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Toxicity of Some New Insecticides Against *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) Under Laboratory and Extended Laboratory Conditions

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Abstract.- Studies on the toxicity of some new insecticides against *Trichogramma chilonis* were carried out under laboratory and extended laboratory conditions following the guidelines recommended by International Organization for Biological Control (IOBC). Field Recommended Concentrations (FRCs) of six insecticides *viz.,* emamectin benzoate, lufenuron, flubendiamide, spinosad, indoxacarb and neem oil were tested against all the life stages of the parasitoid under laboratory conditions. Persistent toxicity of the insecticides against the most susceptible life stage (adult) of the wasp was also determined. Results regarding the harmful effects of the insecticides on the different life stages of *T. chilonis* revealed that flubendiamide was the most selective of all the tested insecticides for the development, survival and fecundity of the wasp. Spinosad, closely followed by emamectin benzoate severely curtailed adult survival and fecundity and to a lesser degree, the development of immature stages inside host eggs. Indoxacarb was also recorded as "slightly harmful" to all, except the egg stage of the wasp. Lufenuron exhibited significantly higher level of toxicity against the larval stage. Neem oil was "harmless" to the eggs, pupae and adults but exerted "slightly harmful" effects on larval development and female fecundity. Persistency test showed that flubendiamide and lufenuron were "short lived", indoxacarb was "slightly persistent" and spinosad and emamectin benzoate were classified as "moderately persistent". On the basis of this study, it could be concluded that flubendiamide is considerably safe, neem oil, indoxacarb and lufenuron are mildly toxic for the bio-control agent, while spinosad and emamectin proved highly toxic of all the insecticides.

Key Words: *Trichogramma chilonis*, Spinosad, neem oi, emamectin.

INTRODUCTION

Several species of *Trichogramma* are reared and released around the world annually on an estimated 80 million acres of agricultural crops and forests in 30 countries (Li, 1994; Olkowski and Zhang, 1990). *Trichogramma* spp. parasitize the eggs of over 400 species belonging to at least seven insect orders (Bao and Chen, 1989). In spite the important role of the biological control agents in agriculture, chemical control is still indispensable but, the use of nonselective insecticides greatly reduces the beneficial potential of the biocontrol agents, particularly parasitic Hymenoptera that are often far more susceptible to insecticides than their hosts. A range of harmful effects of insecticides on *Trichogramma* spp. have been described by different workers (Hewa-Kapuge *et al.,* 2003; Desneux *et al.,* 2007; Vianna *et al.,* 2009). Apart from direct toxic effects, insecticides may interfere with the feeding behavior as repellents, inhibitors, or olfaction disruptors (Desneux *et al.,* 2007). These compounds also cause disruption of sex pheromone communication (Delpuech *et al.*, 1998) and an increase in the arrestment behavior of treated trichogramma males (Delpuech *et al.*, 1999). In majority of studies, mortality due to acute toxicity is the only effect that insecticides are screened for, while sub lethal effects on development, behavior and reproduction are overlooked. However, studies showed that sub lethal effects can severely reduce the performance of biological control agents (Desneux *et al*., 2007). In addition to the negative effects of fresh insecticide residues, some persistent compounds can exert their lethal and sub lethal effects on the trichogramma for a longer period of time post application (Hewa-Kapuge *et al.,* 2003).

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One major purpose of Integrated Pest Management (IPM) strategies is to unify the safe and sustainable use of chemical and biological control methods. Therefore, the side-effects of pesticides on biocontrol agents should be carefully evaluated for induction in IPM programs (Stark *et al.,* 2007). *Trichogramma chilonis* is an important parasitoid species of lepidopterous pests in Pakistan and it is therefore, imperative to assess its compatibility with chemical control for induction in integrated pest management programs. Thus, the objective of this study was to determine the effect of six insecticides, commonly used for lepidopterous pests' suppression, on the development, survival and parasitic efficiency of *T. chilonis* and determine the persistency of these insecticides.

MATERIALS AND METHODS

Insects and insecticides

The experiments were carried out in the Insecticide Toxicology Laboratory Department of Plant Protection, Khyber Pakhtunkhwa (KPk) Agricultural University Peshawar, Pakistan. *T. chilonis* was collected of the parasitized eggs of *Helicoverpa armigera* from tomato crop in Mardan and Swabi, KPk province, Pakistan. The strain was then raised in the laboratory on the same host. Field Recommended Concentrations (FRCs) of the insecticides *viz*, emamectin benzoate (Emamectin benzoate® 1.9 EC, 3.2 mg a.i. L^{-1}), lufenuron (Match® 50EC, 83.3 mg a.i. L^{-1}), flubendiamide (Belt 480® SC, 80.0 mg a.i. L^{-1}), spinosad (Tracer® 240 SC, 120.0 mg a.i. L^{-1}), indoxacarb (Steward® 150 EC, 186.7 mg a.i. L^{-1} and a botanical product (Neem oil, 1500 ppm) were tested initially against all the life stages (egg, larva, pupa and adult) of the bio-control agent in laboratory and later against the adults under extended laboratory conditions. These insecticides are locally used by the farmers against lepidopterous pests in various crops. The experiments were carried out using standard methods developed within the framework of International Organization for Biological Control-West Palaearctic Regional Section (IOBC-WPRS) working group "pesticides and beneficial organisms" (Sterk *et al.*, 1999). Stock solutions of formulated insecticides were prepared in distilled water according to their respective doses. A control treatment (distilled water) was included in each test to assess the natural mortality of the test insects. Paper cards ($1cm^2$) with approximately 50 *H. armigera* eggs each were offered for 24 h to freshly emerged *T. chilonis* adults in glass tubes (10 cm × 2.5 cm). The number of adults used was enough to ensure close to 100% parasitization of the offered host eggs. All the test units were kept in controlled conditions (27±2°C, 60±5 % RH and 14:10 L:D). These experiments were carried out in completely randomized design (CRD) with five repeats.

Laboratory tests

The above mentioned cards were placed separately in vials (5 cm × 2.5 cm) and treated at appropriate post parasitization time for the different immature stages that were: 72 h (eggs), 144 h (larvae) and 192 h (pupae) (Manzoni *et al*., 2007). The cards having the parasitoids eggs, larvae and pupae were dipped in their respective insecticide solutions for three seconds**.** Excessive amount of insecticide residues was air dried at room temperature. The parasitoid adults (five pairs) were put to trial by offering previously treated less than 24 h old 50 *H. armigera* eggs (treated in the same manner as mentioned above) for parasitization in glass tubes (10 cm × 2.5 cm). The parameters evaluated were; *T. chilonis* adult emergence (from the treated eggs, larvae and pupae), adult mortality and parasitization (for the adult stage) from the different treatments.

Extended laboratory tests

Field Recommended Concentrations (FRCs) of the insecticides were applied on potted tomato plants (variety; Roma) with a hand sprayer till run off point. Assays were done in April and plants were maintained in the field under a transparent polyethylene rain cover (approximately 200 cm high). Samples of the treated leaves, taken at different time intervals *i.e*., 0, 5, 15 and 25 days after pesticide application (within the interval proposed by the members of the IOBC/WPRS Working Group for the evaluation of insecticide persistency against predators and parasitoids under

laboratory conditions) (Sterk *et al.*, 1999) were brought to the laboratory and placed in 5cm vented plastic petri dishes. Moistened filter papers were placed beneath the leaves to keep it fresh for longer period of time. In order to enhance the chances of insect contact with the treated surface, fresh (less than 24 h old) *H. armigera* eggs were placed in the middle of the aforementioned testing arena. The experiment was repeated five times with 20 *T. chilonis* adults in each case. Assessment of treatment effect was made on the basis of adult mortality, 24 h post exposure.

Data collection procedure

The effect of the insecticides compared to the untreated was calculated by the formula: E (%) = (1-Vt/Vc) 100, where E is the effect of the pesticide on the biological control agent being measured as the reduction of parasitism viability (Adult Emergence in this case) compared to the untreated, Vt is the parasitism viability observed on each pesticide treatment and Vc is the parasitism viability observed on the control (untreated) (Manzoni *et al*., 2007). Parasitism was determined by counting the number of parasitized eggs per card under stereomicroscope. The reduction in parasitism (RP) was determined for each insecticide by the equation RP (%) = (1-f/t) x 100 where f = average number of parasitized eggs in the insecticide treatment and t = average number of parasitized eggs in the control treatment (Hassan *et al*., 2000). The value E and RP calculated for each pesticide treatment were classified according to the International Organization of Biological Control (IOBC) where: class 1 – harmless (E<30%), class 2 – slightly harmful (30 ≤ E ≤ 79%), class 3 – moderately harmful (80 ≤ E ≤ 99%) and class 4 – harmful (E>99%) (Sterk *et al*.,1999). Classification of the insecticide persistency was determined according to the duration of the toxic activity of the compounds, that is, the interval of time in which its residues caused 30% mortality which is the minimum level of toxicity as described for laboratory tests by IOBC. Accordingly, categories developed by members of the IOBC/WPRS Working Group for the evaluation of harmful activity duration (persistence) of insecticides against predators and parasitoids under laboratory conditions include: A, short lived (<5 days); B, slightly persistent (5–15 days); C, moderately persistent (16–30 days) and D, persistent (>30 days) (Sterk *et al.,* 1999).

Statistical analysis

The results were analyzed using MSTATC and means were separated by using LSD at 5% level of probability (Gomez and Gomez, 1980).

RESULTS

Insecticide toxicity against immature stages

The results (Table I) showed varying degrees of toxicity for the insecticides used against the different immature stages of *T. chilonis* inside host eggs. Spinosad ranked first in terms of reducing adult emergence followed by emamectin benzoate, flubendiamide, indoxacarb and lufenuron when the wasp was treated at egg stage. Despite the statistically significant differences among the treatments, only spinosad and emamectin were categorized as “slightly harmful” (Class 2, 30-79% mortality) according to the IOBC ranking. Rest of the insecticides, though reduced adult emergence to some extent as compared to the control but were still safe enough to be ranked as harmless (< 30% mortality).

Larval stage of the parasitoid appeared more vulnerable to the insecticides as compared to the egg stage. Significantly lower adult emergence was observed in case of lufenuron as compared to the rest of insecticides and control and was therefore, categorized as “slightly harmful” (Class 2). Spinosad, emamectin benzoate, indoxacarb and neem oil followed lufenuron in terms of reducing adult emergence rates and were also ranked as “slightly harmful” (Class 2). Flubendiamide, though significantly more toxic to the larval stage as compared to control, yet much safer than the rest of insecticides thus was labeled as “harmless”. Spinosad, indoxacarb and emamectin benzoate reduced adult emergence to some extent as compared to the control when the pupal stage of *T. chilonis* was treated, thereby placing these compounds in the “slightly harmful” category. Flubendiamide, neem oil and lufenuron exerted minimal degree of negative effects on the pupal stage of the wasp thus were ranked as “harmless”.

Table I.- Effects of different insecticides on the adult emergence of *T. chilonis* when treated at egg, larval and pupal stages under laboratory conditions.

Treatment	Eggs			Larvae			Pupae		
	AE(%)[1]	E(%)[2]	Class[3]	AE(%)[1]	E(%)[2]	Class[3]	AE(%)[1]	E(%)[2]	Class[3]
Emamectin benzoate	56 ef	34	2	47 c	42	2	61 c	31	2
Lufenuron	70 bc	18	1	19 d	77	2	76 b	14	1
Flubendiamide	62 de	27	1	60 b	26	1	80 b	10	1
Spinosad	51 f	40	2	51 c	38	2	56 c	37	2
Indoxacarb	66 cd	22	1	53 c	35	2	58 c	34	2
Neem oil	72 b	14	1	53 c	35	2	78 b	12	1
Control	84 a	-	-	82 a	-	-	88 a	-	-
$LSD_{0.05}$	6.33	-	-	6.01	-	-	6.38	-	-

[1]AE (%) = total adult emergence from treated eggs, larvae and pupae; [2]E (%)=(1-Vt/Vc) 100, where E is the effect of the pesticide on the biological control agent being measured as the reduction in adult emergence compared to the untreated, Vt is the parasitism viability(AE) observed on each pesticide treatment and Vc is the parasitism viability(AE) observed on the control (untreated). [3]Class: 1, harmless (E<30%); 2, slightly harmful (30%<E<79%); 3, moderately harmful (80%<E<99%); 4, harmful (E>99%). Means followed by the same letter in column do not differ statistically by Least Significant Difference (LSD) test ($P \leq 0.05$).

Table II.- Toxic effects of different insecticides on the survival and fecundity of *T. chilonis* adults.

Treatment	Adult mortality			Fecundity		
	AM (%)[1]	E(%)[2]	Class[4]	Parasitization female^{-1}	RP[3]	Class[4]
Emamectin benzoate	80 b	79	2	1.68 e	82	3
Lufenuron	46 c	43	2	4.20 d	54	2
Flubendiamide	28 d	23	1	7.48 b	18	1
Spinosad	100 a	100	4	0.76 f	92	3
Indoxacarb	80 b	79	2	4.60 d	50	2
Neem oil	16 e	11	1	6.44 c	30	2
Control	6 f	-	-	9.16 a	-	-
$LSD_{0.05}$	9.3	-	-	0.67	-	-

[1]AM (%) = total adult mortality; [2]E (%)=(1-Vt/Vc) 100, where E is the effect of the pesticide on the biological control agent being measured as the adult mortality compared to the untreated, Vt is the adult survival observed on each pesticide treatment and Vc is the adult survival observed on the control (untreated); Reduction in parasitism. [4]RP(%)=(1-f/t) x 100 where RP is the reduction in parasitism, f = average number of parasitized eggs in the insecticide treatment and t = average number of parasitized eggs in the control treatment [4]Class: 1, harmless (E<30%); 2, slightly harmful (30%<E<79%); 3, moderately harmful (80%<E<99%); 4, harmful (E>99%). Means followed by the same letter in column do not differ statistically by Least Significant Difference (LSD) test ($P \leq 0.05$).

Lethal and sub lethal effects of insecticides on adults

The data (Table II) revealed that the insecticides showed a varying degree of effects on *T. chilonis* adult mortality as compared to the control. Spinosad caused 100% *T. chilonis* adult mortality 24 hours after exposure to the insecticide treated eggs, hence, proving "harmful" (Class4). Spinosad was followed by emamectin and indoxacarb with significantly higher mortalities as compared with the rest of insecticides and control. Flubendiamide and neem oil were significantly more lethal to the adults than control, but were safe enough to be ranked as "harmless" (Class4). The data (Table II) regarding ovipositional preference of *T. chilonis* females for the host (*H. armigera*) eggs treated with different compounds showed that highest rate of parasitization (9.16 eggs/ female) was recorded in control (water only) followed by flubendiamide (7.48 eggs/female), neem oil (6.44 eggs/female), indoxacarb (4.60 eggs/ female) and lufenuron (4.20 eggs/female). Lowest rate of parasitization (0.76 eggs/female) was recorded for spinosad followed by emamectin

treated eggs (1.68 eggs/female). *T. chilonis* females were able to parasitize a higher number of indoxacarb treated host eggs (4.6/ female) despite the fact that it had caused 80 % mortality of the adults within 24 h. On the basis of IOBC ranking of the toxicity levels for reduction in the beneficial capacity (parasitization in this case) of the biological control agents, flubendiamide was ranked as "harmless" (Class 1), spinosad and emamectin were categorized as "moderately harmful" (Class 3), while the rest of the compounds were labeled as "slightly harmful" (Class 2).

Table III.- Mortality (%) of *T. chilonis* adults after exposure to 0, 5, 15 and 25 days old insecticide residues for 24 h under laboratory conditions.

Treatments	Mortality (%) of *T. Chilonis* adults				Class[1]
	Age of pesticide residues (days)				
	0	5	15	25	
Emamectin Benzoate	67	50	41	11	C
Lufenuron	50	25	15	10	A
Flubendiamide	34	21	11	9	A
Spinosad	100	82	53	13	C
Indoxacarb	85	60	14	9	B
Neem oil	25	20	12	8	A
Control	7	8	10	10	A

[1]IOBC persistency ranking; A, short lived (<5 days); B, slightly persistent (5–15 days); C, moderately persistent (16–30 days); D, persistent (>30 days) (Sterk *et al.,* 1999).

Persistent toxicity of the insecticides

Results regarding the effects of insecticide persistency on *T. chilonis* adults (Table III) revealed that all the insecticides were significantly more toxic as compared to the control when offered fresh. All the *T. chilonis* adults exposed to spinosad residues were recorded dead 24 hrs post exposure and the compound was therefore, labeled as "harmful" (Class 4). Indoxacarb was observed as "moderately harmful" (Class 3), while the rest of the insecticides, except neem oil proved "slightly harmful" (Class 2). A variable degree of reduction in potencies of the chemicals was recorded when the adults were exposed to 5 days old residues. Minimum mortality was induced by 5 days old neem oil, flubendiamide and lufenuron residues and these were therefore, ranked as "short lived" (IOBC, persistency Class A). Indoxacarb toxicity showed a drastic decline as it was recorded "harmless" in case of 15 days old residues and was therefore, ranked as "slightly persistent" (IOBC, persistency Class B). Spinosad and emamectin though recorded a considerable decrease in their toxicities but were still potent enough to be classified as "moderately persistent" (IOBC, persistency Class C). Though recorded as "slightly harmful" to the test insects when offered in the form of fresh residues, emamectin benzoate remained so even 15 days after its application. It is further evident from the data that none of the insecticides remained potent after 25 days of their application.

DISCUSSION

Limited literature is available on the effects of pesticides on immature life stages of *T. chilonis* because the IOBC sequential scheme for testing side-effects of pesticides recommends the testing of most susceptible life stage *i.e.*, adults of parasitoids. Majority of the researchers have reported that the immature stages of the wasp are tolerant to insecticides. This high level of tolerance could be made possible by the chorion of the host eggs. Our study revealed that emamectin benzoate proved slightly harmful to the egg, larval and pupal stages on the basis of adult emergence. Similar observations were made by Hewa-Kapuge *et al.* (2003) who tested seven different insecticides including emamectin benzoate for their compatibility with *T. brassicae* under laboratory conditions and reported that the egg and pupal stages were not severely affected by the insecticide. We observed lufenuron to be 'harmless" for the eggs and pupae and "slightly harmful" for the larval stage of the wasp. Somewhat similar trend was observed by Carvalho *et al.* (2005a) while investigating the lethal and sub-lethal effects of lufenuron to *T. pretiosum*. They determined that lufenuron proved "harmful" and "harmless" against the larvae and pupae, respectively. The escalated level of toxicity that

Carvalho *et al.* (2005a) reported for the larval stage might have been caused by the difference in the tested species. Somewhat similar trend but a higher degree of toxicity caused by lufenuron to the eggs and larvae was determined by Cristina *et al.* (2006) and Consoli *et al.* (2008). Our conclusions regarding the safety of the chemical to the pupal stage of the insect are also in concurrence with that of Cristina *et al.* (2006). The harmlessness of flubendiamide to the immature stages of *Trichogramma* spp. is confirmed by similar observations reported by Carvalho *et al.* (2005b) and Rezende *et al.* (2005) for the larval and pupal stages of *T. pretiosum* and *T. atopovirilia,* respectively. Somewhat similar trend but a higher degree of spinosad lethality was reported by Consoli *et al.* (2008) who tested all the life stages (adult, larvae, pre-pupae and pupae) of *T. halloi* against spinosad under laboratory conditions. They concluded that spinosad was harmful when tested against any stage whether immature or adult. We recorded neem oil as significantly toxic as compared to the control when the parasitoid was treated at different immature stages. But according to the IOBC ranking it could be categorized as "slightly harmful" only for the larval stage of the parasitoid. The possible reason for the escalated toxicity level of the chemical for the larval stage as compared to the egg and pupal stages could be the fact that it acts as a feeding deterrent and growth inhibitor (Schmutterer, 1990). Much higher toxicity level of the chemical was reported by Klemm and Schmutterer (1993) who applied NSKE (2.5% and 3%) against *Trichogramma* spp. and reported that the two treatments completely prevented adult emergence. A much lower concentration (0.2%) of neem oil reduced the emergence of *T. principium* from treated eggs by 45.1 %.

Being relatively a modern insecticide, the selectivity of flubendiamide for parasitoids in general and trichogrammatids in particular is still a subject to be explored. Nevertheless, workers like Carvalho *et al.* (2005b), Rezende *et al.* (2005) and Somchoudhury *et al.* (2007) have investigated and confirmed its safety to the different stages of the wasp. Our results about the chemical are completely in agreement with the aforementioned workers as we found flubendiamide "harmless" for both, the survival and parasitism capacity of the wasp. Spinosad is considered primarily as a stomach poison with limited contact toxicity (Bret *et al.,* 1997) and requires bioactivation after ingestion (Michaud, 2003). On the contrary, Lia *et al.* (2008) reported that survival of the parasitoid *Diachasmimorpha longicaudata* was more severely affected when exposed to dried residues as compared to wasps that were offered spinosad bait. We strongly agree with Lia *et al.* (2008) regarding the contact activity of spinosad since females remained on the treated host eggs during the whole host selection and parasitization process after exposure. In addition to the contact activity, the high adult mortality suggests that ingestion of the product could not be completely ruled out, since after the host chorion is drilled, the parasitoid female feeds on the egg contents that ooze out of the puncture hole,enabling the ingestion of insecticide residues (Klomp and Teerink, 1962). Our observations are also supported by that of Williams *et al.* (2003) who published a comprehensive review on spinosad toxicity to the biocontrol agents and concluded that among 25 parasitoid species tested, 78 and 86% of the laboratory and field studies, respectively recorded spinosad as "moderately harmful" or "harmful" to the parasitoids. Consoli *et al.* (2008) also recorded it as harmful for the adults of *T. halloi* in laboratory assays. Emamectin benzoate emerged as the second most toxic insecticide in terms of adult mortality and reduction in parasitization capacity of the wasp in our laboratory bioassay. These observations are partially in agreement with that of Hewa-Kapuge *et al.* (2003) who concluded that the compound caused 23–64% mortality of *T. brassicae* adults in residual toxicity assay in addition to affecting parasitism on the day of spraying. Indoxacarb appeared equally toxic to the parasitoid adults as emamectin in terms of adult mortality but was much safer in case of oviposition. Contrary to our findings, the compound was observed as "harmless" to *T. brassicae* adults when tested through direct application and residual effects by Hewa-Kapuge *et al.* (2003). The difference in both the studies could be due to the fact that they used a

much lesser dose (10 g a.i ha^{-1}) relative to 56 g a.i ha^{-1} that we tested. The reduction in parasitization rate caused by neem oil could be attributed to the ovipositional deterrence that the chemical causes as Raguraman and Singh (1999) have reported it to be an ovipositional deterrent at a much lower rate (0.3%) as compared to ours (1.65%). Similarly, Gandhi *et al.* (2005) recorded only 59.3% parasitization of the host eggs treated with 2% neem oil. We recorded lufenuron to have exerted low level of negative effects on *T. chilonis* adult survival but, it affected parasitization capacity more severely, thereby, highlighting its activity as an ovipositional deterrent. Similar effects were reported by Carvalho *et al.* (2005a) who determined that lufenuron caused 61.8% reduction in the parasitism capacity of *T. pretiosum* under laboratory conditions. Concurring opinions about reduction in parasitism capacity as induced by different concentrations of the IGR on *T. pretiosum* have been published by various workers (Grutzmacher *et al.,* 2005; Junior *et al.,* 2005; Cristina *et al.,* 2006). On the contrary, Consoli *et al.* (2008) declared it safe to the parasitization capacity of *T. galloi,* while Vianna *et al.* (2009) recorded a higher parasitism rate of *T. pretiosum* for lufenuron treated host eggs.

It is evident from the results regarding insecticide persistency that spinosad, followed by indoxacarb and emamectin benzoate had high initial toxicity against the wasp. Indoxacarb toxicity eroded quickly and was therefore, ranked as "slightly persistent" while spinosad and emamectin benzoate were labeled as "moderately persistent". Very few specifically relevant published references for the persistent activity of the insecticides are available; therefore, information regarding the persistency of these insecticides against other insects has also been considered here. Nevertheless, the findings about the toxicity of fresh residues of these insecticides are almost parallel to those of our laboratory bioassays (Tables I and II) and are discussed in detail in the foregoing pages. Our findings regarding the persistent toxicity of emamectin benzoate against *T. chilonis* adults are somewhat similar to that of Hewa-Kapuge *et al.* (2003) who reported that emamectin benzoate remained moderately toxic to the wasps throughout the experimental period (7 days). Concurring opinion regarding the persistency of emamectin-benzoate (10.6 d) spinosad (8.9 d) and indoxacarb (3.7d) were published by Brevault *et al.* (2009) who evaluated these insecticides for their initial and residual activity against *Helicoverpa armigera*. Sunlight intensity, high temperature and foliage growth are the main factors responsible for the decay/dilution of the insecticides (Wilson *et al.*, 1983, 1986).

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