Original Research Article

Bioefficacy Potential Of *Andrographis paniculata* Burmf. Against *Agrotis spinifera* Hubn. Larvae (Lepidoptera: Noctuidae)

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Received: August 2, 2015; revised: October 27, 2015; accepted: November 6, 2015

Abstract: *Agrotis spinifera* is a challenging insect pest belonging to the family Noctuidae, the larvae of which cause severe damage to cabbage, cauliflower and potato plants. Crude petroleum ether extract of the leaf and tender shoot of *Andrographis paniculata*, (Acanthaceae) a common medicinal plant of North-East India with bitter principles was applied using both topical application and food leaf soaking/dip methods against the larvae of this common cut worm, *Agrotis spinifera*. The extract not only found to have its effect on growth and metamorphic processes but also have fatal effect on the larval stages of this polyphagous pest species. On topical application in lower dose (30mg/g of larval weight), the crude extract of *A. paniculata* appeared to have no effect on normal moulting process while in moderate dose (35mg, 40mg and 45mg/g of larval weight) the extract caused delay in moulting to successive developmental stages and also showed some pupae with deformities. In higher dose ($\geq$50mg/g of larval weight) a number of the treated larvae died without moulting. When the crude extract was applied to soak the food leaves and fed the larvae, in low dose the larvae showed delayed ecdysis, but the higher dose led the larvae to avoid food. The present study showed that petroleum ether extract of leaf and tender shoot of *A. paniculata* may be highlighted as an ecofriendly phytoproduct in botanical control strategy of *A. spinifera*..

Keywords: *Andrographis paniculata*, Polyphagous, Ecdysis, Food leaf rejection, Botanical control

Introduction

Agronomists and chemists have been giving importance and priority in utilizing the plant derived products for controlling insect pests and pathogens of agricultural and horticultural fields as the synthetic chemical insecticides and pesticides are not only hazardous to the consumer animals but also pose as serious pollutants of air, water and soil. It has also been observed that many of the insect pest species develop resistance to the synthetic pesticides (Ramasubramaniam and Regupathy, 2004). Hence, it is the most urgent matter of investigation to find out the alternatives which are biologically rational and ecofriendly (Sahayaraj and Paulraj, 2001; Opender *et al*., 2002; Subhasini *et al*., 2004; Balasubramaniam *et al.,* 2008).

A. spinifera, the common cut worm has its cosmopolitan distribution that widely occurs in all states of India including Assam. Larvae of this Lepidopteran insect are polyphagous which damage not only the rabi/winter crops in Assam but also pose as a major pest of cabbage, potato and cauliflower (Chandel *et al.,* 2008). The nocturnal larvae are found in the upper layer of soil and come out during night cutting the plant shoot at the base.

A. paniculata is an erect and branched shrub locally known as 'Kalmegh' or 'Chirata' (Fig. 1A). This annual plant with racemose inflorescence has its distribution throughout India, mainly in the plain areas, and also in other Asian countries. Every part of this plant is extremely bitter in taste for which it is also known as 'king of bitter'. Crude extract of

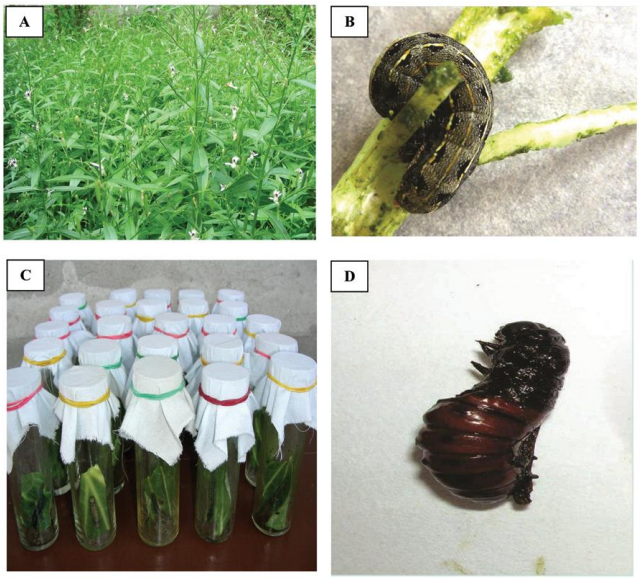

Fig.1. Photographs showing (**A**) The plant *Andrographis paniculata* (**B**) A 6th instar larva of *Agrotis spinifera* (**C**) *A spinifera* cultured in the laboratory (**D**) A deformed pupa from *A paniculata* extract treated larva of *A spinifera*

this plant is known to be used by a number of farmers against Lepidopteran pests and are found effective against some agricultural pests in laboratory conditions (Bora *et al.,* 2012). Ramya *et al.,* (2011) reported larvicidal activity of *A. paniculata* while Jagajothi and Martin (2010) found the effect of Andrographolid, a terpenoid in *A. paniculata* on pupal and adult transformation of *Corcyra cephalonica*, a Lepidopteran pest. Considering the importance of the plant and the pest, the present investigation was designed to study the effect of the leaf and tender shoot extract of *A. paniculata* on mortality, ecdysal behavior and food leaf acceptance behavior of *A. spinifera* larvae.

Materials and methods

Culture of insects

Mature larvae and adult females of *A. spinifera* were collected from the natural population in potato and cabbage fields at Pathsala and Guwahati area of Assam. Insects were reared in laboratory conditions with room temperature at 19°C ±2°C, relative humidity of 75% ±5% and in laboratory light conditions adopting the modified technique originally described by Uspenskaya and Kozhaeva (1974). Upto the 3rd instar stage the larvae were reared in groups while the 4th, 5th and 6th instar larvae (Fig. 1B) were reared individually in small insect rearing glass tubes (100mm x 25 mm) and were fed with fresh cabbage leaves grown locally on organic fertilizers. Glass tubes were plugged with pieces of porous cotton cloth to provide proper ventilation and to avoid escape of the larvae (Fig. 1C).

Test plant collection and extract preparation

For the present study *A. paniculata* was collected from Basistha area of Guwahati in Kamrup district, Assam. The test plant *A. paniculata* was identified taking help of the taxonomic literature and was authenticated by a plant taxonomist from the Department of Botany, Gauhati University.

Leaves and tender stems of *A. paniculata* plants were washed with clean water and shed dried for one week. The dried parts of the plant were then powdered using an electric grinder and stored in an airtight container in room temperature for further use. The powdered content is then extracted with petroleum ether (40°C-50°C) in a Soxhlet apparatus (4-5 runs). The crude extract was then dried in a rotary vacuum evaporator. Dry extract is obtained as black coloured flakes which were stored in glass container. The dry extract was then dissolved in acetone (10% W/V) to prepare the stock solution. The stock solution was further diluted with acetone to 7 different concentrations. These were 30mg, 35mg, 40mg, 45mg, 50mg, 55mg and 60mg/g of larval body weight (ranged from 137mg to 162mg).

Treatment of insects

The 4th, 5th and 6th instar larvae of *A. spinifera* are voracious feeders that are known to cause maximum damage to the crop plants. Therefore these three instars were selected for treatment with *A. paniculata* extract. During treatment the larvae of each of the above mentioned instars were classified into groups of which one group was for controlled/acetone treated while the remaining groups were used for treatment with different doses of *A. paniculata* extract. For each group three replicates of 10 larvae were taken.

Treatment was done in two ways – topical application and by soaking the food leaf with *A. paniculata* extract. In

topical application required amount of leaf extract was taken in a micro syringe and was spread over the larval body. In soaking the food leaf with plant extract fresh cabbage leaves were cut into small circular size of 4cm diameter, washed thoroughly with clean water and allowed to dry in air for half an hour. Selected doses of extract were spread uniformly over the leaf surface and then shade dried before leaf pieces were served to the overnight starved larvae.

After treatment the duration of moulting, and pupation were recorded. Food leaf rejection by the larvae was recorded after 24 hours of observation. The larvae that avoid feeding of soaked leaves for 24 hours but consumed fresh food leaves subsequently when supplied with, were considered to reject the treated leaves. Visual observation on larval mortality was made within 0.5 hour and 24 hours of post treatment period.

Results

The average weight of each of the 4th, 5th and 6th instar larvae of *A. spinifera* which were taken for treatment of plant extracts were 137mg, 150mg and 162mg, respectively. All the selected doses of extract were applied to these instars both topically as well as by soaking the food leaves. While the controlled/ acetone treated larvae neither showed any abnormality in development nor mortality, the larvae treated with *A. paniculata* extract found to have some significant effects on moulting and pupation, and even the extract exhibited lethal effect in high doses.

Effect of topical application

Results of the topical application of *A. paniculata* extract dissolved in acetone showed significant changes in the larval development, ecdysis and mortality of *A. spinifera.* At the concentration of 30mg/ g larval weight, the larvae of all the three selected instars found to have effect neither on moulting nor on mortality. At 35mg/g concentration 9.0% and 5.0% of the early instars i.e. 4th and 5th instar larvae, respectively failed to maintain normal duration of larval period exhibiting delayed ecdysis and metamorphosed to deformed pupae (Table 1). In this concentration mortality of 4th and 5th instar larvae within 24 hours was found as 4.0% and 11.7%, respectively. However, the 6th instar larvae were not affected in this dose. Normal moulting was not recorded in 4th and 5th instar larvae with increase in concentration of extract above 45mg/g body weight. While 17.9% of the last instar larvae in the concentration of 50mg/g and only 3.7% of larvae in 55mg/g concentration succeeded to show normal pupation, few were pupated with deformities. Such pupae exhibited incomplete metamorphosis in abdominal region (Fig. 1D). With increasing concentration of extract, the larval mortality was found increasing within 24 hours of treatment. However, the 4th instar larvae treated with plant extract ≥ 50mg/g body weight concentration exhibited 100% mortality. The concentration of extract in 55mg/g body weight found to be lethal to 96.6% and 86.0% of 5th and 6th instar larvae, respectively. Similarly, 100% of 5th instar and 90% of 6th instar *A. spinifera* larvae in 60mg/g concentration died within 0.5 hour during topical treatment.

Table 1. Effects of *A paniculata* crude extract (applied topically) on moulting/ pupation and mortality of *A spinifera* larvae.

Groups	Normal larval moulting/pupation (%±SE)			Deformed pupation (%±SE)			Larvae which died within 24 hours of treatment (%±SE)			Larvae which died within 0.5 hour of treatment (%±SE)		
Larval Instars	4th	5th	6th	4th	5th	6th	4th	5th	6th	4th	5th	6th
A) Control/ Acetone treated B) Extract dose (mg/g BW)	100±00	100±00	100±00	0	0	0	0	0	0	0	0	0
30 mg	100±00	100±00	100±00	0	0	0	0	0	0	0	0	0
35 mg	87.0±1.15	83.3±0.81	100±00	9.0±1.15	5.0±0.52	0	4.0±0.87	11.7±1.10	0	0	0	0
40 mg	20.0±1.15	76.7±0.69	100±00	9.6±0.35	1.6±0.23	0	28.2±0.12	21.7±0.75	0	42.2±1.04	0	0
45 mg	3.0±0.87	5.1±0.03	58.5±0.58	1.0±00	3.3±0.40	0	16.0±0.58	30.6±0.35	3.9±0.06	80.0±00	61.0±0.29	37.6±1.50
50 mg	0	0	17.9±1.67	0	0	2.2±0.12	0	8.3±0.40	7.7±0.12	100±00	75.0±0.06	72.2±0.40
55 mg	0	0	3.7±0.12	0	0	1.0±00	0	3.4±0.23	9.3±0.17	0	96.6±0.92	86.0±0.58
60 mg	0	0	0	0	0	0	0	0	10.0±0.58	0	100±00	90.0±2.89

Table 2. Effects of *A paniculata* crude extract on moulting/ pupation and feeding behaviour of *A spinifera* larvae after treatment with soaked food leaves.

Groups	Normal larval moulting (%±SE)			Larvae which showed delayed moulting/pupation (%±SE)			Larvae which rejected treated food leaves (%±SE)			Larvae which died without moulting/ pupation (%±SE)		
Larval Instars	4th	5th	6th	4th	5th	6th	4th	5th	6th	4th	5th	6th
A) Control/ Acetone treated B) Extract dose (mg/gof larval BW)	100±00	100±00	100±00	0	0	0	0	0	0	0	0	0
30 mg	100±00	100±00	100±00	0	0	0	0	0	0	0	0	0
35mg	98.0±0.29	100±00	100±00	2.0±0.43	0	0	0	0	0	0	0	0
40 mg	70.2±0.12	83.3±0.98	100±00	14.0±0.40	6.6±0.29	0	15.8±0.46	10.1±0.12	0	0	0	0
45 mg	30.1±0.06	41.6±0.94	73.2±0.64	15.3±0.23	10.6±0.23	9.8±0.12	54.6±0.23	47.8±0.23	17.0±0.29	0	0	0
50 mg	28.1±0.23	28.3±0.75	25.6±0.35	0	19.0±0.29	12.4±1.50	61.0±0.29	75.6±2.94	62.0±1.04	0	0	0
55 mg	13.4±0.52	10.0±0.58	10.5±0.87	0	5.0±1.44	22.0±1.15	86.6±0.46	85.0±1.15	67.5±1.15	0	0	0
60 mg	0	0	0	0	0	8.2±0.07	100±00	100±00	91.8±0.46	0	0	0

Effect of soaking food leaves

When the 4th, 5th and 6th instar larvae were fed with food leaves soaked with *A. paniculata* extract dissolved in acetone, the extract showed effect on moulting and pupation process as well as food taking behaviour of the larvae. It was observed that the larvae moulted normally after consuming *A .paniculata* extract with food in the concentration of 30mg and 35mg/g of body weight. But above that concentration a fraction of the treated larvae showed delayed moulting and pupation by 2-3 days while the remaining larvae rejected food. Number of larvae showing delayed moulting and rejecting food gradually increased together with increase in dose concentration (Table 2). At the dose of 60mg/g body weight no larvae of 4th and 5th instar stages took the soaked food leaves. However, no lethal effect on the *A. spinifera* larvae was seen during soaked leaf feeding treatment for all the three instars as they either rejected the food leaves or underwent ecdysis in delayed manner.

Discussion

Results of the present investigation showed that the crude extract of *A. paniculata* not only affect the normal moulting and pupation of *A. spinifera* larvae but also can act as a lethal phytoproduct to this pest insect when applied in high concentration ($\geq$ 50mg/g of body weight). Such results support (Koul 1985) the findings of other workers like Ruscoe (1972). Ahmed (2007), Bobbarala *et al.*, (2009) and Ramya *et al.*, (2011). Similarly, the result on rejection of food leaves soaked with *A. paniculata* extract was in conformity with the works of Jermy (1990), Simmonds *et al.*, (1990), Dodia *et al.,* (1998), Janardhan *et al.*, (1999) and Choudhury (2009) who found anti-feedant activity of different plant extracts to certain pest insects. Delayed larval moulting and inability of a number of larvae for complete pupation may be indicative of the hormone-like effect of *A paniculata* extract on this pest insect species as reported by Slama and Williams (1965) that certain plant extract which contain JH analog can cause abnormality in moulting and metamorphosis in insects. Jagajothi and Martin (2010) attributed JH like activity of andrographolide, an active compound of *A. paniculata* on *Corcyra cephalonica*. Appearance of deformed pupae after plant extract treatment was also obtained by Gujar and Mehrotra (1983) and Koul *et al.,* (1987). Supporting all these explanations it could be assumed that the *A. paniculata* leaf extract may has its JH analog like activity, especially in moderate dose. Possibly the crude extract of this plant somehow influence the activity of the endocrine system of the larvae in producing respective hormones for growth and metamorphosis of the pest larvae.

Results showing no larval death during treatment with soaked food leaves at any one of the given dose has its important significance. Because food rejection by the pest larvae in a very high percentage can not only be beneficial to the crop population but also be significant in keeping the pest population under control, which may be an important aspect of IPM. Death of larvae either shortly or gradually after topical

application of plant extract is clearly suggestive of the pesticidal efficiency of *A. paniculata* leaf extract. This mode of treatment can be more efficient and advantageous over the other mode of treatment in bringing the sudden outbreak of this pest larval population under control.

It has been reported by Jermy (1990), Ahmed (2007) and Ramya *et al*., (2008, 2011) that the plant extracts with both insecticidal and behavioral effects have successful practical application, the *A. paniculata* leaf and tender shoot extract may be projected as an efficient phytochemical for both pesticidal as well as anti-feedent activities. However, further investigation will be necessary to find out the bioactive compound and its action at molecular level.

Acknowledgements

This study was supported by the University Grants Commission, New Delhi. I gratefully acknowledge the UGC for its financial assistance. I also thank the Department of Zoology, B.Borooah College for providing laboratory facilities till completion of the study.

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