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Estimation of Toxicity of Spinosad Using Two Different Bioassay Methods Against Cotton Bollworm, *Helicoverpa armigera* (Hub.)

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Abstract.- Two different bioassay methods; larval dip bioassay and surface diet bioassay were tested for the contact/ingestion toxicity and to see the advantage of one method over the other using spinosad against second instar *Helicoverpa armigera.* Little difference was observed in LC_{50} values which were 170 and 130ppm for both the methods, however ingestion/contact toxicity (surface diet bioassay) as compared to contact toxicity only (larval dip bioassay) remained comparatively but not significantly different. Results also proved that surface diet bioassay had advantage over larval dip bioassay as it provided better estimate of potential toxicity.

Key words: Spinosad, Bioassay, LC_{50}, *H. armigera.*

INTRODUCTION

Cotton is attacked by a number of sucking and chewing pests in Pakistan; amongst the latter, cotton bollworm, *Helicoverpa armigera* is currently a very important pest. It is currently the most important in economic terms and highly polyphagous agriculture pest. Host plants of *H. armigera* belongs to a broad spectrum of plant fauna and include important agricultural crops such as cotton, maize, chickpea, pigeonpea, sorghum, sunflower, soybean and groundnuts (Fitt, 1989). Due to wider host range, multiple generations, migratory behavior, high fecundity and existing insecticide resistance this became a difficult pest to tackle (Hussain *et al.,* 1991; Khan *et al.,* 1993; Ahmad *et al.,* 2000). Like other cotton producing countries, Pakistan largely relied on chemical control of this pest. The indiscriminate use of insecticides, particularly during 80s and 90s contributed to the emergence of insecticide resistance in *H. armigera.* Control of this pest was not always adequate probably due to the development of resistance. Moderate to high level of resistance to pyrethroides and organophosphorus insecticides were recorded in field population of *H armigera* (Ahmad *et al.,* 1995).

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Hence newer molecules with novel mode of action are currently essential for the management of pyrethroid resistant of *H. armigera.* Spinosad is a reduced risk insecticide based on metabolites of a soil bacterium *Saccharopolyspora spinosad* (Mertz and Yao, 1990). This bacterial insecticide has a unique mode of action with a very low mammalian toxicity compared with other insecticides (Bert *et al*., 1997; Salgado, 1997; Thompson *et al*., 1997). In the present study the toxicity of spinosad was studied against *H. armigera* using two bioassay methods and also to see suitability of bioassay method for better estimates of results.

MATERIALS AND METHODS

Test insects

Cotton bollworms were reared in the laboratory on modified semi-synthetic diet (Ahmad and McCaffery, 1991), consisting of Chickpea flour, Sorbic acid, Wesson's salt, Vitamin (ABDE), Ascorbic acid, Yeast (Brewer's), Choline chloride, Agar agar, Formaldehyde, Streptomycin sulphate and Methyl para hydroxy benzoate, under laboratory

conditions of 27±2°C, 65±5% RH and 14:10 L:D h.

Larval dip bioassay

Aqueous dilutions of formulated (emulsion concentrates) insecticide were prepared and batches of second instar larvae were submerged for 5 sec. as described by Watkinson *et al.* (1984). A group of 50 larvae were dropped into 100 ml of the appropriate dilution in 500 ml beaker and gently swirled for 5 sec. to ensure complete wetting. The solution plus larvae were then poured through a fine nylon mesh suspended over an empty beaker. The solution was decanted and larvae separated by this process. After shade drying for about 5 min. the treated larvae were then transferred individually into semi synthetic diet. Control insects were treated with water alone.

Surface-treated diet bioassay

The surface-treated diet bioassay methods were similar to those described by (Joyce *et al.*, 1986). Three ml of a chickpea based artificial diet was pipetted into 30 ml vials and allowed to cool at room temperature for approximately 1 h. For each insecticide tested, serial dilutions of formulated material (100 µl aliquots) were pipetted onto the diet surface, agitated to distribute evenly, and allowed to dry for approximately 30 min. Second instar larvae were placed into a series of vials that contained 4 different concentrations of formulated insecticide *viz*., 100, 200, 300, and 500 ppm along with untreated controls to determine the LC_{50} for a given insecticide. Each vial contained one larva. A minimum of 50 larvae per dose were tested for each insecticide concentration. Larvae were considered dead if no movement was observed after prodding with blunt forceps for 10s. Control vials were treated with distilled water only.

Laboratory conditions and test insecticides

Bioassays were carried out at 26±1°C under approximately 12:12 (L:D) h photoperiod. Mortality was recorded at 48 h interval. A serial dilution of the formulated test insecticide was prepared as ppm of the active ingredient.

Statistical analysis

Larval mortalities were assessed after 48 h. Results were expressed as percentage mortalities. Data were analyzed using computer based probit analysis programme (Finney, 1971).

RESULTS AND DISCUSSION

Results of bioassays revealed non-significant variation in response of *H. armigera* to spinosad using two different bioassay methods in Table I. The LC_{50} values of spinosad for larval dip bioassay, surface diet bioassay on *H. armigera* with 95% confidence limit (lower and upper limit) are also presented in Table I. After 48 hrs of application of spinosad LC_{50} value was found as 170 ppm using larval dip bioassay, whereas 130 ppm for surface diet bioassay. Chi-square values shows that there was insignificant difference of mortality rate of *H armigera* among the concentrations of spinosad. The R^2 value and regression equation for log dose and probit mortality has been shown in Figure 1. The R^2 value and regression equation for larval dip bioassay was found as 0.93 and Y=140 x +1.85, whereas Y=1.82 x +1.14 for surface diet bioassay. In both the above mentioned cases the slopes were positive. Similar to our findings Ramasubramanian and Regupathy (2004) recorded low LD_{50} against early instar larvae of *H. armigera.*

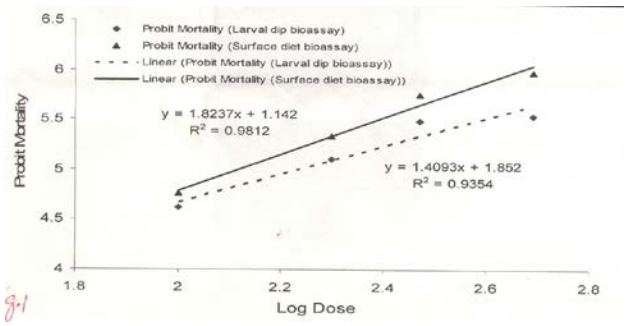

Fig. 1. Relationship of log doses and probit mortalities of spinosad using two bioassay methods on second instar larvae of *H. armigera.*

Spinosad's high effectiveness is even against pyrethroid resistant strains is attributed to its novel mode of action as mentioned by (Watson, 2001) With the increasing resistance that *H. armigera* is

exhibiting towards a wide range of insecticides especially pyrethroids (Ahmad *et al*., 1997) the need for the use of more effective insecticides is increasing. Spinosad in our studies showed more toxicity for surface diet bioassay probably because of both contact and ingestion action of spinosad. Tabashnik and Cushing (1987) also proved that leaf residue bioassay which is similar to surface diet bioassay in our experiment has advantage over topical application, as it is time and labour saving method and provide more accurate estimates.

Table I.- Toxicity of spinosad against *Helicoverpa armigera* using two different methods of bioassay.

Insecticide conc. (ppm)	Log conc.	Sample size (n)	Number dead (Larvae)	Mortality (%)	Corrected mortality (%)	LC_{50} (ppm) (95% FL of LC_{50})	Slope (SE)	Chi-square (df)
				Surface diet bioassay				
100	2.0	50	19	38	35.42	170.23	1.39	0.94 (2)
200	2.30	50	28	56	54.17	(36.42-275.48)	(0.37)	
300	2.47	50	35	70	68.75			
500	2.69	50	36	72	70.83			
				Larval dip bioassay				
100	2.0	50	21	42	40.82	129.91 (81.23-168.34)	1.81 (0.38)	0.42 (2)
200	2.30	50	32	64	63.27			
300	2.47	50	39	78	77.55			
500	2.69	50	42	84	83.67			

It is concluded from the results that spinosad is effective against *H. armigera* as direct and indirect application. Furthermore, the use of surface diet bioassay provides better estimates of potential toxicity for laboratory bioassay studies.

REFERENCES

AHMAD, K., QURESHI A.S. AND KHALIQUE, F., 2000. Effect of environmental factors on pheromone trap catches of chickpea pod borer, *Helicoverpa armigera* (Hub.) from 1983 to 1998. *Proc. Pakistan Acad Sci.*, **37**: 227-238.

AHMAD, M., ARIF, M.I. AND AHMAD, Z., 1995. Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptra: Noctuidae) in Pakistan. *J econ. Ent.*, **88**: 771-776.

AHMAD M., ARIF, M.I. ATTIQUE, M.R., 1997. Pyrethroids resistance *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bull. entomol. Res*., **87**: 43-347.

AHMAD, M. AND McCAFFERY, A.R., 1991. Elucidation of detoxification mechanisms involved in resistance to insecticides against third instar larvae of a field selected strain of *Helicoverpa armigera* with the use of synergists. *Pestic. Biochem. Physiol*., **41**: 41-52.

BERT, B.L., LARSON, L.L., SCHOONOVER, J.R., SPARKS, T.C. AND THOMPSON, G.D., 1997. Biological properties of spinosad. *Down to Earth*, **52**: 6-13

FINNEY, D.J., 1971. *Probit analysis*. 3rd ed. Cambridge, Cambridge University Press.

FITT, G.P., 1989. The ecology of *Heliothis* in relation to agro ecosystems. *Annu. Rev. Ent.*, **34**: 17-52.

HUSSAIN, T., TALPUR, M.A. AND TUNIO, G.D., 1991. Relative toxicity of pyrethroid insecticides to gram pod borer. *Proc. Pakistan Congr. Zool.*, **11**: 119-122.

JOYCE, J.A., OTTENS, R.J., HERZOG, G.A. AND BASS, M.H., 1986. A laboratory bioassay for thiodicarb against the tobacco budworm, bollworm, beet armyworm and fall armyworm. *J. agric. Ent.*, **3**: 207-212.

KHAN, M.M., RUSTAMANI, M.A., TALPUR, M.A., BALOCH, H.B. AND CHUTTO, A.B., 1993. Effect of different insecticides against *Helicoverpa armigera* on gram. *Pakistan J. Zool.*, **25**: 117-119.

MERTZ, E. P. AND YAO, R. C., 1990. *Saccharopolyspora spinosa* sp. novo isolated from soil collected in a sugar rum still. *Int. J. Syst. Bact.*, **40**: 34-39.

RAMASUBRAMANIAN, T. AND REGUPATHY, A., 2004 Magnitude and mechanism of insecticide resistance management. in *Helicoverpa armigera* Hub. population of Tamil Nadu, India. *Asian J Plant. Sci.*, **3**: 94-100.

SALGADO, V.L., 71997. The modes of action of spinosad and other insect control products. *Down to Earth*, **52**: 35-43.

TABASHNIK, B.E. AND CUSHING, N.L., 1987. Leaf residue vs. topical bio assays for assessing insecticides

resistance in diamondback moth, *Plutella xylostella* Linn. *FAO Plant Protect. Bull*., **35**: 1.

THOMPSON, G.D., MICHEL, K.H., YAO, R.C., MYNDERSE, J.S., MOSBURG, C.T., WORDEN, T.V., CHIO, E.H., SPARKS, T.C. AND HUTCHINS, S.H., 1997. The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. *Down to Earth*, **52**: 1-5.

WATSON, G. B., 2001. Actions of insecticidal spinosyns on c-aminobutyric acid responses from small-diameter cockroach neurons. *Pestic. Biochem. Physiol.*, **71**: 20-28.

WATKINSON, I.A., WIREMAN, J. AND ROBINSON, J., 1984. A simple test for field evaluation of the susceptibility on insect pests to pesticides. In: *Proceedings, British Crop Protection Conferences Pests and Diseases*, pp. 559-564. British Society of Chemical Industry. Brighton, UK.

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