Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 20(2), May-August, 2022]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 20(2) May-August, 2022

CONTENTS

Laboratory evaluation of Dashparni extract against bollworm complex of cotton

RACHNA PANDE, RAMKRUSHNA GI, NEELKANTH HIREMANI and SUNITA CHAUHAN[1]

ICAR- Central Institute for Cotton Research, Nagpur-440010 (Maharashtra),[1]KVK Nagpur, ICAR- Central Institute for Cotton Research, Nagpur-440010 (Maharashtra)

ABSTRACT: Bollworm complex of cotton is major yield limiting factor of cotton. In the present study, the larvicidal activity of Dashparni extract were evaluated against the bollworm complex of cotton *viz*., *Helicoverpa armigera* and *Pectinophora gossypiella* using diet bioassay. The ratio of toxicant and diet was standardized. The study was conducted under the controlled laboratory condition with 1%, 2%, 4%, 6%, 8% and 10% concentration and compared with control. The result was presented with values of % mortality on each day. All the tested concentrations had larvicidal activity which were significant over control. The increase in % mortality data showed clear relation with the increase in concentration and increase in duration of the feeding time period within the same concentration. At higher concentration (8% and 10%) the effect of extract on % mortality (91.67%) was at par in case of pink bollworm, however in case of american bollworm at highest concentration (10%) the mortality (45.83%) was significant with rest of the treatments. The LD_{50} value of Dashparni extract for pink bollworm was calculated as 1.01. The Dashparni extract had proved its effect on growth and development of both the bollworm. However, the field efficacy of the same needs the further experimentation.

Key words: American bollworm, Dashparni, diet bioassay, mortality, pink bollworm

Cotton, *Gossypiun species* is the most important fiber crop grown in India. Many pests including insects and pathogens attack the cotton plants at various stages and cause damage to the crop. Bollworm complex of cotton is the major limiting factor in cotton production. In central India, pink bollworm *Pectinophora gossypiella* (Saunders) and American bollworm *Helicoverpa armigera* (Hübner) are the most prevailing bollworms. The *P. gossypiella* (Lepidoptera: Gelechiidae) is the most destructive and cosmopolitan pest of cotton. It usually feeds on flowers, bolls and seeds which cause premature or partial dropping of the bolls. This pest has also developed the resistance against Bt cotton (Dhurua and Gujar, 2011; Naik *et al.,* 2018) which was considered as the promising option to keep the population of this menace under check. Similarly, *H. armigera* (Lepidoptra: Noctuiidae) is distributed all around the world and polyphagous in nature. Larvae cause severe yield loss as it prefers to feed on all the part of the cotton plants including young leaves, squares, flowers and bolls (Pande *et al*., 2019). Similar to pink bollworm incidence, it is also reported that the larvae of *H. armigera* are able to feed on *Bt* cotton (Prasad *et al*., 2009), even they survived up to adult stage and did successful reproduction (Ranjith *et al*., 2010). Due to the failure of *Bt* cotton against the bollworm complex, farmers are bound to rely on insecticidal spray which is hazardous to environment. So, it is high time to search some alternative or complementary management tactics for the management of bollworms. Many researchers have tested the efficacy of different indigenous products including plant or animal origin against the various insect pests *viz*., neem seed kernel extract (NSKE) and biopesticides (plant and animal origin) against the pests of soybean (Pande *et al*., 2010; Das *et al*., 2018); indigenous plant product against mustard aphid (Debnath *et al*., 2018); plant extract against *H. armigera* (Shalaby and Dhafar, 2019), neem extract with entomopathogenic fungi against *P. gossypiella* (Farooq *et al*., 2020), animal urine on *H. armigera* (Ajaykumara and Tiwari, 2019) and cow dung-cow urine on bollworm complex of cotton (Shalaby *et al*., 2019) to reduce the use of pesticide. But it is observed that most of the researchers relied on the single plant extract or animal origin biopesticide which have specific compounds with limited insecticidal activity. The idea behind in this experiment was to take cocktail of a greater number of plant extract with animal origin biopesticide like cow urine and cow dung. So, keeping the above fact in mind in the present experiment 'Dashparni' extract (Dash means ten; Parni means leaves-extract of ten plant) was tested against the target bollworm complex under the laboratory condition.

MATERIALS AND METHODS

Insect culture collection and maintenance

Larvae of American bollworm and pink bollworm were collected from Nagpur ($21^0$04'48.39" N $78^0$06'58.02E), Maharashtra India from the non-*Bt* variety of cotton (*Gossypium hirsutum*) during 2020. Larvae were reared

on chickpea based artificial diet under controlled condition (65±5% relative humidity, 27±1^{0}C temperature, 14L:10D photoperiod) in insectary of ICAR-Central Institute for Cotton Research, Nagpur. Culture maintenance of American bollworm was done by collecting the egg laying on muslin cloth and for pink bollworm cotton twigs with square were used as oviposition substrate (Pande *et al*., 2019; Shah *et al*., 2020). Cotton plug dipped in 10% honey solution was used as a source of food for adult moth of bollworm. Moths were allowed to mate for egg laying. Newly hatched larvae were collected on the same chickpea-based diet for further diet bioassay studies.

Preparation of Dashparni extract

Water extract of Dashparni was prepared by taking ten plants leaves as a prominent component of extract. Plants used in preparation of Dashparni extract may vary from location to location on the availability in local condition. Similarly, in ICAR-CICR, Nagpur plants which have proven pesticidal properties were selected for the preparation of Dashparni are listed in Table 1.

Other ingredients are cow dung and cow urine (desi cow). The water extract of Dashparni was prepared by mixing of these ingredients in a specific quantity. For water extract of Dashparni 10 gm of all the leaves except neem were mixed in 1 liter of water than 25 g of neem leaves, 25 g of cow dung and 25 milliliter cow urine were further added. All the freshly collected ingredients were mixed in a jar and kept for 30-45 days. The liquid was stirred regularly at morning and evening hours and was covered with muslin cloth to prevent egg laying by the flies on the extract. Once ready, extract can be used for six months. In laboratory condition, the extract was tested at 1%, 2%, 4%, 6%, 8% and 10%. In the control treatment water was applied on the diet.

Diet bioassay

Chickpea-based diet was prepared and used for the diet bioassay. Mixing of extract in hot diet was not done to avoid the degradation of active compound of extract. In both the bioassay, the extract was applied with micropipette on the diet when the diet temperature reached to room temperature. The bioassay was conducted in 24 well tissue culture plates (make: Tarsons). The ratio of diet and extract was standardized by adding the extract with micropipette. The ratio of diet and extract was finalized as 6:1 for pink bollworm and 11:1 for American bollworm. It was made assured that after application of extract on diet it should be fully absorbed by the diet otherwise larva will be drowned in the liquid of extract. Care was taken that larva should not get stick with the wall of plate or surface, the treated diet was kept for 15 minutes at room temperature for drying. Same method was repeated for all the treatments including control. After drying, each well was released with 3-4 days old single larva or first instar larva. Single treatment was represented by one plate and numbers of replications were three.

Data recording and analysis

Observation was recorded every 24 hours. Per cent mortality was calculated using simple arithmetic formula. Duration of bioassay period was different for both the insects depending on the larval period. Pink bollworm data was recorded up to 15 days after release as pink bollworm larval period is upto 21 days (Malthankar and Gujar, 2016) and 3-4 days old larvae would complete its larval period after 15 days of treatment. In case of American bollworm, the larval period is up to 12-15 days (Nasreen and Mustafa, 2000). So, in diet bioassay of *H. armigera* the data was recorded up to 8 days after treatment because 3-4 days old larvae will complete its larval period within 8 days of treatment. The benefit of the larval period-based bioassay duration is that it gives the idea about the delayed development. If larva completes its larval stage and turns to pupa within this period it would reflect that treatment had no effect on larval development period. The experiment was conducted in controlled condition similar to culture maintenance. Completely Randomized Design (CRD) was used to compute the variance (Snedecor and

Table 1: List of shrubs used in preparation of Dashparni

Sl No.	Name	Scientific name	Family
1.	Custard apple	*Annona squamosa* Linnaeus	Annonaceae
2.	Papaya	*Carica papaya* Linnaeus	Caricaceae
3.	Karanj	*Pongamia pinnata* (Linnaeus)	Fabaceae
4.	Nirgundi	*Vitex negundo* Linnaeus	Lamiaceae
5.	Kaner	*Nerium indicum* Mill	Apocynaceae
6.	Gudbel (Giloy)	*Tinospora cordifolia* (W) Mier. Ex Hook.	Menispermaceae
7.	Castor	*Ricinus communis* Linnaeus	Euphorbiaceae
8.	Calotropis	*Calotropis procera* (Ait.) Ait.	Asclepiadaceae
9.	Ghaneri (lantana)	*Lantana camara* Linnaeus	Verbenaceae
10.	Neem	*Azadirachta indica* A. Juss	Meliaceae

Cochran, 1968). Means were calculated to represent the data and were compared by Tukey's honest significant difference test at 5% level of Significance. All analyses were conducted using the SPSS software (SPSS, 2007). LC50 value was also calculated by using polo plus software, 2013.

RESULTS AND DISCUSSION

The per cent mortality data of both the bollworm are discussed separately. Every 24 hrs. (day wise) detailed data on % mortality of larvae of *H. armigera* is presented in Table 2. It was observed that increase in concentration of Dashparni extract caused significant effect on % mortality of larvae except during initial days of bioassay. Day wise data within the same concentration revealed that as duration of diet exposure increased mortality was also increased. In the diet bioassay of *H. armigera* after 8 days of bioassay maximum mortality of 45.83% were observed at concentration of 10% which was the highest concentration. The mortality at 10% concentration is significant with rest of the concentrations. Concentration 1% and 2% were at par with each other and caused 25 per cent mortality after 8 days of treatment. Per cent mortality was ranged from 25 to 45.83 from lowest concentration to highest concentration. The per cent mortality in control was 4.17%. In case of *H. armigera* the maximum mortality of the highest concentration was less than 50% hence analysis in polo plus for LC_{50} value was not valid for the present experimental setup.

In case of pink bollworm days wise data on % mortality is presented in Table 3 which showed that increase concentration had positive effect on % mortality. Similar to previous pattern, within the concentration the % mortality had increased as the exposure of diet in terms of days was prolonged. In diet bioassay of *P. gossypiella*, the maximum mortality of 91.67% was observed at concentration of 10% and 8% after the 14 days of treatment. The performance of both the concentration were significant with rest of the concentration in the experiment. Per cent mortality was ranged from 58.33 to 91.67 from

Table 1: Larval mortality of *Helicoverpa armigera* in diet bioassay with various concentration of Dashparni extract

Treatments	Days after treatment							
	1	2	3	4	5	6	7	8
1%	0.00^{a}	0.00^{a}	0.00^{a}	4.17^{b}	12.50^{b}	12.50^{b}	20.83^{b}	25.00^{b}
2%	0.00^{a}	0.00^{a}	4.17^{b}	8.33^{c}	16.67^{c}	20.83^{c}	25.00^{c}	25.00^{b}
4%	4.17^{b}	4.17^{b}	4.17^{b}	12.50^{d}	16.67^{c}	20.83^{c}	25.00^{c}	29.17^{c}
6%	4.17^{b}	4.17^{b}	7.33^{c}	12.50^{d}	16.67^{c}	20.83^{c}	33.33^{d}	33.33^{d}
8%	4.17^{b}	4.17^{b}	12.50^{d}	25.00^{e}	29.17^{d}	33.33^{d}	33.33^{d}	41.67^{e}
10%	4.17^{b}	8.33^{c}	20.83^{e}	37.50^{f}	41.67^{e}	41.67^{e}	41.67^{e}	45.83^{f}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	4.17^{a}	4.17^{a}	4.17^{a}	4.17^{a}
SeM	0.44	0.44	0.49	0.53	0.58	0.58	0.58	0.58
CV (%)	31.7	25.3	12.0	6.4	5.0	4.5	3.8	3.4
CD (p=0.05)	1.32	1.32	1.48	1.62	1.75	1.75	1.75	1.75

(Note: value followed by the same letters not significant different at p= 0.05 after Tukey's HSD test)

Table 2: Larval mortality of *Pectinophora gossypiella* in diet bioassay with various concentration of Dashparni extract

Treatments	Days after treatment														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1%	8.33^{b}	8.33^{b}	8.33^{b}	8.33^{b}	20.83^{b}	20.83^{b}	25.00^{b}	29.17^{b}	29.17^{b}	29.17^{b}	29.17^{b}	37.50^{b}	54.17^{b}	58.33^{b}	58.33^{b}
2%	12.50^{c}	12.50^{c}	12.50^{c}	12.50^{c}	25.00^{c}	29.17^{c}	29.17^{c}	29.17^{b}	33.33^{c}	33.33^{c}	50.00^{c}	50.00^{c}	54.17^{b}	58.33^{b}	62.50^{c}
4%	20.83^{d}	20.83^{d}	20.83^{d}	20.83^{d}	29.17^{d}	33.33^{d}	33.33^{d}	33.33^{c}	33.33^{c}	62.50^{d}	62.50^{d}	62.50^{d}	66.67^{c}	66.67^{c}	79.17^{d}
6%	20.83^{d}	25.00^{e}	20.83^{d}	20.83^{d}	33.33^{e}	37.50^{e}	37.50^{e}	37.50^{d}	41.67^{d}	62.50^{d}	62.50^{d}	70.83^{e}	79.17^{d}	83.33^{d}	83.33^{e}
8%	20.83^{d}	33.33^{f}	33.33^{e}	37.50^{e}	45.83^{f}	50.00^{f}	50.00^{f}	54.17^{e}	66.67^{e}	70.83^{e}	75.00^{e}	79.17^{f}	83.33^{e}	91.67^{e}	91.67^{f}
10%	33.33^{e}	33.33^{f}	33.33^{e}	37.50^{e}	45.83^{f}	58.33^{g}	58.33^{g}	58.33^{f}	66.67^{e}	75.00^{f}	79.17^{f}	79.17^{f}	87.50^{f}	91.67^{e}	91.67^{f}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	4.17^{a}	4.17^{a}	4.17^{a}	4.17^{a}	4.17^{a}	8.33^{a}
SeM	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.58	0.58	0.58	0.58	0.58	0.58
CV (%)	5.5	4.8	5.0	4.7	3.2	2.8	2.7	2.6	2.1	2.1	2.1	2.1	2.1	2.1	2.1
CD (p=0.05)	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.62	1.75	1.75	1.75	1.75	1.75	1.75

(Note: value followed by the same letters not significant different at p= 0.05 after Tukey's HSD test)

lowest concentration to highest concentration. Per cent mortality of 8.33% was observed in control treatment after 14 days of treatment. The LC_{50} value of Dashparni extract was calculated as 1.018 (conc 6, df 4, fiducial limit 0.60 to 1.42) at 95% confidence for pink bollworm.

It was observed in both the insect's bioassay that per cent mortality was increased with the increase in concentration and with the duration of the feeding time period. After the completion of the bioassay both the bollworms were in larval stage which confirmed the effect the Dashparni extract on growth and development of larvae.

Hence, the above results confirmed the efficacy of Dashparni extract on bollworm complex of cotton. All the plant and animal origin product utilized in present Dashparni preparation have pesticidal properties may be due to deterrence and antifeedant activity (Kasarkar *et al*., 2021). The present findings were supported by Raskar and Wani (2014) who claimed that Dashparni extract was an excellent organic bioagent against the insect pests of paddy in field condition. A study done in by Kumbhar *et al*. (2018) supported the present results as they found the efficacy of Dashparni against gram pod borer, *H. armigera*; bean aphid, *Aphis craccivora*; aphid, *Rhopalosiphum maidis*; thrips, *Megalurothrips sjostedti*; white fly, *Bemisia tabaci*; shoot fly, *Atherigona soccata*; stem borer, *Chilo partellus*; ear head bug, *Calocoris angustatus* on green gram and sorghum. Similarly, Nikam *et al*. (2021) reported the efficacy of Dashparni against the insect pests of brinjal and also stated that extract was safer to the natural enemies. Efficacy of Dashparni was also supported by Gaikwad *et al*. (2020) against the aphid of okra *Aphis gossypii* where the performance of Dashparni extract was better than the microbial insecticides. Likewise, insecticidal effect of Dashparni was also corroborated by Sharma *et al*. (2014) against the mustard aphid under organically grown crops.

CONCLUSION

Dashparni extract has effects as toxicants against bollworm of cotton. Higher concentrations reduced the population of American bollworm up to 45% and pink bollworm population up to 91% which showed its efficacy as a larvicidal in laboratory condition. However, under natural field condition these pests are concealed inside plant tissue and hence they may show reduced effectiveness. After the 8 days and 15 days of treated diet exposure to American bollworm and pink bollworm respectively, alive individuals were still in larval stage which confirm the effect of Dashparni extract on growth and development of insects which needs to be tested. The results indicate the possibility of using Dashparni extract in field application as an option in ecofriendly IPM programs and further evaluation is in progress.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, ICAR-CICR Nagpur for providing the required facilities during this research work.

REFERENCES

Ajaykumara, K. M. and Tiwari R. (2019). Preliminary Bioassay Studies on the Efficacy of Animal Urine against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Chemical Science Review and Letters*, 8: 173-178.

Das, A., Dey, U., Baiswar, P., Pande, R., Ramkrushna GI, Layek, J., Suting, D., Babu, S., Yadav, G.S. and Prakash, N. (2018). Development of spray schedule involving commercial and indigenous biopesticides for insect pest and disease management in soybean crop. *Innovative Farming,* 3: 11-18.

Debnath, P., Pande, R., Patra, S., Layek, J., Ramkrushna, G.I., Bamon, R. N. and Majumdar, D. (2018). Evaluation of botanicals against mustard aphid, *Lipaphis erysimi* (Kaltenbach) in Mid Hills of Meghalaya. *Journal of Oilseeds Research,* 35: 283-288.

Dhurua, S. and Gujar, G. T. (2011). Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. *Pest Management Science*, 67: 898-903.

Farooq, M. A., Atta, B., Gogi, M. D., Arif, M. J. and Arain, Q. A. (2020). Compatibility of entomopathogenic fungi and *Azadirachta indica* extract against the cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) under controlled conditions. *Egyptian Journal of Biological Pest Control*, 30:63.

Gaikwad, B. B., Bhosle, B. B. and Bhede, B. V. (2020). Evaluation of different bio-pesticides against aphid on okra. *Journal of Entomology and Zoology Studies*, 8: 339-345.

Kasarkar, A. R., Kulkarni, D. K. and Salokhe, S. P. (2021). Phytochemicals investigated in Dashparni ark. International *Journal of Agriculture and Plant Science*, 3: 48-51.

Kumbhar, N., Gaikwad, P. and Wani A. (2018). Climate resilient agricultural technologies for green gram and Rabi Sorghum in parner block Ahmednagar

district of Maharashtra. WOTR centre for resilience studies, Pune ISBN 978-81-941116-0-3, 64 p.

Malthankar, P. A. and Gujar, G. T. (2016). Toxicity of *Bacillus thuringiensis Cry2Ab* and the inheritance of *Cry2Ab* resistance in the Pink bollworm, *Pectinophora gossypiella* (Saunders). *Indian Journal of Experimental Biology*, 54: 586–596.

Naik, V. C. B., Kumbhare, S., Kranthi, S., Satija U. and Kranthi K. R. (2018). Field evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (*Bt*) cotton expressing crystal 1Ac (*Cry1Ac*) and *Cry2Ab* in India. *Pest Management Science,* 74: 2544-2554.

Nasreen, A. and Mustafa G. (2000). Biology of *Helicoverpa armigera* (Hubner) Reared in Laboratory on Natural Diet. *Pakistan Journal of Biological Sciences*, 3: 1668-1669.

Nikam, J. P., Lande, G. K., Bisen, A., Naikwadi, B. V. and Bhure, K. (2021). Evaluation of botanicals and synthetic insecticides against major sucking pests of brinjal and its impacts on natural enemies. *Journal of Entomology and Zoology Studies,* 9: 2099-2104.

Pande, R. Shah, V. and Verma, P. (2019). First report on identification of volatiles from egg and larval frass of Indian strain of the American bollworm *Helicoverpa armigera* (Hübner). *African Entomology,* 27: 403–409.

Pande, R., Firake D. M. and Karnatak, A. K. (2010). Various activities of Neem seed kernel extract (Methanolic and Water) at different concentration against Bihar hairy caterpillar, *Spilarctia obliqua* (Walker). *Journal of Ecofriendly Agriculture,* 5: 73-77.

Prasad, N. V. V. S. D., Mallikarajuna, R. and Hariprasad Rao, N. (2009). Performance of *Bt* cotton and non-*Bt* cotton hybrids against pest complex under unprotected condition. *Journal of Biopesticides*, 2:107–110.

Ranjith, M.T., Prabhuraj, A. and Srinivasa, Y. B. (2010). Survival and reproduction of natural populations of *Helicoverpa armigera* on *Bt*-cotton hybrids in Raichur, India. *Current Science,* 99: 11.

Raskar, S. S. and Wani, A. G. (2014). Promotion of organic farming in tribal farmers of Akole with relation to climate change. *International Journal of Current Research,* 6: 4697-4701

Shah, V., Pande, R., Verma, P., Gokte-Narkhedkar N. and Waghmare V. N. (2020) Identification of oviposition deterrents from pink bollworm, *Pectinophora gossypiella* (Saunders). *Journal of Environmental Biology,* 41644-649

Shalaby, A. and Dhafar, Al. (2019). Some Natural Plant Extracts Having Biocide Activities against the American Bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Advances in Entomology,* 7: 10-20.

Shalaby, M. A. M., Adly, A. M. and Ahmed, A. F. (2019). Cow's Urine-Dung Extract Foliar Spraying as a Complementary Pest Control Method against BollWorm Complex on the Cotton Plant. *International Journal of Science and Research,* 8 (7): 212-219.

Sharma, S. K., Punam, Saini, J. P. and Chadha, S. (2014). Management of aphid pests by using organic inputs in organically grown crops. *International Journal of Agricultural Sciences and Veterinary Medicine*, 2: 116-121.

Snedecor, G. N. and Cochran, W. G. (1968). Statistical Methods, 6th edn, Oxford & IBH Publishing Co. New Delhi.

SPSS. (2007). Inc. SPSS for windows, Released version 16.0. Chicago, SPSS Inc.

Received: July 30, 2022
Accepted: August 17, 2022