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TOXICITY OF SPINOSAD TO THE PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* (COLEOPTERA: BRUCHIDAE) AND ITS PARASITOID, *DINARMUS BASALIS* (HYMENOPTERA: PTEROMALIDAE)

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ABSTRACT

Toxicity of Spinosad 45SC against adults of pulse beetle, *Callosobruchus chinensis* and its hymenopteran parasitoid, *Dinarmus basalis* was determined using dry film contact toxicity method under laboratory conditions. Spinosad was found to have contact toxicity against *C. chinensis* and the median lethal concentration (LC_{50}) values at 24, 48 and 72 hours post-treatment were 51.05, 11.99 and 1.92 ppm, respectively. Contact toxicity of spinosad to *D. basalis* was higher with LC_{50} values of 0.130, 0.062 and 0.015 ppm at 24, 48 and 72 hours post-treatment, respectively. Field evaluation of Spinosad 45SC in mungbean and urdbean revealed that the insecticide was effective in reducing pod (82.9 to 84.9% reduction over control) and seed (76.5 to 78.1% reduction over control) damage due to the pulse beetle and comparable with conventional insecticide Dichlorvos 76EC (81.8 to 90.2% and 82.4 to 84.4% reduction in pod and seed damage, respectively). The present results provide informative data on susceptibility of pulse beetle and its parasitoid to spinosad for developing efficient pest management programs.

KEY WORDS
Spinosad
Callosobruchus chinensis
Dinarmus basalis
Mungbean, Urdbean

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INTRODUCTION

Post harvest damages by insect pests have been an increasingly important constraint to food legume supplies worldwide. Pulse beetles or Bruchids belonging to *Callosobruchus* spp. (Coleoptera: Bruchidae) are major storage pests of legume crops grown in the tropics and sub-tropics. Among the different species of pulse beetle, *Callosobruchus chinensis* L. is the most destructive in India and the post harvest seed losses due to the beetle can reach even up to 100% during severe periods of infestation (Srinivasan *et al*., 2010). The infestation of these beetles originates in the crop field and carried to store. Synthetic insecticides or fumigants have been extensively used all over the world to check infestation of the beetle in stored seeds. However, their application is being discouraged globally because of handling hazards, adverse environmental impacts and development of resistance against them in insects. In recent years, spinosad an eco-friendly biorational insecticide derived from naturally occurring soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae) has been found to be effective against stored pests (Vayias *et al*., 2009; Hertlein *et al*., 2011). The US Environmental Protection Agency has classified spinosad as a reduced risk insecticide due to its low effective use rate and safety to the environment and mammals. It is considered a natural product suitable for use in organic agriculture by numerous national and international certification bodies (Racke, 2007). Spinosad is currently registered in the USA as a grain protectant on several agricultural commodities including wheat, corn, rice, millets, oats, sorghum and barley (Huang and Subramanyam, 2007). It is reported that the insecticide spinosad will be used more widely in many countries for the management of storage pests (Vayias *et al*., 2009). In India, spinosad has been registered for use on redgram, cotton, chillies, cabbage and cauliflower against lepidopteran pests. Information on the toxicity of spinosad against the pulse beetle and its parasitoids is limited. The present study was undertaken to evaluate the toxicity of spinosad against adults of pulse beetle, *C. chinensis* and its hymenopteran parasitoid, *Dinarmus basalis* (Rond.) on major grain legumes, mungbean [*Vigna radiata* (L.) Wilczek] and urdbean [*Vigna mungo* (L.) Hepper].

MATERIALS AND METHODS

Test insects and maintenance

Pure culture of pulse beetle (*Callosobruchus chinensis*) and the larval parasitoid of pulse beetle, *Dinarmus basalis* (Hymenoptera: Pteromalidae) maintained at Crop Protection Division of Indian Institute of Pulses Research, Kanpur were used for the experiment. The pulse beetle was mass reared on fresh mungbean (*V.*

radiata) seeds under laboratory condition following Strong *et al*. (1968). Subculturing of this beetle was done at regular intervals by releasing 50 pairs of adults in wide-mouth plastic jar (30 cm x 15cm) containing 500g of mungbean seeds. The mouth of the jar was covered with muslin cloth and fastened tightly with the help of rubber band.

The parasitoid *D. basalis* was mass reared on the pulse beetle that infested *desi* chickpea seeds (*Cicer arietinum* L.) in laboratory as per the method described by Islam (1998). Fifty pairs of pulse beetle (*C. chinensis*) adults were released for 72h in wide-mouth plastic jar (30cm x 15cm) containing 500g of chickpea seeds. The mouth of the jar was covered with muslin cloth and fastened with rubber band. After 20 days, ten pairs of *D. basalis* adults were introduced into the jars containing larvae of *C. chinensis* for parasitization. A cotton swab soaked in 50% honey solution was kept in the jar as feed for the adult parasitoids. Seeds containing the parasitized larvae were held in the jar until the emergence of adult parasitoid. The adult parasitoids emerged from the seeds were used for the experiments. Both *C. chinensis* and *D. basalis* culture were maintained at room temperature (28 ± 2ºC) and 70 ± 5% RH throughout the period of study (April to October, 2011). One-day-old adults of *C. chinensis* and *D. basalis* were used in the experiments.

Insecticides

Commercial formulation of Spinosad (Tracer 45SC) obtained from Dow Agro Sciences was used in this assay. The insecticide formulation was diluted in distilled water to make solutions of different concentrations for bioassay. For field evaluation, the commercial formulations of spinosad 45SC along with four conventional insecticides recommended for pod borer complex in legume crops *viz*., methomyl (Lannate 40SP), profenofos (Curacron 50EC), dichlorvos (Nuvan 76EC) and triazophos (Hostathion 40EC) were used.

Bioassay Technique

Dry film contact toxicity method described by Sadat and Asghar (2006) was used to determine the toxicity of spinosad to adults of pulse beetle (*C. chinensis*) under laboratory conditions. Bioassays were performed on filter paper placed inside Petri-dishes (90 mm x 14 mm, diameter x height). The required dilutions were prepared from the commercial formulation of spinosad with a distilled water control. Preliminary range determining tests were conducted to attain series of doses which give a range of kills from 10 to 100 per cent. Five concentrations based on preliminary range determining test were used for the experiment. Filter paper discs were treated with 2mL each of different concentrations of spinosad solutions along with control placed in the bottom and the lid of 90-mm Petri-dishes and allowed to dry. After shade drying of the Petri-dishes, fifteen one-day-old adults of *C. chinensis* were separated from cultures and introduced into each dish of different concentrations and control. The experiment was replicated four times. The Petri-dishes were secured with rubber band and maintained at 28 ± 2ºC and 70 ± 5% RH. Observations on the mortality of the beetles were recorded after 24, 48 and 72h. The same method *i. e.,* dry film contact toxicity bioassay was followed to determine the toxicity of spinosad to the parasitoid, *D. basalis,* except that a cotton swab soaked in 50% honey solution was kept in the side of the Petri-dishes as feed for adults.

Field efficacy of spinosad against pulse beetle on mungbean and urdbean

Field experiments were conducted during *kharif* 2011 (July to October) at the Research Farm, Indian Institute of Pulses Research, Kanpur (26º27′ N, 80º14′E and 152.4 m above MSL) to test the efficacy of spinosad 45SC against pulse beetle (*C. chinensis*) on mungbean (cv. Meha) and urdbean (cv. Shekar 1). The soil was sandy loam, with pH 8.16, EC 0.21 dS/m, organic carbon 0.24%, available P 11.88 kg/ha and available K 126 kg/ha. The rows and plants were spaced 30 cm and 10 cm apart, respectively. Recommended crop management practices were adopted. The efficacy of spinosad 45SC @ 0.018% and four conventional insecticides recommended for pod borer complex in pulse crops *viz*., Methomyl 40SP @ 0.06%, Dichlorvos 76EC @ 0.11%, Profenofos 50EC @ 0.10% and Triazophos 40EC @ 0.06% (Saxena *et al*., 2010) were tested against *C. chinensis* along with untreated control. The experiments were conducted in a randomised block design with a plot size of 5 x 4 m with five replications. The treatments were imposed during pod development stage of the crops using a backpack knapsack sprayer while in untreated control, water spray was given. The data on 100 pods (10 pods from 10 randomly selected plants) was recorded at the time of harvest from each treatment. Sampled pods were kept in plastic jar (30cm x 15cm) and examined in the laboratory for pulse beetle emergence. The percent infestation of the pulse beetle was assessed on the basis of presence of circular hole on pod/seed and emerged adults of pulse beetle from the field collected pod samples (Fig. 1). The number of healthy and damaged pods and seeds were recorded. The data were computed to determine the extent of percentage pod damage and seed damage.

Statistical analysis

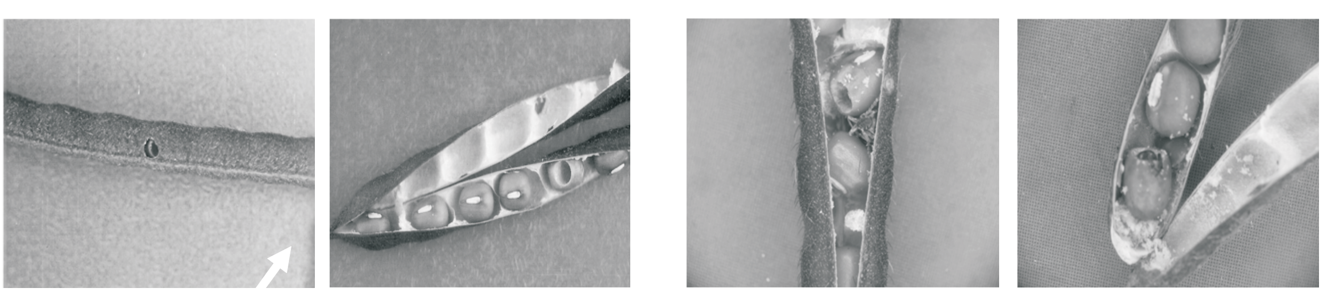

Figure 1: Field infestation of pulse beetle (*C. chinensis*) in mungbean and urdbean

Control mortality was corrected by using Abbott's formula. Based on the adult mortality in different treatments, the median lethal concentration (LC_{50}) value of spinosad was calculated using probit analysis with SPSS advanced version 10.0. In the randomized block design analysis, the data expressed in terms of percentage in different experiments were converted to arcsine percentage values. The ANOVA was done using Agres statistical software. Following ANOVA, differences between datasets were determined using least significant difference at P = 0.05 in all instances. Data are presented as means and compared as DMRT.

RESULTS AND DISCUSSION

Toxicity of spinosad to pulse beetle, *C. chinensis*

The results of the bioassay revealed that spinosad was toxic to the beetle. The median lethal concentration (LC_{50}) of spinosad to *C. chinensis* was 51.05 ppm at 24 hours after treatment with the upper and lower fiducial limits of 81.51 and 32.29 ppm, respectively. The LC_{50} values decreased with increase in period of exposure and the values were 11.99 and 1.92 ppm after 48 and 72 hours of treatment, respectively. Ninety five per cent adults of pulse beetle died at 431.25, 111.37 and 32.82 ppm at 24, 48 and 72 hours after treatments, respectively (Table 1). Spinosad is tetracyclic macrolide compound which is primarily a stomach poison with some contact activity. It has been registered in many countries against a wide range of pests and has excellent insecticidal activity against Lepidoptera (Gadhiya *et al*., 2014), Diptera (Daharia and Katlam, 2013) and some species of Coleoptera (Hertlein *et al*., 2011). The present report is the first of its kind on toxicity of spinosad to pulse beetle, *C. chinensis* (Coleoptera: Bruchidae) and supports the reports of Subramanyam *et al*. (2003); Sadat and Asghar (2006); Vayias *et al*. (2009) and Hertlein *et al*. (2011) who reported the toxicity of spinosad to coleopteran stored-grain pests *viz*., cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae); red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae); lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) and larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). The LC_{50} values of spinosad to *C. chinensis* recorded in the present study was lower when compared to its LC_{50} value on *Callosobruchus maculatus* (F.) (Sadat and Asghar, 2006). This indicates that *C. chinensis* is comparatively less susceptible to spinosad as compared to *C. maculatus*.

Toxicity of spinosad to parasitoid, *Dinarmus basalis*

The data revealed that spinosad was toxic to the parasitoid and the LC_{50} value was 0.130 ppm at 24 hours after treatment (Table 2). After 48 and 72 hours of treatment, the LC_{50} was very low and the respective values were 0.062 and 0.015 ppm, which revealed the high contact toxicity of spinosad to the parasitoid. The LC_{95} values of spinosad were found to be 1.395, 0.459 and 0.071 ppm at 24, 48 and 72 hours after treatment, respectively. Spinosad is classified as an environmentally and toxicologically reduced risk material and has been embraced by IPM practitioners as a biorational pesticide. Conventional toxicity tests indicate that spinosad has virtually no toxicity to birds and mammals and it has also been reported to be practically non-toxic to natural enemies of the insects such as *Orius* spp., *Chrysopa* spp., coccinelids, and the predaceous mite *Phytoseiulus persimilis* Athias-Henriot (Bret *et al*., 1997). Contrarily, the present investigation revealed that spinosad was harmful to *D. basalis* (Hymenoptera: Pteromalidae), an important parasitoid of pulse beetle in store and field. This finding was in confirmation with the report of Williams *et al*. (2003) who stated that hymenopteran parasitoids have significantly high susceptibility to spinosad than the predatory insects with moderately harmful or harmful result. This also corroborates with the reports of Mason *et al*. (2002) who found that spinosad was toxic to the parasitoids *Trichogramma inyoense* Pinto and Oatman (Hymenoptera: Trichogrammatidae), *Microplitis mediator* Haliday (Hymenoptera: Braconidae) and *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae).

Field efficacy of spinosad against pulse beetle on mungbean and urdbean

The effect of foliar spray of spinosad and conventional insecticides was evaluated against pulse beetle (*C. chinensis*) during the pod development stage and the pulse beetle damage

Table 1: Contact toxicity of spinosad to pulse beetle, *Callosobruchus chinensis* exposed for 24, 48 and 72 hours

Hours After Treatment (HAT)	Regression equation Y = a + bx	χ^2	LC_{50}(ppm)	Fiducial limits (ppm)		LC_{95}(ppm)	Fiducial limits(ppm)	
				Upper limit	Lower limit		Upper limit	Lower limit
24 HAT	Y= -3.03 +1.77 x	7.84	51.05	81.51	32.29	431.25	2763.69	202.74
48 HAT	Y= -1.83 + 1.69 x	4.99	11.99	15.33	8.46	111.37	172.09	82.99
72 HAT	Y= -0.38 + 1.33 x	4.09	1.92	4.53	0.20	32.82	54.04	23.15

Table 2: Contact toxicity of spinosad to bruchid parasitoid, *Dinarmus basalis* exposed for 24, 48 and 72 hours

Hours After Treatment (HAT)	Regression equation Y = a + bx	χ^2	LC_{50}(ppm)	Fiducial limits (ppm)		LC_{95} (ppm)	Fiducial limits (ppm)	
				Upper limit	Lower limit		Upper limit	Lower limit
24 HAT	Y= 1.41 +1.59 x	7.70	0.130	0.208	0.076	1.395	8.230	0.646
48 HAT	Y= 2.29 + 1.89 x	13.46	0.062	0.092	0.041	0.459	1.490	0.253
72 HAT	Y= 4.47 + 2.46 x	1.81	0.015	0.018	0.013	0.071	0.094	0.057

Table 3: Field efficacy of spinosad on pulse beetle (*C. chinensis*) damage in mungbean and urdbean

Treatments	Mungbean				Urdbean			
	Pod damage (%)	Reduction over check (%)	Seed damage (%)	Reduction over check (%)	Pod damage (%)	Reduction over check (%)	Seed damage (%)	Reduction over check (%)
Spinosad 45SC @ 0.018%	0.7(4.8) [ab]	82.9	0.16 (2.3) [a]	76.5	0.5(4.1) [a]	84.9	0.14(2.1) [a]	78.1
Methomyl 40SP @ 0.06%	1.2(6.1) [b]	70.7	0.44 (3.8) [b]	35.3	1.1(5.9) [b]	66.7	0.34(3.3) [b]	46.9
Dichlorvos 76EC @ 0.11%	0.4(3.6) [a]	90.2	0.12 (1.9) [a]	82.4	0.6(4.3) [a]	81.8	0.10(1.8) [a]	84.4
Profenofos 50EC @ 0.10%	2.6(9.1) [c]	36.6	0.44 (3.8) [b]	35.3	2.0(8.0) [c]	39.4	0.32(3.2) [b]	50.0
Triazophos 40EC @ 0.06%	2.8(9.6) [c]	31.7	0.54 (4.2) [b]	20.6	2.2(8.4) [c]	33.3	0.52(4.1) [c]	18.8
Untreated check	4.1(11.6) [d]	-	0.68 (4.7) [c]	-	3.3(10.5) [d]	-	0.64(4.6) [c]	-
SEd	0.69	-	0.22	-	0.66	-	0.24	-
CD (P=0.05)	1.4	-	0.45	-	1.4	-	0.50	-

Means in a column followed by different letter(s) are significantly different at P=0.05; Figures in the parenthesis are arc sine values

assessed from the field collected pods at harvest in different treatments has been presented in Table 3. In both mungbean and urdbean, application of insecticides reduced the pod and seed damage due to *C. chinensis* as compared to that in the untreated check. The per cent reduction in pod and seed damage ranged between 31.7 to 90.2% and 18.8 to 84.4% over the untreated check, respectively. Among the insecticides, dichlorvos 76EC @ 0.11% recorded significantly lower damage in pods (0.40% and 0.60% in mungbean and urdbean, respectively) and seed (0.12% and 0.10% in mungbean and urdbean, respectively). The damage was found at par with spinosad 45SC@0.018% in both mungbean (0.7 and 0.16% pod and seed damage, respectively) and urdbean (0.5 and 0.14% pod and seed damage, respectively). Application of methomyl 40SP, profenofos 50EC and triazophos 40EC was found less effective against pulse beetle (31.7 to 70.7% and 18.8 to 50.0% reduction in pod and seed damage over untreated check, respectively) as compared to dichlorvos and spinosad (81.8 to 90.2% and 76.5 to 84.4% reduction in pod and seed damage over untreated check, respectively).

The infestation of pulse beetle usually originates in the crop when it is in the field and is carried over to the storage (Srinivasan *et al*., 2010; Mandal and Roy, 2012). Female *C. chinensis* lay their eggs on developing pods and the hatching larva bores into the seed beneath the oviposition site. The new generation of *C. chinensis* adults emerges in the stores after the harvest of seed, reproduces on the stored seeds and causes huge losses in quality as well as quantity of the seed. Insecticides play an important role in preventing field infestation of pulse beetle. If insecticides are applied in the standing crop for the control of other insects at a stage which is vulnerable to pulse beetle also, there is no need to repeat applications for pulse beetle. In the present investigation, conventional insecticides recommended for pod borer complex in legume crops and spinosad 45SC were tested against *C. chinensis* in mungbean and urdbean. The results indicate that spinosad was effective and comparable with conventional insecticide dichlorvos in reducing pod and seed damage due to pulse beetle. This is in confirmation with the reports of Ozar and Genc (1993) who reported that application of pesticides resulted in low oviposition and seed damage due to pulse beetle in cowpea. These findings were in conformity to those reported by Daglish *et al*. (2008), who found that the field application of spinosad was found effective against lesser seed borer (*Rhyzopertha dominica*) infesting wheat.

The results of our laboratory and field study showed that the biologically derived insecticide, spinosad, can be preferred for efficient pest management programs against pulse beetle (*C. chinensis*) infesting seeds of legumes under field and storage conditions. As the pulse beetle parasitoid (*D. basalis*) was found susceptible to spinosad, safer delivery technique and integration with physical and botanical methods need to be studied in future to conserve the parasitoid.

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Cont. .... P 26