Efficacy of imidacloprid and *Beauveria brongniartii* on green peach aphid, *Myzus persicae*, and its predator, *Coccinella undecimpunctata* in tomato

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ABSTRACT

Imidacloprid and the fungus *Beauveria brongniartii* ($1x10^2$ to $1x10^8$ conidia/ml) were evaluated against the green peach aphid, *Myzus persicae* (second instar) and their predator *Coccinella undecimpunctata* (day-old adults) in tomato plantations in two different climatic Governorates Dakahlia and EL Behira. Using a spray technique to evaluate contact effect and a feeding technique to evaluate oral toxicity. Our results showed that under laboratory conditions, the LC_{50} values of Imidacloprid and *B. brongniartii* were 66 ppm and 72 conidia /ml, respectively against *M. persicae*. Under field conditions, the percentages of infested plants with *M. persicae* were significantly decreased after treatments with both Imidacloprid and *B. brongniartii* as compared with the corresponding controls. In El- Esraa Nobaryia (Behira) and El Dakahlia, the weights of tomato yield were 4978± 74.31 kg/feddan (F) and 4597±65.12 Kg/F in Imidacloprid plots treatments. The percentages of yield loss ranged between 24, 49 and 22, 50 in El- Esraa Nobaryia (Behira) and El Dakahlia, respectively. The study showed that *C. undecimpunctata* exhibit relatively high and reasonable resistance to Imidacloprid and *Beauveria brongniartii* at their highest lethal concentration for tested prey.

Keywords: Imidacloprid, *Beauveria brongniartii, Myzus persicae, Coccinella undecimpunctata,* tomato.

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INTRODUCTION

Tomato *Lycopersicon esculentum* is an imortant vegetable crop world-wide. It is usually infested with many destructive pests, including the green peach aphid, *Myzus persicae* (Blackman and Eastop, 2006, and Sabbour and Nayera Solieman 2018, 2019, 2020a,b). This pest transmits several viruses to many economic crops and fruits, leading to decreasing growth, shriveling of the leaves and the death of various tissues (Namba and Sylvester 1981; Berry 1998; Namba and Sylvester 1981; Berry 1998; Filotos *et al.*, 2004).

Entomopathogenic fungi including a groups of pathogens which kill hexapods (Roberts and Humber, 1981. *Beauveria bassiana* and *B. brongniartii* used to control many pests (Lacey *et al.*, 2001; Sabbour and Shadia, 2020). In opposite part, the fungi species always causing a high infestations to pests. The fungi causing a white muscardine to insects after infest them (Wilding and Lauckner, 1974; Carruthers *et al.*, 1985; Eilenberg and Philipsen, 1988).

Coccinella undecimpunctata consider as an important biological control agent attacks many harmful pests and feed on them. Imidacloprid make as a neonicotinoid which cause block to the neuronal pathway. Infestations with imidacloprid causing the insect death. Imidacloprid inter to insect nervous system and block the neurons pathways (Howe and Jander, 2008; Husseien *et al.*, 2019; Jitamoni Hashem *et al*., 2019). This block accumulations to the acetylcholine in the nervous system then the insects paralyses and dead. Imidacloprid

also, affect and poison on contact and via stomach (Kidd and James, 1994; Sabbour and Nayera Solieman, 2019). The objective of the present work is to evaluate the efficacy of Imidacloprid and *B. brongniartii* against *M. persicae* and their main predator *C. undecimpunctata*, in tomato fields in Egypt.

MATERIALS AND METHODS

Insect culture

M. persicae was reared on small potted tomato plants inside cylinderical glass cages, with 15cm in diameter and 40 cm in height, covered with muslin, and kept at 26 ± 2 ºC and 65± 5 % R.H.. The stock culture of the ladybird, *C. Undecimpunctata*, was started with adults that were collected from aphid-infested tomato cultivars in Nobaryia, Egypt. Each five adults were kept in 2L glass jar.

Predator

The stock culture of *C. undecimpunctata* adults were collected from aphid-infested tomato cultivars in Nobaryia , Egypt. Each five adults were kept in 2L glass jars , coverd with muslin cloth, and supplied with fresh lettuce (*Binomial name*) leaves infested with aphids for feeding. Food was renewed every other day. The jars were checked daily for egg deposition. The deposited eggs were collected and transferred to Petri dishes (20 cm in diameter) till hatching. Neonate larvae were transferred individually to plastic cups with ample amount of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs till reaching the second larval instar). Unused larvae were left in 2L glass jars (5/ each) with small duranta branches carrying different stages of aphids till maturation.

Culture of the Entomopathogenic fungus

B. brongniartii (Apopka strain 97) was kindly obtained from Prof. Dr Alain Vey, Mycology unite, National De La Research Scientifique, Univ. Montpellier. And reproduced in Microbiology Dept., National Research Center, Cairo, Egypt. The fungus was primarily purified using the mono-spore technique and propagated on potato dextrose agar medium enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 °C according to Sabbour (1992). Seven-day-old cultures with well-developed spores were harvested, used as stock suspension with known spore concentration and kept at 4 °C. The culture was then adjusted to $1X10^8$ conidia /ml.

Treatment protocol-Imidacloprid

Six aqueous concentrations of Imidacloprid (Shanghai Fuang Agrochemical Co. Ltd, 99.9% purity) were prepared (2.000, 1.500, 0.750, 1.000, 0.500, 0.250, and 0.125 ppm). Tomato leaves were dipped in each concentration separately for 10 seconds and air-dried at room temperature. The treated leaves were offere to 2^{nd} nymphal instars of *M. persicae*. Each treatment consisted of 20 nymphs / concentration. Each treatment was replicated five times. A parallel control of non-treated insects, fed on leaves dipped in distilled water only, was also run. Each control experiment was replicated five times. After seven days of treatment, the percentage of mortality was recorded and then corrected using Abbott's formula (Abbott, 1925). Corrected mortality was then subjected to probit analysis (Finney, 1971) to estimate the LC_{50} value.

B. brongniartii

Two ($1x10^2$ to $1x10^8$ conidia/ml) condidial concentrations of *B. brongniartii* were prepared. Fresh tomato leaves were sprayed with the fungual concentrations (3 shots as spurts / leaf) (Matter *et al.* ,1993), left to dry and placed in individually in 1 L plastic containers. Then, twenty third-instar nymphs of *M. persicae* were placed on each leaf. Each container was covered with muslin and incubated at 25 ºC. Each treatment was replicated five times. A parallel control experiment of non-treated aphids was aslso conducted and replicated five times. The percentage of mortality was calculated after seven days and corrected according to Abbott's formula (Abbott, 1925), and the subjected to propit analysis (Finney, 1971).

Spray technique

A day-old *C. undecimpunctata* adult or *M. persicae* second instar nymphas were placed

in a petri dish (19 cm in diameter) and sprayed with 300 ppm of *B. brongniartii* three shots as spurts)(Matter *et al.,* 1993). The shots were directed to the insects at 15 cm distance, then the insects were individually transferred genetly, using tweezers, to plastic cups (5 cm in diameter and 12 cm in height) with small water moistened filter paper and aphid-infested tomato leaf. The cups were coverd with muslin as and incubated at 25° C. The filter paper and branches carrying aphids were renewed every other day. Each treatment was replicated five times. The cups of each group were checked daily for insects showing signs of fungal infection. The death toll was recorded for two weeks post - treatment and mortality percentage was calculated in each case.

Obligatory and free-choice feeding techniques

C. undecimpunctata adults (20 no.) were exposed either obligatory to *B. brongniartii* -infected second nymphal instars aphids or selectively to *B. brongniartii*- infected and non-infected aphids for 24-h. In case of free-choice feeding, five groups were used/ pathogen/ predator. The predators were starved for 4 and 6 h for nymphes and adults of *M. persicae*, respectivelly. Then, eachgroup was introduced in the middle of 5L- glass jar with two branches of tafla (*Nerium oleander*) carrying ample amounts of the pest. One branch was previously sprayed with the destruxins while the other branch was sprayed with water only. The two branches were placed on both sides of the glass jar facing each other to allow the predators free-choice to feed on either treated or untreated aphids. Five glass jars were used as replication for each pathogen. Regarding obligatory feeding, the same number of predators in each of the five glass jars were used, as mentioned above, but offered only treated aphids. In both trials , exposure period was 24 h. Then, predators of each treatment and the control as well were transferred indiviually to plastic cups, offered untreated aphids, and checked daily for 14 days.

Field assay

Experiments were carried out to studying the efficacy of the tested two pathogens imidacloprid and *B. brongniartii* against the target insect pests in El Esraa; El – Nobaryia, with dry weather and sandy soil, and in El- Dakahlia, with wet weather and clay soil. Tomatoes (Var. Bio-Bride) were planted at the first of April in an area of about 1200 m^2, divided into 12 plots of 100 m^2each. Four plots were assigned for each pathogen, while four plots were treated with water and used as the controls. Imidacloprid and *B. brongniartii* were applied at 5ppm concentration and 5L / plot. Treatments were performed in a randomized plot design at the sunset with a 5L- sprayer. Three applications were made at one-week interval at the commencement of the experiment. Then 20 samples of plants were randomly collected every week from each plot and transferred to laboratory for examination. Average number of each of the tested pests / sample / plot / treatment was 20, 50, 90- and 120-days post 1^{st} application. The infestation of aphids was then determined in each case. After harvest, yield of each treatment was weighed as kg/F Yield loss was calculated according to the following equation Yield loss = Potential yield- Actual yield x 100/

Potential yield

Potential yield was that of which gave the best results among the tested pathogens imidacloprid and was taken as a base for comparison with the other treatments.

Seedlings of tomato plants were sown in raws (50 cm apart) located in Dakahlia Governorate. One month-old plants were found to be highly infested with *M. persicae.* The cultivated area was divided longitudinally into 3 areas (50 M^2 / each), separated from each other by uncultivated bare and (4 m wedth). One area was used for each entomopathogen toxin tested and the check as well. Each pathogen was sprayed at the rate of 5ppm of the pathogen tested, using high pressure hand held gun. Five ppm Imidacloprid and $1X10^8$ conidia/ml *B.*

brongniartii were applied. These concentration induce >80 % mortality in both pests in laboratory experiments, as shown above). Three applications were made first at one- week interval**.** Then**,** *C. undecimpunctata* on *M.presicae* (both nymphs and adults) were carefully counted on site in all tomato plots, hand picked and finally, sweeping net (25 cm dia). The counts were made just, 1, 2 and 3 wk post last application. The predators were placed again after each count on their previous location in the corresponding plant site. Fifty tomato shrubs (10 from each of 5 raws) per each treated area and the cotrol as well were arbitrary chosen / per each time interval. The average number of predators / 50 plants / time interval was calculated in each case. The increase or decrease in the population denesity of the predator / 50 plants as compared with the check was calculated according to the equation of Hendrson and Tilton (1955).

RESULTS

Results shows that the LC_{50} of *M. persicae* treated with Imidacloprid and *B. brongniartii* was 72 ppm and 66 conidia/ml with confidential limits of 33-99, respectively. Under field conditions in El Esraa, Nobaryia, Behira Governorate and Mansoura, Dakahlia, Dakahahlia, the infestation rate with *M. persicae* was significantly ($P<0.05$) decreased. compared to the controls. The infestation rate was progressevily decreased with the increase in the period following application of the *M. persicae* (Table 1).

Table 1. The infestation of tomato plants with *M. persicae* after treatment with the Two tested pathogens under field conditions in two regions during season 2018.

Treatments	Days after 1st application	Means number of *M. persicae* in application areas During 2018	
		El Esraa Nobaryia (Behira)	Dakahlia (Mansora)
Control	20	66±6.9	69±9.9
	50	79±8.8	183±7.8
	90	96±8.9	198±8.9
	120	159±9.8	169±4.9
Beauveria brongniartii .	20	16±8.7	13±8.9
	50	19±9.8	24±8.9
	90	36±4.9	38±9.8
	120	45±7.5	49± 9.6
Imidacloprid .	20	0	2±1.3
	50	5±1.6	7±3.1
	90	15±3.3	19±9.4
	120	21±9.5	26±6.7
F test		27.7	28.8
LSD 5%		13.7	18.7

Effects of Imidacloprid and *B. brongniartii* the predator on *C. undecimpunctata* were shown in table (3), all of the experiments results cleared that the predator *C. undecimpunctata* not affected to both treatments. This predator *C. undecimpunctata* proved a higher resistance against treatments (Table 3). Results show that the predator pray on nymphs are more susceptible to that on the adult stages of *C. undecimpunctata*. under laboratory conditions the nymph's mortality of *M. persicae* obtained 66.11±5.61 and 19.2±9.45 after treated with Imidacloprid and *B. brongniartii*, respectively (Table 3).

Table 3. Determination of the effect of Imidacloprid and *Beauveria brongniartii* on developmental stages of *C. undeciumpunctata* after feeding *M. persicae* nymphs and adults.

Pathogen	Mortality of infected *M. persicae* M±SE					
	Nymphs			Adults		
	Direct spray	Ingetion of the treated food		Direct spray	Ingestion of thetreated food	
		Obligatory (no-choice)	Selection (choice)		Obligatory (no-choice)	Selection (choice)
Imidacloprid *B.brongniartii*	66.11±5.61 19.2±9.45	22.6±7.41 33.2±8.45	10.8±4.76 20.4±9.90	28.6±9.19 39.6±9.71	16.3±8.71 29.2±8.49	6.5±8.51 19.8±6.82
F- test LSD 5%	23.32 13.25					

In case of adult stages significantly decreased by 0.6 times treated with the following the same order.

In general, the indirect treatments by feeding by either of the treated of the prey *M. persicae* only (required) or by free-choice feeding on either treated or untreated prey (selectivity), revealed that the required ingestion of Imidacloprid. – infected prey of *M. persicae* caused a mortality percentage of 2.92 and 0. 17 times that obtained from those of the given free-choice ingestion (selection treatment) for adult and nymph of *C. undecimpunctata* predators, respectively. The corresponding ratios for *B. brongniartii* were about 2.74 and 1.35, respectively. This indicated that the predator, particularly the adult predator, has a greater ability to recognize between the Imidacloprid treated prey and non-treated ones than *B. brongniartii* fungus, which indicates that the adult predator can avoid Imidacloprid which infected the prey much more the often than of the adult predator *C. undecimpunctata* avoids the *B. brongniartii* infected prey. It is worth mentioning that no death from both Imidacloprid infection was encountered in the check within the experimental period.

Table 4. The effect of two tested imidacloprid and *B. brongniartii* on *C. undecimpunctata* (all stages) / 50 tomatos shrubs after successive post two toxins application period.

post application (Weeks)	Treatments Average number of *C. undecimpunctata* ± SE				
	Control	Imidacloprid	*Beauveria brongnia*	% increase(+) or decrease (-)**	
Just before Application	19.75± 3.36	22.00± 2.55	20.00± 1,58	Imidacloprid	*Beauveria brongniartii*
One Week after application	23.75±1.70	17.25±2.25	7.5±1.71	-37.32	-65.39
Two Weeks after application	20.25±1.54	18.29±1.66	11.5±0.96	-18.61	-44.89
Three Weeks after application	18.00±8.18	21.25±1.65	14.25±1.93	+6.46	-22.10

*percentages of increase or decrease in *C. undeciumpunctata* population density as compared with the check according to Hendrson and Tilton (1955).

Under field conditions, the population density of *C. undecimpunctata* in the Imidacloprid-treated area showed 37.32 and 18.61% reductions, one and two weeks after the last application, respectively, compared to the controls. In contrast, After 3 weeks of the last application, the population of *C. undecimpunctata* showed a 6.46% increase compared to the control (Table 4). In the *B. brongniartii* – treated area, severe reductions in the population densities were estimated in the 1st (-65.39 %) and 2nd weeks (-44.89%) after the last application, compared to the controls. There was a less reduction (-

22.10%) 3 weeks post the last application, compared to the control.

Field application of both the two bioinsecticides, showed that in the control plots, the estimated yield weights were 2367± 30.82 and 2230±82.50 kg/F in El Esraa Nobaryia (Behira) and EL Dakahlia governorate during 2018 respectively. While in Imidacloprid and *B. brongniartii* treated plots, the estimated weights of the tomato yields were 4978± 74.31 and 4597± 65.12 kg/F respectively in the El Esraa El-Nobaryia (Behira) region. In EL Dakahlia , the untreated plots recorded 2230±80.50 kg/F but the weight showed a significant increase after the Imidacloprid. and *B. brongniartii* treatments. The percentages of yield loss in the untreated plots were 24 and 50% in the El Esraa El- Nobaryia (Behira) region and the EL Dakahlia, respectively (Table 5).

Table 5. Weight of harvested tomatoes and percentage of yield loss during season 2018 after the two pathogens treatment on *M. persicae* in two governorates.

Treatments	El Esraa Nobaryia (Behira)		Dakahlia	
	Weight tomatoes (Kg/F)	% yield loss	Weight tomatoes (Kg/F)	% yield loss
Control	2367± 30.82	49	2230±82.50	50
Imidacloprid	4978± 74.31	-	4597±65.12	-
B. brongniartii.	3759± 82.57	24	3460±49.13	24
F values	36.40		34.44	
LSD 5%	77		83	

DISCUSSIONS

The present study showed that *C. undecimpunctata* exhibit relatively high and reasonable resistance to the tested EPF *B. brongniartii* and Imidacloprid infections, respectively, even when exposed to a lethal concentration for the prey insects. Thungrabeab and Tongma (2007) concluded that some genera of fungi could be specific and might inflict only on certain types of hosts. They reported the work of James and Lighthart (1994), Matter and Sabbour (2013) whom declared that the fungus *Nomuraea rileyi* exhibits host preferential infection in lepidopterous larvae. Also they found that (*Metarhzium anispliae; B.bassiana*) fungi have potential to infect *Hyppodomia converges* (coccinellidae) where as *N. rileyi* did not. Goettel *et al.* (1990) Hassan et al. (2012) and Sabbour and Nayera (2020 a,b) found that some commercial formulation of the fungi can control aphids and thrips with low impact on non target insects. Sabbour and Shaurub (2018 a,b) and Todorova *et al.* (1994), found that bio insecticides imidacloprid control *Spodoptera littoralis* also, olive pests. found that different strains of *B. bassiana* fungus showed different effects on the two Coleopterous predatory insects due to host response of the insects. (Sabbour and Sahab, 2007; Mahmoud *et al.*, 2014; Sabbour *et al.*, 2012; and Sabbour and Abdel-Raheem, 2016) control *Agrotis ipsilon* and *Heliothis armigera* by the entompathogenic fungi. Sabbour and Abdel-Rahman (2007) found that the two microbial control agents, reduce the number of sugare beet pests under laboratory and field conditions. Sabbour (2007a,b), Sahab and Sabbour (2011) found that the entomopathogenic fungi *Nomuraea rileyi* and *Isaria fumosorosea*, proved highly pathogenic to aphids and the natural enimies *Coccinella* spp not affect by the fungi treatments. Poprawiski *et al.* (1998), found that *Serangium parcestosrum* (Coccinellidae) had lower survival potential when sprayed with *Beauveria bassiana* fungus than that with (*Paecilomyces fumosoroseus*) fungus Sabbour *et al.* (2012). Shanthakumar *et al.* (2010) and Magda ad Shadia (2010) considered that in spite the great virulence of (*N. rileyi*) against *Spodoptera littura*, the pathogen proved reasonable safety to *trichogramma chilonis.* It did not cause reduction in their parasitisation percentages.

The present results also indicated that the predator, *C. undeciumpunctata*, particularly adult predators can distinguish between fungus infected from non-infected preys and they almost avoid treated ones, especially if given free choice

feeding. This, however, was more pronounced in case of (*N.rileyi*) than (*P. fumosoroseus*), Sabbour and Shadia (2017 and 2018) Such observed phenomenenn in our investigations, was viewed by many authors. It was mentioned that predators, when given free choice to feed upon fungus –treated or untreated aphids, predation on infected preys was less than uninfected ones (Baverstock, *et al.,* 2007). Also, Roy *et al.* (2010); Goettel *et al.* (1990); Husseien *et al.* (2015) proved that *C. septempunctata* adults avoid contacting with leaf and soil surface innoculated with (*B.bassiana*) fungus and mycosed cadavers. The predator was positioned away from mycosad cadaver than uninfected ones. Neverthless, some researches indicated several adverse effects of some entomopathogenic fungi against some natural enemies. It was considerd that *C. septempunctata* was somewhat susceptible to (*B.b*) (Haseeb and Murad,1997; Delete *et al.,* 1995; Zaki and Abdel-Raheem, 2010). Sabbour (2008); Sabbour and Nayera (2016 a,b,c); Sahab *et al.* (2015) consider that some entomopathogenic formulations of *B. bassiana* shows promising impacts againts pests. Sabbour and Singer (2016) recorded the deletorious effects on *C.udecimpunctata* if applied at high concentration levels. However different views about the safety of entomopathogenic fungi, declared by many authors, might be due to the relative efficacy of the fungus or its isolates on pests exhibit different susceptibilities,bionomics and characters as well as types of assessment and application rates (Nayera *et al*., 2016).

Imidacloprid, *B. brongniartii* give a promising control against *M. persicae* they reduce the pest number laboratory conditions Sabbour and Shadia (2016). Under field conditions the pathogen significantly causing a decrease in the target pests. The experiments showed that pathogen Imidacloprid and *B. brongniartii* tested not effect on the *C. undecimpunctata* populations.

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