Compatibility of Bio and Natural Compounds with *Trichogramma evanescens* (Westwood) for *Pieris rapae* management in Cauliflower

Abstract: The efficacy of a bio compound, Protecto (*Bacillus thuringiensis*) and botanicals extract (mixture of ginger, garlic, hot red pepper extracts and aloe vera) either in combination with *Trichogramma evanescens* releases or alone was evaluated in comparison to an insecticide, Uphold 36% SC (Methoxyfenozide/ Spinetoram) on *Pieris rapae* on cauliflower *Brassica oleracea*. Field experiments were conducted at Atfih District, Giza Governorate, Egypt in September 2020 and October 2021. Results revealed that, all the tested compounds combined with *Trichogramma* releases were more effective in reducing *P. rapae* population than when used alone. *B. thuringiensis* with *Trichogramma* releases caused 96.91% and 95.32% reduction in larval population in 2020 and 2021 respectively while the reduction in *Pieris* pupal pupation was 70.87% and 71.59% respectively during these two trials. Similar results were observed with botanical extracts. Parasitization by *T. evanescens* ranged between 91.95-92.77% in the plots sprayed with bio- and natural compounds during the trials in two years. Meanwhile, the insecticide treatment was the least effective in reducing *P. rapae* population (37.85% and 47.85% respectively). It could be concluded that either *B. thuringiensis* or the botanicals extract could be integrated with *T. evanescens* releases for the sustainable production of Cauliflower.

Keywords: Compatibility, *Trichogramma evanescens*, *Bacillus thuringiensis*, botanicals extract, cauliflower, *Pieris rapae*, sustainable Production.

1- Introduction

Agriculture faces several challenges like rapid exploitation of the natural resources, the huge use of insecticides and the sudden changes in the global climate, in addition to the need for the production of safe, pesticides-free agricultural products mainly the edible vegetables. All these challenges push researchers and those responsible for public health to work on new, safe, available and eco-friendly alternatives to the synthetic pesticides for suppressing pests invading agricultural crops [36]. One of the important vegetable crops is the cauliflower(*Brassica oleracea*). This economically important crop is seriously attacked by the cabbage white butterfly *Pieris rapae* L. (Family: Pieridae). The pest is the most abundant during all the seasons, where the female lays solitary, yellowish eggs, hatching into nasty, green larvae which considered the most serious and destructive stage causing great economic loss in the yield [20] and [5]. Continuous application of broad spectrum synthetic insecticides has resulted in the development of the pest resistance, destruction of beneficial insects and contamination for the environment [6]. Currently, great attention has been drawn to the use of various biological agents such as the parasitoids and entomopathogens instead of synthetic insecticides [37] , [15], [14] and [16]. *Trichogramma* parasitoids are considered the most efficient and promising organisms in biological control programs [46]. As the damage caused by *P. rapae* reduces the crop production, the intervention with *Trichogramma* parasitoids with compatible bio and natural compounds is imperative to conserve naturally occurring bioagents and to harvest healthy and safe produce. The entomopathogen *Bacillus thuringiensis* is considered as an efficient biological control agent resulting in significant reduction in the pest and in the use of chemical insecticides [2]. The components of a botanical extract [39] such as ginger, garlic, and hot red pepper, and aloe vera possess an insecticidal effect against many insects [32], [43],[30] and [21]. The botanicals mixture was found to be effective against certain pests [39] and was safe to *Trichogramma evanescens* Westwood under laboratory conditions [40]. The integration of the parasitoids with bio and natural compounds was earlier evaluated by [31] and [26] who found that, *T. evanescens* with *B. thuringiensis* was effective against *Helicoverpa zea* Boddie and *Ostrinia nubilalis* (Hubner) . Because of the legal restrictions on the edible fresh vegetables and the periodical sprays of different insecticides, alternatives have to be introduced to suppress *P. rapae*. Therefore, this work was carried out to evaluate the efficacy of *B. thuringiensis* , the botanicals mixture extract and combinations with *T. evanescens* releases against *P. rapae* infesting cauliflower crop.

2-Materials and Methods

Experiments were carried out to evaluate the integration of *T. evanescens* Westwood releases with certain bio and natural compounds for the management of *P. rapae* on cauliflower. Comparisons were made with an insecticide. Preparation of the botanicals extract and the rearing processes were carried out at the *Trichogramma* Mass Rearing Laboratory of Fayoum Governorate, Plant Protection Research Institute, Agricultural Research Center,Egypt.

2.1. Rearing T. evanescens

The rearing of the host *Sitotroga cerealella* and the parasitoid *T. evanescens* were conducted at 25±2 °C and 70±5% R.H. The host *S. cerealella* Olivier was reared as the modification of [17]. The parasitoid was multiplied on *S. cerealella* eggs.

2.2. Preparation of the botanicals extract

The botanicals extract was prepared as per the methods described by [39]. About one kilogram of garlic bulbs, dry hot pepper, fresh ginger rhizomes were cleaned, ground well, soaked in ten liters water for 48-72 h and filtered About one kilogram of aloe vera leaves were cut into small pieces, blended and added to the filtrate . This mixture was diluted to 100 liters water for spraying.

2.3. Field experiments

Field experiments were carried out at Atfih District, Giza Governorate,Egypt in cauliflower seasons of 2020 and 2021 . The experimenta carried out as completely randomized block design with six treatments and the control with three replications. Each replicate was ¾ feddan and each plot was separated with three rows as a buffer zone from the adjacent plot to reduce interference of the treatments. The experimental plots were planted with cauliflower seedlings (Colonia variety) on 25th September. Each plot had 10 rows of cauliflower plants which was 50 cm a side within the rows. Plants were inspected for the presence of eggs, larvae and pupae of *P. rapae* every 10 days until harvest on 5th December. Pre-treatment population count was made randomly from half of the rows. Plants had eggs of *P. rapae* was marked with coloured paper cards. Larvae and pupae were also counted. The first release of *T. evanescens* was done after 20 days of transplanting the seedlings. Hard paper envelopes housing the parasitoids were hung in the canopy of the cauliflower plants at the rate of 60 envelopes/ feddan (about 90000 parasitoids/each release). Each envelope had three pieces of different developmental stages of *T. evanescens*. The envelopes were hung every 10 days at the sunset. Normal cultivation practices were followed in cauliflower field.

The treatments were: Releasing *T. evanescens* alone, spraying *B. thuringiensis* alone, spraying the botanicals extract alone, combination of *T. evanescens* with *B. thuringiensis*, combination of *T. evanescens* with the botanicals extract, the chemical insecticide Uphold 36% SC and the control (without any treatment). Back motor sprayer (Agranomodo- China) of 20 liters was used for spraying the compounds. Unparasitized and parasitized *Pieris* eggs, number of both larvae and pupae of *P. rapae* were the criteria used for the evaluation of the treatments. Upon appearance of *Pieris* larvae, sprays of the tested compounds were applied in the early morning with the recommended rates as following:

1) *Bacillus thuringiensis* sub sp *kurstaki* 9.4% WP at the rate of 300 gm/200 L. (Product of Plant Protection Research Institute Agricultural Research Center, Egypt.)
2) The prepared botanicals extract at the rate of 1L/100L.
3) Uphold 36% SC at the rate 250cm/200L. (Commonly used by farmers in the neighboring fields in case of heavy infestation with the pest)

2.4. Data collection and statistical analysis

The *Pieris* eggs collected from the plots were observed every 10 days to record the parasitism (the black color of the eggs). The efficacy of the treatments was assessed through the percent reduction of *Pieris* population. It was calculated according to [18].

$$\text{Reduction percentage} = \left[1 - \left(\frac{\text{Population of pest after treatment}}{\text{Population of pest before treatment}} \times \frac{\text{Population in control before treatment}}{\text{Population in control after treatment}}\right)\right] \times 100$$

Obtained data of treated plots were compared with those of Uphold insecticide and control plots. Statistical analysis was carried out by IBM-SPSS for windows (v.25). Variance Analysis (ANOVA) was used to process the obtained data. Duncan's multiple range test [7] was used for separation of means [41].

3. Results

3.1. Efficiency of tested compounds on P. rapae

a. Reduction percentages of P. rapae larvae

The bio compound *B. thuringiensis* combined with *T. evanescens* releases was significantly more effective in reducing *P. rapae* larvae population in 2020 and 2021($P<0.05$) with reduction percentages of 96.91% and 95.32% respectively. This was followed by the natural compound the botanicals extract combined with the parasitoids releases causing the reduction in *Pieris* larvae population by 96.31% and 94.89% in 2020 and 2021 respectively. The chemical insecticide Uphold was the least effective compound in reducing the larvae population with respective reductions of 37.85% and 47.85% (Figure 1, 3).

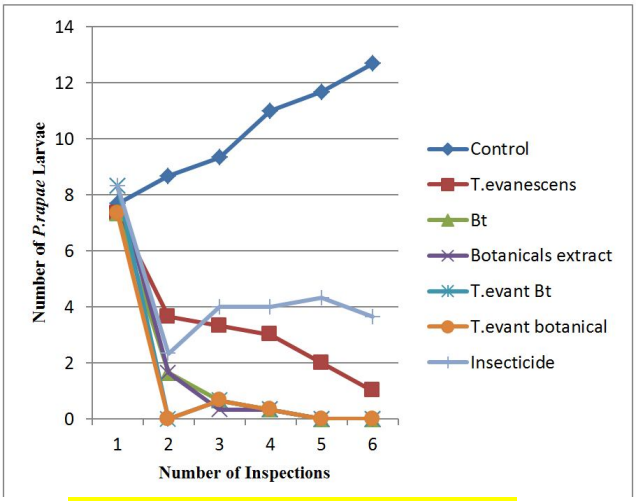

Figure 1. *Population of P. rapae larvae on Cauliflower in 2020 growing season.*

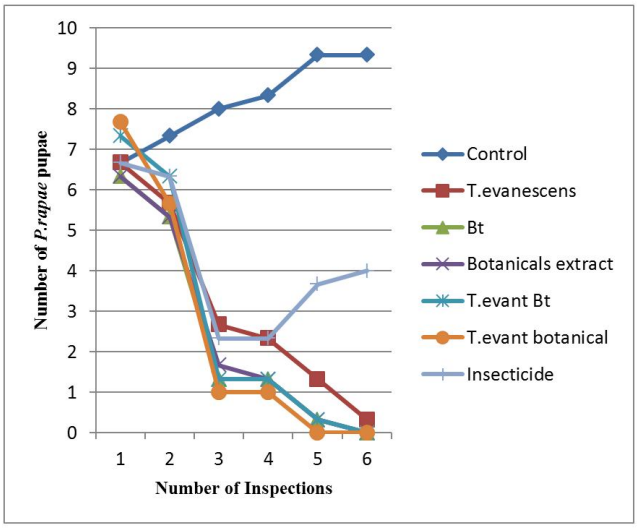

Figure 2. *Population of of P. rapae pupae on Cauliflower in 2020 growing season.*

b. Reduction percentages of P. rapae pupae

The tested compounds significantly differed in its effectiveness against the population density of *P. rapae* pupae ($P<0.05$). The botanicals extract accompanied with *T. evanescens* releases was effective in reducing pupae of *P. rapae* on cauliflower plants in 2020 and 2021 growing seasons with reduction percentages of 77.4% and 72.63% respectively, followed by the treatment of combination of *B. thuringiensis* with *Trichogramma* releases with reduction of 70.87% and 71.59% in 2020 and 2021 respectively. The least reductions of 26.12% and 14.04% in *P. rapae* pupal population in 2020 and 2021 respectively were recorded in plots sprayed with the chemical insecticide Uphold. (Figure 2, 4)

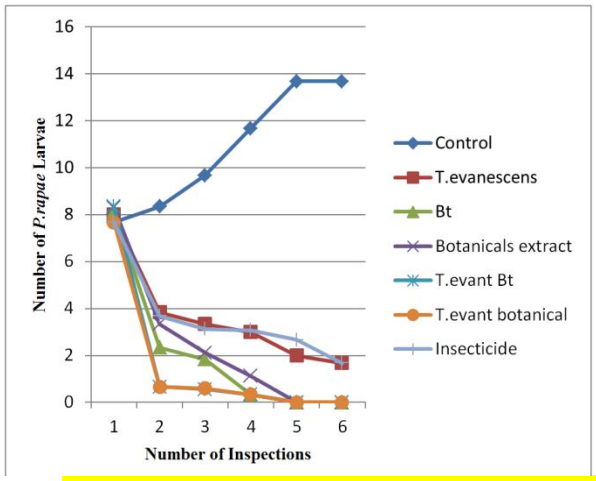

Figure 3. *Population of P.rapae Larvae on Cauliflower in 2021 growing season.*

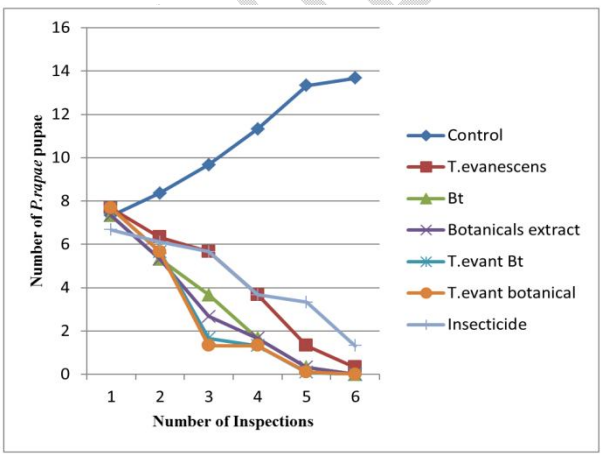

Figure 4. *Population of P. rapae pupae on Cauliflower in 2021 growing season.*

c. Parasitism percentage

There was no parasitism rate recorded before any treatment in 2020 and 2021. With the successive releases of *T. evanescens*, increase in the parasitism was observed in parasitoid- released plots. Results presented in Tables 1, 2 revealed that the plots treated with *T. evanescens* and *B. thuringiensis* had the parasitism rates of 92.56% (2020) and 92.17% (2021). Also, *T. evanescens* releases with the botanicals extract recorded parasitism rates of 91.95% (2020) and 92.77% (2021). Results revealed that releases of *Trichogramma* parasitoids alone without any compound recorded parasitism rates of 91.03% (2020) and 89.99% (2021). The remaining treatments plots showed no parasitism rate as there no released parasitoids (Tables 1, 2) proving no natural parasitism.

Table 1 Parasitim by T.evanesces on P.rapae eggs (2020).

T.evanescens			*B.thuringiensis*			Botanicals extract			*T.evanescens*+ Bt			*T.evanescens* +botanicals extract			Insecticide			Control		
unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras
7.66	0	0	8.33	-	-	8	0	0	8.67	0	-	8.33	0	0	8	-	-	7.66	-	-
3	8.66	74.27	5.67	0	0	5.66	0	0	2.33	9	79.44	2.33	9	79.44	8.67	0	0	9.33	0	0
2	12.33	86.04	6.33	0	0	6.67	0	0	2	12.33	86.04	2.33	11.33	82.94	9.33	0	0	10.33	0	0
0.67	12.33	94.85	5.67	0	0	6	0	0	0.33	12	97.32	0.33	12.33	97.39	9.33	0	0	10.67	0	0
0	12.33	100	6	0	0	6	0	0	0	13	100	0	13	100	9	0	0	13.33	0	0
0	13.33	100	2	0	0	1.67	0	0	0	13	100	0	13	100	9.33	0	0	12.67	0	0
2.22	9.83	91.03	5.67	0	0	5.67	0	0	2.22	11.87	92.56	2.22	11.73	91.95	8.94	0	0	10.67	0	0

Table 2. Table 1 Parasitism by T.evanesces on P.rapae eggs (2021).

T.evanescens			*B.thuringiensis*			Botanicals extract			*T.evanescens* +Bt			*T.evanescens* +botanicals extract			Insecticide			Control		
unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras	unp.	P.	%Paras
8.33	-	-	7.67	-	-	8	0	0	8.33	-	-	7.67	-	-	7.67	-	-	7.33	-	-
3.33	7.67	69.73	7.33	0	0	7.67	0	0	2.67	8.33	75.73	2.33	8.67	78.82	8.33	0	0	9.67	0	0
2.67	13.11	83.08	7.11	0	0	8.33	0	0	2.03	13.33	86.78	2	13.67	87.24	9.67	0	0	11.33	0	0
0.33	15.33	97.89	6.67	0	0	5.67	0	0	0.23	13.67	98.35	0.33	14.67	97.8	11.33	0	0	15.03	0	0
0	15.76	100	7.33	0	0	6.67	0	0	0	15.33	100	0	15.67	100	13	0	0	18	0	0
0	16.33	100	7.33	0	0	7.67	0	0	0	16.67	100	0	16.33	100	13.67	0	0	19	0	0
2.44	13.62	90.14	7.24	0	0	7.34	0	0	2.21	13.47	92.17	2.01	13.8	92.77	10.61	0	0	13.39	0	0

4. Discussion

The impact of integration between synthetic or natural compounds with bio control agents like *Trichogramma* parasitoids on the latter should be considered. The present work in 2020 and 2021 growing seasons indicates that incorporating either *B. thuringiensis* or the botanicals mixture extract might be compatible with *T. evanescens* for suppressing *P. rapae* populations infesting cauliflower plantations. Obtained results revealed that releasing *T. evanescens* with *B. thuringiensis* could suppress *P. rapae* larvae and pupae populations and resulted in the highest parasitism rates of *Pieris* eggs than those treated by releasing *Trichogramma* parasitoids alone, that's because *Trichogramma* females parasitizes fresh eggs of the pest and *B. thuringiensis* kills larvae of the pest. Our results are in concurrence with those of [27] who evaluated releasing *Trichogramma* parasitoids with and without *B. thuringiensis*; their results confirmed that releasing only *Trichogramma* parasitoids was less effective than releasing them with *B. thuringiensis* for controlling Lepidopteron pests. Also, [42] supported our observations with their work on controlling *P. rapae* in organic cabbage crop. They reported that *B. thuringiensis* with *Trichogramma* releases was the most effective treatment as it caused high parasitism rate of *Pieris* eggs with a remarkable reduction in larvae populations which resulting in a significant increase in the crop yield over the treatment with *Trichogramma* alone or controls. Other authors worked on the combined use of different species of *Trichogramma* parasitoids and *B. thuringiensis* on different crops aiming at the control of different Lepidopteron pests. [26] proved high efficiency of the combination of *T. nubilalis* and *Bt.*, for controlling *Ostirnia nubilalis* H. (Lepidoptera: Pyralidae) infesting sweet corn. The combination of *T. evanescens* with Agerin (*B. thuringiensis*) was highly effective against *Cryptoblabes gnidiella* Mill infesting grape orchards as it caused the highest parasitism than those with the parasitoids only [44]. [3] reported that *B. thuringiensis* was not harmful for the parasitoids as proven by the highest parasitism rates of two species of *Trichogramma* parasitoids on *Tuta absoluta* infesting tomato in greenhouse. *T. evanescens* was highly effective on suppressing the *Cydia pomonella* (L.) in apple plantations when the parasitoid releases were combined with *B. thuringiensis* than either of the agents being used alone[33]. The results from the current study revealed that *B. thuringiensis* had no adverse effect on *Trichogramma* . Earlier studies agreed with our findings as those of [19] who stated that, the combination of *Trichogramma ostriniae* (Peng and Chen) and *Bt* reduced the pest population significantly over either of the bio agent alone. [25] found that among *B. thuringiensis*, deltamethrin and Spinosad, only *Bt* was harmless to the immature developmental stages of *T. cacoeciae* Marchal, *T. bourarache* Pintureau and *T. evanescens* Westwood in pomegranate orchard. Spore mixture of *B. thuringiensis* did not affect the parasitization performance or longevity of the *T. evanescens* adults[4] .Similarly, [38] found that *B. thuringiensis* was safe to *T. evanescens* and was able to suppress *T. absoluta* infesting tomato fields at Fayoum Governorate, Egypt by releasing *T. evanescens* and spraying *B. thuringiensis*. The present study declared that, the use of *B. thuringiensis* as alone recoded reduction in *Pieris* larvae and pupae populations. Our findings are consistent with an earlier studies by [45], [22] , [11] and [28] who reported that *B. thuringiensis* was significantly effective against *Pieris* larvae while [8]reported that Bio Guard (*B. thuringiensis*) was the least effective compound against *P. rapae*.

In this work, the combination of the botanicals extract with *T. evanescens* releases did not affect the parasitism despite its lethal effect on *Pieris* larvae. [40] reported that the botanicals

extract was safe to the emergence, fecundity and the general productivity of the *T. evanescens*. The same botanicals extract had a bio pesticide property [39], effective against mango scale insect and was recommended as safe , easy prepared extract and an alternative to synthetic insecticides.

5- Conclusion,

Either *B. thuringiensis* or the botanicals extract in combination with *T. evanescens* releases has the potential to suppress *P.rapae* populations on cauliflower plants and could be used as alternative for synthetic insecticides.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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