was the most efficient in reducing the infestation level and its activity was optimal after 48 hr from the treatment, time needed for the larvae of *Tuta absoluta* to ingest NOVOSECT SC21[®] metabolites. These results agree with the results of Derballa et al. (2012), who reported that the Bt metabolites having potential insecticidal activity against Lepidopterans pests. ATO BED BUG was also effective in reducing the infestation level by *Tuta absoluta*, but its efficacy decreased with time due to its volatility. Similar results were observed by Ebadah et al. (2006) showing a reduction of *Tuta absoluta* infestation between 50-60% when treated with clove oil under semi field conditions. Similarly, Mouawad et al. (2013) recorded that clove oil caused highly reduction percentage of penetration and accumulative mortality of larvae and caused ovipositional deterrence reaction towards adult stage of *T. absoluta* under laboratory conditions.

4.2 Toxicity of Biopesticides on Larvae of *Tuta absoluta*

NOVOSECT SC21[®] was the most effective in controlling *T. absoluta* by causing 92.40% mortality at 48 hr after the treatment: the larvae ingest the crystal inclusions (Cry toxins) which will be dissolved and activated in the alkaline environment of the insect gut, where it binds to specific receptors and cause the lyse of larvae midgut (Bravo et al., 2005). Similar observation was obtained with Derbalah et al. (2012) where a combination of *Bacillus thuringiensis* filtrate and Indoxacarb, have showed a reduction of larvae and mine blotch count in treated plants, and where Bt filtrates has exhibited satisfactory effectiveness against *T. absoluta* in greenhouses. ATO BED BUG[®] was effective in increasing the mortality of *T. absoluta* larvae (82.77% at 48 hr after the treatment), similarly, Mouawad et al. (2013) affirmed that clove oil gave satisfactory results against *T. absoluta*.

4.3 Effect of Biopesticides on the Level of *Liriomyza trifolii* Infestation

The effect of all the biopesticides was optimal at 48 hr after the treatment. Similar results were reported by Ebadah et al. (2016) where the efficiency of three recommended insecticides (Acetamiprid 20% SP, Chlorpyrifos 48% and Lambda-cyhalothrin 5% EC) were compared to two natural oils (clove oil and bitter orange) against some of tomato insects (*Liriomyza trifolii*, *Tuta absoluta* and *Bemisia argentifolii*) under semi field conditions. The study showed a 48.7% reduction in tunnels after being treated with clove oil by between 3 and 5 days compared to a 52.5% reduction of tunnels after being treated with chlorpyrifos.

4.4 Biopesticides Toxicity Against *Liriomyza trifolii* Larvae

Ebadah et al. (2016) reported that clove oil caused 56.8% mortality of *L. trifolii* compared to 59.2% and 69.1% mortality when treated with Chlorpyrifos and Lambda-cyhalothrin, respectively after three days. While after 5 days of the treatment, the mortality percent was increased to 61.4% when treated with clove oil compared to 43.7% by Acetamiprid and 40.1% by bitter orange. Based on the 215 day mortality mean, clove oil was the produced the greatest mortality of *L. trifolii* (60.5%). These results are in agreement with those obtained by Sabbour and Abd-El-Aziz (2010), who reported that clove oil and mustard revealed a strong repellent activity after 7 days (71 and 89%, respectively) against *Bruchidius incarnates*. Clove oil was also effective against other Dipteran's insects, such as *Anopheles dirus* mosquitoes (Trongtokit et al., 2005) and clove oil displays an insecticidal activity against this mosquito (Chaeib et al., 2007a). Cikman et al. (2006) reported that Bt is effective in controlling the larvae of *L. trifolii* and should be treated once every 2-3 weeks for effective control. According to Cikman (2006), Bt treated leaves have significantly fewer live larvae than non-treated leaves and that during the whole production period (15 weeks) the number of live larvae was still fewer than the number at which treatment is recommended (4-5 larvae per leaf). Ramirez-Godoy et al. (2018) showed that soil and foliar silicate applications enhanced plant resistance against the Asian Citrus Psyllid in Tahiti lime. Our results suggest that foliar NEO-BOOST applications should be considered by growers because it has an impact on *Liriomyza* populations.

4.5 Effect of Biopesticides on *Alternaria solani* Infection

ATO BED BUG[®] was efficient in controlling *Alternaria solani*. According to Manohar et al. (2001), the phenolic components of clove oil: eugenol and carvacrol possess fungicidal characteristics on the cellular membrane. Also, clove oil has shown an antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (Tampieri et al., 2005). Other studies have shown that a mixture of clove with concentrated sugar solution produced a strong fungicidal effect by reducing the fungi inoculum size (Nunez et al., 2001). Also, Pawar et al. (2006) affirmed that clove oil inhibited the growth of *Asparagillus niger*. The activity of clove oil against fungi is exerted on the cellular membrane and depends on the presence of aromatic ring and the presence of free phenol hydroxyl group and the lipophilic features of the components present in the oil (Tampieri et al., 2005; Cox et al., 2001; Chaeib et al., 2007b).

5. Conclusion and Recommendation

Our research indicates that NOVOSECT SC21[®] and ATO BED BUG[®] has potential for controlling *Tuta absoluta* and *Liriomyza trifolii* by reducing the infestation level and increasing the larval mortality. Additional formulation research should be conducted to optimize the benefits of each material. This formulation research should involve investigating the impact of surfactants or other additives of the efficacy of the biopesticides and maximize their effective longevity once applied to the plants. Biopesticides might compete and replace conventional pesticides for the control of tomato leafminer *Tuta absoluta*, *Lyromyza trifolii* and *Alternaria solani* fungus. An economical study should be conducted in order to provide a better understanding of these biopesticides as alternatives to the conventional chemicals used by Hrajel regional farmers. Thus, these biopesticides are highly recommended to be implemented with other types of control measures as biological control agents or other biorationals in sustainable agro-ecosystems, such as organic farming and in integrated pest management (IPM) programs for *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani*.

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