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Resistance Development To Selected Insecticide In Spodoptera litura F., 1775 (Lepidoptera: Noctuidae)

Sule Rupa[1] and *Kumar Dolly[2]

[1 2]Department of Zoology, Facutly of Science, The Maharaja Sayajirao University of Baroda, Vadodara-390002, Gujarat,India

ABSTRACT

This study takes into account, the evaluation of development of resistance to the selected bio pesticide, Spinosad (Tracer®) in a laboratory culture of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). The pest was brought from nearby agricultural fields of Vadodara (Gujarat). The parental colony i.e. mother culture was not exposed to any type of insecticide and it was ensured by keeping it separately. The bioassay was performed in laboratory conditions by traditional leaf dip methodology. The pest was exposed to Spinosad (Tracer®), continuously for five generations. The results showed that LD50 of the selected resistant lines was higher as compared to the parental colony which indicated toxicity level of the selected insecticide. Exposure to bio-pesticide for five generations indicated very low amount of resistance to Spinosad. The live larvae from each bioassay were continued to next generation. Results showed very low levels of resistance i.e. LC50 values as 1.68, 1.33, 2.44, 1.51 and 1.38. This study can prove to be useful when co-related to field conditions where there is much usage of pesticides and less knowledge about their pattern of rotations. Many farmers are now focussing on such bio pesticides which can be a promising alternative to toxic insecticides.

KEY WORDS: Bio pesticide, Spinosad, *Spodoptera litura***,** Resistance

***Corresponding Author**

Prof. Dolly Kumar

Department of Zoology,

Faculty of Science

The Maharaja Sayajirao University of Baroda,

Vadodara-390001Gujarat, India

Email id: dollymsu@gmail.com

INTRODUCTION

The cutworm *Spodoptera litura* Fab. (Lepidoptera, Noctuidae) is well known as a serious cosmopolitan pest with extensive host range of economically important crops such as cotton, groundnut, soybean, tomato, sweet potato and many other crops.[1] The present study was planned of a bio-pesticide Spinosad (Tracer®). Spinosad contains a mix of two spinosoids, spinosyn A, the major component, and spinosyn D (the minor component), in a roughly 17:3 ratio.[2] The spinosyns are a unique family of fermentation-derived insecticides having potent activity and lower environmental effect.[3] Bio-pesticides have the potential to control crop losses and reduce negative environmental externalities. Bio-pesticides constitute around 3 per cent of pesticide market in the country. So far 14 bio-pesticides have been registered under the Insecticide Act 1968 in India.[4] They can play an important role in shifting the focus from chemical pesticides to reliable, sustainable and environment friendly options. The usage of bio-pesticides forms one of the vital step in integrated pest management program. The insecticide resistance is a serious problem which has gained impetus because of extensive use of insecticides. Baseline data on the susceptibility of the target pest to the toxicant is the most important factor for insecticide use especially for monitoring the development of resistance.[5] Resistance to insecticides is a major problem associated with the chemical control of insect pests.[6] Previous exposure and selection with insecticides can confer cross resistance to newly introduced insecticides. The presence of pests on different crops throughout the year has widely exposed it to insecticides and resulted in the rapid development of resistance to a range of these insecticides.[7] The insecticides are classified into various groups according to the toxicity levels i.e extremely toxic, highly toxic, moderately toxic and slightly toxic. Hence keeping in mind the indiscriminate use of various types of insecticides and toxicity levels conferred by insecticides, this study has been planned to observe the effect of a bio pesticide against *Spodoptera litura*, in controlled laboratory conditions. Most studies were performed on populations collected from nearby locations, but this study takes into account the level of resistance developed in laboratory conditions. *Spodoptera litura* was reared for at least three generations on artificial diet to ensure the robustness and infection free condition of the culture. It was continuously monitored for six months and observations were recorded on daily basis for natural mortality occurring. The results of this study will prove to be helpful in understanding the role and pattern of insecticides rotation in field. Thus, newer and conventional insecticides, which have different resistance mechanisms, can serve as effective insecticides in the rotation program for *Spodotpera litura*.[8]

EXPERIMENTAL SECTION

Collection and Preservation

A site survey was done in some parts of Vadodara, Gujarat and populations of *Spodoptera litura* were collected from fields nearby regions of Vadodara in Gujarat. The information on sprays occurring in these fields was recorded beforehand, taking help of the local farmers at the time of collecting the populations of the pest. A mixed culture containing mostly smaller instars like second and third instars were collected in separate bowls along with healthy leaves for survival. Pupae and adults were collected in plastic jars with holes. Tissue papers were kept in these containers and moist conditions were maintained so that the collected culture did not desiccate due to dry conditions. The collection of pest was done in the early morning between 6:00 am to 8:00 am. The pest was then reared in laboratory conditions at 27±2°C and 65-70% relative humidty(RH). Incubators were used, to maintain constant conditions for the survival of test insect before conducting bioassays. The culture was reared for at least three generations so as to ensure the health and infection free nature. After successful rearing, next generation was selected for testing of insecticide through monitoring bioassay.

Rearing in laboratory conditions

Larvae of *Spodoptera litura* were collected from castor and cotton fields (200-300 larvae). They were reared in controlled laboratory conditions i.e. 25±2°C, 65-70% RH and a photoperiod of L: D, 14:10. It was reared on artificial diet.[9] The diet was poured in a plastic container which had partitions in it. The larvae were carefully transferred on diet by using brush. The diet was changed at regular intervals.

Figure 1: Rearing of Spodoptera litura in lab conditions (27±2°C, 65% RH)

All the lab paraphernalia used for the whole process was pre- sterilized to avoid fungal and bacterial infections. The transferring of larvae becomes very crucial; hence utmost care was taken. Until pupation, the larvae were kept on artificial diet. Rearing in container was feasible as there was no cannibalism observed in *Spodoptera litura.* After complete formation of pupae, they were

transferred to bowl. The completion of pupal stage leads to the beginning of adult emergence. As soon as adult emergence started, healthy male and female adults were released in oviposition pots in the ratio of 2:2(male:female). Adult diet was also provided by using honey solution. The moths were provided with water and honey solution. Another method was used for rearing i.e. rearing on natural diet. The freshly laid egg masses from fields along with the leaves. Adults and larvae of *Spodoptera litura* were also collected and were bought to the laboratory in perforated polythene bags along with infested leaves. The eggs were kept in Petri dishes (11 cm dia.) and were covered with fine muslin cloth and secured with rubber bands. The larvae were kept in rearing jars (15 cm × 13 cm) covered with muslin cloth and secured with rubber bands. They were daily supplied with fresh cabbage leaves for feeding. The adults were also kept in rearing jars (15 cm × 13 cm), supplied with a piece of folded paper for oviposition and a cotton swab dipped in 50 % honey solution was hanged from the top in order to provide feeding material for adults. The honey solution was renewed after every 48 hours. The Petri dishes having *Spodoptera litura* eggs and rearing jars containing larvae and adults were kept in B.O.D. incubator maintained at 27 ± 2ºC temperature and 65 ± 2 %RH. The culture was maintained and the second generation adults (male and female) were kept in jars.[10] Both types of rearing i.e natural diet and artificial diet was done, so as to ensure the survival of larvae for the testing against insecticide. The larvae which were reared on natural diet had many challenges like plant health, virus in plant material, fungus development, changing of diet on regular intervals. In contrast, those reared on artificial diet had different challenges like, diet developing infections, larvae preference for artificial diet etc. The larvae reared on artificial diet were then selected for second generation. The larval health was observed on regular intervals by different parameters like larvae weight, pupal height, type of wings in adult, number of egg masses and longevity of adult. Table 1 indicates the health parameters indicating healthy and infestation free culture. Such type of reared culture was then continued to next generation for conducting bioassays.

All these conditions were observed and recorded for three generations. After ensuring of susceptibility of culture, they were reared to the fourth generation in which testing against insecticide was to be done.

Table 1: Health Parameters for rearing of *Spodoptera litura*

Larvae (Total 10 nos)	Days for completion of life cycle	Larval weight (g) Early instar	Larval weight(g) Late instar	Weight of food consumed (g)	Adult longevity (days)
Set-1	27	0.12	0.42	1.88	4
Set-2	25	0.13	0.58	1.96	5
Set-3	26	0.14	0.65	1.85	5
Set-4	27	0.11	0.55	2.10	4
Set-5	28	0.15	0.49	1.95	5
Set-6	27	0.12	0.50	2.00	4

Insecticide selection

Commercial formulations of Spinosad (Tracer®, Dow Agro Sciences), was used for the efficacy studies. Spinosad is a novel mode-of-action insecticide derived from a family of natural products obtained by fermentation of *S. spinosa*. Spinosyns occur in over 20 natural forms, and over 200 synthetic forms (spinosoids) have been produced in the lab.[11] Spinosad is highly active, by both contact and ingestion, in numerous insect species Spinosad has high efficacy, a broad insect pest spectrum, low mammalian toxicity, and a good environmental profile, a unique feature of the insecticide compared to others currently used for the protection of grain products . Its labelled use rate is set at 1 ppm (1 mg a.i./kg of grain) and its maximum residue limit (MRL) or tolerance is set at 1.5 ppm. Spinosad widespread commercial launch was deferred, awaiting final MRL or tolerance approvals in a few remaining grain-importing countries. It is considered a natural product, thus is approved for use in organic agriculture by numerous nations.[12]

Selection of larvae for bioassay

As described in the rearing procedure, the larvae of fourth generation were selected for resistance monitoring bioassay. The larvae selected for the experiment were pre-checked for any type of infections. After ensuring the healthy nature of larvae, third instar was selected for the bioassay. These were separated from mother culture and kept for starvation before initiating the experiment.

Leaf dip bioassay

The traditional leaf dip bioassay was conducted in laboratory conditions. Primary stock solutions of insecticides were calculated and bracketing was done to arrive the different concentration on third instar larvae of S. litura.[13] Test solution was prepared by using the commercial formulation of Spinosad (Tracer®, Dow Agro Sciences). Different ppm concentrations were made,

using serial dilution process. Healthy and infestation free cotton leaves were collected from field and they were washed in laboratory using distilled water. Leaf discs of five centimetres were cut. These leaf discs were dipped in the test solutions for ten seconds with gentle agitation and were placed on tissue papers for drying with adaxial surface. Natural drying was performed by giving enough time. After ensuring, the leaf discs were placed in petri plates having moist filter paper to avoid desiccation of leaves in ten replicates. The larvae was kept for starvation for one hour before exposing it to testing. On each leaf disc, three 3rd instar larvae (F1 generation) were released, using fine camel hair brush. All the test units were kept in controlled environmental conditions, humidity chamber (25±2°C, 65-70%). The humidity chamber was properly checked to ensure the correct working according to the parameters set inside. Untreated check was also kept in which the leaf discs were treated with distilled water. After 72 hours, the test units were taken out of the chamber and brought to laboratory conditions. Anything unusual was captured in data sheet. At 96 hours,the observation was taken using camel hair brush, which was pre-sterilized. Table 2 indicates the mortality values for five generations when exposed to Spinosad (Tracer®, Dow Agro Sciences) at different concentrations, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 ppm.

Table 2: "Mortality (%) when treated with Spinosad (Tracer®)"

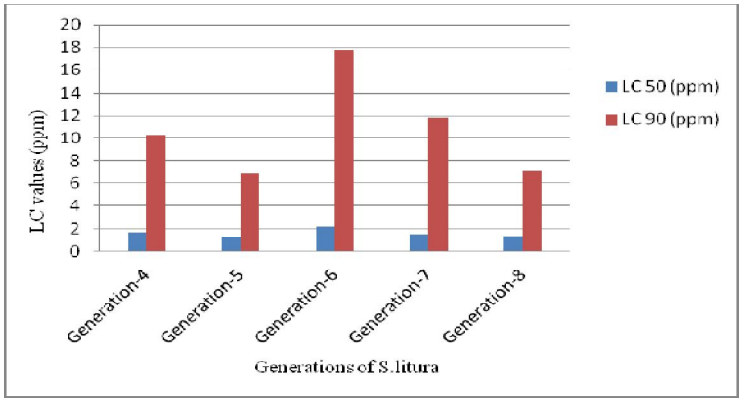
Sr. no	Concentration(ppm)	Percent Mortality (%)				
		Generation 4	Generation 5	Generation 6	Generation 7	Generation 8
1	0.01	0.00	0.00	0.00	0.00	0.00
2	0.03	0.00	0.00	0.00	3.33	0.00
3	0.1	3.33	0.00	3.33	6.67	3.33
4	0.3	6.67	10.00	3.33	6.67	6.67
5	1	46.67	56.67	43.33	46.67	50.00
6	3	63.33	66.67	60.00	66.67	70.00
7	10	83.33	90.00	76.67	86.67	90.00
8	30	100.00	100.00	93.33	96.67	100.00
9	Untreated Check	0.00	0.00	0.00	0.00	0.00

Separate brushes were used for untreated check unit and treated units so as to avoid contamination. The test units were first checked for any kind of fungal/bacterial infections. Observations were recorded for mortality in each petri plate. Larvae were considered to be dead, if there was no movement after contact with brush. Larvae was considered to be moribund, if it showed less and uncoordinated movement as compared to untreated check. Larvae were considered live if it showed normal movement when compared to untreated check. All these observations were recorded and if there were any special findings, there were also recorded. The larvae which survived to the

different concentrations of testing insecticide were then mixed and continued to fourth generation for testing on the same concentrations. The whole process was repeated for seven generations and observations were recorded keeping all the parameters constant. Table 3 indicates the Lethal Concentration (LC) values for subsequent generations. The rearing process was done on artificial diet, while the testing was done by leaf dip. The mother culture was kept intact and the testing culture was kept separately as they were previously exposed to insecticidal concentrations. Both these were kept in different chambers of laboratory and proper care was taken for non- mixing of both the culture.

Table 2: "LC 50 values of Spinosad exposed to *S.litura*"

Treatment (Spinosad)	Hetero geneity (Chi square)	LC 50 (ppm)	LC 90 (ppm)	Fiducial limit (Upper)	Fiducial limit (lower)	Slope± Error
Generation-4	5.12	1.68	10.31	2.42	1.17	2.79± 0.35
Generation-5	6.71	1.33	6.87	1.88	0.94	3.08 ± 0.40
Generation-6	5.64	2.24	17.78	3.34	1.52	2.44 ± 0.31
Generation-7	3.44	1.51	11.82	2.23	1.02	2.45± 0.30
Generation-8	3.65	1.38	7.06	1.95	0.98	3.10 ± 0.40



Graph 1: Comparison of LC values in five generations of *S.litura*

RESULTS AND DISCUSSION

As the mother culture was brought from nearby fields of Vadodara, the health parameters of the larvae were recorded before keeping the bioassay to ensure robustness and pesticide free culture. Total six sets of larvae were kept at 27±2ºC and 60-65% relative humidity (Rh). The larval weight indicated by values 0.42, 0.58, 0.65, 0.55, 0.49 and 0.59 g. The adult longevity was average 4-5 days. All these parameters indicated good health of culture and showed no symptoms of resistance. It becomes very important to record the type of sprays of insecticides done previously in the fields from where the mother culture is collected. Thus the history of sprays in the fields before collection

of the pest was also recorded in order to compare the results. The leaf dip bioassay was kept at different concentration 0.01, 0.03, 0.1, 0.3, 1, 3, 10 and 30 ppm in accordance with the field rates. The whole setup was kept in controlled conditions maintain the temperature 27±2ºC and 60-65% relative humidity (Rh). The same test was repeated for seven generations. Table 2 indicates the data obtained for all the eight generations. The data was corrected using Abbott's formula (1925) (Abbott, 1925) where necessary, and were analyzed by probit analysis to obtain LC50 values and their 95 % Fiducial limits (FLs). As Graph 1 indicates, in the first generation, the LC 50 and LC 90 values were 1.68 and 10.31 respectively. The second generation indicated values 1.33 and 6.87 respectively. In the third and fourth generation 2.24, 3.34 and 1.51, 11.82 indicated low amount of resistance developing in the culture. In the fifth generation, 1.38 and 7.06 values indication onset on resistance. The upper and lower Fiducial limits were found to be 1.17 and 2.24 for first generation. Subsequently, the second third, fourth and fifth generation upper and lower fiducial limits were 1.88 and 0.94, 3.34 and 1.52, 2.23 and 1.02, 1.95 and 0.98 respectively. There was 50% mortality found in one of the concentrations i.e. 1 ppm which indicated onset of resistance in laboratory conditions. Spinosad is a biopesticide and known for its less toxicity and lower residual values.

CONCLUSION

Repeated use of the same class of pesticides to control a pest can cause undesirable changes in the gene pool of a pest leading to a form of selection pesticide resistance. Spinosad being a bio pesticide may prove to be useful by keeping it one of the insecticides in rotation. It would help in reducing environmental impact and in avoiding development of resistance in insects. The study will be continued for more generations, as a part of whole studies. Hence overall results on insecticide resistance will be concluded after the complete study. The results of the above study indicate onset of resistance in laboratory conditions. Studies indicate that Spinosad shows 8.2% and 23.8% after 3 and 24 hours of insecticide application at 960ppm and 15360ppm respectively. Also, it proved as most toxic insecticide against *Spodoptera litura* larvae with LC50 19.53 before Indoxacarb (Avaunt 15.8% EC) and after Methoxyfenozide (Runner 240 SC) with 21.85 and 16.04.[14] The studies which tested the different insecticides included thiodicarb, chlorpyriphos, endosulfan, indoxacarb, profenophos, Spinosad, cypermethrin, deltamethrin and Neem extract against tobacco caterpillar, Spodoptera litura in laboratory, concluded that spinosad is poor insecticide as control agent against S. litura.[15] Some of the studies indicated that Spinosad resistance in S. litura has a high reversion rate (-0.15) which indicates that Spinosad resistance in *Spodoptera litura* is unstable and can be easily managed by switching off the selection pressure for a few generations or alternating with insecticides having different modes of action.[16] Information regarding the correct application of pesticides and the use of

advanced technologies for target delivery of pesticide, as well as intensive training on selective application of the correct pesticides at the correct time for the correct pests, should be disseminated to the user group.[17] The current studies showed onset of resistance which may be considered as an alarming situation. At the same time, due to its less toxicity and biological properties, this type of bio insecticide can be used in the rotation of insecticide programmes

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