Journal of Applied and Natural Science 8 (1) : 191-195 (2016)

JANS

Bioefficacy of plant extracts on stem rot, *Macrophomina phaseolina (*Tassi) Goid and Bihar hairy caterpillar, *Spilosoma obliqua* Walker in jute crop

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Received: May 28, 2015; Revised received: September 18, 2015; Accepted: February 11, 2016

Abstract: *In vitro* study was conducted to test mycellial growth inhibition effect of plant extracts on *Macrophomina phaseolina* causing stem rot of jute as well as for feeding inhibition and mortality on 3rd instar larvae of Bihar hairy caterpillar, *Spilosoma obliqua* Walker. The result revealed, at 2000 ppm, acetone extracts of sunnhemp and *Azeratum conyzoides* exhibited maximum of 34.44% and 41.85% mycellial growth inhibition respectively. Whereas, 83.32% and 66.67% spore germination inhibition of the fungus was observed respectively. At 5000 ppm, methanolic extracts of *Crotolaria quinquefolia*, garlic, curry leaf and turmeric oil recorded 35.55%, 44.44%, 50.00% and 70.00% mycellial growth inhibition of the fungus. Methanolic extracts of *C. juncea*, *C. quinquefolia*, curry leaf and garlic recorded above 80.00% feeding inhibition on *S. obliqua* and larval mortality of 20.00%-44.44%. It is clear that neem, sunnhemp, garlic and turmeric extract possess antifungal, insecticidal, antifeedant properties and may be integrated for management of stem rot as well as *S. obliqua* in jute crop.

Keywords: Feeding inhibition, Larval mortality, *Macrophomina phaseolina*, Plant extracts, *Spilosoma obliqua*, Spore germination

INTRODUCTION

Indiscriminate use of synthetic pesticides to ensure higher crop yield have adversely affected both biological and physical environment, leading to the pollution of biosphere and rapid build-up of resistance and resurgence of insect pests and diseases (Chowdhury *et al.*, 2012b). The damage is caused due to high toxicity and non-biodegradable nature of the pesticides and due to the residues in soil, water and crops that affect human health. Thus, efforts are needed to search new selective and biodegradable pesticides. In the move towards green pesticides and the continuing need for developing new crop protection tools, phytochemicals derived from various bio-active plant species offer a promising source of safer agrochemicals (Isman, 2006). There are many botanical products that have been reported as antifungal compounds (Chowdhury *et al.*, 2008; Koul *et al.*, 2008). These antifungal compounds present in higher plants are the well-known factors to disease resistance. They are biodegradable and selective in toxicity.

Various compounds isolated from plants have been studied for insecticidal activity globally (Dev and Koul, 1997) and majority of them are insect antifeedants (Jermy, 1990; Koul, 2005). More than 140 compounds, which are chemically diverse and structurally complex, have been isolated from the leaves, seed oil and bark of neem (Koul, 2005). Phytochemicals are often distasteful and toxic to many pests. They can modify behaviour of an insect by acting directly on the chemosensilla resulting in feeding deterrence (Isman, 1994).

Jute (*Corchorus olitorius* L. and *C. capsularis* L.) is an important fibre crop next to cotton. It is mainly cultivated in India, Bangladesh, China, Nepal and Thailand. Its fibre is used for making bags, decoratives, textiles and geotextiles and its sticks are used for fuel, door panels of automobiles, and for making false ceiling boards. Its production and productivity is hampered by number of abiotic and biotic stresses. Among them stem rot, *Macrophomina phaseolina* (Tassi) Goid and Bihar hairy caterpillar, *Spilosoma obliqua* Walker (Arctiidae: Lepidoptera) are devastating and often causing yield losses. *M. phaseolina* causing stem rot in jute is the most economically important disease. Besides stem rot, the pathogen causes damping off, seedling blight, leaf blight, tip blight, collar rot and root rot (Roy *et al.*, 2008). The disease is reported from all the jute growing countries affecting equally both the cultivated species of jute with 10.00% loss in fibre yield. Fibre yield loss reported even up to 35.00-40.00% under severe infection in hot (34 ± 1ºC) and humid weather (Mandal, 1990).

The *S. obliqua* is a polyphagus pest attacking wide range of crops including bast fibre crops like jute and mesta. Previously it was considered as sporadic and irregular pest of jute in less rainfall areas and gradually

ISSN : 0974-9411 (Print), 2231-5209 (Online)

this pest has turned to a major pest of jute in heavy rainfall areas like North Bengal and Assam (Das, 1948). In the present study, efforts were made to explore the naturally available various plant extracts as antifeedant against *S. obliqua* and antifungal agent against *M. phaseolina* affecting jute crop.

MATERIALS AND METHODS

Collection and preparation of plant extracts: Jute (*C. capsularis*), mesta (*Hibiscus sabdarrifa*), sunnhemp (*Crotolaria juncia*), *C. quinquefolia*, ramie (*Bohemea nivea*), sisal (*Agave sisalana*), curry leaf (*Murraya koenigii*), goat weed (*Azeratum conyzoides*) and marigold (*Tagetis* sp.) plants were grown at ICAR-Central Research Institute for Jute and Allied Fibres research farm, Kolkata, West Bengal. Plant leaves were washed properly in cold water and dried under shade. Dried leaves were grinded in a mechanical grinder to fine powder. Leaf powder were put into different solvents (Hexane, Acetone, Methanol, Ethanol, Ethyl Acetate) and kept in room temperature for a week as per (Sindhan *et al.,* 1999). Garlic cloves were peeled off and then macerated in a mortar and pestle to make it paste and then dipped into methanol for a week in room temperature. Solvent extracts were filtered through activated charcoal bed to remove pigments and then passed through sodium sulphate bed to remove moisture. The solvent was evaporated under vacuum to obtain concentrated plant extracts. Hexane extract of turmeric powder after evaporation of solvent yielded 3.45% oil (w/w). Other plant samples extracted in solvents when passed through charcoal bed and then through sodium sulphate bed clear and dry solvent extracts were obtained. Evaporation of solvents under vacuum yielded 4.5-16.6% (w/w) concentrated extracts. Required quantities of the plant extracts were dissolved appropriate solvents to prepare test solutions (5,000 ppm and 2,000 ppm) for bioassay on the fungus and test insect i.e Bihar hairy caterpillar, *Spilosoma obliqua*.

Fungal bioassay: Effect of plant extracts (5,000 ppm and 2000 ppm) on radial growth and spore germination inhibition of *M. phaseolina* was studied up to seven days from inoculation by poisoned food technique using potato dextrose agar (PDA) medium (Nene and Thapliyal, 1979). For the mycellial growth inhibition studies, seven day old growth of *M. phaseolina* was used. The four replicated PDA Petriplates were inoculated with 5 mm disc of *M. phaseolina* at the center of each of the plates. Both treated and untreated (only solvent) Petriplates were incubated at 27 ± 1^0C in biological oxygen demand (BOD) incubator and the mycelial growth of the fungus was measured diametrically in three different directions after 7 days and antagonistic potential of plant extracts was calculated as per the formula of I=C-T/C*100, where I= inhibition in mycelial growth; C= growth of mycelium in control (mm); T= growth of mycelium in treatment (mm). The data obtained were analyzed following completely randomized design (CRD).

Seven day old culture of test fungus was taken from PDA slant and spore suspension was made by addition of sterilized distilled water taking small bit of fungal culture in 20 ml of sterilized distilled water. Haemocytometer was used to get standardized spore suspension (1×10^6 spores/ml). Small droplets (0.02 ml) of the test solution and spore suspension in equal amount were seeded in the cavity slides. These slides were kept in Petriplates lined with moist filter paper and then incubated for 24 hrs at 25 ± 1^oC. Germination of the spores was recorded on 0-4 scale and per cent spore germination was calculated.

Bioassay against *S. obliqua:* The first instar larvae of *S. obliqua* were collected from the field and reared in the laboratory on jute leaves (*C. olitorius*) in glass jars (20 x 15 cm) at 27 ± 1^0C and 70 ± 5% relative humidity. After 24 hrs of feeding, the larvae were transferred to fresh leaves in another disinfected container. Full grown and about to pupate larvae were transferred to glass jars having a thick layer of sterilized soil. The moths emerging after a week were collected and transferred to clean jars containing a suspended cotton swab soaked in honey solution and pieces of folded papers at the bottom for oviposition. The eggs laid were separated and observed for hatching every day. The freshly hatched larvae of the same batch were removed and kept separately on fresh and tender jute leaves in a glass jar in order to have 3rd instar larvae of uniform weight (30-40 mg) were selected for bioassay of plant extracts for feeding inhibition activity and larval mortality of *S. obliqua* as per Chowdhury *et al* (2012b). Larval mortality after 24 hrs of feeding was also counted and corrected larval mortality was calculated as per Abbott (1925). Corrected mortality (%) = (T-C)/(100-C) × 100 where, T= mortality in treatment and C= mortality in control. The per cent feeding inhibition and larval mortality (corrected) data were arcsine transformed.

Antifeedant activity against *S. obliqua:* Forced feeding method was followed for testing the antifeedant activity of the plant extracts on 3rd instar larvae of S. *obliqua* (Abdelgaleil and Nakatani, 2003). Jute leaves of uniform size were plucked from insecticides free plots, washed thoroughly with distilled water and dried under shade. For each treatment five leaves were treated with the botanicals, air dried and placed inside the Petriplates (15 mm × 1.5 mm) followed by ten 3rd instar larvae of *S. obliqua* pre-starved for 6 hrs were released and all treatments were replicated thrice. The outer margin of each leaf offered for feeding was traced in the graph paper. The extent of consumption of leaves after 24 hrs was measured from the graph paper (Sarma and Kalita, 2001). Consumption data of solvent treated leaf disc was taken as control. Feeding inhibition (%) was calculated as described by Pande and Shrivastav, 2003). Feeding inhibition (%) = (C-T)/(C+T) x 100 where C= consumption of leaf in control and T= consumption of leaf in treatment. Larval mor-

tality after 24 hrs of feeding was also recorded.

RESULTS AND DISCUSSION

Effect of plant extracts on growth and spore germination on *M. phaseolina:* The mycellial growth of *M. phaseolina* was recorded in 2000 ppm test solutions of marigold, goat weed, mesta, sunnhemp and sisal extracts treated petriplates at seven days after treatment. However, sunnhemp extracts (acetone, ethanol) along with the acetone extract of *A. conyzoides*, inhibited mycellial growth of the fungus by about 33.00-41.85% over control (Table 1). Previous study also revealed that the garlic extract inhibited maximum mycelial growth (73.00%) as well as sclerotial formation followed by rhizome extract of turmeric (63.98%) (Dhingani *et al.,* 2013). The sporulation inhibition of *M. phaseolima* was recorded to be 66.67% and 83.32% in acetone extract of *A. conyzoides* and to be normal sunnhemp recorded 33.32 % spore germination inhibition. Similar study was also carried out by Tandel *et al.* (2010) and results obtained were in confirmation of the present investigation and it was revealed that onion bulb extract resulted maximum inhibition (98.14%) of *M. phaseolina* followed by extract of acacia, ginger, neem, garlic and karanji. Less than 10.00% sporulation inhibition was recorded by rest of the extracts. Present results are also in confirmation with those described earlier and who found the fungitoxic properties of *Acacia arabica*, *Allium cepa*, *A. sativa* against vegetative growth and sclerotial viability of *M. phaseolina* (Dubey and Dwivedi, 1991).

At 5000 ppm, methanolic extract of curry leaf and turmeric oil recorded about 50.00% and 70.00% radial growth inhibition respectively, however, methanolic extract of *C. quinquifolia* and garlic recorded 35.55% and 44.44% radial growth inhibition of *M. phaseolina*, respectively. Similarly, Cowdhury *et al.* (2012a) studied the pathogenicity of turmeric oils against *M. phaseolina* and found bioactive turmerones in the oil. Methanolic extract of ramie leaf recorded less than 20% radial growth inhibition in the fungus (Table 2). The present study also supported by the previous workers that *Crotalaria* leaf extract are having fungicidal action (Rao, 1957; Vernier *et al.,* 2005; Gomes *et al.,* 2005). *M. phaseolima* sporulation inhibition 80.50% and 56.75% in hexane extract of turmeric and methanol extract of *C. quinquifolia*, respectively at 5000 ppm was recorded. The acetone extract of *A. conyzoides* and alcoholic extract of sunnhemp was found to have inhibitory effect on mycelial growth of *M. phaseolina* and similar studies on plant extracts were reported by Appleton and Tansey (1975) who found the growth inhibitory effect of garlic extract on pathogenic fungi.

Effect of plant extracts on feeding inhibition and mortality of *S. obliqua:* In the present study, sunnhemp, *C. quinquifolia*, curry leaf, garlic extracts were also found active in causing feeding inhibition in *S. obliqua* and some of them caused larval mortality. Methanolic extracts (2000 ppm) of neem, sunnhemp,

Table 1. Effect of plant extracts on mycellial growth and sporulation of *M. phaseolina* at 2000 ppm.

Plant extracts	Mean radial growth (cm)	Growth inhibition over control (%)	Mean sporulation (0-4 scale)	Spore germination inhibition over control (%)
Jute (Ethyl acetate)	8.90	0.74 (4.93)*	3.67	8.25 (16.69)
Marigold (Acetone)	9.00	0.00 (0.00)	3.67	8.25 (16.69)
A. conyzoides(Acetone)	5.20	41.85 (40.31)	1.33	66.67 (54.74)
Mesta (Ethanol)	9.00	0.00 (0.00)	3.67	8.25 (16.69)
Mesta (Acetone)	9.00	0.00 (0.00)	3.67	8.25 (16.66)
Sunnhemp (Acetone)	5.70	34.44 (35.93)	0.67	83.32 (65.89)
Sunnhemp (Ethanol)	6.10	33.33 (35.26)	2.67	33.32 (35.26)
Sisal (Acetone)	9.00	0.00 (0.00)	3.67	8.25 (16.69
Control (no spray)	9.00	0.00 (0.00)	4.00	0.00 (0.00)
LSD (P=0.05%)	0.80	---	1.52	---

*Figures in parenthesis are arc sine transformed values

Table 2: Effect of plant extracts on mycellial growth of *M. phaseolina* at 5000 ppm.

Plant extracts	Mean radial growth (cm)	Growth inhibition over control (%)	Mean sporulation (0-4 scale)	Spore germination inhibition over control (%)
Turmeric oil (Hexane)	2.700	70.00 (56.79)	0.78	80.50 (63.79)
Curry leaf (Methanol)	4.50	50.00 (45.00)	2.37	40.75 (39.67)
Ramie leaf (Methanol)	7.50	16.66 (24.09)	3.79	5.25 (13.25)
Garlic (Methanol)	5.00	44.44 (41.81)	2.82	29.50 (32.90)
C. *quinquifolia* (Methanol)	5.80	35.55 (36.60)	1.73	56.75 (48.88)
Control (no spray)	9.00	00.00 (0.00)	4.00	0.00 (0.00)
LSD (P=0.05%)	1.37	---	0.48	--

*Figures in parenthesis are arc sine transformed values

Table 3: Effect of plant extracts on feeding of 3rd instar larvae of Bihar hairy caterpillar at 2000 ppm.

Plant extract	Leaf area fed /insect (%)	Feeding inhibition (%)	Larval mortality (%)
C. quinquifolia (Methanol)	5.45 (13.45)*	83.48 (66.02)	33.33 (35.26)
C. juncea (Methanol)	3.10 (9.72)	90.60 (72.15)	20.00 (26.57)
Garlic (Methanol)	2.00 (8.13)	93.94 (75.75)	44.44 (41.81)
Curry leaf (Methanol)	4.13 (11.67)	87.48 (69.28)	0.00 (0.00)
Neem (Methanol)	2.00 (8.13)	98.29 (82.49)	58.67 (49.99)
Control (no spray)	33.00 (34.12)	0.00 (0.00)	0.00 (0.00)
LSD (P=0.05%)	14.21	---	---

garlic and curry leaf limited the leaf consumption by the starved 3rd instar larvae of *S. obliqua* in comparison to the control (untreated). In previous studied the *S. obliqua* survivorship of larvae at 1000, 2000 and 3000 ppm of neem was significantly reduced to 51.00%, 49.00% and 42.00% (Kapinder and Singh, 2014). In present study in control, 33.00% leaf area was consumed whereas in treated leaves only 2.00-5.45% leaf area was consumed by insect. Similarly, inhibitory activities of plant extracts have been screened against *S. obliqua* (Dubey *et al.,* 2004). Thus above 98.00% feeding inhibition over control was recorded in all the plant extracts tested (Table 3). This feeding inhibition may be due to antifeedant activity possessed by plant extracts like neem oil (Mosaddaque, 1995). In a previous studies *Adhatoda vasica* caused the maximum mean mortality (93.33%) which was significantly superior to *Azadirachta indica*>*Curcuma domestica*>*Cleome monophylla*>*Alpinia galanga*> the control against *S. obliqua* (Chandel *et al*., 2009).

In present studies, the larval mortality recorded 58.67% in neem extract treatment with 98.27% feeding inhibition. Though in control no insect mortality was observed, in case of garlic extract treated leaves the larval mortality was 44.44%. *C. juncea* and *C. quinquifolia* extracts treated leaves recorded 20.00% and 33.33% larval mortality, respectively. No larval mortality in curry leaf extract was observed. Kumar and Ali (2010) observed that the standard check insecticide, endosulfan 35 EC at 0.07% had the best performance, followed by 5% NSKE, 2% NLE, 0.15% nimbecidine, 2% neem oil, 2% mahuwa oil and 5% KSKE, with 23.0, 17.6, 16.4, 15.3, 14.3 and 11.5% larval population reduction, respectively against *S. obliqua.*

Conclusion

The methanolic extracts of *C. juncea, C. quinquefolia*, curry leaf and garlic showed upto 70% growth inhibition of *M. phaseolina* and 80.00% feeding inhibition of *S. obliqua* under *in vitro* condition. The methanolic plant extracts at 5000 ppm can be used effectively for the control of jute stem rot pathogen, *M. phaseolina*. Thus, the plant extract may have their greatest impact in future integrated pest management (IPM) programmes due to their safety to non-target organisms and the environment. Detailed investigation needs to be isolate the active constituents of the plant extracts responsible for the antifungal and insecticidal activities of the plant extracts which may lead to development of suitable botanical formulations that can be incorporated into the IPM programme for controlling the fungal disease and the insect pest in jute crop. The findings of this experiment may be helpful for management of *M. phaseolina* and *S. obliqua.*

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR-Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, for providing the necessary facilities for conducting the experiments**.**

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