Influence of botanicals in total head protein of *Spodoptera litura* (Fab.)

F. Brisca Renuga[1] and K. Sahayaraj*

ABSTRACT

Both *Ageratum conyzoides* and *Ageratum vulgaris* have been used to manage pest throughout the world. *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is one of the pests of field crops and has been developed resistance against most of the modern classes of synthetic pesticides worldwide. *S. litura* third and fifth instar larvae treated topically with 0.1µg of A. *conyzoides* and *A. vulgaris* were found to be significantly reduce total head protein after 24, 48 and 72 hours of treatment application over control. Similar trend was also observed when these plant extracts injected at 0.01 µl/ insect. Furthermore both *A. conyzoides* and *A. vulgaris* have similar impact on *S. litura* third and fourth instar larvae. These two plants can be used as botanical insecticide in pest management programme.

Keywords : Botanicals, head protein, *Spodoptera litura*

INTRODUCTION

India is endowed with rich tropical flora, which is considered as store house of plant protection chemicals. Even before the advent of the synthetic insecticides in plant protection scene botanicals were in use. There is a renewed interest in botanicals now because of the adverse effects of synthetic insecticides. Till, recently only the plants with toxic chemicals were considered as suitable candidates for plant protection. The discovery of antifeedant, insect growth regulatory and other behavior modifying chemicals in plants against insect pest that fit into the concept of insect pest management assume importance. It has been estimated that only 5 – 15 per cent of the existing plant species meal, one and most of them were screened for a specific activity, did not cover the full spectrum (Balandrin *et al*., 1985).

The worldwide demand for natural insecticides is increasing. Among the plants found to contain insecticidal or growth regulatory effects of insects, plants from the genus *Ageratum* and *Artemisia* were reported to have insecticidal activity. *Ageratum conyzoides* L., is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from this species. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities (Adewole L. Okunade, 2003; Anjoo Kamboj and Ajay Kumar Saluja, 2008). It was already reported that *A. conyzoides* (Sharada Singh and Rao, 2000) possess hormone antagonist precocene I and II and *Ar. vulgaris* commonly known as 'sage bush' contain artemesin as an active principle (Sharada Singh and Rao, 1999). *Artemisia vulgaris* (Compositae) fresh or the dried plant repels insects, it can be used as a spray but caution is advised since it can also inhibit plant growth (Riotee, 1978). A weak tea made from the infused plant is a good all-purpose insecticide (Allardice, 1993). An essential oil from the plant kills insect larvae (Duke and Ayensu, 1985). Despite the increasing interest in botanicals, we still do not fully understand manifestations of botanicals -feeding on insects, besides its digestion in the insect midgut (Babu *et al.*, 1996). An examination of the effect of the phytochemical on insect metamorphosis with special reference to the nervous system is important. This study aims at determining the effect of *Ageratum conyzoides* and *Artemisia vulgaris* on head protein levels in *S.litura* was determined.

MATERIALS AND METHOD

Plants were collected from Western Ghats in and around Coonoor. *Ageratum conyzoides* plants were freshly collected and shade dried. After drying they were ground to powder in an electric grinder and whole plant powder was soxhlet extracted with ethanol as solvent for 24 hours.

Table 1. Topical application (0.1 µg) and injection (0.01 µl) of ethanol plant extracts of*A.conyzoides* and *A.vulgaris* on head protein content (mg/100 mg) of third instar and fifth instar larvae of *S.litura*

Treatments	Larval stage	Topical application			Injection Method
		24 hrs	48 hrs	72 hrs	
Control	III instar	6.23 ± 0.02	6.88± 0.8	6.11± 0.02	6.28± 0.3
A.conyzoides		5.01± 0.02(20)	5.42± 0.9(21)	5.74± 0.09(6)	5.08± 0.05(19)
A.vulgaris		5.22± 0.01(16)	5,32± 0.8(23)	5.41± 0.3(12)	5.25±0.3(16)
Control	V instar	25.23± 0.01	26.01± 0.9	26.11± 0.4	25.33±0.4
A.conyzoides		15.34± 0.02(39)	19.32± 0.9(26)	20.54±0.4(21)	15.44±0.7(39)
A.vulgaris		15.224± 0.02(40)	17.32± 0.8(33)	18.41± 0.4(30)	15.92±0.07(37)

Value in parentheses indicated reduction (in per cent)

The solvent was evaporated under pressure to collect the residue. *S. litura* was maintained at 25 ± 1° C and 65 ± 5 relative humidity and subsequently they were transformed to round plastic tough of 29 cm × 8 cm size. For each experiment 25 pre-starved third (23 mg) and fifth instar (55.0 mg) larva of *S.litura* were introduced in to the plastic troughs containing castor leaves and covered with muslin cloth. 0.1µg of ethanol extracts of. *A. conyzoides and A. vulgaris* were applied topically on the dorsal side of the thoracic region of the anesthetized third and fifth instar larvae. Each larva was treated only once. Larvae were allowed to feed on diet for 24 hrs, 48 hrs and 72 hrs of post treatment.

In another experiment, the plant extract was dissolved in 70 % ethanol and 0.01 µl of 1% extract was injected into the anesthetized third and fifth instar larvae at the base of the second abdominal proleg using 10 µl Hamilton Syringe (Hamilton Company, Reno Nevada) fitted with a 26S pointed needle. Each larva was injected only once. The larvae were fed on castor leaves for 24 hrs post treatment followed by head dissection. For each treatment 10 larval heads were hand homogenized in an extraction medium consisting of Tris HCL (pH 7.5) 1% Triton – x 100 and 107 DMSO using a pestle and mortar. The homogenate was centrifuged at 15,000 g for 15 minutes. At 4° C and supernatant was precipitated with equal volume of trichloro acetic acid (12%) or cold acetone (-20° C). Precipitated protein was collected by centrifugation and dissolved in 0.1 N Sodium hydroxide. Protein concentration in the solution was estimated using Lowry *et al.* (1951) method with BSA standard and per cent reduction was calculated.

RESULT

Head protein concentration of the control larvae increased with the advancement of age from an initial value of 6.23 ± 0.021 mg/100 mg at 24 hrs of third instar to 26.11 ± 0.353 mg/100 mg at 72 hrs of fifth instar. In the control group the concentration of head protein is steadily increasing during the active feeding stage of third instars larvae and it is dropped during the wandering stage (72 hrs). When comparing the third with fifth instars a steady increase in protein level at 24 hrs, 48 hrs and 72 hrs occurred and the protein concentration reached the maximum of 26.11 ± 0.353 mg/100 mg fresh weight of the larvae.

Protein concentration of *Ag.conyzoides and* Ar.vulgaris treated third instar and fifth instar larvae were significantly increased at the active and wandering stage. It is observed considerable reduction in mean value of protein level from 6.40 ± 0.338 mg/100 mg to 5.39 ± 0.298 mg/ 100 mg and 5.31 ± 0.077 mg/100 mg in the third instar and at fifth instar from 25.78 ± 0.398 to 18.4 ± 2.220 and 16.98 ±0.323 in *A. conyzoides* and *A. vulgaris* treated animals respectively (Table 1). Injected administration of plant extracts at the rate of 0.01 µl at third and fifth instar larvae showed further increase in the reduction in per cent of head protein level when compared to topical application.

DISCUSSION

The present study reveals that plant extracts of *Ag. conyzoides and Ar.vulgaris* influenced the total head protein profile of *S.litura*. The influence of azadirachtin on head protein of *Helicoverpa armigera* was reported by Neoliya *et al.* (2005, 2007). The protein content was higher during the active feeding stage and dropped at later stage of second larval instar suggesting epidermal programming. In the control group, high protein content was observed in the fifth larval instar when larval transformation was nearing the completion. In the treated larvae, the trend was similar but the protein content was very low when compared to the control groups. Maximum per cent of reduction is observed in the topical application of plant extracts on third instar larvae at 48 hrs after the

tratement and for the fifth instar the maximum reduction is observed at 24 hrs i.e 39% and 40% for *Ag. conyzoides and Ar.vulgaris* respectively (Table 1). Similar decrease in protein of *S. litura* after azadirachtin treatement and in the cuticle of plumbagin treated *H.armigera* (Josephraj Kumar and Subramanyam, 2000). On the contrary cyclic changes in concentration have been in *Schistocerca gregaria* (Rao and Subramanyam, 1986) and *Manduca saxata* (Levenhook,1985).

Though the protein content was highest at the last stages of each instar it was still lower in the treated when compared to the control groups. Epidermal reprogramming and synthesis of new cuticular proteins at metamorphosis moults are regulated by the moulting hormone (Anderson *et al.*, 1995). Infact the treatment drastically depleted the critical ecdysteroid level in azadirachtin and plumbagin treated also observed in *H.armigera leading* to defective metamorphic moult (Josephraj Kumar *et al.*, 1999). According to Saxena *et al.* (1992) *A. conyzoides* showed growth inhibitory and JH mimicking activity in the treated larvae of *Culex quinquefasciatus.*

Injection of extracts in the third and fifth instar larvae at the minimum dosage resulted in decreased protein level. The per cent of reduction is more in injection than in topical application of plant extracts i.e. 19% and 16% in third instar and 39% and 37% in fifth instar at 0.1 µg/g body weight concentration of plant extracts (Table 1). Maintenance of reduced level of protein in 24 hrs, 48 hrs and 72 hrs may imply the existence of extract in the neuroendocrine system up to 72 hrs in both ingestion and injection method of application. Similarly ingested azadirachtin reduce the protein in the head and other tissue of *Periodoma sausia* (Koul, 1993). Head protein of *S.litura* is derived from storage proteins in the insect body or is synthesized by neurosecretory cells. Observed changes in the head protein may arise through several possible pathological perturbations including modulation of protein synthesis or turn over. It is also assumed that this plant extracts altar the feeding in larvae towards food avoidance and maintain body metabolism at the expense of storage of cellular proteins.

A. conyzoides possesses hormone antagonist precocene I and II and *Ar.vulgaris* contain a substance sesquiterpenoid lactone named as 'vulgain' (Sharada Singh and Rao, 1999). Padmaja *et al.(*1999) reported the interference of oils of *Ag. conyzoides and Ar.vulgaris* in the protein synthesis of *H. armigera.* Sharada Singh and Rao (2000) also reported the growth inhibitory effect of these two plant extracts. Thus in general, it appears that the factors responsible for affecting growth and development are widely spread in the genus *Artemisia and Ageratum.* Ferrolino and Padolino (1985) found that the leaf extract of *Artemisia vulgaris* was highly toxic to *Tribolium castaneum* and *Sitophilus zeamis.*

REFERNCES

Adewole L. Okunade. 2002. *Ageratum conyzoides* L. (Asteraceae). *Fitoterapia,* **73** (1): 1-6.

Allardice.P. 1993. *A - Z of Companion Planting.* assell Publishers Ltd. ISBN 0-304-34324-2.

Anjoo Kamboj and Ajay Kumar Saluja. 2008. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy*, **2** (2): 59 – 68.

Anderson, S.O., Rafin, K. Krogh, T.N., Hojrup, P. and Roepstroff, P. 1995. Comparison of larval and pupal cuticular proteins in *Tenebrio moliter*. *Insect Biochemistry and Molecular Biology,* **25**: 177-187.

Ayyangar,G.S.G. and Rao, P.J. 1990. *Indian Journal of Entomolology.* **52**: 69-83.

Balandrin, M.F., Klocke, J.A., Wurtle, L.S., Bollinger, W.M. H. 1985. Natural Plant chemicals: Source of industrial and medicinal material science, 228 -1154.

Babu, R., Murugan, K and Vanithakumari, G. 1996. Interference of azadirachtin on the food utilization efficiency and midgut enzymatic profiles of *Helicoverpa armigera*. *Indian Journal of Environment and Toxicology,* **6**: 81-84.

Duke, J. A. and Ayensu, E. S. 1985. *Medicinal Plants of China* Reference Publications, Inc. ISBN 0-917256-20-4.

Ferrolino-Calumpang, S.M. and Padolino, W.G. 1985. Insecticidal activity screening and isolation of the major crystalline fraction of *Artemisia vulgaris* L. *Philippine Agriculturist,* **68**(2): 249-261.

Josephraj Kumar, A., Subramanyam, B. and Srinivasan. 1999. Plumbagin and azadirachtin deplete haemolymph ecdysteroid levels and alter the activity profile of two lysosomal enzymes in the fat body of *Helicoverpa arimgera* (Hubner). *European Journal of Entomology,* **96**: 347-353.

Josephraj Kumar. A. and Subramanyam B. 2000. Effect of plumbagin and azadirachtin on cuticular proteins of Helicoverpa *arimgera* (Hubner). *Entomon.* **25**(3): 185-192.

Koul, O. 1993. World Neem Conference, India, 307-313 **PP.**.

Levenhook, L.1985. In: *Comprehensive Insect physiology, Biochemistry and Pharmacology* (Kerkut, G.A. and Gibert L.I eds.), Pergamon Press, New York. 1985. 307-346 **PP.**.

Lowry, O.H., Rosen Brough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin- phenol reagent. *Journal of Biological Chemistry*, **193**:265-275.

Neoliya, N.K., Dwijendra Singh and Sangawan. R. S. 2005 Azadirachtin influences total head protein content of *Helicoverpa armigera* Hub. larvae. *Current Science*, **88** (12): 1889-1890.

Neoliya, N.K., Dwijendra Singh and Sangawan, R. S. 2007. Azadirachtin-based insecticides induce alternation in *Helicoverpa armigera* Hub. head polypeptides. *Current Science*, **92** (1): 94 - 99.

Padmaja, P.G. and Rao, P.J. 1999. Effect of plant oils on the fat body protein of *Helicoverpa armigera*. *India Journal of Entomology*, **61**(4): 330 -336.

Rao, P. J. and Subrahmanyam, B. Z. 1986. *Angew Entomol*, **102**: 217 - 224.

Otte. L. 1978. *Companion Planting for Successful Gardening*. Garden Way, Vermont, USA. ISBN 0-88266-064-0.

Sharada Singh and Rao, P. J. 1999. Effect of *Artemesia vulgaris* on development and reproduction of *Spodoptera litura* and *Schistocerca gregaria. Indian Journal of Entomology*, **61**(4): 305 – 311.

Sharada Singh and Rao,P.J. 2000. Effect of Ageratum conyzoides on development and reproduction of *Spodopera litura*. *Entomology*, **102**: 217-224.

Saxena, R.C., Dixit, O.P. and Sukumaran, P. 1992. Laboratory assessment of indigenous plant extracts for antijuvenile hormone activity in *Culex quinque fasciatus. Indian Journal Medical Research*, **95**: 204-206.

F. Brisca Renuga[1] and K. Sahayaraj*

Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College, Palayamkottai – 627 002, Tamil Nadu, India.

[1]Department of Advanced Zoology and Biotechnology, Holy Cross College, Nagercoil, Tamil Nadu, India.

*Communication author Email: ksraj42@gmail.com