Efficacy of some selected biopesticides against *Helicoverpa armigera* (Hub.) using detached leaf bioassay in chickpea

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ABSTRACT

A laboratory experiment was conducted to evaluate the efficacy of different biopesticides against the second instar larva of *Helicoverpa armigera* at International Crops Research Institute for the Semi-Arid Tropics during 2016-2017. Varied doses of biopesticides were used during experiment against second instar larvae of H. armigera and recorded the per cent morality. Among the selected biopesticides neem seed powder, HaNPV and Spinosad showed superior and recorded maximum per cent morality at 24 hr and 48 hr day after release. The *metarhizium anisopliae, Streptomyces* sp. and consortia were at par with each other.

Key words: Soybean, Biopesticides, Sprays, Population, Insect pests.

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INTRODUCTION

The pod borer *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is among the most threatening plant pests, cosmopolitan in distribution and polyphagous in herbivorous nature (Wakil *et al*., 2009a, b; 2010).The larvae of *H. armigera* feed on leaves and stems but, they prefer buds, inflorescences, fruits and pods, thus causing significant damage to both vegetative and reproductive plant parts (Moral Garcia, 2006). A total of 500 US$ million worth of soybean and cotton has been lost in Brazil by *H. armigera* where it has been introduced in recent past (Czepak *et al.,* 2013). The *H. armigera* is the key production constraints in several crops including chickpea, pigeonpea, pea, lentil chilies, sunflower, tomato, tobacco and cotton crops. A viable and sustainable method for this polyphagous pest using the conventional approach of relying primarily on chemical pesticides has become increasingly costly nowdays, and resistance in several pest species, environmental impact, safety and accumulation of residues has been the primary cause of concern. Hence, there is an urgent need for the development of environment-friendly management by adopting insect pathogens, antagonist or competitor populations of a third organism and botanicals to suppress the pest population, thus making it less abundant and less damaging to main crop (Gopalakrishnan *et al.*, 2010, 2011a, 2011b; Murray *et al*., 2000). Microbial based insecticides spinosad has become so popular that it is now widely used by the organic farmers of Europe and America to manage *H. armigera* larval population under field conditions. Excessive use of synthetic insecticides worldwide warrants environmental and human health concerns, and urges researchers to develop safer alternatives for eco-friendly pest management (Cherry *et al*., 1997). The promising alternatives of insecticides would be *Nucleo polyhedron virus* (NPV), plant based products and new chemistry molecules which can be successfully included in the integrated pest management (IPM) program to lessen the resistance issues in the lepidopterous insects. Botanical pesticides act as a synergistic component in several IPM strategies and have the potential to help in the management of these pests as safe alternatives to synthetic insecticides (Schmutterer, 1995; Elshafie and Basedow, 2003; Lowery *et al.*, 1993; Basedow

et al., 2002). The bioagent *Beauveria bassiana* and *Metarhizium anisopliae* constitute about 68 per cent of the entomopathogenic fungi as microbial pesticides (Faria and Wraight, 2007). Among the alternatives, entomopathogenic fungi are getting serious attention due to their environmental safety and selectivity pest (Carner & Yearian 1989). The efficacy of entomopathogenic fungi is well documented by Nguyen *et al.* (2007), who reported promising results obtained from seven strains of *M. anisopliae, B. bassiana* and *P. fumosoroseus* against different larval stages of *H. armigera*. The fungal spores germinate and penetrate the cuticle by making germ tubes and proliferate in the hemolymph, which later produce new propagules (Zimmermann, 2007). The performed lab tests for the isolates of *M. anisopliae* and *B. bassiana* on larvae of *H. armigera* and reported mortality rates ranging from 58% to 74 % (Douro Kpindou *et al.,* 2012b). The use of synthetic insecticides to protect crops leads to some unfortunate consequences such as environmental pollution, pest resistance and toxicity to other non-target organisms. The limited success rates of these control methods explains the need for developing alternatives that are more effective, healthy and respectful of the environment and human health and more economically profitable. The spinosad can be used in any IPM programme for the control of *H. armigera* because they are considered among the best ecofriendly insecticides to control the lepidopteran pests (Ahmad *et al.,* 2005). The main aim of this study was to reduce the load of synthetic chemical insecticides and evaluate the efficacy of some effective biopesticides *viz., M. anisopliae, Streptomyces sp*, HaNPV, Neem seed powder, consortia and spinosad under laboratory conditions against *H. armigera* for identifying best bio-agents which act as an alternate component of pest management for environmentally safe approaches.

MATERIAL AND METHODS

The chickpea grown seedlings of greenhouse were used for bioassays experiment with similar environmental conditions (27 ± 2^{o}C, 65-75% RH, and a photoperiod of 12:12 [L: D] h) at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, Telangana, India.

Biopesticides

The experiment was conducted in the biocontrol laboratory with four replication and seven treatment of biopesticides including control, the biopesticides like *M. anisopliae, streptomyces*, HaNPV, neem seed powder, consortia and spinosad which were prepared in the laboratory except for synthetic insecticide. The *M. anisopliae* (4.3 X 10^3, 3.9 X 10^4 and 2.9 X 10^5), *Streptomyces* (12.6 X 10^4, 5.8 X 10^5 and 5 X 10^7), HaNPV (10.10 X 10^6, 4 X 10^7 and 3 X 10^8) and neem seed powder (2.5gm, 5gm and 10gm) were performed by serial dilution with three different dilutions. The counting of spores was made after the serial dilution of the suspension by using doubled ruled Neubauer haemocytometer for determining the number conidia in 1ml of suspensions. The consortia were prepared with the combination of the above four treatment and concentrations like 0.1mL/Lit, 0.3mL/Lit and 0.5mL/Lit for spinosad against second instar larvae of *H. armigera.*

Rearing and maintenance of *H. armigera*

Larvae of *H. armigera* were reared using chickpea-flourbased semisynthetic diet, as per the standard protocols of Narayanamma *et al.* (2007) and were maintained at a temperature of 27 ± 3 °C, with a relative humidity of 65–70 %.

Detached leaf bioassay

The detached-leaf bioassay was performed as per Sharma *et al.* (2005) the 10 mL of 3 % agar-agar was poured into plastic bioassay cups positioned at an angle of 45° and the chickpea terminal branches with four leaflets along with the terminal bud were washed thoroughly in distilled water to avoid interference of exudates released by the plant. The branches were dipped in 5 mL of the each dilution for 5 min, then allowed to dry, and inserted into the agar containing bioassay cups and healthy larvae (pre-starved for 6 hrs) of similar weight were released for each experiment bioassay cups. There were four replications per treatment on each dilution or

concentrations. For each replication, twelve larvae were used and observations were recorded on 24hr, 48hr 72 hrs and days after release.

$$\text{Per cent mortality} = \frac{\text{No of dead larvae}}{\text{Total no of larvae}} \times 100$$

Statistical analysis

For statistical analysis of efficacy of biopesticides to *H. armigera* mortality due to the different biopesticides were analyzed using the programme SPSS 8.0 ANOVA.

RESULT AND DISCUSSIONS

Biopesticides on *H. armigera* larvae

The efficacy of six biopesticides (*M. anisopliae* and *Streptomyces sp.* HaNPV, Consortia, one botanical insecticide (neem fruit powder) and one novel insecticide (spinosad) was tested against 2nd instar larvae of *H. armigera* with three doses or dilutions of each treatment. The mortality was observed at 24, 48 and 72 hrs after treatment. The laboratory studies showed the significant differences in efficacy among the biopesticides at different concentrations or dilutions against 2nd instar larvae of *H. armigera. M. anisopliae* did not cause the mortality of larvae at 24 hrs after treatment with different concentrations. The infectivity of *M. anisopliae* increased after 48 hrs of treatments and the mortality was 50 percent with the highest concentration (4.3 x 10^3 conidia / mL). There was no significant difference with other two concentrations of *M. anisopliae*. There was no mortality of larvae observed in control (Fig. 1.) The results are in contrary to the findings of Kulat *et al.*, 2003 who found the highest larval mortality (97.50%) of 2nd instar larvae of *H. armigera* with 2.28 X 10^{10} conidia/rnl of *M. anisopliae.* Similarly, Gundannavar *et al.* (2006) found that young larvae were more susceptible than older after application of different dilutions of *B. bassiana* on larvae of *H. armigera.* In case of *Streptomyces sp.* the highest per cent mortality 66.67% was recorded in the concentration of (12.6 x 10^4 colonies / mL) after 48 hrs of treatment and least 50 per cent mortality was found in concentration of (5 x 10^7 colonies / mL). There was no mortality of larvae after 72 hrs of treatment (Fig.1**)** The result were confirmation with Gopalakrishnan *et al.* (2016) who shows that purified metabolite of *Streptomyces* sp. showed 70–78% mortality in 2nd instar larvae of *H. armigera* by detached leaf assay. The three different dilutions of HaNPV showed the highest percent of mortality which was 91.67 percent in dilution of 10.10 X 10^6 POB / mL. Among three treatments, HaNPV showed higher mortality than *M. anisopliae* and *streptomyces* sp. (Fig. 1) Qayyum *et al.* (2015) observed that the susceptibility of *H. armigera* larvae decreased with later stage as greater mortality was recorded in second instar larvae in comparison to fourth instars larvae. Cherry *et al.* (2000) reported that the susceptibility of *H. armigera* depends on the larvae instars. Cowgill and Bhagwat (1996) reported HaNPV was more effective in killing *H. armigera* when applied to the *H. armigera* susceptible genotype (ICCC 37) of chickpea than on a *H. armigera* resistant genotype (ICC 506EB).

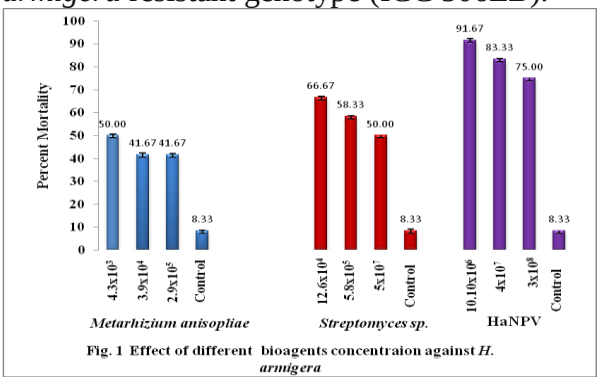

Fig. 1. Effect of different bioagents concentraion against *H. armigera*

Neem seed powder did not cause mortality of larva upto 2 days whereas after 72 hrs of treatment it resulted in 100 per cent mortality at the concentrations of (10gm). The lowest concentration (2.5 gm) recorded the least mortality which was on par with 5gm concentration (Fig. 2). Neem seed kernel extract (NSKE 5%) was found most effective in reducing the larval population and pod damage by Prasad and Roy (2011). Azadirachtin interaction with development of *H.armigera* showed growth inhibitory and antifeedant activity of extracts from *Melia dubia* which was by Koul *et al.* (2000)

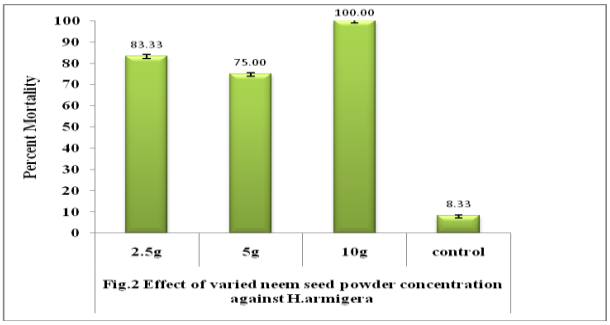

Fig. 2. Effect of varied need seed powder concentraion against *H. armigera*

The experimental results with spinosad after 24hrs of treatment indicated 100 per cent larval mortality at a dose of 0.3mL/L and 0.5mL/L which was significantly higher than the mortality obtained with lower concentrations *viz.*, 0.1 mL/Lit (Fig. 3). Khan *et al*. (2010) reported that the different concentrations of Tracer 240 SC were tested under laboratory conditions against first and second instar larvae of *H. armigera* the result showed that spinosad is very effective. Maximum mortality was observed and they can be used in the IPM program of any crop. The present findings are in conformity with reports of Babar *et al.* (2012) who evaluated the larvicidal action of Spinosad against *H. armigera* and recorded more than 90 per cent larval mortality in the laboratory experiment and found it to be the most effective as a larvicide.

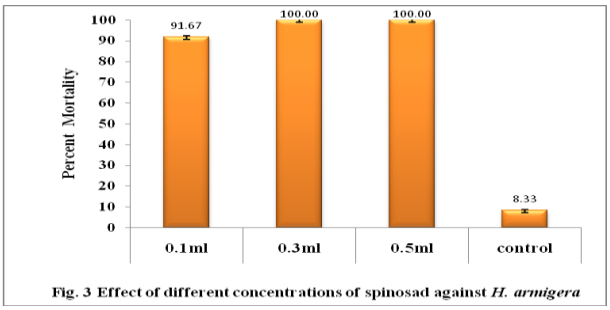

Fig. 3. Effect of different concentrations of spinosad against *H. armigera*

The experimental results with consortia (combinations of *M. anisopliae*, *Streptomyces* sp. HaNPV and neem seed powder with their respective dilutions) after 48 hrs of treatment showed 91.67 percent mortality. This treatment shows the significant differences among the treatments. There was no mortality in control (Fig.4). The present findings are in conformity with those of Kulkarni *et al.* (2005), Ali *et al.* (2008) and Kale and Men (2008). They reported *M. anisopliae,* neem seed powder and their combinations as the most effective treatment in reducing *H. armigera* damage.

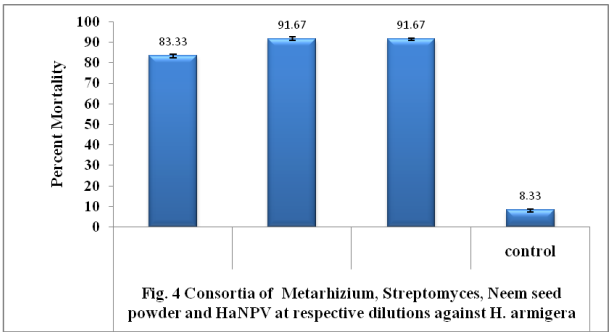

Fig. 5. Consortia of Metarhizium, Streptomyces, neem seed powder and Ha NPV at various doses on *H. armigera*

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