

# Biorational insecticides against the potato tuber moth (Lepidoptera: Gelechiidae) on stored potatoes

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**Key words:** larvicidal, ovicidal, *Phthorimaea operculella*, potato storage, reduced-risk insecticides.

**Abstract:** This study was conducted to evaluate the residual activity and efficacy of spinosad, emamectin benzoate, and chromafenozide on potato tuber moth, *Phthorimaea operculella*. Almost 0% egg hatch of 1-1.5 and 4-4.5-day-old eggs occurred when eggs were treated topically with spinosad at a concentration of 216 mg/L. No ovicidal activity was observed when emamectin benzoate and chromafenozide were tested against the eggs at concentrations of 5, 10, 15 and 37.5, 75 mg/L. Spinosad and emamectin benzoate were equally highly toxic to larvae (100% mortality) even when they were used at low rates. A relatively small proportion of F<sub>1</sub> adults (≈11 to 20%) emerged in the chromafenozide treatment at concentrations of 37.5 and 75 mg/L. One hundred percent larval mortality was noted when potato tubers were sprayed with spinosad and emamectin benzoate and stored for at least 90 days after application. Whereas, chromafenozide applied at 75 mg/L was effective in reducing moth emergence, exhibiting activity for 14 days only after application; thereafter a similar number of F<sub>1</sub> adults occurred in chromafenozide and control treatments. Thus, spinosad and emamectin benzoate could be used to efficiently protect potato tubers from *P. operculella* infestation for three months in unrefrigerated rustic potato stores.

## 1. Introduction

The potato tuber moth, *Phthorimaea operculella* (Zeller) is a widely distributed oligophagous pest of solanaceous crops, including potato, tomato, tobacco, and other cultivated or uncultivated Solanaceae (Cameron *et al.*, 2002). Due to the economically important damage it causes, this pest has a long history of exposure to a broad array of synthetic insecticides. Not surprisingly, *P. operculella* has developed resistance to many of these insecticides, including chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids (Dillard *et al.*, 1993; Symington, 2003).

In the past decade, several classes of conventional insecticides have emerged that show great promise for controlling *P. operculella* (Edomwande *et al.*, 2000; Saour, 2008; Clough *et al.*, 2010). Spinosad is a naturally-derived biorational insecticide with a relatively benign toxicology profile (Aydin and Gürkan, 2005). It is comprised primarily of two macrocyclic lactones, spinosyn A and D, secondary metabolites produced by the actinomycete, *Saccharopolyspora spinosa*, under natural fermentation conditions (Thompson *et al.*, 2000). Insects ingesting spinosad experience paralysis caused by rapid excitation of the nervous system through binding to the nicotinic acetylcholine and/or gamma-aminobutyric acid (GABA) receptors (Salgado, 1998). Currently, spinosad is registered in

over 60 countries and is used to control Lepidoptera pests in many vegetables, fruits, and field crops (Legocki *et al.*, 2010; Wang *et al.*, 2013).

Emamectin benzoate (an epi-methyl amino derivative of abamectin) is a second-generation avermectin analog with exceptional activity against lepidopterous on a variety of vegetable crops worldwide (Ioriatti *et al.*, 2009). Avermectins (a 16-membered family) are naturally occurring macrocyclic lactones isolated from fermentation products of the soil micro-organism *Streptomyces avermitilis* (Ishaaya *et al.*, 2002). Emamectin benzoate causes irreversible activation of chloride channels in the nervous system of insects. Shortly after contact or feeding exposure, the insect larvae stop feeding, become irreversibly paralyzed and die in three to four days. Due to its rapid photodegradation by sunlight, contact activity of emamectin benzoate against insect predators or parasites is limited to a very short period, allowing selective control of some Lepidoptera pests (Sechser *et al.*, 2003).

Chromafenozide is a relatively novel insecticide against lepidopteran larvae characterized by a methyl-chromane moiety in its dibenzoylhydrazine structure. Its mechanism of action is similar to nonsteroidal ecdysone agonists known as an insect-specific ecdysis hormone (Nakagawa, 2005). Although this compound is very toxic to insects, it is safe for mammals and is environmentally benign (European Food Safety Authority, 2013). Chromafenozide on plants is ingested orally by insect larvae to exhibit an ecdysis-promoting activity and lead to death. It is effective in controlling various lepidopterous pests

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(i.e. Tortricidae, Pyralidae, Noctuidae) on vegetables or other agricultural plants (Yanagi *et al.*, 2006).

In reviewing the literature, no experimental data were found related to the direct contact or residual activity of these insecticides against *P. operculella* under storage conditions. Thus, the current study was performed to determine the potential effects of three insecticides - spinosad, emamectin benzoate, and chromafenozide - on the embryonic and early larval stages of the potato tuber moth. Moreover, this study was designed to evaluate the residual activity of the insecticides tested at various intervals up to 90 days after application.

## 2. Materials and Methods

### *Insects*

Insects used in the experiments were obtained from a laboratory stock culture, which is supplemented each year with field-collected *P. operculella* larvae (infested tubers). Larvae were reared on wax-coated potato slices placed in plastic containers (40x25x10 cm). The newly emerged moths were collected and confined in 800 ml transparent plastic jars (10-12 pairs in each jar). A band of filter paper was added to the bottom of each jar for oviposition and 10% sucrose solution was presented as a food source. The rearing procedures were conducted at a constant temperature of  $25\pm1^{\circ}\text{C}$  with  $70\pm5\%$  relative humidity (RH) and a photoperiod of 12:12 h (L:D).

### *Insecticides*

The commercially formulated insecticides used in the present study were spinosad (Spintor® 2 SC, 240 mg [AI]/ml), emamectin benzoate (Proclaim® 05 SG, 50 g [AI]/kg), and chromafenozide (Matric® 5% SC, 50 g [AI]/l).

### *Ovicidal effect*

After the copulation and oviposition period, the first batch of eggs deposited on the oviposition support (white filter paper) from each pair were removed, counted and approximately divided into two groups. The eggs were counted with the aid of a binocular stereo microscope (Kyowa Optical SDZ-PL, Japan). The first group was used for insecticide treatments and the second served as a control. Eggs were kept in a small transparent plastic box (4x3x2 cm) and held at a constant temperature of  $25\pm1^{\circ}\text{C}$  with  $70\pm5\%$  RH, until used.

Treatments were prepared by diluting each commercial formulation of the aforementioned insecticides in 1 L water. *P. operculella* eggs aged 1-1.5 and 4-4.5 days deposited on the oviposition support were dipped for 30 s in three different concentrations 72, 144, 216 ml/L and 5, 10, 15 mg/L for spinosad and emamectin benzoate, respectively (0.3, 0.6, 0.9 ml/L of Spintor and 0.1, 0.2 and 0.3 g/L of Proclaim); while chromafenozide was applied at two concentrations of 37.5 and 75 mg/L (0.75 and 1.5 ml/L of Matric). The concentrations used did not exceed the manufacturer's recommended application rates. A non-ionic or-

ganic surfactant (Agral® 600 g/L nonyl phenol ethylene oxide condensate) was added at 0.15 ml/L as a wetting agent to ensure good dispersal of the preparation. After treatments, the eggs were air-dried and held for incubation. A 0.15 ml/L surfactant solution was used as a control in the experiments. Seven days following treatment the percentage of eggs hatch was recorded. The experiment consisted of three replicates for each concentration of each insecticide with a set of 1000 eggs per replicate for each age group.

### *Larvicidal effect*

*Experiment 1.* Healthy, medium-sized potato tubers of 130-150 g weight ( $n\approx150$ ) were sprayed to runoff with spinosad, emamectin benzoate, and chromafenozide including surfactant at the above-mentioned concentrations, while the untreated tubers (control) were misted with surfactant solution. All tubers were then allowed to air dry at room temperature. After treatments, tubers were deposited over a layer of sand in transparent plastic containers (20x12x10 cm, three tubers per container). First-instar of *P. operculella* larvae (<16 h old) were gently placed on the treated and untreated potato tubers (three larvae per tuber) using a fine camel-hair brush and held at the rearing conditions described above. The number of emerged  $F_1$  adults was recorded. The experiment was conducted three times using a total of 1080 larvae (45x3 larvae for each concentration of each insecticide).

*Experiment 2.* The experiment was carried out to determine whether or not larvae hatched from insecticide-treated eggs are able to survive if they are allowed to develop on untreated tubers (from a practical point of view this is only possible when insecticide-treated and untreated potato heaps were stored together). As described above, 1-1.5-day-old eggs on the filter paper bands were dipped for 30 s in the respective insecticide preparations, air-dried, cut to small sections containing ten eggs and fixed on the surface of uninfested potato tubers using mini paper pins (one egg section per tuber). Eggs of the control were treated with surfactant-added water solution (0.15 ml/L). The tubers were placed in transparent plastic containers (three tubers per container) with a layer of sand completely covering the bottom of the container and held at the rearing conditions. The tubers were visually inspected and the number of emerged  $F_1$  adults was noted. The experiment was repeated three times for each concentration of each insecticide with 120 eggs per replicate.

### *Residual activity*

Four heaps ( $\approx50$  kg each) of healthy, medium-sized potato tubers were thoroughly sprayed with the respective insecticide preparations at the highest concentrations 216, 15 and 75 mg/l for spinosad, emamectin benzoate, and chromafenozide, respectively. Water/surfactant solution was used for the control treatment. After drying, the tubers were stored in the dark at room temperature ( $\approx23^{\circ}\text{C}$ ) in order to reduce tuber weight loss and the accumulation of solanine (Haddadin *et al.*, 2001; Gachango *et al.*, 2008).

Newly hatched larvae were placed on the tubers at 0, 7, 14, 21, 28, 35, 42, 49, 60 or 90 days after insecticide applications (three larvae per tuber and three tubers were placed in each plastic container). The number of emerged  $F_1$  adults was recorded. Three replicates were retained for each stored period of each insecticide with 45 larvae per replicate.

### Statistical analysis

Differences in egg hatchability and emergence of  $F_1$  adults were tested by the analysis of variance (ANOVA) at the 5% level ( $P < 0.05$ ). Significant ANOVAs were followed by the protected least significant difference (PLSD) at  $\alpha < 0.05$ . Differences in egg hatch between 1-1.5 and 4-4.5-day-old eggs were determined using paired-samples  $t$ -test (StatView Version 4.02; Abacus Concepts, 1994). Data were arcsine transformed prior to analysis to stabilize the variance. Dose-mortality responses were estimated by probit analysis (IBM, 2010). Percentages of egg hatching and adult emergence were corrected according to Schneider-Orelli's (Kroschel and Koch, 1996) formula:

$$\% M = (b - k/100 - k) \times 100$$

where  $M$  = corrected %,  $b$  = % observed in the treatment, and  $k$  = % observed in the control.

## 3. Results

Among the insecticides tested, ovicidal activity was observed only in spinosad preparations (Table 1). Spinosad at the median and high concentrations (144 and 216 mg/l) was very effective in controlling *P. operculella*

egg hatch compared to emamectin benzoate, chromafenozide and control treatments. There were no significant differences in egg hatchability between emamectin benzoate and chromafenozide used at 5 and 37.5 mg/L and the control for 1-1.5-days-old eggs ( $F = 476.2$ ;  $df = 8, 261$ ;  $P < 0.0001$ ). The  $LC_{99s}$  for 1-1.5-day-old eggs were 240.81, 97.17 and 530.32 mg/L for spinosad, emamectin benzoate and chromafenozide, respectively. 1-1.5-day-old eggs were more sensitive to emamectin benzoate and chromafenozide than 4-4.5-day-old eggs ( $t = 6.3$ ;  $df = 29$ ;  $P < 0.0001$  for emamectin benzoate used at 15 mg/L concentration).

A drastic reduction in the percentage of  $F_1$  emerged adults was observed when insecticide-treated tubers were offered to *P. operculella* neonate larvae (Table 2). Adult emergence was completely inhibited in spinosad and emamectin benzoate treatments compared with 72.2% in the control ( $F = 474.2$ ;  $df = 8, 126$ ;  $P < 0.0001$ ). However, 20.3 and 11.3% of  $F_1$  adults successfully emerged in chromafenozide treatment at 37.5 and 75 mg/L, respectively.

Significant differences were observed in the mean percentage of  $F_1$  adults emerged from larvae that hatched from insecticide-treated eggs and fed on untreated potatoes compared to the control ( $F = 1616.5$ ;  $df = 8, 99$ ;  $P < 0.0001$ ) (Table 3). However, no  $F_1$  adults emerged with spinosad treatment at 0.6 and 0.9 mg/L, while 13.4 and 18.3% of adult emergence was noted in emamectin benzoate and chromafenozide at 0.1, and 37.5 mg/L, respectively.

The time-dependent efficacy of the tested insecticides against *P. operculella* is presented in Table 4. The residual activity of spinosad and emamectin benzoate used at 216 and 15 mg/L remained unchanged (zero  $F_1$  adults

Table 1 - Mean ( $\pm$ SE) hatchability of potato tuber moth eggs of two age groups treated topically with spinosad, emamectin benzoate, and chromafenozide insecticides

Insecticides	Chemical group	Active ingredient mg/l	% of hatched eggs <sup>(2)</sup>	
			1-1.5 day old egg	4-4.5 days old egg <sup>(3)</sup>
Control	-		91.9 $\pm$ 4.2Aa	94.1 $\pm$ 3.9Aa
Spinosad (Spintor® 2 SC, 240 mg/ml)	Spinosyns	72	12.7 $\pm$ 3.9Ac	15.8 $\pm$ 3.8Ac
		144	0.0 $\pm$ 0.0Ac	6.0 $\pm$ 1.5Bd
		216	0.0 $\pm$ 0.0Ac	1.6 $\pm$ 0.4Ad
Emamectin benzoate (Proclaim® 05 SG, 50g/kg)	Avermectins	5	86.8 $\pm$ 5.3aBb	93.7 $\pm$ 3.3Aa
		10	78.6 $\pm$ 4.0Bab	87.4 $\pm$ 4.5Aab
		15	70.3 $\pm$ 5.1Bb	86.3 $\pm$ 5.1Ab
Chromafenozide (Matric® 5% SC, 50g/l)	Non-steroidal ecdysteroid agonist	37,5	84.6 $\pm$ 5.0Bab	91.6 $\pm$ 4.1Aab
		75	76.9 $\pm$ 3.9Bb	84.7 $\pm$ 4.8Ab

<sup>(2)</sup> Means in row for each egg age followed by the same uppercase letter are not significantly different ( $P < 0.05$ ,  $t$ -test); means in column for each egg age followed by the same lowercase letter are not significantly different ( $P < 0.05$ , Fisher PLSD).

<sup>(3)</sup> Data were corrected according to Schneider-Orelli's formula and arcsine transformed prior to analysis; mean of three replicates, 1000 eggs per replicate for each insecticide and concentration.

Table 2 - Mean percentage ( $\pm$ SE) of potato tuber moth  $F_1$  adults emerged from spinosad-, emamectin benzoate-, and chromafenozide-treated and untreated potato tubers

Insecticides	Chemical group	Active ingredient mg/l	$F_1$ emerged adults (%) <sup>(2)</sup>
Control			72.2 $\pm$ 7.84 a
Spinosad (Spintor® 2 SC, 240 mg/ml)	Spinosyns	72	0.0 $\pm$ 0.0 d
		144	
		216	
Emamectin benzoate (Proclaim® 05 SG, 50 g/kg)	Avermectins	5	0.0 $\pm$ 0.0 d
		10	
		15	
Chromafenozide (Matric® 5% SC, 50 g/l)	Non-steroidal	37,5	20.3 $\pm$ 2.1 b
	ecdysteroid agonist	75	11.3 $\pm$ 2.8 c

<sup>(2)</sup> Means in column followed by the same letter are not significantly different ( $P < 0.05$ , Fisher PLSD); data were corrected according to Schneider-Orelli's formula and arcsine transformed prior to analysis; mean of three replicates, 45 larvae per replicate for each concentration of each insecticide.

Table 3 - Mean percentage ( $\pm$ SE) of potato tuber moth  $F_1$  adults emerged from spinosad-, emamectin benzoate-, and chromafenozide-treated and untreated eggs, the hatched larvae being fed on untreated tubers

Insecticides	Chemical group	Active ingredient mg/l	$F_1$ emerged adults (%) <sup>(2)</sup>
Control			68.9 $\pm$ 8.4a
Spinosad (Spintor® 2 SC, 240 mg/ml)	Spinosyns	72	7.1 $\pm$ 3.1d
		144	0.0 $\pm$ 0.0e
		216	
Emamectin benzoate (Proclaim® 05 SG, 50g/kg)	Avermectins	5	13.4 $\pm$ 1.6c
		10	8.1 $\pm$ 2.0d
		15	0.0 $\pm$ 0.0e
Chromafenozide (Matric® 5% SC, 50g/l)	Non-steroidal	37,5	18.3 $\pm$ 4.6b
	ecdysteroid agonist	75	13.1 $\pm$ 5.1c

<sup>(2)</sup> The treated eggs were at the 1-1.5 day-old egg stage; means in column followed by the same letter are not significantly different ( $P < 0.05$ , Fisher PLSD); data were corrected according to Schneider-Orelli's formula and arcsine transformed prior to analysis; mean of three replicates, 120 eggs per replicate for each concentration of each insecticide.

Table 4 - Mean percentage ( $\pm$ SE) of potato tuber moth  $F_1$  adults emerged from spinosad-, emamectin benzoate-, and chromafenozide-treated and untreated potato tubers several days after treatment

Stored period, days after insecticides application	$F_1$ emerged adults (%) <sup>(2)</sup>			Control
	Spinosad (216 mg/ml)	Emamectin benzoate (15 mg/l)	Chromafenozide (75 mg/l)	
7	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	11.7 $\pm$ 1.5 b	70.3 $\pm$ 8.9 a
14	0.0 $\pm$ 0.0 c	0.0 $\pm$ 0.0 c	23.4 $\pm$ 4.1 b	72.1 $\pm$ 9.9 a
21			62.2 $\pm$ 6.9 a	68.9 $\pm$ 7.2 a
28			65.1 $\pm$ 6.1 a	67.9 $\pm$ 7.8 a
35			68.0 $\pm$ 9.9 a	71.0 $\pm$ 9.9 a
42			65.7 $\pm$ 7.8 a	66.9 $\pm$ 6.9 a
49			68.2 $\pm$ 9.8 a	69.1 $\pm$ 9.0 a
60			70.0 $\pm$ 9.9 a	71.4 $\pm$ 9.7 a
90	0.0 $\pm$ 0.0 b	0.0 $\pm$ 0.0 b	66.3 $\pm$ 7.5 a	68.0 $\pm$ 9.6 a

<sup>(2)</sup> Means in row followed by the same letter are not significantly different ( $P < 0.05$ , Fisher PLSD data were corrected according to Schneider-Orelli's formula and arcsine transformed prior to analysis; mean of three replicates, 45 larvae per replicate for each stored period of each insecticide.

emergence) for up to 90 days after application. In contrast, a severe loss in efficacy occurred at the end of the two-week test period for tubers treated with chromafenozide. Significant differences were obtained when chromafenozide was compared to the other insecticides tested ( $F = 5714.3$ ;  $df = 3, 56$ ;  $P < 0.0001$  after 14 days of storage), indicating that spinosad and emamectin benzoate were more efficient in protecting potato tubers from *P. operculella* infestation than chromafenozide even during 14 days after application.

#### 4. Discussion and Conclusions

In general, there are few studies regarding the effect of insecticides on eggs of Lepidoptera pests and most publications focus on the control of the larval stage, consequently, little information is available about the effect of insecticides on eggs of *P. operculella*. Regardless of egg developmental stages, nearly 100% mortality (or 0% egg hatch) was obtained when eggs were treated with spinosad at high and medium concentrations (216 and 144 mg/L, respectively), which was not the case for emamectin benzoate and chromafenozide. Therefore, it is obvious that spinosad has excellent embryocidal activity and the compound was able to penetrate the chorion (eggshell) and reach the developing embryos. Our results concerning the efficacy of spinosad on *P. operculella* egg hatchability agree with several authors who found an excellent ovicidal activity of spinosad when applied against the eggs of the Egyptian cottonworm *Spodoptera littoralis* (Boisd.), the cactus moth *Cactoblastis cactorum* (Berg), the cranberry fruitworm, *Acrobasis vaccinii* Riley and the diamondback moth *Plutella xylostella* (L.) (Bloem *et al.*, 2005; Temerak, 2005; Wise *et al.*, 2010; Mahmoudvand *et al.*, 2011). On the other hand, El-Barkey *et al.* (2009) reported that a 52% reduction in percentage of egg hatching was obtained when eggs of the pink bollworm *Pectinophora gossypiella* (Saunders) were challenged with Radiant Sc 12% (the second generation of spinosad) at the  $LC_{50}$  level. However, according to Perez *et al.* (2007), spinosad showed no ovicidal properties when applied against eggs of the *Aedes aegypti* (L.) mosquito.

When *P. operculella* eggs were topically treated with emamectin benzoate and chromafenozide either at low and medium, or at high concentrations, egg hatchability was not prevented. However, at the highest concentrations, a weak ovicidal effect was noted and eggs hatching were significantly reduced by 8.3 and 10% at the concentrations of 15 and 75 mg/L for emamectin benzoate and chromafenozide, respectively. Our data corroborate the results that emamectin benzoate used in field experiments at the rate of 13.5 g AI/ha had no ovicidal effect on the minute pirate bug *Orius albidipennis* (Reuter) (Sechser *et al.*, 2003). In fact, emamectin benzoate was registered as lepidopteran larvicidal insecticide, which means that the product must be ingested by Lepidoptera larvae to be effective. Accordingly, Scarpellini (2001) reported that all larval stages of

the cotton leafworm *Alabama argillacea* Hübner died 12 h after they started eating cotton leaves treated with emamectin at 9.6 g AI/ha.

Not surprisingly, we found no effect of chromafenozide on the mean percentage of egg hatch. Chromafenozide, like other inhibitors of ecdysteroid biosynthesis, shows toxic effects against larvae of lepidopteran pests mainly via digestion (Yanagi *et al.*, 2006). In this respect, Slama (1995) reported that the ecdysone agonists were completely ineffective in all methods of topical application against the ligated larvae of *Galleria mellonella* L., *Manduca sexta* (L.) and *Pieris brassicae* (L.). Nonetheless, Kandil *et al.* (2012) reported that the hatchability of 1-d-old eggs of *P. gossypiella* was 53% when they were treated with chromafenozide at the  $LC_{50}$  level compared with 97.0% in the control.

Our data show that the older eggs (4-4.5 days old) were relatively more tolerant to insecticides than the younger ones (1-1.5 days old). In general, the hatchability of Lepidoptera eggs following insecticide treatments depends on the compound, dose, and age of eggs (Gelbic *et al.*, 2011). The relative sensitivity of newly laid eggs to insecticides compared to those of older age classes can be attributed, at least in part, to chorion hardening during the embryo development that obstructs the penetration of external products (Tavares *et al.*, 2011).

*P. operculella* neonate larvae exhibited a high response (100% mortality) to all concentrations tested of spinosad and emamectin benzoate, however low numbers of larvae that challenged chromafenozide-treated tubers achieved their development and emerged as  $F_1$  adults (the percentage of emerged adults was relatively high when the compound was used at the low concentration). These findings agree with the data reported by other researchers regarding the larvicidal efficacy of spinosad, emamectin benzoate and chromafenozide in controlling several lepidopteran pests (Kandil *et al.*, 2012; Abouelghar *et al.*, 2013; Dong *et al.*, 2013; Nasir *et al.*, 2013; Tong *et al.*, 2013).

When *P. operculella* larvae hatched from spinosad-, emamectin benzoate-, and chromafenozide-treated eggs were fed on untreated tubers, the percentages of  $F_1$  emerged adults were completely inhibited for spinosad and emamectin benzoate used at medium and high concentrations and drastically reduced for chromafenozide treatment. It seems probable that treated-egg chorion retained toxic residues to cause the death of newly hatched L1 larvae which puncture the eggshell and eat their way through while hatching. It's worth mentioning that when we evaluated the results concerning the emamectin benzoate and chromafenozide treatments, we found that *P. operculella* neonate larvae had been successful at creating a small opening in the eggshell during the process of exiting the chorion; however, most of the larvae failed to survive and died without feeding in close proximity to the egg. This indicates that although these two compounds do not have ovicidal activity, they have demonstrated ovi-larvicidal activity when applied topically after eggs have been laid. Our data concerning the ovi-larvicidal activity

of emamectin benzoate and chromafenozide are congruent with studies on the effects of these insecticides and other insecticides with ecdysone mode of action on *Helicoverpa armigera* Hübner and *A. vaccinii* (Dhadialla *et al.*, 2005; Wise *et al.*, 2010; Singh and Kumar, 2012).

Determination of residual activity of an insecticide is essential information to protect agricultural products from re-infestation. Spinosad and emamectin benzoate provide, under protected environments (complete darkness), a 100% residual control effect up to 90 days after application, while chromafenozide applied at 75 mg/L showed limited residual activity of 7 to 14 days. Therefore, *P. operculella* larvae exposed to chromafenozide-treated tubers at 21 days following treatment continued their development and reached the adult stage. The extended period of residual activity of spinosad and emamectin benzoate could be related to the rate of their photodegradation, since these two compounds are known to undergo relatively rapid photodegradation via photolysis (primary route of degradation) that ultimately affects their residual toxicity (Liu *et al.*, 1999; Jones *et al.*, 2005; Zhu *et al.*, 2011). Our insecticide-treated potatoes were stored in total darkness and this could explain the slow degradation of spinosad and emamectin benzoate under our experimental laboratory conditions. On the other hand, Ditya *et al.* (2012) found that the dissipation rate (half-life) of chromafenozide applied to different types of soil samples in laboratory conditions was between 15.8 and 23.9 days.

In conclusion, the results of this study are the first published data on the efficacy of spinosad, emamectin benzoate and to a lesser extent chromafenozide against *P. operculella* eggs and neonate larvae and demonstrate that these compounds could be used as replacements for earlier insecticide classes. Moreover, spinosad and emamectin benzoate proved to be highly effective in protecting potatoes from *P. operculella* infestation almost completely for three months and therefore they could be successfully used in unrefrigerated rustic potato stores.

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