Potential of EPN in management of cotton bollworms in Pakistan

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Abstract

Cotton is the most important cash crop of Pakistan and plays a vital role in the economy of country. It is attacked by insect pests including bollworms. These pests are controlled by frequent use of pesticides. However, the indiscriminate use of synthetic pesticides has disturbed agro-ecosystem and costs over US$ 195 million per year to the nation in terms of environmental and social costs. Pathogenicity and efficacy trials of indigenous entomopathogenic nematodes (EPNs) isolates have positive results. Four EPN isolates viz., *Steinernema pakistanense*, *S. asiaticum*, *S. feltiae* and *Heterorhabditis indica* were assessed for their infectivity against the cotton bollworm complex in field. EPNs cultured on *Galleria mellonella* L. and stored in distilled water at 5 °C, were kept at room temperature for 24 hrs before use. The number of bollworms on plants before and 24 hrs after EPN spray @ 1000 and 2000 juveniles/ml water were assessed for mortality percentage. All four species of insects, viz., *Helicoverpa armigera*, *Earias insulana*, *E. vitella* and *Pectinophora gossypiella* were found susceptible to infective juveniles of the four EPN species; *S. pakistanense* was the most virulent EPN species. There is a dire need to focus further research on these EPN isolates to explore and exploit their potential as an alternative to synthetic pesticides in Pakistan, especially in IPM programmes.

Cotton also known as 'white gold' is an important cash crop and lifeline of textile industry in many developing countries including Pakistan. Pakistan is the fifth largest producer of cotton in the world. It accounts for 6.9 percent of value added in agriculture and 1.4 percent of GDP. Cotton crop was cultivated on an area of 2878.8 thousand hectares and production 13030.7 thousand bales (Pakistan Statistical Year Book, 2012-13)**.** The yields however remain relatively low due to a number of factors such as unfavorable weather conditions at the time of sowing which affects germination, incidence of pest attack during the early growth of the crop as well as at the time of flowering and boll formation. Cotton is a pest-loving plant and is attacked from sowing to picking stage of its growth and of 30 most important pests of cotton which are injurious to growth, development and production of crop include; the caterpillars of pink, spotted and American bollworms, aphids, whitefly, jassids, mealy bugs and the spider mites. The bollworm complex is a primary insect pest problem with larvae attacking squares and bolls causing significant yield losses if left uncontrolled (Ali & Khan, 2007).

Insecticides are frequently used to control these pests and during 2007 Pakistan consumed 90,676 metric tons of chemical pesticides at a total cost of Pak Rs. 12290 million, i.e., over US$183 millions. About 80% of the insecticides used in the country are meant for cotton. The indiscriminate use of synthetic pesticides not only has no correlation with yields, but it has also disturbed agro-ecosystem and costs over US $195 million per year to the nation in terms of external costs including the environmental and social costs in Pakistan (FAO, 2001). Increased awareness of environmental hazards and toxicity of insecticide as well as the resistance to insecticides has prompted research for alternative means of pest control. The entomopathogenic nematodes (EPNs) have been recognized as one of the most effective, safe and non-polluting bio-control agents for the control of insect pests that cause serious damage to the major crops and fruit trees.

Entomopathogenic nematodes of the genera *Heterorhabditis* Poinar, 1975 and *Steinernema* Travassos, 1927 have a worldwide distribution and occur in a variety of soil types and habitats. These can be used for insect control as an alternative to synthetic insecticides in agriculture. Infective juveniles (IJs) of these nematodes are capable of killing a wide range of insects within 24-48 hrs (Poinar, 1979) and their pathogenicity is associated with lethal bacteria (*Xenorhabdus* and *Photorhabdus*), which produce nematode toxins (Akhurst & Boemare, 1990) and the ability of the (IJs) to search and penetrate the host (Dadd, 1971, Glazer, 1992). In Pakistan, EPN studies were initiated by Shahina & Maqbool (1996) on the isolation, distribution, taxonomy, insect rearing, mass rearing and storage. So far, three new species viz., *Steinernema pakistanense* Shahina *et al*., 2001, *S. asiaticum* Anis *et al*., 2002 and *S. maqbooli* Shahina *et al*., 2013 while seven known species viz., *S. abbasi* Elawad *et al*., 1997; *S. feltiae* Filipjev, 1934; *S. siamkayai* Stock *et al*., 1998; *S. carpocapsae* (Weiser, 1955) Wouts *et al*., 1982; *S. litorale* Yoshida, 2004, *Heterorhabditis indica* Poinar *et al*., 1992 and *H. bacteriophora* Poinar, 1976 have been described, re-described or reported from Pakistan. In this study, four EPN isolates were assessed for their infectivity against cotton bollworm complex in field trials.

Materials and Methods

Nematode culture: Four entomopathogenic nematode species of the genera *Steinernema* and *Heterorhabditis* viz., *S. pakistanense* Shahina *et al*., 2001, *S. asiaticum* Anis *et al*., 2002, *S. feltiae* Filipjev, 1934 and *H. indica* Poinar *et al*., 1992 were used in the study to assess their infectivity against the cotton bollworms viz., *Helicoverpa armigera*, *Earias insulana*, *E. vittella* and *Pectinophora gossypiella*. EPNs were reared in the laboratory at 25 ± 2 °C on the last instars/larvae of the greater wax moth *Galleria mellonella* L. (Woodring & Kaya, 1988). All nematodes were stored in distilled water at 5 °C before use, except *H. indica* was kept at 15 °C. The nematodes were kept at room temperature (18-22 °C) for 24 hrs before use.

Mass rearing: Mass rearing of these nematodes *in vitro* on solid culture 80 g chicken offal medium on a porous foam substrate was used (Bedding, 1981; 1984) which provides the largest surface-volume ratio and adequate interstitial space (Bedding, 1986) in 500 ml conical flasks. After two weeks of incubation, approximately 5-7 million infective juveniles of EPNs were produced in a single flask stored in distilled water at 20-25 °C for 3-4 months.

Experimentation: Field trials were conducted to evaluate the effectiveness of four EPNs in reducing the bollworm complex infesting cotton bolls. The initial experiment was carried out at the experimental cotton field of Department of Agriculture Extension, Vehari, Punjab, Pakistan. The sites heavily infested with cotton bollworms were selected for experimentation. Cotton plants were sown with around 66 cm (2½ ft) row-to-row distances in a 3x3 m (10 x10 feet) plot size. The plants were at stages of flowering and boll development when treatments were applied. Concentration of the treatment was at the suspension rate of 1000 and 2000 juveniles/ml of water. The experiment was in a split-split-plot design with three replications and with a control (distilled water added in sprayer instead of nematode suspension).

Application method: At the time of application, plants in the trial plots were full-grown and contained all vegetative parts. The number of insect larvae viz., *Helicoverpa armigera*, *Earias insulana*, *E vittella* and *Pectinophora gossypiella* were counted carefully and recorded. All larvae exhibiting normal behavior were recorded as live. EPNs were sprayed on all aerial parts of plants and the control with only water, with the help of hand sprayer at late evening. EPNs were

applied @1000 (T_1) and 2000 (T_2) juveniles per ml using a spray rig with drop nozzles. The suspension of $2x10^{-6}$ IJs per 2.5 lit was applied in each plot, the air temperature ranged between 25 and 30 °C. Immediately after application, the field was irrigated (Wright *et al*., 2005).

Mortality of insect larvae was recorded after 24 hrs; the cadavers were examined under compound microscope every other day for 7 days to detect morphological changes for bacterial or nematode infection. Dead larvae were collected and screened carefully for their colour and then transferred into Petri dishes (50x9 mm) on moistened filter paper. Each insect was dissected to check the development of EPNs progeny. The subject data were analyzed and treatments mean separated using LSD.

Results

It was hypothesized that the four EPN species as bio-pesticides at different dose levels are effective on cotton bollworm complex. The overall analysis of variance agrees with the hypothesis (3way- ANOVA F= 10.8, df= 5, P= 0.05) and accepted it with 5% chances of being wrong. Field trials were conducted for three consecutive/years and no significant differences were found between the years (F= 1.83, df = 3, P = 0.05). The four EPN species were compared for their effectiveness and showed significant differences on the pests (3way- ANOVA F= 8.6, df= 5, P= 0.05), nematodes doses (T_1 and T_2) also differed significantly (3way- ANOVA F= 4.41, df= 2, P= 0.05) and interaction of two doses with four EPN species also marked effect on the bollworms (3way- ANOVA F= 10.8, df= 5, P= 0.05). The least significant differences (LSD) values were also significant.

S. pakistanense caused higher mortality ratio at both dose levels (57.2 and 74%) as compared to other EPN species tested on spotted bollworm *E. insulana* than *E. vitella* (55.6 and 70.8%). The mortality response of American bollworm *H. armigera* was 63.9 and 69 % by the two doses, whereas pink bollworm *P. gossypiella* showed mortality rate of up to 57.6 and 71.2 % by the two doses, respectively.

The infectivity of entomopathogenic nematode *S. asiaticum* varies on different cotton bollworms in T_1 and T_2. The effect of doses on spotted bollworms (*Earias insulana* and *E. vitella*) did not differ significantly. However, *E. insulana* showed slightly higher mortality in T_2 (59.5%) as compared to *E. vitella* for the same treatment (57%). Similarly, mortality of *H. armigera* was slightly higher in T_2 than in T_1 (58.3 and 58%). While, *P. gossypiella* showed similar mortality response (62.8 and 62%), respectively to *S. asiaticum* in both treatments.

There was significant difference in the infectivity of *H. indica* to the two spotted bollworm species at both doses. *E. insulana* was more susceptible (56.3 and 70.5%) to *H. indica* than *E. vitella* (54 and 65 %) in T_1 and T_2 treatments respectively. *H. armigera* showed greater mortality response (67.5%) in T_2 than in T_1 treatment (61%). *P. gossypiella* was less susceptible to *H. indica* than other bollworm species at both dose levels 55.4 and 62.7%, respectively.

The mortality response of cotton bollworm complex was very low to *S. feltiae* at both doses. It was 37.8 and 49% for *E. vitella* while 44 and 45% for *E. insulana*, respectively. Mortality of 39.7 and 47.4% was noted for *H. armigera*. *S. feltiae* did not have any significant effect on *E. insulana* and *E. vitella* at both dose levels. The least effect was observed in *P. gossypiella* at both doses of treatments *S. feltiae* (41.2 and 46.7%) as shown in Fig. 1.

The overall results showed that *S. pakistanense* was highly virulent against all the four species of cotton bollworm complex observed 24 hrs after application. This EPN species was comparatively more active at higher temperatures; while *S. feltiae* was found the least effective.

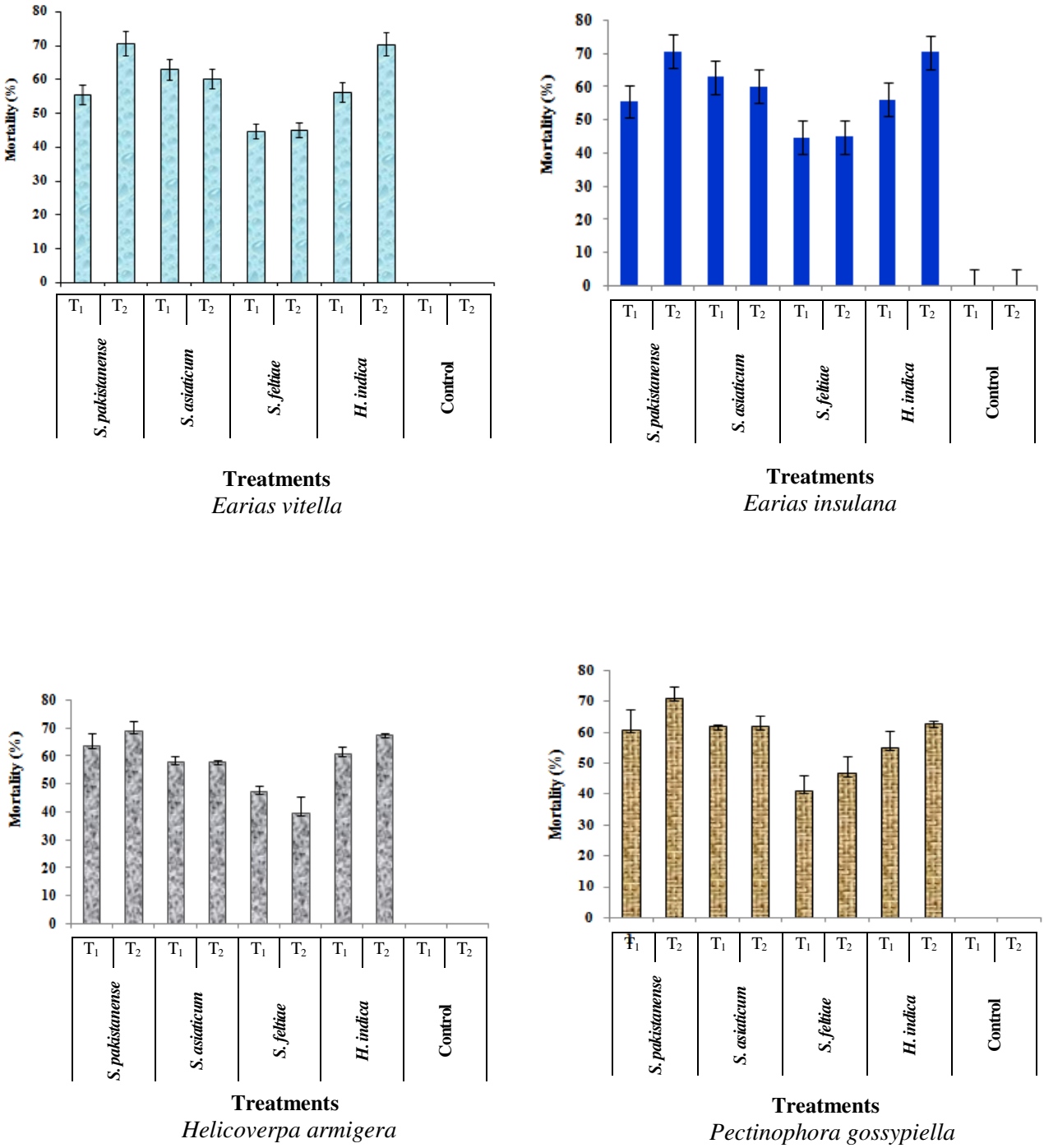

Fig. 1. Mortality (%) of cotton bollworms against 4 EPN strains at two doses.

Discussion

Studies on the efficacy of indigenous EPN species against the cotton bollworm showed the predominance of *S. pakistanense* over other EPN species. The results clearly showed that EPNs could be efficient control agents of cotton bollworm complex, confirming reports of several previous studies (Navon *et al*., 2002, Glazer & Navon, 1989). *S. carpocapsae* caused 100% mortality of *H. armigera* after an exposure to 200 IJs for 48 hrs and similar results were obtained with the *Heterorhabditis* isolate HP 88 by Glazer & Navon (1989). The number of IJs/ml applied as a dose is one of the main factor of the outcome and much depends on the population of the insects and the area sprayed. This study shows that 2000 IJs/ml gave significant mortality of cotton bollworms as compared to 1000 IJs /ml. Gouge *et al*., (1997) demonstrated parasitism of pink bollworm larvae by EPNs resulting in mortality range of 53-79% for different doses. Whereas, the present results showed pink bollworm mortality of 57-71% at a dose of 1000 and 2000 IJs of *S. pakistanense*/ml.

Conclusion

It is concluded that EPNs can be used as bio-control agents on pilot scale. *S. pakistanense* is more virulent to cotton bollworm complex at warmer temperature. However, further research is warranted on nematode species selection and the dynamics of edaphic conditions in relation to field performance.

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