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## THE INTEGRATION BETWEEN *TRICHOGRAMMA EVANESCENS* WEST. (HYMENOPTERA: TRICHOGRAMMATIDAE) AND SELECTED BIOINSECTICIDES FOR CONTROLLING THE POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* (ZELL.) (LEPIDOPTERA: GELECHIIDAE) OF STORED POTATOES

Nasser Said Mandour, Awad Ahmed Sarhan\*, Dina Hussien Atwa

Department of Plant Protection, Faculty of Agriculture Suez Canal University, 41522 Ismailia, Egypt

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**Abstract:** The efficacy of the egg-egg parasitoid *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) and certain bioinsecticides (*e.g.*, Neemix, Virotecto, Agerin, Dipel 2x and Spinosad) for controlling the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) under storage conditions was studied. Single and combined treatments of *T. evanescens* and the bioinsecticides were tested. Neemix and spinosad were evaluated as spray treatments. Virotecto, Agerin and Dipel 2x were evaluated as dust and spray treatments. Data were recorded in terms of rate of infestation, reduction of infestation, number of *P. operculella* pupae, and number of mines per 20 tubers as well as percentage of edible parts. Data revealed that the percentage of infestation in the control treatment was as high as in the Neemix treatment being 96.67 and 90% after one and two months of storage, respectively. Obviously, Spinosad and Dipel 2x were the most effective bioinsecticides in reducing tuber infestation and number of *P. operculella* recovered pupae. Virotecto and Agerin reduced rate of infestation and number of recovered *P. operculella* pupae over that of the control, but their effect was significantly lower than that of Spinosad or Dipel 2x. Moreover, there were significant differences among treatments in the form of the number of *P. operculella* tunnels and percentages of edible parts after two months in storage. Data further indicated that the integration between the tested bioinsecticides and *T. evanescens* enhanced the control of *P. operculella*. Significant differences in percentages of infestation, edible parts, number of recovered pupae as well as number of mines in stored potatoes existed between individual treatments (without *Trichogramma*) and combined treatments.

Key words: Phthorimaea operculella, Trichogramma evanescens, bioinsecticides, infestation, integration, storage

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important vegetable crop in Egypt with 107,953 cultivated hectares, and a total production of 2,760,164 tons in 2007 (Ministry of Agriculture of Egypt, 2007). Under field conditions, potato plants are under attack by a large number of insect pests such as aphids, leafhoppers, and Lepidopterous pests. The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is the most destructive pest. In addition to potato, *P. operculella* also attacks other solanaceous plants such as tomato, tobacco, eggplant and pepper in tropical and subtropical countries, but potato is still the preferred host plant (Shelton and Wayman 1979; Mandour 1997; Keasar and Sadeh 2007).

In potato fields, the female *P. operculella* lays its eggs on the underside of the potato leaves or on the exposed tubers mainly after irrigation where potatoes are cultivated under the flooding irrigation system (Mandour 1997). PTM larvae mine the foliage, stems, and tubers (Islam et al. 1990; Mandour 1997). The larvae of P. operculella also form blotches in leaves and fold leaves over for shelter during feeding and for pupation (Mandour 1997). The new mines are always present in the upper part of the potato canopy. The old blotches, harboring the older larvae of PTM, are likely to be found the lower parts of the potato canopy particularly near the soil surface. Larvae may also bore into potato stem and petioles (Mandour 1997). There is usually a 10% rate of tuber infestation by P. operculella, but when control measures are not used, the infestation rate may reach 100% (Shelton and Wayman 1979; Mandour 1997; Sileshi and Teriessa 2001). In potato stores, infestation of P. operculella also causes partial or complete rotting by the subsequent infestation with fungi and/or bacteria. When rotting is present, it means the infested tubers are unmarketable (Shelton and Wayman 1979; Sarhan 2004).

Until the last 2 decades, the control of *P. operculella* has relied upon the use of the traditional insecticides (Sarhan 2004; Keasar and Sadeh 2007). Recently, biological control

dr.awadsarhan@yahoo.com

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of *P. operculella* using bioinsecticides and natural insect enemies has become the backbone in potato protection, either in the field (Sileshi and Teriess 2001; Agamy 2003) or in potato storage (Farrag 1998; Moawad *et al.* 1998; Sarhan 2004; Keasar and Sadeh 2007; Mandour *et al.* 2009), and has gained more credibility for controlling this pest. Increased research efforts have been taking place for integration using natural enemies (parasitoids, predators and entomopathogens, Mandour *et al.*, 2008).

*Trichogramma evanescens* West. (Hymenopetra: Trichogrammatidae) is a native egg-egg parasitoid in Egypt. It is used worldwide against several lepidopteran pests including *P. operculella* (Agamy 2003; Sarhan 2004; Mandour *et al.* 2008). *T. evanescens* kills the hosts before they reach the plant-feeding or tuber-mining stage. In earlier studies, although there was significant reduction in PTM populations in natural-enemy treatments as compared to the control ones, *P. operculella* still inflicted heavy damage to stored potatoes (Sileshi and Teriess 2001; Sarhan 2004; Keasar and Sadeh 2007; Mandour *et al.* 2009). In this case, integration between an egg parasitoid such as *T. evanescens* and bioinsecticides may result in satisfactory control of *P. operculella* inside potato stores (Mandour *et al.* 2008).

With control of *P. operculella* under storage conditions in mind, the main objective of this study was to evaluate the effectiveness of certain bioinsecticides (Neemix, Virotecto, Agerin, Dipel 2x and Spinosad) and the parasitoid, *T. evanescens* in individual or combined treatments.

### MATERIALS AND METHODS

#### Rearing of P. operculella and T. evanescens

Laboratory cultures of *P. operculella* and *T. evanescens* were taken from the stock colonies kept on potato tubers under the laboratory conditions of  $25\pm2^{\circ}$ C,  $60\pm10\%$  Relative humidity (RH) and 14 h photophase in the Biological Control Center, Suez Canal University, Egypt. The ovipositional cage for PTM moths consisted of a glass jar (30 cm in height x 20 cm in diameter) covered with a piece of muslin cloth. Eggs of *P. operculella* were laid on the cloth sheet. Larvae of *P. operculella* were reared on potato tubers using the method described by Mandour (1997). As for *T. evanescens*, eggs of *P. operculella* were exposed to adult wasps in a glass tube (3x10 cm) for parasitization. *T. evanescens* adults were fed on a honey solution smeared on the inner wall of the glass tube.

#### **Tested bioinsecticides**

Five bioinsecticides were evaluated in this study. These bioinsecticides were Agerin, Dipel 2x (both are *Bacillus thuringiensis* – based insecticides), Virotecto (PTM virus), Spinosad and Neemix. Agerin, Dipel and Virotecto were tested as dust and spray treatments; whereas Spinosad and Neemix were tested only as spray treatments. The rate of application was identical to that described by Mandour *et al.* (2009). Spinosad and Neemix were used at the field rate of 0.5 ml/liter. Virotecto and Dipel 2x were used at 150 g/ton tubers for the dust treatment and at 1 g/ liter for the spray treatment. Agerin was tested at the recommended rate of 250 g/ton tuber for the dust treatment or 250 g/40–60 liter for the spray treatment.

#### Experiments

## Evaluation of selected bioinsecticides against *P. operculella*

This experiment was performed in a laboratory (4 m in width x 4 m in length x 3.5 m in height) at abiotic conditions of 25±2°C, 60±10% RH and 14 h photophase. Potato tubers were purchased, and then sorted out. Spoiled and PTM-infested tubers were removed and discarded. Only non-infested tubers were used. Four kilograms of potato tubers were assigned to form one replicate (each kg contained 6–7 tubers). Each treatment of biocides was replicated 6 times with a total of 24 kg tubers per treatment. The tested treatments were Neemix, Virotecto solution (VS), Virotecto dust (VD), Agerin solution (AS), Agerin dust (AD), Dipel solution (DS), Dipel dust (DD) and Spinosad.

The methodology and experimental setup used in this experiment were similar to that described earlier by Mandour et al. (2009). An assigned weight of potato tubers for each replicate was placed on a clean piece of cloth. Tubers were moistened with tap water using a small handled sprayer. The moistened tubers were then dusted with the assigned bioinsecticide using a small handled duster. Treated tubers were left to dry out for 2-3 hours. After that, the treated tubers were placed in a wooden cage (60 cm in width x 70 in length x 70 cm in height). A fine layer of clean sand (3–5 cm) was placed under the tubers in each cage to serve as the pupation site for PTM larvae. Cages were closed tightly, labeled, and placed on a steel stand under the aforementioned conditions. As for spray treatments, fresh solutions of the tested bioinsecticides were prepared 30 minutes prior application. The experimental setup and protocol including replicates, size of replicates, storage methods and doses were the same as those used in the dust treatment (Mandour et al. 2009). The control treatment was performed using the same protocol and replicates but without bioinsecticide application.

An artificial infestation was made using the rate of 6 PTM pupae/kg tubers, which was sufficient to inflict a 100% infestation rate, thus, the potato tubers were totally unmarketable (emergence rate was 90%, Atwa 2009). PTM pupae were placed in a Petri dish (9 cm), which in turn was placed inside each cage. After that, the tubers were checked weekly to remove the spoiled tubers. When PTM larvae start to pupate, the sand layer (under each treatment) was sieved thoroughly twice a week to collect the developed PTM pupae. Moreover, 20 tubers from each treatment were randomly selected each month to calculate the percentage of infestations, and the edible parts. This experiment lasted 2 months. Data were recorded in terms of rates of infestation, edible parts, and number of PTM pupae as well as number of PTM tunnels/20 tubers in each treatment.

# Integration between *T. evanescens* and bioinsecticides against *P. operculella*

The obtained data in experiment 1 indicated that while some bioinsecticides treatments (*e.g.* spinosad and

Dipel 2x) showed full protection against PTM, others (*i.e.* Neemix, Virotecto and Agerin) did not give satisfactory control of PTM. Therefore, in this experiment the integration of the egg parasitoid *T. evanescens* (TE) and the bioinsecticides Neemix, Virotecto and Agerin was investigated in cages under the previously mentioned conditions.

The methodology used in this experiment was similar to that described in experiment 1. After being treated with the biocides, potato tubers were placed inside the wooden cage. The artificial infestation with PTM pupae was initiated at the same rate used in experiment 1. Once adult moths emerged and started to deposit their eggs, T. evanescens was released in each cage at the rate of 6 T. evanescens black eggs (P. operculella eggs harboring Trichogramma' pupae) per one kg potato tubers. Trichogramma pupae (black eggs) were released to emerge in successive waves. This form of release was done to guarantee the continuous presence of younger parasitoid females throughout the whole ovipositional periods of PTM moths. Data were recorded as percentages of infestation and edible parts, total number of PTM pupae, and number of PTM tunnels in the tubers.

#### Statistical analysis

All obtained data were analyzed using SAS package 8.2 v (SAS Institute 2003). Proportional data were transformed by arcsine square root before analysis. Data of percentages of infestation, reduction in PTM infestation, percentages of edible parts as well as number of mines were compared using ANOVA analysis of variance. When F values were significant, means were separated using Fisher's least significant differences (FLSD) at a 0.05 level of significance (SAS Institute 2003). The T-test was used to compare the results of single and combined treatments.

### RESULTS

## Evaluation of selected bioinsecticides against *P. oper-culella*

As shown in table 1, Spinosad and DD treatments gave satisfactory control of P. operculella. After 2 months of storage, no infestation was observed in the spinosad treatment, but infestation was minimal at 3.33% in the DD treatment (Table 1). Significant differences existed between the tested treatments after a month (F = 11.04; P = 0.0001) and 2 months (F = 8.20; P = 0.0001) of storage. Moreover, the numbers of recovered P. operculella pupae differed significantly between the biocide treatments and the control after a month (F = 194.17; P = 0.0001) and 2 months (F = 39.97; P = 0.0001) of storage. The tested bioinsecticides had a negative impact on the number of P. operculella mines (F = 69.51; P = 0.0001, table 1). The greatest number of mines was recorded in the control at 154 mines/20 tubers whereas the lowest was observed in the DD dust treatment - at 4.67 mines/20 tubers.

Moreover, the percentages of edible parts decreased gradually as the storage period increased. Spinosad showed full protection with an edible part of 100% after 2 months of storage. Significant differences among tested bioinsecticides in percentages of edible parts recorded after a month (F = 11.78; P = 0.0001) and 2 months of storage (F = 15.53; P = 0.0001) (Fig. 1).

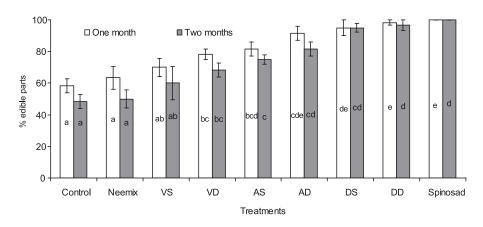
## Integration between *T. evanescens* and bioinsecticides against *P. operculella*

As shown in table 2, the integration between the tested bioinsecticides and *T. evanescens* resulted in a significant reduction in the infestation rates of stored potatoes (F = 102.20; P = 0.0001), with a subsequent reduction in the number of recovered PTM pupae (F = 220.92; P = 0.0001)

Table 1. Mean (±SE) of percentage of infestations, number of *P. operculella* recovered pupae, and number of mines in stored potato tubers treated with different bioinsecticides in cages

Treatments	% of infestation after		No. of recovered PTM pupae after		No. of PTM
	1 month	2 months	1 month	2 months	mines/20 tubers*
Control	96.67±3.33 a	90.00±5.77 a	177.33±2.60 a	31.33±0.88 a	154.00±7.09 a
Neemix	90.00±5.77 a	85.00±7.64 a	132.67±5.70 b	25.33± 2.96 b	111.33±6.89 b
VS	68.33±9.28 ab	71.67±11.67 ab	117.67±6.44 c	23.67±2.91 b	97.00±8.72 b
VD	66.67±8.81 ab	60.00±9.28 ab	67.67±5.55 d	6.00±2.31 cd	53.67±5.49 c
AS	43.33±1.86 b	41.67±8.78 b	54.66±4.81 ed	9.33±2.96 c	49.00±8.08 c
AD	38.33±1.67 bc	36.67±3.33 bc	46.00±5.57 e	2.67± 1.67 de	39.33±4.48 c
DS	3.33±1.67 c	5.00±1.89 cd	14.66±3.18 f	0 e	15.33±2.91 d
DD	1.67±0.67 c	3.33±0.67 cd	3.33±1.86 gf	0 e	4.67±0.45 d
Spinosad	0 c	0 d	0 g	0 e	0 e
P&F values	F = 11.04; P = 0.0001	F = 8.20; P = 0.0001	F = 194.17; P = 0.0001	F = 39.97; P = 0.0001	F = 69.51; P = 0.0001
LSD	33.75	36.65	13.26	5.85	18.36

VS – Virotecto spray; VD – Virotecto dust; AS – Agerin spray; AD – Agerin dust; DS – Dipel solution; DD – Dipel dust; P – P and F values are statistical data of probability and F values; PTM – potato tuber moth; FLSD – Fisher ' Least Significant differences Different letters (column wise) indicate significant differences (FLSD; p < 0.05)



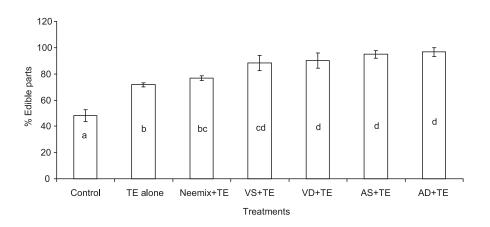
VS – Virotecto spray; VD – Virotecto dust; AS – Agerin spray; AD – Agerin dust; DS – Dipel solution; DD – Dipel dust. Same letters indicate no significant difference (FLSD; p > 0.05)

Fig. 1. Effect of treatment with bioinsecticides on the percentage of edible parts of stored potatoes after one and two months of storage

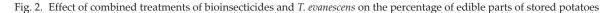
Table 2. Mean (±SE) of percentage of infestations, number of *P. operculella* recovered pupae, and number of mines in stored potato tubers in combined treatments of bioinsecticides and *T. evanescens* after 2 months of storage

Treatments	% infestation No of recovered PTM pupae		No. of PTM mines/20 tubers*	
Control	96.67±3.33 a	208.67±2.96 a	154.00±7.09 a	
TE only	71.67±6.01 b	62.00±7.37 b	51.00±1.53 b	
Neemix + TE	45.00±2.89 c	40.67±5.61 c	50.33±1.86 b	
VS + TE	41.67±4.41 c	25.33±4.84 d	53.33±4.91 b	
VD + TE	13.33±1.68 d	17.67±3.53 de	20.00±3.00 c	
AS + TE	8.33±1.6 d	12.00±3.21 de	21.67±2.85 c	
AD + TE	3.33±1.66 d	7.67±4.33 e	16.67±2.73 c	
F & P values	F = 102.20; P = 0.0001	F = 220.92; P = 0.0001	F = 151.44; P = 0.0001	
LSD	10.47	14.48	11.74	

VS – Virotecto spray; VD – Virotecto dust; AS – Agerin spray; AD – Agerin dust; TE – *T. evanescens* Means followed with the same letters (column wise) are not significantly different (FLSD; p > 0.05) \*based on data after 2 months of storage



VS – Virotecto spray; VD – Virotecto dust; AS – Agerin spray; AD – Agerin dust; TE – *T. evanescens.* Same letters indicate no significant difference (FLSD; p > 0.05)



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Table 3. Comparison between individual and combined treatments of different bioinsecticides and T. evanescens against P. operculella in cages

Treatments		Parameters			
		% Infestation	No. of PTM pupae	No. of PTM mines	
Control	with TE	71.67±6.01	62.00±7.37	51.00±1.53	
	without TE	96.67±3.33*	208.67±2.96*	154.00±7.09*	
Neemix	with TE	45.00±2.89	40.67±5.61	50.33±1.86	
	without TE	86.67±8.82*	158.00±8.62*	111.33±6.89*	
1/0	with TE	41.67±4.41	25.33±4.84	53.33±4.91	
VS	without TE	75.00±12.58*	141.33±6.89*	97.00±8.72*	
1/D	with TE	13.33±1.68	17.67±3.53*	20.00±3.00	
VD	without TE	66.67±8.82*	73.67±6.74	53.67±5.49*	
10	with TE	8.33±1.6	12.00±3.21	21.67±2.85	
AS	without TE	43.33±21.86*	64.00±5.69*	49.00±8.08*	
10	with TE	3.33±1.66	7.67±4.33	16.67±2.73	
AD	without TE	38.33±21.67*	48.67±5.21*	39.33±4.48*	

VS - Virotecto spray; VD - Virotecto dust; AS - Agerin spray; AD - Agerin dust; TE - T. evanescens \*indicated significant differences between the two compared treatments (T-test, p < 0.05)

and numbers of mines in tubers (F = 151.44; P = 0.0001). Obviously, the greatest infestation rate was observed in the control treatment - at 96.67%, and the lowest rate was recorded in the AD + TE treatment - at 3.33%. Moreover, combined treatments caused a significant increase in the percentages of edible parts (F = 17.92; P = 0.0001). The highest percentages of edible parts were recorded for AS + TE and AD + TE treatments at 95 and 96.67%, respectively (Fig. 2).

The T-test was used to compare the difference between the results of single treatments of bioinsecticides (without TE) with those of combined treatments with T. evanescens (with TE). Percentages of infestation showed significant differences between the single or combined treatment as far as control was concerned (t = 3.64; P = 0.4706), Neemix (t = 4.49; P = 0.1935), VS (t = 2.50; P = 0.2188), VD (t = 5.94; P = 0.0690), AS (t = 1.58; P = 0.0127), AD (t = 1.56; P = 0.0107). Significant differences were also observed in the number of recovered PTM as far as control was concerned (t = 18.46; P = 0.2782), Neemix (t = 11.41; P = 0.5945), VS (t = 13.78; P = 0.6614), VD (t = 7.36; P = 0.4299), AS (t = 7.96; P = 0.4844) and AD (t = 6.05; P = 0.8184; table 3). Numbers of PTM tunnels in tubers showed significant differences between single and combined treatments as far as control was concerned (t = 14.19; P = 0.0886), Neemix (t = 8.55; P = 0.1354), VS (t = 4.36; P = 0.4817), VD (t = 5.38; P = 0.4602), AS (t = 3.19; P = 0.2209) and AD (t = 4.12; P = 0.5403, table 3).

### DISCUSSION

Bt-based insecticides (Agerin and Dipel 2x) differed dramatically in their efficacy in controlling PTM. Agerin was not as effective as Dipel in either of the two methods of application (dust or spray). The differences between both biocides might be attributable to: 1) the commercial formulation of both of them, and 2) their recommended doses. In earlier studies, the use of Bt at the recommended dose gave full protection against P. operculella infestation for up to 255 days of storage [Salama et al. (1995) or 3 months (Mandour et al. 2009)]. On the contrary, Salama and Salem (2000) found that Dipel 8 l did not afford acceptable protection against P. operculella. Treatment with Bt-based biocides also caused significant reduction in number of PTM recovered pupae. These findings were also in agreement with those of Mandour et al. (2009) when Agerin and Dipel 2x were tested against PTM in gunny sacks.

Treating potato tubers with Spinosad in this study, showed full protection against P. operculella for up to 2 months. Neither PTM mines nor pupae were observed in the Spinosad treatment. These findings conform those reported earlier by Gomaa and El-Nenaey (2006) who reported 100% protection against PTM with a very long persistence throughout the storage period, and Spinosad was found to be more effective than the organophosphorous insecticide, Tilton (Temerek 2003).

Neemix was ineffective for controlling P. operculella. This conclusion is in agreement with that reported for Neemix (Mandour et al. 2009) or Achook 300 ppm Azadirachtin (Chandel and Chandla 2005). These authors found that neem compounds were not effective against P. operculella during storage. In contrast, neem treatments afforded acceptable protection against P. operculella in storage for up to 3 months (Hossain et al. 1994; Debnath et al. 1998) and neem oil was as effective as the insecticide, Sevin (Salama and Salem 2000).

In the current study, Virotecto was not effective in controlling P. operculella. Islam et al. (1991) came to the same conclusion and also found that granulosis virus (GV) did not protect potato tubers from infestation with P. operculella. On the contrary, GV was reported to be very effective in controlling P. operculella either in storage or in

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the field even as dust, spray or as soil treatments (Chandel and Chandla 2005). Gomaa and El-Nenaey (2006) also found that the application of Virotecto or GV infected larvae more effectively controlled PTM infestation than Bt-based insecticides.

Individual treatment of the T. evanescens caused significant reduction of infestation and decreased number of mines per tubers 3 times as compared to the control. These findings agree with those reported by Saour (2004) who found that Trichogramma spp. caused significant reduction in P. operculella emerged adults and generation survival when wasps were released over potato plants or potato hills. Rubio et al. (2004) also found that a frequent release of T. lopezandinensis at 3 day-intervals in a wooden cage achieved good control of PTM, indicating that the continuous presence of young females of Trichogramma was critical for achieving a better level of parasitization. Mikhael (1999) found that the use of T. minutum caused the lowest number of mines/25 potato tubers. The release of T. evanescens for three times in potato fields caused a drop in the rate of tuber infestation to 6.84% compared to 18.45 and 15.23% in fields sprayed once or twice with Selecron, respectively (Agamy 2003).

The integration of T. evanescens and biocides enhanced the control of PTM in storage over that of single treatment (Trichogramma alone or biocide alone). The number of recovered PTM pupae and number of mines per tubers were 4 and 2 times lower than those of the control, respectively. These results may indicate several facts. First, the tested bioinsecticides (Neemix, Agerin and Virotecto) are not toxic to T. evanescens. Egg parasitoids have been found highly compatible with Bt, as the eggs of the host insect are not the target stage for the microbe. The release of Trichogramma in a Bt-based IPM program in tomato, improved profitability of this program when compared with the use of conventional insecticides (Trumble and Alvarado-Rodriguez 1993). Second, dust treatments of Agerin and Virotecto did not retard the searching parasitoids. Finally, there is an opportunity for further, largescale integration of the biocontrol agents under field or storage conditions. Our findings are in agreement with those reported by Sarhan (2004), who found that biocide Agerin at the recommended dose (150 g/ton) in combination with the predatory anthocorids Blaptostethus piceus, Orius albidipennis or the egg parasitoid, Trichogramma evanescens caused comparable reduction in infestation rate at 96.7, 95.9 and 94.9%, respectively.

To summarize, the application of spinosad and Dipel 2x afforded full protection for stored potato tubers against PTM infestation for up to 2 months. Agerin, Neemix and Virotecto were commercially unacceptable and inapplicable when used as single treatments. The integration between *T. evanescens* and the tested bioinsecticides helped to control PTM as compared to that in single treatments of bioinsecticides.

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