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Compatibility of Spinosad with *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) in Integrated Pest Management of *Sitotroga cerealella*

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Abstract.- With a view to use *Trichogramma chilonis* as a parasitoid in the integrated pest management, the effect of different concentrations of Spinosad (0.2, 0.15. 0.1 0.05 and 0.01%) was studied on the parasitoid under laboratory conditions. The results showed that 0.2% gave the minimum parasitism in egg, larva and pupa and during oviposition preference test. At 0.01% concentration, the spinosad showed less toxicity to the parasitoid in oviposition preference test followed by the larval stage. In egg stage, low percent parasitism showed toxicity of chemical to the parasitoid. Emergence time data followed almost same pattern as observed in case of percent parasitism. The 0.2, 0.15 and 0.10% concentrations increased the emergence time of parasitoid in pupal stage and oviposition preference test. Significantly lower emergence time was observed in 0.05 and 0.01% in larval stage. Significantly lower adult longevity was observed in all stages at all concentrations. The effect of Spinosad on adult *T. chilonis* was found extremely toxic as it gave 100% mortality within 15 minutes of exposure. Spinosad was applied at different concentration *viz*., 0.005% and 0.001%. Individual wasp were observed during approximately three minutes, first in absence and then in presence of spinosad. Movement activity was estimated with the help of computer based software *Abid's Trackmove* on an arena divided in to grids. The wasp exposed to 0.005% took more of the effect than the wasp exposed to 0.001%. It was observed that the total movement of wasp under spinosad effect was decreased while ineffective time period (of the wasp) increased. Although average speed during the whole observation period shows increase, average speed excluding the points of no movement decreased.

Key Words: Spinosad, *Trichogramma chilonis*, insecticide-parasitoid compatibility.

INTRODUCTION

Almost all insecticides are very harmful to *Trichogramma* adults. However, the eggs, larvae, and pupae are afforded some protection from insecticides by the moth egg shell in which they are enclosed. Even so, it is not wise to apply insecticides during this time period when *Trichogramma* are used. Also, it is important that the grower has a good understanding about the residual life of all insecticides that may be used during the growing season. Insecticide should be used only when caterpillar number are "out of control" (Thomson *et al*., 2000).

Spinosad (spinosyn A (R=H) and spinosyn D (R=CH3) are a new chemical class of insecticides that are registered by the United States Environmental Protection Agency (EPA) to control a variety of insects. The active ingredient is derived from a naturally occurring soil dwelling bacterium called *Saccharopolyspora spinosa*, a rare actinomycete reportedly collected from soil in an abandoned rum factory in the Caribean in 1982. Spinosad kills susceptible species by causing rapid excitation of the insect nervous system. Due to this unique mode of action, Spinosad is valued in resistance management programs. Spinosad has both contact and stomach activity. Spinosad is relatively fast acting. The insect dies within 1 to 2 days after ingesting the active ingredient and there appears to be no recovery. It is used to control a variety of insect pests, including fruit flies, caterpillars, leafminers, thrips, sawflies, spider mites, fire ants, and leaf beetle larvae. Spinosad is recommended for use in an Integrated Pest Management program for commercial greenhouses since it will not harm most beneficial insects or predatory mites. Spinosad does not significantly affect beneficial organisms including ladybugs, green lacewings, minute pirate bugs, and predatory mites. Spinosad shows low

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toxicity when ingested by mammals (male rat LD50 = 3738 mg/kg) and no additional adverse effects from chronic exposure. It is a neurotoxin with a novel mode of action targeting the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado, 1998).

The impact of Spinosad on natural enemies has been extensively studied since its introduction. It has been demonstrated that direct application (wet spray) of Spinosad are extremely harmful to parasitoids, including *Aphidius colemani* and *E. formosa*, however, any toxic effects generally decrease as the spray residues age. Spinosad application has shown to be toxic to the eggs of *Trichogramma* spp. (Consoli *et al*., 2001).

Monitoring the walking speed of arthropods is an important component of insect behavior studies. Walking speed and pattern is an indicator of arthropod's response to various physical and chemical stimuli. Studies on walking behavior require the observation of movement pattern, in the presence of such stimuli, either manually or through using electronic monitoring systems. Manual recording does not provide an accurate measurement of the response while electronic recording needs specialized apparatus.

A windows-based computer application 'Abid's Trackmove' was developed for assisting the monitoring of walking speed and pattern of arthropods movement using a simple coordinate system. The application can be used to monitor the response of biological control agents or insect pests to chemical stimuli (attractants or repellents), comparison of laboratory-reared strains with their wild counterparts and see mobility of insect pests at various intervals after getting infected by viral or bacterial agent or any other situation where the study of walking behavior is important. The application was basically developed for the insects and other arthropods but can be used in general zoological studies involving other animals as well. (http://www.nifa.org.pk/ trackmove.htm)

Very little work on the compatibility of Spinosad with *T. chilonis* has been conducted in this part of the world. The present study was conducted to determine the effect of Spinosad on egg, larval, pupal and adult stage of *T. chilonis.* Also to find the effect of Spinosad on the parasitism efficiency of *T. chilonis* and to determine the effect of Spinosad on the walking behavior of the parasitoid.

MATERIALS AND METHODS

A study on the compatibility of Spinosad with *Trichogramma chilonis* (Ishii) was conducted at Nuclear Institute for Food and Agriculture (NIFA), Peshawar during 2008-09.

Completely Randomized Design (CRD) was used in the experiment having 10 replications with 6 treatments. In some cases some replications are discarded because of fungus attack. The data was analyzed by using the SAS program (6.1). The means were separated by using the DMR test (Steel and Torrie, 1980).

Rearing host Sitotroga cereallela (Olivier)

Sitotroga cereallela adults were obtained from the infested grains and kept in oviposition jars having lid on the top while bottom of each jar was cut out and mesh no 25-30 or 35-40 fixed on their bottom. The oviposition jars were kept in a dish containing starch for oviposition. The eggs laid in the starch were separated daily by using a sieve (with mesh no. 50-70).The egg obtained were spread out in sterilized grains and kept in glass jars in host rearing chambers for larval and adult emergence. The host eggs used for mass production of *T. chilonis* were stored at 25-30°C and 70-80% RH.

Rearing of T. chilonis *on* Sitotroga cereallela

Gum was spread uniformly on a piece of hard paper. *S. cereallela* eggs were sprinkled on the sticky cards which were placed in UV radiation for one hour to kill the larvae present inside the eggs. Then the egg cards were transferred to glass jars containing adults of *T. chilonis* for 1-3 hrs for parasitism. After 1-3 hours the cards were removed and kept in incubator at 25-30º C and 60-70% RH for parasitoid development. When the parasitoid emerged, these were used for further parasitization of host eggs.

Preparation of Spinosad *concentrations*

To prepare 0.2% solution of Spinosad, 8ml of Spinosad was dissolved in 970 ml of water that was

also stock solution. The other solutions of 0.15, 0.10, 0.05 and 0.01%were prepared by using the following formula: C1V1=C2V2, where C1 is concentration of stock solution; V1 is required volume of stock solution; C2 is required concentration, and V2 is required volume of required concentration

Determination of oviposition preference

For no choice oviposition preference test, the *S. cereallela* eggs were sprayed with desired concentration of Spinosad. From 50-100 eggs were glued on the cards and placed in vials. The vials had freshly emerged virgin females with individual virgin male which were allowed mating for a day. The females were allowed to remain in vials till death. Blackening of the eggs indicated parasitism while failing to blacken means that eggs were not parasitized. The hollow cream colored eggs showed the infertility of *S. cereallela* eggs which were neither parasitized nor emergence of *S. cereallela* occurred. The data was collected on the number of eggs being parasitized by the parasitoid) number of adults emerged from the parasitized eggs and total life of the adults emerged from parasitized eggs (adult longevity) was recorded. Similar type of data was collected for all other stages.

Determination of effect of spinosad

Egg stage

Eggs of *S. cerealella* were glued on the cards. The cards were exposed to a pair of *T. chilonis* in a jar for 24 hrs. After 24 hrs it was assumed that the *T. chilonis* had laid eggs in *S. cerellela* egg and they were in egg stage now. After 24 hours these cards were sprayed with Spinosad solution. The eggs were left uptill the emergence of adults.

Larval stage

T. chilonis completes its life cycle within the *S. cerealella* eggs and except adult no other stage is visible. After 2 days of exposing the *S. cerealella* eggs to *T. chilonis*, it was assumed that *T. chilonis* was in larval stage. These cards were sprayed with solution of Spinosad. Data was collected as above.

Pupal stage

Blackening of *S. cereallela* eggs show that *T. chilonis* is in the pupal stage. Cards having black eggs were sprayed with different concentrations of Spinosad. The parameters recorded were % adult emergence, mortality during pupal stage and Adult longevity.

Adult

The effect of Spinosad on adult was observed in glass vials. The vials were dipped in different concentrations of Spinosad and left to dry. A single *T. chilonis* was introduced to it. After 24 hrs the mortality of *T. chilonis* was observed.

Walking behavior of T. chilonis

Walking behavior of *T. chilonis* is determined with the help of computer based software Abid's Trackmove. *T. chilonis* were identified with the help of a microscope. Different sizes of grids were made on an arena and the wasps of *T. chilonis* were set free to walk over these grids. Those grids were chosen on which the insect could move and data could easily be taken.

The accuracy of the result depends upon the size of the grids. The smaller the grids size, the accurate would be the result. The size of the grids was appropriate enough for the data to be taken easily. The grids were numbered with a specific pattern on which the computer operates the software. A transparent cover slips of length 5cm and width 6cm having very thin boundaries was used so that wasps could not fly and escape out of the grids. The time for the whole observation was three minutes. Spinosad was sprayed evenly on the wasp in two concentrations *i.e.* 0.001% and 0.005%. Also the same experiment carried out without chemical treatment. As the insects started moving on the grids the software was started and numbers of those grids were entered in the computer from which the wasp passed away. Three replication of the experiments was performed on 0.001, 0.005% and control.

RESULTS

Oviposition preference

The data regarding the effects of different concentrations of Spinosad on the oviposition preference of *T. chilonis* is presented in Table 1.

The table shows that maximum parasitism (48.10%) was observed in the control (untreated eggs) which is almost statistically same as found in 0.01% concentration. It was followed by 0.05% concentration, where 35.90% parasitism was recorded. The lowest percent parasitism (15.40%) was recorded with the use of 0.2% concentration, while 19.80 and 25.00% parasitism was recorded in 0.15% and 0.10% concentrations, respectively.

Data regarding adult emergence (days) is also presented in Table I. Control (untreated eggs) was found superior in terms of adult emergence time (days) as compared to all other different concentrations of Spinosad. Most of the concentrations were found statistically same (P>0.05). Among treatments 0.2% concentration was found comparatively more toxic by recording maximum adult emergence time (11.4 days).

Table I.- Effect of different concentrations of Spinosad on oviposition preference, emergence time and adult longevity of *Trichogramma chilonis* (Ishii) when sprayed before exposing to the adult female.

Concen-trations	Mean number of parasitoid	Adult emergence time (days)*	Adult longevity (days)
0.20 %	15.400 ±1.54e	11.4 ±0.27a	1.31±0.154 b
0.15 %	19.800 ±1.98d	10.7 ±0.26 ab	1.40±0.147 b
0.10 %	25.000 ±2.5c	10.6±0.22 abc	1.53±0.156 b
0.05 %	35.900 ±3.59b	10.6±0.33 abc	1.65±0.144 b
0.01 %	46.300 ±4.63a	10.4±0.24 bc	1.50±0.140 b
Control	48.100±4.81 a	9.9±0.16 c	2.50±0.114 a

* Time taken from parasitism to parasitoid emergence
Means ± SE followed by the same letter(s) (in columns) are not significantly different from each other at (P< 0.05 DMRT method.)

The effect of different concentrations of Spinosad on the adult longevity (days) of *T. chilonis* is presented in Table I. The table shows that in control (untreated) highest longevity (2.50 days) was recorded and was found statistically different from all other treatments (P<0.05). Although same trend was observed just like the % parasitism and adult longevity, where reduced concentration found more effective by recording highest longevity (1.50 days). As the concentration was increased the adult longevity was found decreased. 1.65, 1.53 and 1.40 days longevity was recorded for 0.05, 0.01 and 0.15% concentrations, respectively. The highest concentration (0.20%) was found highly toxic by showing the least longevity (1.31 days).

Egg stage

The data regarding the effect of different concentrations of Spinosad on the egg stage of *T. chilonis* are presented in Table II. The maximum parasitism (41.80%) was observed in the control (untreated eggs), while in 0.01% concentration it was 33.50%. It was followed by 0.05% concentration where 31.60% parasitism was recorded. The lowest percent parasitism, (14.60%) was recorded by using 0.2% concentration which was found statistically at par with 0.10% concentration (P>0.05), while 21.40 and 31.30 percent parasitism was recorded by using 0.15% and 0.10% concentrations, respectively.

Data regarding adult emergence (days) is also presented in Table II. Control (untreated eggs) was found superior in terms of adult emergence as compared to the all other different concentrations of Spinosad. Most of the concentrations showed statistically same results (P>0.05). Among treatments, 0.2% concentration was found comparatively more toxic, as *T. chilonis* adults took the maximum emergence time (10.8 days).

Table II.- Effect of different concentrations of Spinosad on the oviposition preference, emergence time and adult longevity of *Trichogramma chilonis* (Ishii) when sprayed on egg stage.

Concen-trations	Mean no. of parasitoid pupae	Emergence time (days)*	Adult longevity (days)
0.20%	14.600 ±1.46d	10.8±0.27 a	1.25±0.104 d
0.15%	21.400 ±2.14c	10.6±0.25 a	1.35±0.121 cd
0.10%	31.300 ±3.13b	10.4±0.22 ab	1.57±0.133bcd
0.05%	31.600 ±3.16b	10.3±0.22 ab	1.68±0.116abc
0.01%	33.500 ±3.35b	9.9±0.20 b	1.74±0.117 ab
Control	41.800 ±4.18a	9.8±0.18 b	2.06±0.136 a

* Time taken from parasitism to parasitoid emergence
Means ± SE followed by the same letter(s) (in columns) are not significantly different from each other at (P< 0.05 DMRT method.)

The effect of different concentrations of Spinosad on the adult longevity (days) of *T. chilonis*

is presented in Table II. The table shows that in control (untreated) highest longevity (2.06 days) was recorded and was found statistically different from all other treatments (P<0.05). No significance difference (P>0.05) was observed among 0.20, 0.15 and 0.10% concentrations of Spinosad by recording 1.25, 1.35 and 1.57 adult longevity, respectively. Although same trend was observed just like the % parasitism and adult longevity that reduced concentration was found more effective by recording highest longevity (1.74 days). As the concentration was increased the adult longevity was decreased. The longevity of 1.68, 1.57 and 1.35 days was recorded for 0.05, 0.10 and 0.15% concentrations, respectively. The highest concentration (0.20%) was found comparatively more toxic where highest adult longevity (1.25 days) was recorded.

Larval stage

The data regarding the effect of different concentrations of Spinosad on the larval stage of *T. chlonis* is presented in Table III. The maximum parasitism (52.30%) was observed in the control (untreated eggs). The data shows that mean number of parasitoid pupae are inversely proportional with the concentrations. Highest mean number of parasitoid pupae (42.60) was recoded by applying 0.01% concentration which increased with the increase of concentrations by recording 40.60, 25.10, 17.70 and 13.60 mean number of parsitoid pupae for 0.05, 0.10, 0.15 and 0.20% concentrations.

Data regarding adult emergence (days) is also presented in Table III. Control (untreated eggs) was found superior in terms of adult emergence (days) as compared to the all other different concentrations of Spinosad. Among treatments, lowest emergence time (9.8 days) was recorded by applying 0.01% concentration. Emergence time was increased by increasing the concentration of Spinosad. Highest emergence time (10.5 days) was recorded in case of 0.20% concentration of Spinosad.

The effect of different concentrations of Spinosad on the adult longevity (days) of *T. chilonis* is presented in Table III. The table shows that in control (untreated) highest longevity (2.55 days) was recorded and was found statistically different from all other treatments (P<0.05). Same trend was observed just like the % parasitism and adult longevity that reduced concentration was found more effective by recording highest longevity (1.90 days). As the concentration was increased the adult longevity was decreased. 1.78, 1.51 and 1.42 days adult longevity was recorded for 0.05, 0.10 and 0.15% concentrations, respectively. The highest concentration (0.20%) was found comparatively more toxic by showing the lowest longevity (1.32 days).

Table III.- Effect of different concentrations of Spinosad on the oviposition preference, emergence time and adult longevity of *Trichogramma chilonis* (Ishii) when sprayed on larval stage.

Concen-trations	Mean No. of parasitoid pupae	*Emergence time (days)	Adult longevity (days)
0.20%	13.600±1.36e	10.5±0.22a	1.32±0.112d
0.15%	17.700±1.77d	10.4±0.24ab	1.42±0.120 cd
0.10%	25.100±2.51c	10.0±0.19abc	1.51±0.180 cd
0.05%	40.000±4.00b	9.7±0.11 bc	1.78±0.111 bc
0.01%	42.600±4.26b	9.8±0.22bc	1.90±0.102 b
Control	52.300±5.23a	9.5 ±0.12c	2.55±0.114 a

* Time taken from parasitism to parasitoid emergence
Means ± SE followed by the same letter(s) (in columns) are not significantly different from each other at (P< 0.05 DMRT method.)

Pupal stage

The data (Table IV) showed the life history parameters of *T. chilonis* when sprayed with different concentration of Spinosad. Lowest emergence time (9.6 days) was recorded in control (untreated) as compared with all other treatments (P<0.05). As the concentration of Spinosad increased the emergence time was also increased. 10.1, 10.3, 10.9 days (emergence time) was recorded by applying 0.01, 0.05, 0.10, 0.15 and 0.20% concentrations of Spinosad.

The adult longevity (2.64) was found significantly higher in control. The adult longevity at 0.05, 0.10 and 0.15% was recorded as 1.64, 1.63 and 1.62 day respectively which was found statistically same (P>0.05). Lowest adult longevity (1.40 days) was observed by applying 0.20% concentration of Spinosad.

Table IV.- Effect of different concentrations of Spinosad on the emergence time and adult longevity of *Trichogramma chilonis* (Ishii).

Concentrations	Emergence time (days)*	Adult longevity (days)
0.20%	10.9±0.25 a	1.40±0.139 c
0.15%	10.3±0.29 ab	1.62±0.154 bc
0.10%	10.1±0.28 b	1.63±0.135 bc
0.05%	10.3±0.28 ab	1.64±0.140 bc
0.01%	10.1±0.28 b	2.00±0.088 b
Control	9.6±0.16 b	2.64±0.116 a

* Time taken from parasitism to parasitoid emergence
Means ± SE followed by the same letter(s) (in columns) are not significantly different from each other at (P< 0.05 DMRT method.).

Adult stage

When the Spinosad was applied to the adults of *T. chilonis*, 100% mortality occurred within 5-15 minutes in all concentrations, showing that Spinosad is highly toxic to the adults (Table V).

Table V.- Behaviour of *Trichogramma chilonis* females (n=5) after getting exposed to Spinosad

Concen-tration. (%)	n	Total distance traveled (cm)	Pause time (seconds)	Avg. velocity (cm/sec)	Velocity excluding pause (cm/sec)
0.005	4	16.22±3.5 a	77.02±14 a	0.08±0.01a	0.15±0.01
0.001	5	25.4±1.63 ab	50.8±4 a	0.14±0.01ab	0.20±0.01
Control	5	32.8±4.04 b	42.3±0.01a	0.18±002 b	0.24±0.01

Means ± SE followed by the same letter(s) (in columns) are not significantly different from each other at (P< 0.05 DMRT method.)

Walking behavior of T. chilonis

The data in Table V reveal the behaviour of *T. chilonis* when treated with the lowest concentrations of 0.005% and 0.001%. The total distance covered by the parasitoid in 0.005% is 16.22 cm with the pause time of 77.02 sec which gives the average velocity of 0.08 cm sec^{-1}. When the pause time is excluded the average velocity is 0.1 cm sec^{-1}, while in 0.001% total distance covered is 25.4 cm with pause time of 50.8 sec. Average velocity is 0.14 cm sec^{-1} while pause time excluded velocity is 0.2 cm sec^{-1}.

DISCUSSION

The present research work findings show that percent parasitism, emergence time and adult longevity were found higher in control as compared with all concentrations of Spinosad. Highest concentration of Spinosad (0.2%) reduced the percent parasitism, emergence time and adult longevity as compared with all other concentrations. Our results show conformity with Medina *et al*. (2001), who reported that Spinosad at the highest concentrations caused a significant reduction in the adult life span and fecundity. Nasreen *et al*. (2004) also confirms our present findings by recording the toxic effect of Spinosad on different life parameters of *T. chilonis* and *C. carnea*.

Significantly higher number of pupae formation were recorded in control. The chemical proved toxic to the beneficial insect when used in higher concentrations. The same trend can be observed in percent pupation, emergence time and adult longevity. Our results show conformity with Charles *et al*. (2000) who investigated that Spinosad along with other insecticides adversely affect *Trichogramma* emergence from host eggs when exposed at different preimaginal stages of development (larval, prepupal or pupal).

The present findings follow almost the same pattern as observed in case of egg stage. At highest concentration of Spinosad least number of pupae was formed. This gives least % pupation by using the highest concentration. Emergence time was increased and adult longevity was found decreased. Our results are in line with Medina *et al*. (2003) who investigated that Spinosad at the highest concentration tested cause slight significant reduction in the adult life span and fecundity.

The present data show that at highest concentration, the *T. chilonis* took maximum time for emergence which gradually decreased by decreasing the concentrations of Spinosad. Due to the toxic effect the adult longevity was least at highest concentration while maximum adult longevity was recorded in control. Our results are in conformity with Charles *et al* (2000) who investigated that Spinosad when applied on preimaginal stages of development (larval, Prepupal and pupal) of *T. chilonis* adversely affect the

emergence from host eggs. In addition the mean life span of the emerged adults also varied at different concentrations.

According to our results the adults when exposed to surface treated vials, died within 5-15 minutes. It is obvious from the present work that the Spinosad adversely affect the *T. chilonis* behavior. Significantly lower distance (16.22 cm) was covered by the parasitoid when treated with 0.005%. While it traveled 25.4 cm when treated with 0.001%. Similar pattern can be observed in the pause time and average velocity showing that the higher concentrations affect parasitoid behavior and search ability. Nasreen *et al*. (2004) support the present findings who investigated that the Spinosad along with some other insecticides show selectivity to *T. chilonis* adults. Same results were also investigated by Singh and Amandeep (2005), who reported that Spinosad along with some other insecticides showed 100% mortality of *T. chilonis* adults within 15 minutes of exposure.

CONCLUSIONS

Keeping in view the results of the present study it is concluded that the Spinosad was found highly toxic for the adults. Also all other parameters are directly affected by increasing the concentrations of Spinosad.

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