

# Compatibility of Certain Bio and Natural Compounds with *Trichogramma evanescens* (Westwood) Release for Controlling *Pieris rapae* on Cauliflower Crop

**Abstract:** The compatibility of spraying each of the bio compound Protecto (*Bacillus thuringiensis*) and the natural compound botanicals extract (mixture of ginger, garlic, hot red pepper extracts and aloe vera) with *Trichogramma evanescens* releases in the form of sprays either in combination with *Trichogramma* releases or as alone was evaluated in comparison to the insecticide Uphold 36% SC (Methoxyfenozide Spinetoram) on the cabbage white butterfly *Pieris rapae* infesting cauliflower *Brassica oleracea*. Field experiments were conducted at Atfih District, Giza Governorate in September 2020 and October 2021. Results revealed that, each of the tested compounds accompanied with *Trichogramma* releases was the most effective in reducing *P. rapae* population than being alone. *Bt.*, treatment with *Trichogramma* releases caused reduction in *Pieris* larvae with the values (96.91% and 95.32%) in 2020 and 2021 respectively, and the reduction in *Pieris* pupae reached (70.87% and 71.59%) respectively, followed by the botanicals extract treatment with the parasitoid releases, as it caused a reduction in larvae with the values (96.31% and 94.89%) in 2020 and 2021 respectively. Also, *Pieris* pupae reduced to (77.4% and 72.63%) respectively. Results indicated that both the tested compounds permits the *Trichogramma* females to parasitize *Pieris* eggs with the rates (92.56% and 92.17%) in plots of *Trichogramma* with *Bt.*, in 2020 and 2021 respectively, and (91.95% and 92.77%) in plots of *Trichogramma* with the botanicals extract in 2020 and 2021 respectively. Meanwhile, the insecticide treatment was the least effective in reducing *P. rapae* population. Generally, the results revealed that either *B. thuringiensis* or the botanicals extract could be integrated with *T. evanescens* releases for the sustainable production of clean and healthy agricultural products.

**Keywords:** Compatibility, *Trichogramma evanescens*, *Bacillus thuringiensis*, Botanicals extract, Cauliflower, *Pieris rapae*, Sustainable Production.

## 1- Introduction

Conservation of the environment must be ranked first before the effectiveness of any synthetic or natural compound used for pests' control. The current agricultures are affected by many challenges like; the natural resources rapid exploitation, 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 that 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 those important vegetables is the cauliflower crop (*Brassica oleracea*) Family: Brassicaceae, which comprises many crops of nutritive, healthy and economic benefits, like cauliflower, broccoli, cabbage, collard, kale, mustard and rape [10]. Cauliflower has valuable nutritional facts; it is rich in the anti-oxidant compounds and carotenoids which help in the prevention of different kinds of dangerous diseases. Also, it is rich in vitamins C and B6; it comprises water, carbohydrates, and protein, potassium and dietary fibers with low dietary minerals [13] and [34]. That important and economic crop is attacked by the serious cabbage white butterfly *Pieris rapae* L. (Family: Pieridae) is the most abundant 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]. Once farmers noticed the presence of *Pieris* larvae, they immediately controlled it with abroad spectrum of synthetic insecticides, resulted in the development of the pest resistance, destruction of beneficial insects and contamination for the environment [6]. Nowadays, great attention has been directed to the various biological agents as the parasitoids and the beneficial pathogenic microorganisms to be applied instead of the synthetic insecticides [37], [15], [14] and [16]. *Trichogramma* parasitoids, the egg-parasitoids (Family: Trichogrammatidae), are minute wasps, have the ability of pest specify, host finding and a self-increasing population effect [23]. Its tiny female parasitizes many lepidopteron eggs, resulting in destroying and regulating those pests population [24] and [35]. For that, *Trichogramma* parasitoids considered the most efficient and promise organisms in biological control programs [46]. As the damage caused by *P. rapae* reduces the crop production, so, the intervention with *Trichogramma* parasitoids with compatible bio and natural compounds is imperative to conserve *Trichogramma* parasitoids and to maintain healthy and safe products. *Bacillus thuringiensis* are specific pathogens for lepidopteron larvae, it is considered as an efficient biological control agent resulting in significant reduction in the pest and the used chemical insecticides [2]. Taking into consideration the severity caused by chemical insecticides, natural alternatives as certain plant products could be considered as natural pesticides [9] and [29]. The

plant kingdom comprises certain plants possess insecticidal properties [12], so the botanicals extract [39] which is a prepared mixture of ginger, garlic, and hot red pepper extracts with aloe vera. Each ingredient of that mixture possess an insecticidal effect against many insects [32], [43], [30] and [21]. However, this botanicals mixture was effective against certain pests [39] but also; it was safe on *T. evanescens* developmental stages under laboratory conditions [40]. In earlier studies the integration of the parasitoids with bio and natural compounds was evaluated as those of [31] and [26] who found that, *Trichogramma evanescens* with *B. thuringiensis* was effective against *Helicoverpa zeas* Boddie (Lepidoptera: Noctuidae) and European corn borer. So, because of the legal restrictions on the edible fresh vegetables, and the periodically sprays of different insecticides on cauliflower pests mainly *P. rapae*, different alternatives to the synthetic insecticides have been introduced to suppress that serious pest. Therefore, this work carried out to evaluate the efficacy of *B. thuringiensis* and the botanicals mixture extract and their integration with *T. evanescens* releases against *P. rapae* infesting cauliflower crop to ensure sustainability production of that important, healthy and economic crop with the preservation of the natural enemies and the agricultural ecosystem.

## 2- Materials and Methods

This experiment was established to evaluate the integration of releasing *T. evanescens* Westwood with certain bio and natural compounds comparing with synthetic insecticide aiming at suppressing *P. rapae* population infesting cauliflower crop. 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.

### 2.1. Rearing *T. evanescens* Techniques

The rearing of the host *Sitotroga cerealella* and the parasitoid *T. evanescens* were conducted at  $25\pm 2$  °C and  $70\pm 5\%$  R.H. *S. cerealella* moths rearing is reared first to produce the eggs in which *Trichogramma* parasitoid will develop

### 2.2. Rearing the host *Sitotroga cerealella*

The host *S. cerealella* Olivier was reared as the modification of [17].

### 2.3. Rearing *T. evanescens* parasitoids

As described by [1], fresh *S. cerealella* eggs were distributed on self-adhesive paper, then parasitoids released on that eggs in glass jars and provided with a droplet of cane honey as nutrition for the adults. The jars were covered with cloth-wrapped cotton, tied well by rubber bands. The egg cards replaced daily to avoid super-parasitism.

### 2.4. Preparations of the botanicals extract (According to [39])

The botanicals extract was prepared to be used in this work as a biocide for *P. rapae* larvae. 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. Then refined and squeezed well. About one kilogram of aloe vera leaves cut into small pieces, blinded and added to the filtrate and diluted to 100 liters water for spraying.

### 2.5. Field experiments

Field experiments were carried out at Atfih District, Giza Governorate in 2020 and 2021 growing seasons of cauliflower crop. The experimental area was completely randomized block design, six treatments and the control with three replicates per each. Each replicate was  $\frac{3}{4}$  feddan and each plot was separated with three rows as a buffer zone from the adjacent plot to reduced interference of the treatments. The experimental plots were planted with cauliflower seedlings (Colonia variety) on 25<sup>th</sup> 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 every 10 days until harvest on 5<sup>th</sup> December. *P. rapae* eggs, larvae and pupae were counted before any treatment randomly from half of the plants rows. Colored paper cards were tied with plants had eggs of *P. rapae*. Larvae and pupae were also counted. The first release of *T. evanescens* was done after 20 days of transplanting the seedlings. The parasitoids were held in hard paper envelopes and it was hung inside 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 accomplished in cauliflower field. The treatments were: Releasing *T. evanescens* alone, spraying *B. thuringiensis* alone, spraying the botanicals extract alone, releasing *T. evanescens* with *B. thuringiensis*, releasing *T. evanescens* with the botanicals extract, the chemical insecticide Uphold 36% SC and the controls (had neither release of *T. evanescens* nor any compound sprays, so damaged cauliflower plants quickly increased). Unparasitized and parasitized *Pieris* eggs, number of both larvae and pupae of *P. rapae* were the criteria used for the evaluation of *T. evanescens* release as alone or in combination with *B. thuringiensis* or with the botanicals extract or the chemical insecticide spraying. In case of *Pieris* larvae presence, it was immediately sprayed with the tested compounds in the early morning with the recommended rates as following:

- 1) *Bacillus thuringiensis* subsp *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)
- 4) Back motor sprayer (Agranomodo- China) of 20 liters was used for spraying the compounds.

## 2.6. Data collection and statistical analysis

The obtained *Pieris* eggs were observed every 10 days to record the parasitism induction (the black color of the eggs). The evaluation of the treatments based on the reduction of *Pieris* infestation percentages. It was calculated according to [18].

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

Obtained data of treated plots were compared with those of Uphold insecticide and control plots. All 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 tests [7]. was used for separation of means [41].

## 3.1 Results

### 1. Efficiency of tested compounds on *P. rapae*

#### a. Reduction percentages of *P. rapae* larvae

The bio compound *B. thuringiensis* was significantly effective as combined with *T. evanescens* releases in reducing *P. rapae* larvae population in 2020 and 2021 ( $P < 0.05$ ) with reduction percentages of 96.91% and 95.32% respectively, followed by the natural compound the botanicals extract accompanied with the parasitoids releases with the reduction in *Pieris* larvae population of 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)

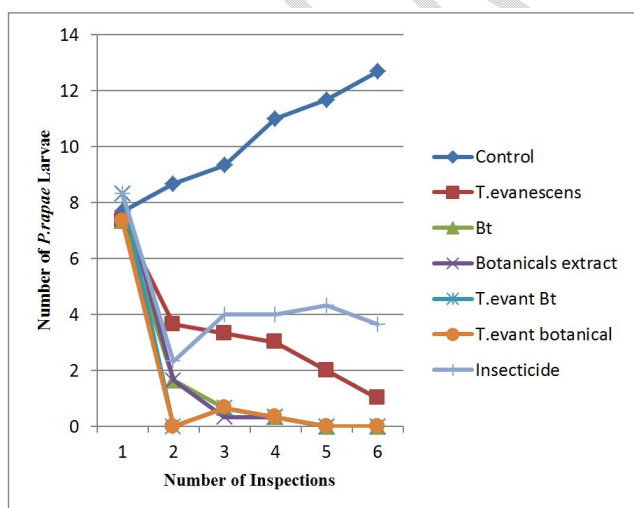


Figure 1. Evolution of *P. rapae* Larvae on Cauliflower Plants in tested treatments plots and control in 2020 growing season.

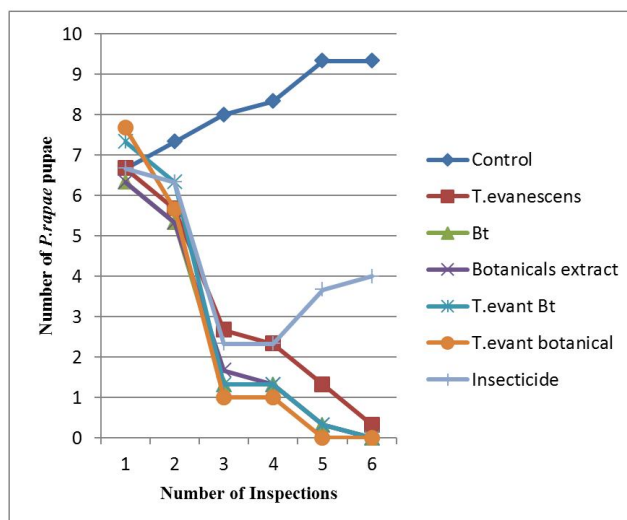


Figure 2. Evolution of *P. rapae* pupae on Cauliflower Plants in tested treatments plots and controls in 2020 growing season.

#### b. Reduction percentages of *P. rapae* pupae

The tested compounds were 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 reduction in *P. rapae* pupae was 26.12% and 14.04% in 2020 and 2021 respectively was recorded with the chemical insecticide Uphold. (Figure 2, 4)

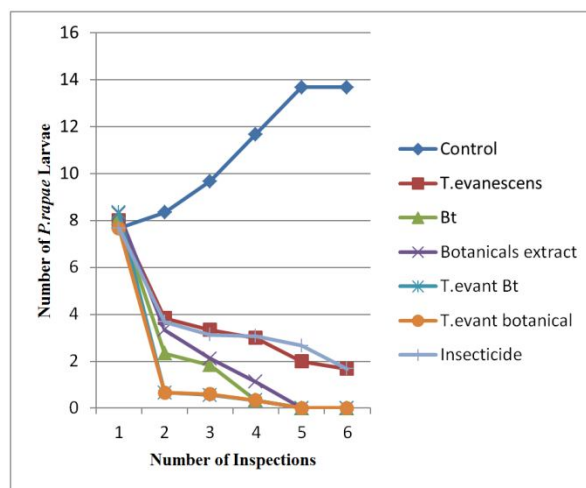


Figure 3. Evolution of *P. rapae* Larvae on Cauliflower Plants in tested treatments plots and control in 2021 growing seasons.

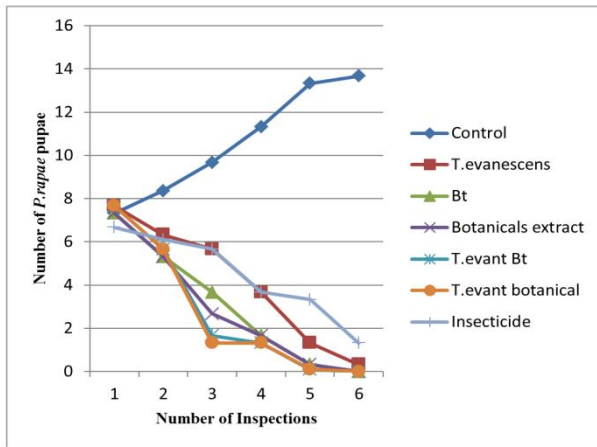


Figure 4. Evolution of *P. rapae* pupae on Cauliflower Plants in tested treatments plots and controls in 2021 growing season.

### c. Parasitism percentage

There was no parasitism rate recorded before any treatment in 2020 and 2021, consequently, with the successive releases of *T. evanescens*, the parasitism percentages raised in plots of releasing the parasitoids. Results presented in Tables 1, 2 revealed that, the treatment of releasing *T. evanescens* accompanied with *B. thuringiensis* raised the *P. rapae* eggs parasitism by 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 only without any compound recorded 91.03% (2020) and 89.99% (2021). The remaining treatments plots showed no parasitism rate as there no released parasitoids. (Tables 1, 2)

Table 1. Evolution of *P. rapae* eggs numbers with parasitism Percentages by *T. evanescens* on Cauliflower plants in treatments plots and the controls (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. Evolution of *P. rapae* eggs numbers with parasitism Percentages by *T. evanescens* on Cauliflower plants in treatments plots and the controls (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

### 3.2 Discussion

The impacts of any integration between synthetic or natural compounds with biological controlling agents like *Trichogramma* parasitoids on that bio agent must be considered. The present work 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 in 2020 and 2021 growing seasons. 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 as alone, that's because *Trichogramma* females parasitizes fresh eggs of the pest and *B. thuringiensis* kills larvae of the pest. Our results are consistence with those of [27] who evaluated releasing

*Trichogramma* parasitoids with and without *B. thuringiensis*; their results confirmed that releasing *Trichogramma* parasitoids only 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 combination of different species of *Trichogramma* parasitoids with *B. thuringiensis* on different crops aiming at the control of different Lepidopteron pests, as those of, [26] who evaluated the combination of *T. nubilalis* and *Bt.*, for controlling *Ostirnia nubilalis* H. (Lepidoptera: Pyralidae) infesting sweet corn, the results of their work showed high efficiency in reducing the pest population with the recommendation of the integration of *Trichogramma* parasitoids with *Bt.* [44] who reported that, 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 in the honey dew moth with the highest mortality for its larvae than those with the parasitoids only. [3] investigated the efficiency of two species of *Trichogramma* parasitoids in combination of *B. thuringiensis* to control *T. absoluta* infesting tomato in greenhouse, they reported that *B. thuringiensis* was not harmful for the parasitoids as recorded from the highest parasitism rates. [33] who evaluated the effectiveness of *T. evanescens* Westwood as alone or combined with *B. thuringiensis* var. *Kurstaki* for controlling *Cydia pomonella* (L.) in apple plantations, they reported that *T. evanescens* was highly effective on suppressing the pest populations when the parasitoid releases was combined with *B. thuringiensis* than their releasing alone or than spraying *Bt* alone. The obtained results revealed that *B. thuringiensis* had no bad effect on *Trichogramma* parasitoids efficiency. Earlier studies agreed with our findings as those of [19] who studied the effect of *B. thuringiensis* on *Trichogramma ostriniae* (Peng and Chen) to produce an eco-friendly control strategy. They stated that, the combination of *Trichogramma* and *Bt* decreased significantly the pest population over any bio agent alone. [25] evaluated the effects of *B. thuringiensis*, deltamethrin and spinosad on the immature developmental stages of *T. cacoeciae* Marchal, *T. bourarache* Pintureau and *T. evanescens* Westwood in pomegranate orchard, they reported that deltamethrin and spinosad were harmful to the immature developmental stages of the parasitoid and *B. thuringiensis* was harmless towards the immature stages of the parasitoids. [4] evaluated the effects of *B. thuringiensis* *Kurstaki* with honey as a nutrition diet for *T. evanescens* adults on its efficiency; they reported that spores mixture had no bad effects on the parasitization performance or longevity of the parasitoids. Also, [38] who evaluated certain bio and chemical compounds on the efficiency of *T. evanescens* under laboratory conditions and her results revealed that *B. thuringiensis* was safe and had no bad impacts on the fitness components of the parasitoid, in addition, she controlled *T. absoluta* infesting tomato fields at Fayoum Governorate, Egypt, by releasing *T. evanescens* with spraying *B. thuringiensis* to suppress the populations density of *T. absoluta*. 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 study by [45], [22] and [11] who reported that *B. thuringiensis* with different concentrations were significantly effective against *P. brassicae* (L.) larvae with 100% mortality after 2-6 days of the treatment, [28] who found that *B. thuringiensis* could control *Pieris* larvae in Cole plantations in Alabama. While, [8] evaluated certain compounds aiming at the reduction of *P. rapae* larvae populations on cabbage plantations and reported that Bio Guard (*B. thuringiensis*) was the least effective compound. In this work, the second tested compound was the botanicals extract, it is a mixture of certain natural plants extracts; the obtained results revealed that, the combination of the botanicals extract with *T. evanescens* releases was not an impediment to *Trichogramma* females to

parasitize *Pieris* eggs, despite its lethal effect on *Pieris* larvae. The results of *T. evanescens* with the botanicals extract revealed that high parasitism rates with remarkable reduction in *Pieris* larvae and pupae populations. From the safety prospective, [40] agreed with our findings when they evaluated the botanicals extract on the fitness components of *T. evanescens* under laboratory conditions, they reported that the botanicals extract was safe on the emergence, fecundity and the general productivity of the resulting parasitoids, however, the same botanicals extract had a bio pesticide property [39], as they evaluated it to control mango scale insect and recommended it as safe and easy prepared extract and an alternative to synthetic insecticides.

#### 4- Conclusion,

*B. thuringiensis* or the botanicals extract had potentials to suppress *P. rapae* populations on cauliflower plants with *T. evanescens* releases. So each of the bio compound *B. thuringiensis* or the botanicals extract was promising and could be integrated with the releases of *T. evanescens* and could be used as alternatives for synthetic insecticides for controlling *P. rapae*.

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