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Comparative Study of The Efficacy Between Some Formulations of Entomopathogenic Fungi with Botanical and Chemical Pesticides Against Onion Thrips, *Thrips tabaci* Lind)

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ABSTRACT

The onion thrips, and attack many vegetable crops causing great economic damage to them especially onion crops. Four entomopathogenic fungi (EPF) formulations, including isolates of *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) were used against *T. tabaci*. Two wettable powders and two emulsions that have been containing *B. bassiana* and *M. anisopliae*, (WPB, WPM, EB and EM respectively) in this study were treated in comparison to a botanical-insecticide Azadiractin (Oikos 3.2 % EC) and a chemical insecticide Thiamethoxam (Actara 25 WG) as a recommended pesticide against *Thrips tabaci*. Field trials were conducted during the 2021 season, at the experimental farm, Faculty of Agriculture, Moshtohor, Banha University, Toukh district, Qalubiya Governorate and in plant protection institute farm, Doki, Giza Governorate. Our results showed that all the tested formulations of EPF and Oikos began to kill insects throughout the period from the 3rd day and the reduction percent increased as the period increased to the 10th day, while, Actara kill insects after 24 hours. After 10th days of treatment, the average general effects were 76.968, 74.53, 73.185, 72.087, 70.4 and 68.625 for EB, WPB, Actara, EM, Oikos and WPM, respectively. These results revealed that four *B. bassiana* and *M. anisopliae* formulations were effective against *T. tabaci* in field conditions, especially *B. bassiana* formulations. So we can suggest that these formulations of entomopathogenic fungi should be applied in integrated pest management (IPM) against *T. tabaci*.

INTRODUCTION

Thrips tabaci (Lindeman) (Thysanoptera: Thripidae) is a significant insect pest of many crops, vegetables and ornamentals that causes economic losses in the open field and greenhouse (Diaz-Montano, *et al.*, 2011; Reitz, *et al.*, 2011). The damage to onion plants by direct nutrition on plant sap consists of silvery scars, decreasing photosynthesis, leading to volume reduction and production loss as long as decreasing the quality and quantity. This infestation cause of decrease the onion yield (Alston and Drost, 2008). The damage is caused by the feeding of nymphs and adults on leaf tissue (Trdan *et al.*, 2005). The onion thrips has been considered as the vector of many plant viruses diseases such as onion Iris yellow spot virus, which reduces bulb size (Gent *et al.*, 2004), tobacco streak virus (Sdoodee and Teakle, 1987), tomato spotted wilt virus (Kritzman *et al.*, 2002; Jenser *et al.*, 2003), and soybean

mosaic virus (Hardy and Teakle, 1992). Also, these insects are transmitters of a tospovirus, which has a prevalence in many important onion-producing areas of the world and causes a crop loss of up to 100% (Diaz-Montano *et al.*, 2011). The uses of synthetic insecticides for thrips control in recent decades have caused environmental pollution of the ecosystem, insecticides residue, and insecticides resistance and are costly (Bielza, 2008; Gao *et al.*, 2012). Due to the difficulties in controlling thrips populations with synthetic insecticides, there is an increasing interest in new and efficient alternatives such as botanical insecticides and microbial biocontrol agents like EPF. It has been claimed that the terpenoid derived from neem oil, azadirachtin, acts as an insecticide, an insect development regulator, and a repellent/antifeedant. It is one of the most widely used bioinsecticides (Roychoudhury, 2016). Products containing azadirachtin are advised for the control of soft-bodied and immature insects, beetles, and caterpillars (Dayan *et al.*, 2009). Entomopathogenic fungi like *B. bassiana* and *M. anisopliae*, which have been the subject of several papers describing their effects, are appealing as bio-pesticides for use in IPM because of their combination of host specificity and safety data (Bateman *et al.*, 1993). Numerous isolates of *B. bassiana* and *M. anisopliae* have recently been found to be extremely potent against a variety of agricultural pest arthropods, such as whiteflies, thrips, aphids, weevils, mealybugs, and psyllids. (Shah and Goettel, 1999; Shiberu *et al.*, 2013; El-Sheikh 2017). Therefore, the purpose of the current study was to assess the effectiveness of *B. bassiana* and *M. anisopliae*, and compared them with Actara insecticide against onion thrips, *T. tabaci* in onion fields, under Egyptian conditions.

MATERIALS AND METHODS

Treatments:

1. Bio-Insecticide:

Four formulations of bio-insecticides were tested. They were prepared in Bio-insecticide Production Unit, Plant Protection Research Institute, Agricultural Research Center, Egypt.

Two wettable powders that have been prepared in the Bio-insecticide Production Unit laboratory, containing the EPF *B. bassiana* and *M. anisopliae*, in this study, it is symbolized by (WPB and WPM), respectively. The wettable powder has been prepared according to Sahar, (2016) in which the final concentration of each wettable powder is 1×10^8 conidia/gram.

Also, two laboratory emulsions were prepared, containing the EPF *B. bassiana*, (EB) and *M. anisopliae*, (EM), each separately. The emulsion has been prepared according to Sahar, (2016) but some components of the emulsion were modified in these formulations, as Span 60 was added as an emulsifier instead of the used emulsifiers in the previous study, to become the final concentration is 1×10^8 conidia/ml.

2- Botanical Insecticide:

The fifth tried treatment was a botanical-insecticide Oikos 3.2 % EC containing Azadiractin as an active substance obtained from Lotus for agricultural development, Egypt, and it was used to control the whitefly on tomatoes.

3- Chemical Insecticide:

Common name: Actara 25% WG (Thiamethoxam) was obtained from Syngenta, Egypt.

Table (1): List of treatments and application concentrations:

Treatments			Rate of application
1- entomopathogenic fungi	Wettable powders (WP)	<i>B. bassiana</i> (WPB)	250 g /100 L.
		<i>M. anisopliae</i> (WPM)	250 g /100 L.
	Emulsions (E)	<i>B. bassiana</i> (EB)	250 ml /100 L.
		<i>M. anisopliae</i> (EM)	250 ml /100 L.
2-Botanical insecticide	Oikos 3.2 % EC	Azadiractin	100 ml /100 L.
3-Chemical insecticide	Actara 25% WG	Thiamethoxam	20 g /100 L.

Experimental Sites:

The present study was carried out on two plots of land, each one in a different governorate in Egypt. The first one was conducted at Moshtohor, Toukh district, Qalubiya Governorate and the second was in plant protection institute farm, Doki, Giza Governorate, during the 2020/2021 season. The objectives of this research were to evaluate and compare the role of native entomopathogenic fungi as two formulations, a botanical-insecticide of the neem substance, and a chemical insecticide on the growth in the onion thrips population in the Onion (*Allium cepa* L.) field.

Crop Establishment and Experimental Activities:

Onion cultivar, 'Red Creole' was transplanted in four replicates of a complete randomised block design with four rows each replicate (5m-long). To prevent contamination from spraying drift, plants were placed 10 cm apart inside rows and 30 cm apart between rows. Each block was also given a 1 m-long separation. The other 4 plots were left without any treatment as a control. The seedlings of onion were sown in early December 2020, using the recommended agriculture practices.

The experimental area was divided into seven treatments including control. The trial started on March 9th season 2020/2021, when the population of *T. tabaci* (adult and nymph) is known to be high.

Samples were randomly chosen and directly inspected as 30 plants from each plot with counted alive nymphs and adults on a white paper before spraying, after 24 hours for Actara and also at 3, 5, 7 and 10 days after treatment for all formulations. To avoid affecting the fungi, the foliage treatment was made in an open field one hour before dusk (about 5:00 PM).

Data Analysis:

In the current investigation, the percentage reduction values (percent mortality) were corrected according to the equation of Hinderson and Tilton (1955).

$$\% \text{ Reduction} = \left\{ 1 - \frac{\text{Treatment after x control before}}{\text{Treatment before x control after}} \right\} \times 100$$

The main effects significance was analyzed using Costat- program methods software version 2005 by analysis of variance two-way (ANOVA). Based on data transformation, and effectiveness analysis for Arcsine was carried out (Gomez and Gomez 1984). Least Significant Differences (LSD) test for comparison between Duncan's multiple range test and formulations ($P \leq 0.05$).

RESULTS**Effect of Entomopathogenic Fungi, Okios and Actara Applications Against Onion Thrips Population:**

The present study was conducted using four formulations of entomopathogenic fungi (WPB, WPM, EB and EM), Okios and Actara against the onion thrips population in Qalubiya Governorate and Giza Governorate, during the 2020/2021 season.

Data presented in (Tables 2 & 3) showed the significant effect of all treatments on the population density of thrips. The results indicate the correct percent reduction of thrips in Qalubiya Governorate was more than Giza Governorate in all treatments. This deferent in results may be back to deferent in the population density of *T. tabaci*.

Data in (Table 4) demonstrate the mean respective reduction percentages of onion thrips which were caused after 24 hours of using Actara, furthermore at 3-, 5-, 7- and 10-days following treatment spraying in the two governorates. After 24 hrs of spraying, Actara caused a 78.2% reduction. After three days of treatment, all the evaluated formulations against thrips were significantly compared with the control plots. Actara was recorded the highest reduction percent (79.025%) followed by WPB (64.125 %), EB (61.325 %), WPM (61.175 %) and EM was (60.7 %). While Oikos recorded the lowest reduction percent (59.75 %). After 5 and 7 days of treatment, significant differences in reduction percent were high in plots treated with EB and WPB (80.8 and 77.076%, respectively). However, the remaining therapies with decreased percentages were 75.32, 75.075, 72.75 and 71.525 for EM, Actara, Oikos and WPM, respectively. Moreover, at ten days, the highest reduction percentage was also recorded from EB treatment (83.97) while the lowest reduction percent was observed for Actara (58.55).

Moreover, these results showed that all the tested entomopathogenic fungi formulations and Oikos began to kill insects throughout the period from the 3rd day and the reduction percent increased as the period increased to the 10th day, while, Actara kill insects after 24 hours (table 4). Results showed that the combined efficacy of treatments in two area were in the order of *B. bassiana* (EB, WPB) (76.96, 74.53, respectively) > Actara (73.185) > *M. anisopliae* (EM) (72.0875) > Oikos (70.4) > *M. anisopliae* (WPM) (68.625) Table (4).

Table 2: Corrected % reduction after spraying at various intervals Qalubiya Governorate during the season (2020/2021).

Treatment	Pre-spray (No. of thrips/plant)	Corrected % reduction in thrips population over control				
		24 h.	3 days	5 days	7 days	10 days
WPB	89.8		70.46	83.88	85.217	80.58
WPM	90.5		67.2	81.54	88.59	78.09
EB	88.6		66.08	84.23	89.69	85.12
EM	91.35		68.75	86.79	87.17	84.36
Oikos	90.25		65.02	84.52	88.54	82.21
Actara	90.15	85.67	83.67	79.57	79.53	67.218
Cont.	92.5	0.00	0.00	0.00	0.00	0.00

Table 3: Corrected percent reduction after spraying at various intervals Giza Governorate during the season (2020/2021).

Treatment	Pre-spray (No. of thrips/plant)	Corrected % reduction in thrips population over control				
		24 h.	3 days	5 days	7 days	10 days
WPB	44		57.87	70.27	70.49	79.3
WPM	44.7		55.9	61.49	63.76	62.8
EB	43.4		56.3	77.55	77.36	83.22
EM	45		52.09	64.18	67.56	70.94
Oikos	45.5		55.2	61.32	70.62	70.8
Actara	45	70.53	74.73	70.57	69.84	50.48
Cont.	41.1	0.0	0.0	0.0	0.0	0.0

Table 4: Average percent reduction after spraying at various intervals in two areas, Qalubiya Governorate and the second were Giza Governorate, during the season (2020/2021).

Treatments	% Reduction in thrips population over control					the combined efficacy of treatments
	24 h.	3days	5 days	7 days	10 days	
WPB		64.125 ^b	77.07 ^b	77.05 ^b	79.875 ^b	74.53
WPM		61.175 ^c	71.525 ^d	71.525 ^d	70.276	68.625
EB		61.325 ^c	80.8 ^a	80.8 ^a	83.975 ^a	76.96875
EM		60.7 ^c	75.325 ^{bc}	75.325 ^{bc}	77 ^{bc}	72.0875
Oikos		59.75 ^c	72.75 ^{cd}	72.75 ^{cd}	76.35 ^c	70.4
Actara	78.2	79.025 ^a	75.075 ^{bc}	75.075 ^{bc}	58.55 ^e	73.185
LSD		2.51	3.0077	3.0077	3.1012	

DISCUSSION

These results are similar to Ain *et al.*, (2021). They evaluated formulations of *B. bassiana* (strain GHA) and *M. anisopliae* (strain ESC) at four concentrations (10^8 , 10^9 , 10^{10} and 10^{11} conidia/ ml) against *T. tabaci* on onions for two years under field conditions. They assessed the efficacy at 3, 5, 7 and 10 days after spray application. After 10 days of application, they noticed that the highest corrected percent decrease in onion plants treated with *B. bassiana* 10^{11} conidia/ ml was 86.62, 84.59, and 86 percent in 3 different onion cultivars. While they showed minimum corrected percent reduction in plants treated with *M. anisopliae* 10^8 conidia/ ml was 69.42, 68.45 and 69.11% in different onion varieties after 10 days of spray.

Also, these results are consistent with earlier reports of El-Sheikh (2017) who study the effectiveness of *B. bassiana* and *M. anisopliae* isolates compared with Actelic 50% as a recommended pesticide against *T. tabaci* in the onion field. He found that the reduction in insect number was 62.87, 74 and 87.08 for *M. anisopliae*, *B. bassiana* and Actelic 50%, respectively. Like that, Shiberu *et al.*, (2013) evaluate *B. bassiana* (PPRC-56) and *M. anisopliae* (PPRC-6) and some botanical extracts to control *T. tabaci*. They claimed that there was no discernible difference between entomopathogenic fungus and the untreated control after the first day of treatment of the various agents. Nevertheless, they showed that the mortality rate was highly significant on the third, fifth, and seventh days following the administration of *B. bassiana* and *M. anisopliae* treatments. On the other hand, they reported that the applied botanical extracts recorded mortality ranging from 26.09 to 74.75% on 3rd day of application.

These findings concur with Neil *et al.*, (2004)'s research, which stated that *B. bassiana* can kill the insect from 3 to 7 days after infection. They mentioned that leaving of *B. bassiana* mass of conidia can spread to other insects. Also, in laboratory bioassays, Shengyong *et al.*, (2013) evaluated 20 isolates of *B. bassiana* against *T. tabaci*. They discovered that strain SZ-26 was the most lethal, and kill 83 to 100% of adult insects at a rate of 10^7 conidia /ml⁻¹ after 4 to 7 days. Meditate of data indicates that by that time, the efficacy of entomopathogens has increased. This is because entomopathogens unlike insecticides, do not have the ability to be quick effect knock down, but they take time to kill insects (Fargues *et al.*, 1994). The mode of action includes the following actions: After the spore is attached to the insect's cuticle, spore germination on the cuticle begins after approximately 10 hours and is completed in 20 hr. at 25°C. When a spore germinates, it produces proteases and chitinases that break down the proteinaceous and chitinous

components of the cuticle at non-stiff places like mouthparts, joints, and between segments, permitting hyphal penetration. After successful penetration, the fungus spreads to the host insect's other tissues and produces toxic secondary metabolites, which causes the host to die and triggers saprophytic outgrowth from the dead host as well as the generation of new conidia (Logrieco, 2002, Fariaa and Wraight, 2007).

On the contrary, Reddy *et al.*, (2019) evaluated *M. anisopliae* and *B. bassiana* against thrips, *Scirtothrips dorsalis* Hood in grapes and compared with an insecticide (thiamethoxam 25% WG) as a stander check. They showed that the pooled corrected percent reduction in two years was thiamethoxam 25% (89.18) > *M. anisopliae* (83.22) > *B. bassiana* (66.93) after 14 days of spray.

Additionally, using different formulations of EPA, a substantial variation in the percentage reduction in onion thrips population was seen, *B. bassiana* (EB) (76.96) > *B. bassiana* (WPB) (74.53), *M. anisopliae* (EM) (72.0875) > *M. anisopliae* (WPM) (68.625) Table (4).

These results indicate that the emulsion formulations may be to improve adhesion to the insect cuticle. The effectiveness of this fungus is improved by the formulation in oil, according to these findings, which suggests that using species with lipophilic conidia in less humid agricultural conditions may be possible (Bateman, 1993). Spray drops of conidia-containing oil stick to insects' waxy cuticles more firmly than drips of water do, and by rapidly spreading upon contact. The intersegmental folds and other protected parts of the host, where the microenvironment supports quick germination and penetration, are thought to be the locations where the oils convey conidia (Ibrahim, *et al.*, 1999; Luz, Rodrigues, & Rocha, 2012).

CONCLUSION

From this study, we recommend that enter these formulations of entomopathogenic fungi especially *B. bassiana* in integrated pest management against *T. tabaci* when used before high huge thrips populations, at the economic threshold level. But with a large thrips population, we can spray any recommended pesticides to reduce the population and then use entomopathogenic fungi.

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ARABIC SUMMARY

دراسة مقارنة لفعالية بعض مستحضرات الفطريات الممرضة للحشرات مع المبيدات النباتية والكيميائية ضد تريبس البصل، *Thrips tabaci* (Lind)

حسن محمد السعدنى، سحر سيد على، عبد الحليم السيد خليل
معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - جيزة - مصر

يهاجم تريبس البصل العديد من محاصيل الخضروات مسبباً أضراراً اقتصادية كبيرة عليها خاصة محصول البصل. تم استخدام أربع مستحضرات من الفطريات الممرضة للحشرات لعزلات من فطرى *Beauveria bassiana* و *Metarhizium anisopliae* ضد حشرة تريبس البصل. في هذه الدراسة تم استخدام اثنين من المساحيق القابلة للبلل للفطرين *B. bassiana* (WPB) و *M. anisopliae* (WPM)، اثنين من المستحلبات المحتوية عليهما (EB و EM على التوالي) بالمقارنة مع مبيد حشري نباتي أذرختين (أويكوس 3.2٪ و مبيد حشري كيميائي ثياميثوكسام (أكتارا 25٪) كمبيدات حشرية موصى بها ضد حشرة تريبس البصل. أجريت هذه الدراسة خلال موسم 2021 بكل من المزرعة التجريبية بكلية الزراعة بمشتهر جامعة بنها بمركز طوخ بمحافظة القليوبية ومزرعة معهد وقاية النبات بالدقي بمحافظة الجيزة. أظهرت النتائج أن جميع المستحضرات المختبرة من الفطريات الممرضة للحشرات و أويكوس بدأت في قتل الحشرات بداية من اليوم الثالث وزادت نسبة الخفض في تعداد الحشرات مع زيادة الفترة إلى عشرة أيام، بينما قتل مبيد الأكتارا الحشرات بعد 24 ساعة فقط من المعاملة. بعد عشرة أيام من المعاملة كانت متوسط نسب الخفض 76.968 و 74.53 و 73.185 و 72.087 و 70.4 و 68.625 لكل من EB و WPB ومبيد الأكتارا و EM وأويكوس و WPM على التوالي.

أظهرت هذه النتائج أن الأربعة مستحضرات من *B. bassiana* و *M. anisopliae* كانت فعالة ضد حشرة تريبس في الظروف الحقلية وخاصة تركيبات *B. bassiana*. لذلك يمكننا أن نقترح أنه ينبغي تطبيق مستحضرات الفطريات الممرضة للحشرات في الإدارة المتكاملة للأفات ضد حشرة تريبس البصل.