African Journal of Biotechnology Vol. 10(73), pp. 16667-16673, 21 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2475
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Efficacy of botanical extracts and entomopathogens on control of *Helicoverpa armigera* and *Spodoptera litura*

Subramaniam Gopalakrishnan*, G. V. Ranga Rao, Pagidi Humayun, V. Rameshwar Rao, Gottumukkala Alekhya, Simi Jacob, Kanala Deepthi, Meesala Sree Vidya, V. Srinivas, Linga Mamatha and Om Rupela

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Accepted 19 October, 2011

Interest in biological control of insect-pests of economically important plants has been stimulated in recent years by trends in agriculture towards greater sustainability and public concern about the use of hazardous pesticides. Botanicals and microorganisms have the capability to synthesize biologically active secondary metabolites such as antibiotics, herbicides and pesticides. In this investigation, washings of herbal vermicompost (called biowash; viz. Annona, Chrysanthemum, Datura, Jatropha, Neem, Parthenium, Pongamia, Tridax and Vitax) and plant growth promoting (PGP) bacteria [viz. *Bacillus subtilis* (BCB-19), *Bacillus megaterium* (SB-9), *Serratia mercescens* (HIB-28) and *Pseudomonas* spp. (SB-21)] and fungus (*Metarhizium anisopliae*) were evaluated for their efficacy against *Helicoverpa armigera* and *Spodoptera litura*. When the feed was treated with crude biowash for healthy larvae (4-day old), 42 and 86% mortality and 32 and 71% weight reduction over control was reported for *H. armigera*, while in the case of *S. litura*, it was between 46 and 74% larval mortality and 47 and 77% weight reduction over control. When healthy larvae were treated with PGP bacteria and fungus, the mortality rate varied between 59 and 73%, with 55 and 92% weight reduction over control on *H. armigera*, while for *S. litura*, 54 and 72% larval mortality and 44 and 79% weight reduction over control was reported. The results of the compatibility studies (entomopathogenic potential biowash of the botanicals with PGP bacteria and fungus) indicate that there was no definite sign of suppression of any of the botanicals on the PGP bacteria and fungus, except Datura with *B. subtilis* BCB-19, whereas, there was definite sign of enhanced growth of *B. megaterium* SB-9 with all the botanicals. Compatibility studies between PGP bacteria and fungus showed that all bacteria are compatible with each other except *M. anisopliae*. Crude biowash of the promising botanicals (Annona, Datura, Jatropha, Neem, Parthenium and Pongamia) were further fractionated on C18 solid phase extraction cartridge (SPE) and the resultant adsorbed and non-adsorbed fractions were tested against *H. armigera*. Results indicate that both adsorbed as well as non-adsorbed fractions showed significant mortality on *H. armigera*. Adsorbed fractions of all the six biowash showed mortality between 81 and 93% (64 and 73% for non-adsorbed fraction) over control and the weight reduction of the larvae was found between 73 and 91% (80 and 97% for non-adsorbed fraction) over control. It was therefore concluded that the aforementioned six botanicals and five entomopathogens has great potential in the management of *H. armigera* and *S. litura*.

Key words: Botanicals, entomopathogens, biowash, secondary metabolites, *Helicoverpa armigera*, *Spodoptera litura*.

INTRODUCTION

Helicoverpa armigera (Hubner) and *Spodoptera litura* (Fab.) are the key production constraints in several crops including chickpea, pigeonpea, pea, lentil chilies, sunflower, tomato, tobacco and cotton. Global crop losses due to *H. armigera* and *S. litura* have been estimated to be over 2 billion US$ annually, of which 80%

*Corresponding author. E-mail: s.gopalakrishnan@cgiar.org. Tel: +91 40 3071 3610. Fax: +91 40 3071 3074.

loss occurs in India, causing wide spread misery and frequent crop failures (Grzywacz et al., 2005). Viable and sustainable control of these polyphagous pests using the conventional approach of relying primarily on chemical pesticides has become increasingly costly, and resistance in several 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 methods such as 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 than it would be otherwise (Gopalakrishnan et al., 2010, 2011a, 2011b; Murray et al., 2000).

There is a growing interest in the use of secondary metabolites, such as toxins, proteins, hormones, vitamins, amino acids and antibiotics from micro-organisms and herbals for the control of plant pathogens as these are readily degradable, highly specific and less toxic to nature (Doumbou et al., 2001). Plants produce a range of chemical compounds to protect themselves from various insect pests. These include alkaloids, terpenoids, flavonoids and acetogenins, such as azadirachtin, isolated from the foliages of Neem. Secondary metabolites from plants such as *Pyrethrum*, *Sabadilla* and *Carvone* were shown to have biological activity, protecting the plant from pathogens and at the same time non-toxic to mammals, fish and pollinators (Dubey et al., 2010). Compounds derived from such plants in general, possess no mammalian toxicity and hence may be exploited for controlling pathogens and insect pests of agriculturally important crops. Secondary metabolites from microbes are also known to kill various insects including *H. armigera* and *S. litura*. Moreover, among the bacteria, *Bacillus thuringiensis* (Bt) is the most studied due to the efficacy of the toxins (cry toxins) it produces to kill the larvae of *H. armigera* (Kaur, 2000). Among the actinomycetes, Spinosad (a product of soil actinomycete *Saccharopolyspora spinosa*) contains a mixture of spinosyn A and spinosyn D as active ingredients (Thompson and Hutchins, 1999), which when sprayed on the larvae of *H. armigera* cause significant reduction in the population (Mandour, 2009; Wang et al., 2009). Spinosad has become so popular that it is now widely used by the organic farmers of Europe and America to control *H. armigera*.

The biological degradation of agricultural wastes by earthworms and microorganisms, called vermincomposting, is becoming a favored method of recycling organic wastes. Application of vermicompost prepared from botanicals not only benefit crop plants as it contains beneficial microorganisms that help the plants to mobilize and acquire nutrients, but also promote plant growth and inhibit many plant pathogens (Perner et al., 2006; Postma et al., 2003). The water extract of vermicompost called bio-wash, extracted in the presence of rich population of earthworms and microbes contains several enzymes, plant growth promoting hormones, vitamins along with micro and macronutrients (Shield, 1982) and beneficial microbes that increase the resistance of crops against various diseases and enhances the growth and productivity of crops (Gopalakrishnan et al., 2010; Nath and Singh, 2009; Suthar et al., 2005; Yadav et al., 2005). This study explains the efficacy of various botanical extracts and plant growth promoting (PGP) bacteria and fungus for their entomopathogenic potential against *H. armigera* and *S. litura*.

MATERIALS AND METHODS

Preparation of herbal vermicompost samples

Foliages of nine different plant species (*Annona squamosa*, *Chrysanthemum morifolium*, *Datura metel*, *Jatropha curcas*, *Azadirachta indica*, *Parthenium hysterophorus*, *Pongamia pinnata*, *Tridax procumbens* and *Vitex negundo*) were collected from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) farm and air-dried at room temperature (30 ± 2°C). The container for vermicomposting was constructed by cutting a 200-L plastic barrel into two halves. A metal grill was placed at the bottom of the barrel [10 cm clearance from the base (floor) of the barrel] and the air-dried foliages of herbals (bedding material) were composted on top of the grill with earthworms (*Eisenia foetida*). The bedding material was moistened with water before it was added to the barrel. The barrel was then covered with a lid to keep the moisture intact and to avoid light as earthworms grow well in moist environment and darkness. A layer of foliage was added once a week as feed for the earthworms. The whole set-up was left for two months until all the foliages of herbals were digested. When the herbal compost was ready, about 100 g of the sample was collected and stored in a refrigerator at 4°C for further studies.

Extraction of crude biowash

At the end of two months, when the foliages of herbals were completely composted, biowash was extracted. To the completely prepared compost, water was sprinkled slowly and uniformly. The quantity of water sprinkled was determined by the volume of compost in the tank. The water extract of vermicompost drained out of the container was collected (1 L) at the bottom of the drum and called as biowash. The crude biowash collected from the vermicompost was centrifuged at 9000 *g* for 20 min, in order to remove the solid particles and microorganisms, and the supernatants collected were evaluated further for their efficacy against *H. armigera* and *S. litura*.

Extraction of bacterial culture filtrate

Four plant growth promoting bacterial strains namely, *Bacillus subtilis* BCB-19, *Bacillus megaterium* SB-9, *Serratia mercescens* HIB-28 and *Pseudomonas* spp SB-21, and one fungus *Metarhizium anisopliae*, were acquired from the Biocontrol Division, ICRISAT, Patancheru, India. Culture filtrates (1 L) of the aforementioned five microbial cultures were prepared by centrifuging a 24-h-old cultures for bacteria and 96-h-culture for *M. anisopliae* (grown in Luria broth and potato dextrose broth [PDB], respectively) at 10000 *g* for 20 min. The supernatants were then collected and further evaluated for their efficacy against *H. armigera* and *S. litura*.

Rearing of larvae in laboratory

Larvae of *H. armigera* and *S. litura* were multiplied using the

protocols suggested by National Research Institute (NRI), United Kingdom (UK) and Overseas Development Administration (ODA), UK (Armes et al., 1992) in the insect rearing laboratory at ICRISAT, Patancheru. Artificial diet for neonates, growing larvae and adults were prepared according to the protocol by NRI and ODA (Mc Caffery, 1992). The artificial diets, soaked chickpea seeds and sucrose solution (10%) were fed to 1st and 2nd instar larvae, 3rd instar onwards larvae and adults, respectively. The insects for these experiments were reared at laboratory temperature (27 ± 3°C) with relative humidity (RH) of 65 to 70%.

Evaluation of the crude biowash and microbial culture filtrates for management of *H. armigera* and *S. litura*

Artificial chickpea flour based diet was prepared and transferred (one piece of 1 × 1 cm) into a Camphor bottle (4 cm diameter × 4.5 cm height). The crude biowash or microbial culture filtrates (50 µl) were added to the diet. In each camphor bottle, four days old single larva was released. Each bottle served as one treatment with single larvae and replicated 25 times. Observations were taken on number of live larvae and their body weight at 96 h after release of the larvae. The larval weights in various treatments were compared with controls and the percent weight gain was calculated as:

$$\text{Percent weight gain} = \frac{\text{Difference in mean weight of the larvae between control and treatments}}{\text{Mean weight of the control}} \times 100$$

Compatibility of the biowash of the botanicals with PGP bacteria and fungus

Compatibility studies were carried out using the method developed by Gopalakrishnan et al. (2009). In brief, pure cultures of the microbes were serially diluted and spread plated onto media containing microbial free biowash samples (1%; the biowash were filter sterilized before applying into the culture medium). The plates were incubated at 28°C in the dark depending on the requirement of the individual cultures and the number of colony forming units (CFU) was calculated. Control plates contained no botanical extracts. Counting was done at the beginning and at the end of eight days of incubation.

Compatibility studies were also carried out among the four entomopathogenic bacteria and fungi using the method developed by Sriveni et al. (2004). In a previous unpublished study, we established that both entomopathogenic bacteria as well as fungus grew on potato dextrose agar (PDA). The four entomopathogenic bacteria (BCB-19, SB-9, SB-21 and SB-28) and one fungus (*M. anisopliae*) were streaked on PDA in a 10-cm diameter plate in a specific pattern that was first drawn on a paper as template. The template was prepared as follows: 5 cm long vertical line at a distance of 5 mm from the margin was drawn on one side of the circle. Five lines, each 5 mm away and as perpendicular to the vertical line, were drawn. Each perpendicular line was 5 cm long and 1 cm apart from each other. Using this as a template below the Petri dish, the four different entomopathogenic cultures and *M. anisopliae* were streaked over the vertical and the perpendicular lines. However, care must be taken that the cultures did not touch wall of the Petri plate. *M. anisopliae* was streaked 24 h prior to the inoculation of the bacterial isolates because of slow growth. The plates were incubated for 24 h at 28°C and observed for compatibility. If zone of inhibition was found between the entomopathogens, both of them were considered as not compatible, whereas if no zone was found, these were considered as compatible.

Fractionation of the crude biowash samples

Fractionation was done with C18 solid phase extraction cartridges (SPE; Sep-Pak® Plus C18 Environmental cartridges, Waters, Ireland). Before injecting the biowash, SPE cartridges were solvated with 20 ml of 100% methanol (MeOH) and equilibrated with 20 ml of 5% MeOH in ultra pure distilled water. The crude biowash (1 L) collected from the vermicompost was centrifuged at 10000 *g* for 20 min and the supernatant was collected. The supernatants were added with MeOH (in order to make the final concentration of 5% MeOH) and slowly injected into the aforementioned C18 SPE cartridge. The cartridge was washed with 20 ml of 5% MeOH in order to remove the residues of non-adsorbed samples. Non-adsorbed biowash was collected separately, evaporated on a rotary evaporator (Buchi V850, Switzerland) at 35°C and finally collected in minimal volume of MeOH (6 ml; called as non-adsorbed fraction). Furthermore, the cartridge was eluted with 6 ml of 100% MeOH (2 ml at a time) and called as adsorbed fraction. The non-adsorbed as well as adsorbed samples were passed through a Millipore filter (Millex® filter unit; 0.22 µM) and further evaluated for their efficacy against *H. armigera* as described earlier.

Statistical analysis

All the data were analyzed statistically by ANOVA (Genstat 10.1 version) to evaluate the different treatments and the mean values were compared at significant levels of 1 and 5%.

RESULTS

Evaluation of the crude biowash and bacterial culture filtrates for management of *H. armigera* and *S. litura*

When the chickpea flour based diet was treated with crude biowash and fed to four days old larvae of *H. armigera*, not only do the mortality of the larvae varied between 42 and 86% (higher mortality being noted in Jatropha 86%, followed by Pongamia 76%, Neem 71% and Parthenium 69%) over control, but the weight reduction of the larvae also varied between 32 and 71% (higher weight reduction being noted in Vitex 71%, followed by Neem 60% and Annona 60%) over the control (Table 1). In the case of *S. litura*, the mortality of the larvae varied between 46 and 74% (higher mortality was noted in Pongamia 74%, followed by Jatropha 62% and Neem 60%) over control and the weight reduction of the larvae varied between 47 and 77% (higher weight reduction being noted in Datura diet (77%), followed by Annona diet (70%) over control (Table 1).

When the PGP bacteria and fungus were treated with chickpea flour based diet and fed to four days old larvae of *H. armigera*, the mortality of the larvae varied between 59 and 73% (highest being found in treatment with *M. anisopliae*) over control, while the weight reduction of the larvae varied between 55 and 92% (all the PGP bacteria showed more than 89% weight reduction except *M. anisopliae*, which showed only 55% reduction) over control (Table 2). In the case of *S. litura*, the mortality of the larvae varied between 54 and 72% (highest being

Table 1. Influence of various botanical extracts on *H. armigera* and *S. litura*.

Treatment	*H. armigera*		*S. litura*	
	% Mortality (over control)	% Weight reduction (over control)	% Mortality (over control)	% Weight reduction (over control)
Annona	58	60	57	70
Chrysanthemum	43	43	49	47
Datura	42	56	58	77
Jatropa	86	53	62	53
Neem	71	60	60	52
Parthenium	69	32	57	59
Pongamia	76	58	74	56
Tridax	45	54	46	54
Vitex	45	71	52	58
SE±	13.1**	9.6**	5.5***	8.3***
CV%	42	34	24	36

** = Statistically significant at 0.01 (*P* values); *** = statistically significant at 0.001 (*p* values); SE = standard error; CV = coefficient of variance.

Table 2. Influence of plant growth promoting bacteria and fungus (*Bacillus subtilis* BCB19, *Metarhizium anisopliae*, *B. amylofaciens* HiB28, *B. megaterium* SB9 and *Pseudomonas* spp. SB21) on *Helicoverpa armigera* and *Spodoptera litura*.

Treatment	*H. armigera*		*S. litura*	
	% Mortality (over control)	% Weight reduction (over control)	% Mortality (over control)	% Weight reduction (over control)
BCB-19	61	92	63	44
M. anisopliae	73	55	70	60
HiB-28	60	90	54	72
SB-9	64	89	72	79
SB-21	59	90	70	72
SE±	6.8***	4.9***	4.2***	9.9***
CV%	30	13	17	41

***= Statistically significant at 0.001; SE = standard error; CV = coefficient of variance.

found in three cultures namely *M. anisopliae*, *B. megaterium* SB-9 and *Pseudomonas* spp. SB-21) over control, while the weight reduction of the larvae varied between 44 and 79% (all the tested cultures, except *B. subtilis*, showed more than 60% reduction in weight of the larvae) over control (Table 2).

Compatibility of the biowash of the botanicals with PGP bacteria and fungus

Compatibility studies between biowash, PGP bacteria and fungus revealed that there was no definite sign of suppression of any of the botanicals (at the rate of 1%) on the PGP bacteria and fungus except Datura, which significantly inhibited the *B. subtilis* BCB-19. Hence, it was concluded that BCB-19 was not compatible with Datura (Table 3). Interestingly, all the botanicals significantly enhanced the growth of *B. megaterium* SB-9 (Table 3). Compatibility studies among PGP bacteria and fungus showed that all the bacteria were compatible with each other but not with *M. anisopliae* (Table 4).

Fractionation of the promising crude biowash samples

When the promising crude biowash samples were further fractionated on C18 SPE cartridge, both adsorbed as well as non-adsorbed fractions showed significant mortality of the larvae of *H. armigera* (Table 5). Adsorbed and non-adsorbed fractions of all the six biowash samples showed significant mortality between 81 and 93% and between 64 and 73%, respectively over control (Table 5). The weight reduction of the larvae was also found between 73 and 91% for adsorbed fractions and 80 and 97% for non-

Table 3. Compatibility of the various biowash of the botanicals with entomopathogens on day 1 and 8 (microbial counts were expressed in log10 cfu ml^{-1}).

Treatment	Day 1				Day 8			
	Annona	Datura	Neem	Parthenium	Annona	Datura	Neem	Parthenium
BCB-19	5.31	5.15	5.24	4.49	5.41	3	5.48	4.57
HiB-28	5.04	5.28	5.84	5.47	5.87	5.39	6.39	5.53
SB-9	4.57	4.97	5.09	5.05	6.38	5.66	6.45	5.59
SB-21	5.75	4.81	6.66	6.19	6.62	5.04	7.04	6.3
M. anisopliae	4.54	4.71	4.22	4.15	4.93	4.95	4.27	4.46
SE	0.083**				0.061***			
CV%	5				4			

= Statistically significant at 0.01 (*P* values); * = statistically significant at 0.001 (*P* values); SE = standard error; CV= coefficient of variance.

Table 4. Compatibility among the bacterial and fungal entomopathogens.

Treatment	BCB-19	SB-9	SB-21	HiB-28
BCB-19	+	+	+	+
SB-9	+	+	+	+
SB-21	+	+	+	+
SB-28	+	+	+	+
M. anisopliae	-	-	-	-

+ = Compatible; - = not compatible.

Table 5. The effect of adsorbed and non-adsorbed fractions of potential crude biowash samples on *Helicoverpa armigera* larvae.

Treatment	Adsorbed Fraction		Non-adsorbed Fraction	
	% Mortality (over control)	% Weight reduction (over control)	% Mortality (over control)	% Weight reduction (over control)
Annona	91	89	65	80
Datura	88	76	64	89
Jatropha	87	84	72	97
Neem	81	79	69	89
Parthenium	93	73	65	91
Pongamia	93	91	73	91
SE±	1.6*	6.0^{NS}	1.7*	2.9*
CV%	3	13	4	6

*= Statistically significant at 0.001; SE = standard error; CV = coefficient of variance.

adsorbed fractions over control (Table 5).

DISCUSSION

The use of botanicals and microbes in crop protection has now gained a popular ground in the world of agriculture as an alternative to toxic, persistent and synthetic compounds. At present, serious attention is drawn to extracts from vermicomposts of plants that contain substances in the form of alkaloids or inhibitors, which help in managing the agriculturally important pests. One of the advantages of using earthworms in composting is that it creates aerobic conditions, thus inhibiting the action of anaerobic microorganism that causes foul odor and release coelomic fluids in the decaying biomass, which may have antibacterial properties that kills pathogens such as *Escherichia coli*, *Salmonella typhi* and *Serratia marcescens* (Prabha, 2009). Earthworms also promote microbial activity and diversity in organic wastes to levels even greater than those in thermophilic composts (Edwards, 1998).

Incorporation of vermicomposts can have a direct impact not only on soil health and crop productivity, but also can be an alternative for the chemical fertilizers and pesticides (Hameeda et al., 2006). Hence, there seems to be an even greater potential for suppression of plant pests and diseases by vermicomposts than by composts, probably due to stimulatory effects of soil microbial activity (Perner et al., 2006; Postma et al., 2003). Number of references are available in support of this hypothesis for example, vermicompost at 25% provided good control of damping off in Patience-plant (*Impatiens wallerana*) caused by *Rhizoctonia solani* (Asciutto et al., 2006). Entomopathogens, antagonistic microbes and botanicals isolated from vermicompost serve as an alternative to chemical pesticides and fertilizers (Murrey et al., 2000; Lacey and Shapiro-Ilanan, 2008).

In this investigation, when the crude biowash of nine vermi-composts and five PGP microbes were evaluated for their efficacy against four day old larvae of *H. armigera* and *S. litura*, more than 50% mortality was found over control in the biowash of Jatropha, Neem, Pongamia, Parthenium and Annona. The biowash of all the nine botanicals significantly reduced the larval body weight of both the species. Biowash of the botanicals such as Jatropha, Parthenium and Annona were shown to inhibit Fusarium wilt and collar rot of chickpea, as well as charcoal rot of sorghum (Gopalakrishnan et al., 2010). In this study, all the four PGP bacteria and *M. anisopliae* were found to cause significant larval mortality and growth inhibition in both *H. armigera* and *S. litura*. Studies related to PGP bacteria and actinomycetes isolated from vermicomposts and rhizosphere soils were also reported to inhibit plant pathogens of chickpea and sorghum (Gopalakrishnan et al., 2011a, 2011b). The usefulness of PGP bacteria are well documented in the literature. For instance, PGP bacteria stimulate growth of host plants directly by nitrogen fixation (Han et al., 2005), solubilization of nutrients (Rodriguez and Fraga, 1999), production of growth hormones, (Correa et al., 2004) and indirectly by antagonizing pathogenic fungi and pests of agriculturally important crops by production of siderophores, chitinase, β-1, 3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal et al., 2001).

The entomopathogenic potential biowash and the four entomopathogenic bacteria and fungus were further tested for their compatibility against each other in order to know whether the biowash of the botanicals and microbes can be applied together, so that the efficacy against the *H. armigera* and *S. litura* can be enhanced. The compatibility studies revealed that there was no suppression of any of the botanicals on the PGP bacteria and fungus except Datura on *B. subtilis* BCB-19. Hence, it was concluded that biowash prepared from Datura was not compatible with BCB-19. It was also observed that all the botanicals enhanced the growth of *B. megaterium* SB9. These studies also revealed the compatibility of the various bacterial strains with themselves but these bacteria were not compatible with the fungus, *M. anisopliae*.

Furthermore, in order to fractionate the metabolite(s) responsible for the mortality of the larvae of *H. armigera*, the promising crude biowash samples were fractionated on C18 SPE cartridge and the resulting adsorbed and non-adsorbed fractions were evaluated for their efficacy on the larvae of *H. armigera*. The results clearly indicate that both adsorbed and non-adsorbed fractions showed significant mortality (81 to 93% and 64 to 73%, respectively) and weight reduction (73 to 91% and 80 to 97%, respectively) over the control larvae of *H. armigera*. Hence, it was concluded that both polar as well as non-polar compounds were present in the biowash of herbals that involved in the inhibition of the larvae of *H. armigera*. The adsorbed fractions of C18 SPE cartridge of botanicals such as Jatropha, Annona and Parthenium were also found to inhibit the pathogens of charcoal rot of sorghum and *Fusarium* wilt and collar rot of chickpea (Gopalakrishnan et al., 2010). Hence, it is concluded that there were some secondary metabolites present in the biowash of Annona, Datura, Jatropha, Neem, Parthenium and Pongamia that were capable of inhibiting the larvae of *H. armigera*.

Mortality and weight reduction of the larvae of *H. armigera* might be due to soluble compounds (such as soluble nutrients, free enzymes, soluble phenolic compounds and a wide range of microorganisms) passing from the solid botanical vermicompost into the biowash. Suppression by soluble nutrients and microorganisms can be ruled out since the biowash was passed through SPE cartridge and Millipore filter which filters microorganisms. It is possible that some free enzymes could influence the mortality of the larvae of *H. armigera*, but not on the scale realized in these studies. Hence, the most likely reason for the larval mortality could be the soluble phenolic substances taken up into biowash from vermicomposts. Exploitation of such allelochemicals needs to be done in the integrated management of pest and pathogens. Further research in this area has the potential to exploit the usefulness of vermicomposts as biopesticide.

ACKNOWLEDGEMENTS

We thank the National Bureau of Agriculturally Important Microorganisms for providing financial support. We also thank all the staff of biocontrol unit of ICRISAT including M/s PVS Prasad, P. Manohar, B. Nagappa, M. Satyam, D. Barath and A. Jabbar for their significant inputs in the sample preparation and laboratory studies.

REFERENCES

Armes NJ, Bond GS, Cooters RJ (1992). The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No. 57. Chatham, UK: Natural Resources Institute.

Asciutto K, Rivera MC, Wright ER, Morisigue D, Lopez MV (2006). Effect of vermicompost on the growth and health of *Impatiens wallerana*. Int. J. Exp. Bot. 75: 115-123.

Correa JD, Barrios ML, Galdona RP (2004). Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant Soil, 266: 75-84.

Doumbou CL, Salove MK, Crawford DL, Beaulieu C (2001). Actinomycetes, promising tools to control plant diseases and to promote plant growth. Phytoprotection, 82: 85-102.

Dubey NK, Shukla R, Kumar A, Singh P, Prakash B (2010). Prospects of botanical pesticides in sustainable agriculture. Curr. Sci. 98(4): 479-480.

Edwards CA (1998). The use of earthworms in processing organic wastes into plant growth media and animal feed protein. In: Edwards CA (ed.). CRC press: Boca Raton, Florida, Earthworm Ecol. pp. 327-354.

Gopalakrishnan S, Humayun P, Kiran BK, Kannan IGK, Vidya MS, Rupela O (2011a). Evaluation of bacteria isolated from rice-rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. World J. Microbiol. Biotechnol. 27: 1313-1321.

Gopalakrishnan S, Kannan IGK, Alekhya G, Humayun P, Meesala SV, Kanala D (2010). Efficacy of Jatropha, Annona and Parthenium biowash on *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina*, pathogens of chickpea and sorghum. Afr. J. Biotechnol. 9: 8048-8057.

Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O (2011b). Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. Crop Prot. 30: 1070-1078.

Gopalakrishnan S, Watanabe T, Pearse SJ, Ito O, Hossain ZAKM, Subbarao GV (2009). Biological nitrification by *Brachiaria humidicola* roots varies with soil type and inhibits nitrifying bacteria, but not other major soil microorganisms. Soil Sci. Plant Nutr. 55: 725-733.

Grzywacz D, Richards A, Rabindra RJ, Saxena H, Rupela OP (2005). Efficacy of biopesticides and natural plant products for *H. armigera* control. In *Heliothis/Helicoverpa* management- Emerging Trends and Strategies for Future research (Sharma HC Ed.), New Delhi: Oxford &IBH, pp 371-389.

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2006). Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res. 163: 234-242.

Han J, Sun L, Dong X, Cai Z, Sun X, Yang H, Wang Y, Song W (2005). Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst. Appl. Microbiol. 28: 66-76.

Kaur S (2000). Molecular approaches towards development of novel *Bacillus thuringiensis* biopesticides. World J. Microbiol. Biotechnol. 16: 781-793.

Lacey LA, Shapiro-Ilan DI (2008). Microbial control of insect pests in temperate orchard systems: Potential for incorporation of IPM. Annu. Rev. Entomol. 53: 121-144.

Mandour NS (2009). Influence of spinosad on immature and adult stages of *Chrysoperla carnea*. Biocontrol, 54: 93-102.

Mc Caffery (1992). The laboratory culure and development of *H. armigera*. Bulletin No: 57, National Research Institute and Overseas Development Administration, United Kingdom.

Murray DAH, Lioyd R, Buddington J (2000). Potential in Australia for a *Helicoverpa baculovirus*. Abstract. International Congress of Entomology, 21-25 Aug 2000, Igassu Falls, Brazil.

Nath G, Singh K (2009). Utilization of vermiwash potential on certain summer vegetable crops. J. Cent. Eur. Agric. 10(4): 417-426.

Pawar CS (1998). Wasps- predators of *Heliothis* in pigeonpea. Int. Pigeonpea Newsletter, 2: 65-66.

Pal KK, Tilak KVB, Saxena AK, Dey R, Singh S (2001). Suppression of maize root disease caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth-promoting rhizobacteria. Microbial. Res.156: 209-223.

Perner H, Schwarz D, George E (2006). Effect of mycorrhizal inoculation and compost supply on growth and nutrient uptake of young leek plants growth and nutrient uptake of young leek plants growth on peat-based substrates. Hort. Sci. 41: 628-632.

Postma J, Montanari M, Van den Boogert PHJF (2003). Microbial enrichment to enhance disease suppressive activity of compost. Eur. J. Soil Biol. 39: 157-163.

Prabha ML (2009). Waste management by vermitechnology. Ind. J. Environ. Prot. 29: 795-800.

Rodriguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 17: 319-333.

Shield EB (1982). Raising earthworms for profit. Shields publication. PO Box 669. Eagle River Wisconsin, p. 128.

Sriveni M, Rupela OP, Gopalakrishnan S, Krajewski M (2004). Spore-forming bacteria, a major group among potential antagonists from natural sources such as termitaria soil and composts used by organic farmers. Ind. J. Micro. 44(2): 95-100.

Suthar S, Choyal R, Singh R, Sudesh R (2005). Stimulatory effect of earthworm body fluid on seed germination and seedlings growth of two legumes. J. Phytol. Res. 1(2): 219-222.

Thompson G, Hutchins S (1999). Spinosad. Pestic. Outlook, 10: 78-81.

Wang D, Qiu X, Ren X, Zhang W, Wang K (2009). Effects of spinodad on *H. armigera* from China: tolerance status, synergism and enzymatic responses. Pest Manage. Sci. 65: 1040-1046.

Yadav AK, Kumar K, Singh S, Sharma M (2005). Vermiwash- A liquid bio-fertilizer. Uttar Pradesh J. Zoo. 25(1): 97-99.