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Comparative Bioefficacy of Pyridalyl Nanosuspension and its Commercial Formulation against *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

The in vitro *insecticidal activity of pyridalyl nanosuspension and commercial formulation was evaluated against 1st instar larvae of* Helicoverpa armigera *by diet incorporation method. The insecticidal activity of pyridalyl nanosuspension increased, with LC_{50} values of 44 µg mL^{-1} in comparison to commercial product (LC_{50} = 69 µg mL^{-1}). Further, bioassay results showed that the EC_{50} of pyridalyl (commercial 10 EC) and nano pyridalyl was 51.26 µg mL^{-1} and 16.74 µg mL^{-1}, respectively. Activity of nano pyridalyl was three-fold than commercial formulation. Thus, nanosuspension of pyridalyl performed better against 1st instar larvae of* H. armigera *as shown by LC_{50} and EC_{50} values. Pyridalyl nano-capsule suspension prepared using sodium alginate was sufficiently stable with higher bioefficacy against* Helicoverpa armigera.

Keywords: pyridalyl, nanoformulation, *Helicoverpa armigera*, diet incorporation

Introduction

Helicoverpa armigera, one of the most serious agricultural insect pests worldwide, alone causes huge losses due to its high reproductive potential and polyphagy. This pest has been recorded from at least 160 cultivated and 67 wild host plants. The production and productivity of the tomato crop is also affected by the fruit borer causing 20 to 60% yield loss (Lal and Lal, 1996). Due to wider host range, multiple generations, migratory behavior, high fecundity and existing insecticide resistance this insect became a difficult pest to tackle (Ahmed *et al*., 1999). It has developed resistance against several conventional insecticides from the organophosphate, pyrethroid and carbamate groups due to indiscriminate use.

An insecticide having novel mode of action and its Nano formulation was tested against larvae of *H. armigera* under laboratory conditions to tackle problem of the increasing resistance. Due to high cost of protecting crops there is growing interest in the use of such pesticides having new mode of action. These groups like Spinosad® have different mode of action from conventional products (Thompson *et al*., 1999) and their properties may differ considerably from the conventional chemicals with which growers are familiar. It is therefore important to generate information on the likely differences in the performances of these products to educate growers and facilitate adoption.

However, this new chemical insecticide, pyridalyl, has no cross-resistance with any other class of insecticide (Isayama *et al*., 2004). Pyridalyl could be useful for building up IPM programs, especially in greenhouse cultivation systems (Isayama *et al*., 2005). In spite of high activity of pyridalyl against target pests, its toxicity against mammals, important beneficial arthropods such as natural enemies or bees is minimal (Sakamoto *et al*., 2003). Pyridalyl is cytotoxic and acts by selectively inhibiting the protein synthesis in insect cells which might contribute significantly to the insecticidal activity and the selectivity of this compound; it acts through dermal exposure as well as ingestion (contact and stomach poison). After treatment, skin of larvae becomes black and shows necrosis and larval body becomes flaccid, followed by death. Various studies have shown the selective inhibition of cellular protein synthesis by pyridalyl (Powell *et al*., 2011)

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Nanoparticles in natural ecosystems have different biological responses than those observed in laboratory cell-based toxicity assays. Properties of nanoparticles can be exploited in the production of new insecticides (Owolade *et al*., 2008). These particles are released slowly but efficiently to a particular host plant against an insect pest (Scrinis and Lyons, 2007). Nano sulphur is potent fungicide against *Erysiphe cicorecaerum* and food pathogen *Aspergilus niger* (Gogoi *et al*., 2013). Because of various advantages of nano formulation, extensive research for its commercialization and wide application in agriculture is beings encouraged. Out of a number of bioassay techniques available (*viz*., topical assay, leaf residue/ leaf disc, foliar application bioassay, thin layer exposure bioassay/surface residue vial bioassay, sticky card technique, slide dip bioassay and glass vial technique), the topical assay, leaf disc and oral feeding methods are more commonly employed (Regupathy, 2001).

Materials and Methods

Materials

Double distilled water, ethanol, pyridalyl commercial formulation (10 EC), nano capsule suspension of pyridalyl and technical material (94.1% purity), cabbage, solvent emulsifying water.

Test insect

Cotton bollworm, *H. armigera* was collected from field of IARI and reared on artificial diet containing chick pea powder as main ingredient under laboratory conditions set at 26±2°C temperature and 65±5% Relative Humidity (RH). The muslin cloth having eggs were kept and when these eggs were hatched, the cultures were maintained by transferring 10-12 neonates per crystal vial having artificial diet. For bioassay by diet incorporation method, 1st instar larvae were taken. Larvae were starved for 12 h before all bioassay experiments.

Rearing of H. armigera

A nucleus culture of *H. armigera* was maintained at 25±1°C temperature and 65±5% RH on artificial diet. An artificial diet of the composition, given in Table 1, was used in the study. Bengal Gram (*Cicer arietinum*) seeds powder was procured from market and grounded in an electric grinder thoroughly with all the ingredients of diet except formaldehyde, agar-agar and multivitamin capsules using 400 mL warm double distilled water. Agar-agar was boiled with remaining double distilled water with constant stirring till it attained necessary consistency and then ground with the rest of the ingredients once again. Formaldehyde, multivitamin capsule and agar-agar were also mixed properly and whole mixture was poured in a large Petri plate and covered with thin plastic film.

Table 1: Composition of Semi-synthetic diet for *H. armigera*

S. No.	Content	Quantity
1	Bengal Gram	84.00 g
2	Yeast	11.00 g
3	Casein	5.00 g
4	L-ascorbic acid	3.00 g
5	Sorbic acid	1.00 g
6	Methyl paraben	2.00 g
7	Cholesterol	0.2 g
8	Multivitamin capsules	0.5 g
9	Tocopherol (vitamin E tablet)	1
10	Streptomycine sulphate	0.20 g
11	Formaldehyde 40%	1 ml
12	Agar-agar	11.00 g
13	Double distilled water	625 ml

After the diet solidified, it was cut into small pieces, when required. After cooling, the diet was kept in refrigerator and used after 24 h of ageing. After hatching from egg, 10-12 neonates were transferred to each crystal vials containing artificial diet. Two-three-day old larvae were then transferred to separate culture tubes containing pieces of diet. Boxes were cleaned daily using ethanol and larvae were fed with fresh diet. When the larvae exhibited gut purge and entered into non feeding wandering stage, they were transferred to boxes containing saw dust for pupation.

Pupae were collected after four to five days and disinfected with 0.02% sodium hypochlorite. Upon emergence of adults, they were transferred to oviposition cages. All the containers used for rearing were periodically disinfected. This enabled to maintain a disease-free and healthy stock culture for further experiments. Larvae for experimental purposes were reared on artificial diet in plastic boxes. Care was taken to avoid any infection.

Bioassay diet incorporation method

Laboratory bioassay experiments were carried out by diet incorporation method to evaluate the relative toxicity of the pyridalyl and its nanoformulation against

larval stage of *H. armigera.*

Insecticidal activity of pyridalyl formulations (nano and commercial) was compared by diet incorporation method using one day old larvae of the F1 generation of *H. armigera.* Pyridalyl and nano pyridalyl were tested using 6 concentrations each (250 µg mL^{-1}, 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 10 µg mL^{-1} and 5 µg mL^{-1}). A total of 168 larvae were exposed to both formulations of pyridalyl to test their toxicity against target insects. Three mL of the solution was taken into a 40 mL plastic cup and lukewarm diet (approx 60 °C) was poured, into the cup to a total volume of 30 mL. After placing the lid cup was shaken vigorously for a minute to mix properly. Then diet was poured to 0.5 cm height, into wells of a 24-cell insect rearing tray and allowed to cool in laminar airflow under UV lamps for 1 h to surface sterilize the diet. The strength of stock solutions taken for preparation of diet was 10-fold of required concentration because the final concentration of insecticide got diluted 10-fold in diet. First instars larvae of *H. armigera* were released into the diet rearing trays at the rate of one larva per well. Diet tray was covered with semi-permeable wrap and lid was closed. The lids were tightly secured with rubber bands, to prevent the larvae from escaping. Control larvae were released on untreated diet. Observations were recorded after 24 h. The LC_{50} and EC_{50} were calculated using POLO PC (Anonymous, 1987).

Results and Discussion

Bioassay by diet incorporation method

In diet incorporation method, pyridalyl recorded an LC_{50} of 68.74 µg mL^{-1} while nano pyridalyl recorded an LC_{50} of 43.88 µg mL^{-1} against 1st instar larvae of *H. armigera.* On the basis of LC_{50} values laboratory made nanoformulation of pyridalyl was found to be 1.57 times more toxic than commercial 10 EC formulation while nanoformulation is 1.22 times more toxic when compared their LC_{90} values (Table 2).

For determining $EC_{50,}$ weight gain at the end of F7 was observed. The weight gain in control larvae was substantially high when compared to treatment. Further the EC_{50} of pyridalyl (commercial 10 EC) and nano pyridalyl was 51.26 µg mL^{-1} and 16.74 µg mL^{-1}, respectively. Activity of nano pyridalyl was three-fold than commercial formulation of pyridalyl (Table 3)

Conclusion

Increased toxicity of nano sized formulation on larvae is probably due to increasing penetration of pyridalyl in the larval body. Our results get support

Table 2: LC_{50} values and relative toxicity of nano and commercial product of pyridalyl against 1st instar larvae of *H. armigera* by diet incorporation method

Insecticide	?2	Slope	SE(M)	LC50 (µg mL^{-1}) Min.	Max.	RT	Fiducial limits		LC90 (µg mL^{-1})	RT
Nano pyridalyl	2.81	4.81	0.48	43.88	1.57	30.33	59.43	80.94	1.22	
Commercial	7.31	5.14	1.03	68.74	1	58.39	77.86	98.80	1	

SE(M) (standard error of mean)
RT (relative toxicity)

Table 3: EC_{50} values and relative toxicity of nano and commercial product of pyridalyl against 1st instar larvae of *H. armigera* by diet incorporation method

Insecticide	?2	Slope	SE(M)	EC50 (µg mL^{-1}) Min.	Max.	RT	Fiducial limits		EC90 (µg mL^{-1})	RT
Nano pyridalyl	9.43	4.27	0.57	16.74	3.06	5.51	26.61	38.37	1.49	
Commercial	11.4	7.51	1.82	51.26	1	23.62	58.91	57.06	1	

SE(M) (standard error of mean)
RT (relative toxicity)

from similar results reported by other authors. Sasson *et al*. (2006) did a series of experiments on cotton white fly (*Bemisia tabaci*) and cotton leafworm (*S. litturalis*) to compare the toxicity of nano sized novaluron to that of standard EC and SC formulation and reported better toxicity of nanoformulation. In another experiment with *S. litturalis* larva, higher toxicity to the pest was explained due to higher penetration of nano insecticide in to the digestive tract and its faster reach to biochemical sites than the standard commercial formulation. Imidacloprid nano crystals encapsulated with natural polysaccharides, chitosan-alginate also showed higher toxicity against the adult stage of *Martianus dermestoides*, when compared to 95% imidacloprid (Guan *et al*., 2008b).

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