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Biological effect of sweet wormwood, *Artemisia annua* methanol extracts and essential oil against *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae)

Neelima Anshul, Alok Kalra and Dwijendra Singh

Abstract

Methanolic extract of different parts of *Artemisia annua*, (Leaf, shoot, and seed) and essential oil were evaluated for their effect on growth and development of larvae of *Helicoverpa armigera*. The experiments were done by the incorporation of different extracts and essential oil in semi-synthetic diet at two percent concentration. Among leaf, stem, seed extract and essential oil treatments the larval weight was reduced by diet containing essential oil (69.71%) and leaf extract of *A. annua* (60.21%) as compared to control. The prolongation of larval and pupal period and an average more than fifty percent IGR activity was recorded by the treatments of *A. annua* extract and essential oil. The growth retardation, including reduced weight and prolongation of larvae may possibly interrupt the production of insect hormone-ecdysone level in insect body which also delayed the pupation. Physiological effects found in treated larvae included formation of larva pupa intermediate and adultoids.

Keywords: *Artemisia annua*, Bio-pesticide, Essential oil, Growth inhibition, *Helicoverpa armigera*, Methanol leaf extract.

1. Introduction

Pod borer, *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae) is one of the cosmopolitan and injurious pests of various agricultural crops namely food, fibre, ornamental, medicinal and aromatic plant species. It is commonly called as pod borer, has a wide host range of 300 plant species throughout the world^[1]. Initially larvae feed on tender leaves and thereafter, bore into the pod and feed therein. The total loss of agricultural production due to this pest alone is estimated for 29.2% in chickpea in India^[2]. Total reliance on the application of synthetic insecticides to control *H. armigera* has not achieved the desired success, and induced resistance to several groups of chemicals has been one effect^[3]. Among various plant extracts/Phyto-molecules evaluated in past, neem seed kernel (NSKE) extracts containing azadirachtin obtained from Indian neem, *Azadirachta indica* have been commercialized world over as plant bio-pesticide for controlling various agricultural pests^[4]. However, commercial NSKE products have shown low efficacy, short supply of raw materials, plantation block the land for several years and synthesis of active molecule – azadirachtin have been found uneconomical for industrialization. Thus, attempts are within the broad ambit of integrated pest management, entomologists the world over are concentrating on the use of plant products, especially neem products, to tide over the menace of insect pests. Of which chemical control have been found easy in application, quick results oriented with low cost of application that have posed serious threat to man and environment through residue problem, development of resistance and harm to beneficial insects etc^[5]. Therefore, requirement of potential plant bio resource and novel insecticidal principles are of high demand, and need regular supply globally.

The genus *Artemisia* belongs to the large family of Asteraceae, encompassing more than 300 species. *Artemisia annua* L. (Asterales: Asteraceae), commonly known as sweet wormwood or annual wormwood, grows widely in Europe and America and is planted to a large extent in China, Turkey, Vietnam, Afghanistan, and Australia^[6]. In various insect pests of agriculture several studies relating to *A. annua* extracts have been reported, for the growth regulatory and toxicity and feeding deterrence activity^[7, 8, 9]. There has been no report on extract of various parts and essential oil of *A. annua* effects on *H. armigera*. In the present study we have tried to elucidate the effect of the different parts of *A. annua* extract and essential oil on growth and

development of most economically important key pest– pod borer, *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae).

2. Materials and Methods

The neonate larvae has been collected from the field of chick pea during the year 2011-12 and reared under laboratory conditions to maintain the culture and further used for biological studies.

2.1 Insect culture

The field collected larvae reared continuously since their collection on artificial semi-synthetic diet as method described by Singh and Rembold [10]. Vitamins were purchased from HIMEDIA separately and required quantities of ingredients were weighed through electronic balance and homogeneous paste were made by gradually adding water and mixing in ingredients of part-I, all weighed separately in 50 ml beaker in which the paste of vitamins was added. Water was boiled in a non-sticking Teflon coated pot or beaker and agar powder was gradually added, stirred and mixed so as to make a homogenous solution and then cooked for 2-3 minutes. It was then poured into the dough of part -I ingredients and mixed continuously with the hand stirrer, part -all ingredients were added and blended, again rearing containers were kept at room temperature until the diet settled. The diet was stored for drying and stored in the refrigerator. Freshly emerged adults were transferred into jar (25x20 cm) containing cotton swab dipped in 10% honey solution and covered with muslin cloth. Freshly hatched larva was transferred to Petri dishes containing artificial diet. After three days larva were transferred to multi rearing trays to avoid cannibalism.

2.2 Collection and processing of plant parts

Artemisia annua, herb was collected from the field of CSIR-Central Institute of Medicinal and Aromatic Plants Research Farm, Lucknow, stem and leaves was separated and chopped in pieces, shade dried and powdered. The Powdered plant parts were separately added to conical flask and sequentially extracted with methanol at room temperature 30 °C for 24-h and filtered with the help of What Man filter paper and again the marc was dissolved in respective solvent consecutively for three days. After repeating this process pooled filtrate was then filtered using filter paper and evaporated the solvent and condensed through rotary Rotavapor R-200 M/S BUCHI Labortechnik, Switzerland. The essential oil from *A. annua* was hydro distilled by Clevenger apparatus.

2.3 Bioassay

For bioassay studies, the third instar larvae of *H. armigera* reared on semi synthetic diet were fed containing 20,000 ppm concentration of *A. annua* methanol leaf, stem, seed extract and essential oil mixed into the semi synthetic diet after it had cooled. Treated diet was stored and refrigerated (6 °C). The experiment was carried out in multi-rearing trays group of ten larvae selected and individually placed in rectangular multi rearing tray. Untreated diet was also provided to individual larvae as control counterpart. The treated and untreated diet was replaced periodically as and when necessary until all larvae had completed the pupal moult.

2.4 Observations

After five days of treatment application larval weight were recorded in laboratory.

Data on larval mortality, pupal mortality, larval –pupal intermediate, and adultoids were recorded till the adult emergence of each treated and untreated (control) individuals for insect growth regulator activity.

2.5 Statistical analysis

The statistical analyses were performed by SPSS software (version 17.0) and data were expressed as means with standard deviation (\pm SD). The data were subjected to one way analysis of variance (ANOVA) and significant differences between means were determined by Tukey's multiple range test ($P < 0.05$).

3. Results

3.1 Effect on growth of *H. armigera*

In dietary utilization experiments, the results revealed that among all treatments, the methanol leaf extract showed significant reduction in larval weight subjected to oil treatment over untreated control (0.284 g) at two percent concentration ($P = 0.05$) (Fig 1a). A considerable reduction in mean larval weight was caused by methanol leaf extract 60.21% and essential oil 69.71% respectively at 2% concentration as compared to control (Table 1). Hence larvae exhibited a strong growth inhibition. Pupal weight also reduced significantly ($P = 0.05$) 25.76% with methanol leaf extract and 34.23% with essential oil as compared to (0.284 g) control (Fig 1b). Apparently also, the larval size was observed smaller as compared to control.

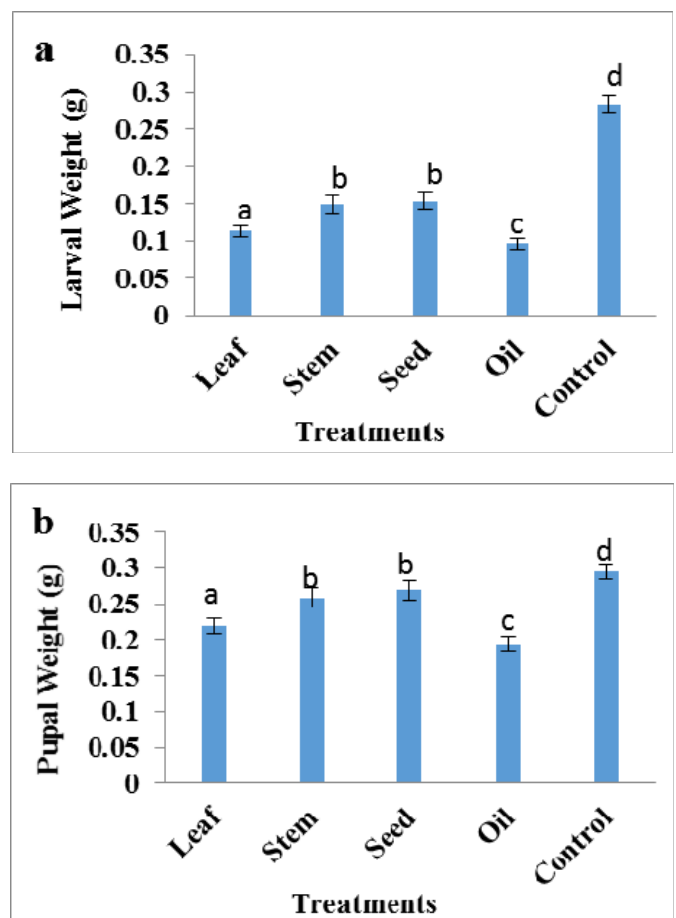


Fig 1: Effect of different parts and essential oil of *A. annua* on mean larval (a) and pupal (b) weight of *H. armigera*

*Means (\pm SE) followed by the same letters above bars indicate no significant difference at $P < 0.05$ (Tukey's test)

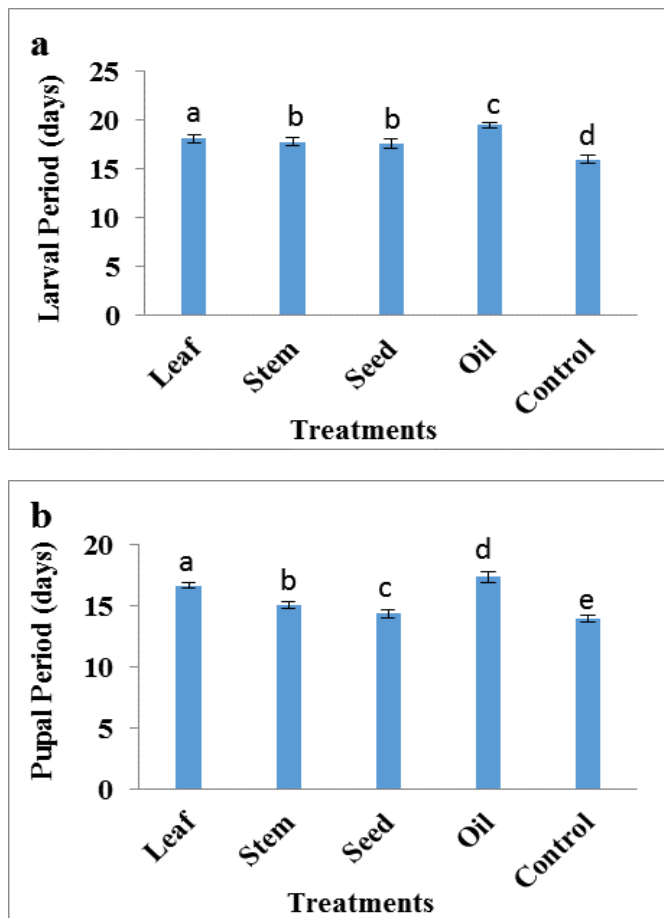
Table 1: Mean Antifeedant Index of different parts and essential oil of *A. annua* on *H. armigera*

Treatments	Mean Antifeedant Index (%) *
Methanol Leaf extract	60.21
Methanol Stem extract	47.18
Methanol Seed extract	45.77
Essential Oil	69.71

*Result observed after five days, Antifeedant Index (AI) = $(1 - T/C) \times 100$, using ten replications.

3.2 Effect larval and pupal development of *H. armigera*

The result showed that larval period prolonged up to 18.1 days and 19.5 days with treatment of methanol leaf extract and essential oil respectively, as compared to control ($P < 0.05$) (Fig 2a). Among treated different part of *A. annua*, the pupal period also increased significantly ($P < 0.05$) up to 16.7 days and 17.4 days by treatment of methanol leaf extract and essential oil respectively, from control 14 days (Fig 2b).

**Fig 2:** Effect of different parts and essential oil of *A. annua* on mean larval (a) and pupal (b) period of *H. armigera*

*Means (\pm SE) followed by the same letters above bars indicate no significant difference at $P < 0.05$ (Tukey's test)

3.3 Effect on adult development of *H. armigera*

Out of different part of *A. annua* extract and essential oil tested, essential oil of *A. annua* caused 60% growth regulator activity against third instar larvae of *H. armigera*. Result showed that larvae treated with methanol leaf extract at 2% percent concentration were severely affected i.e. some died in larval stage, some formed larval pupa intermediate and abnormal adults. The maximum total 50% growth regulator activity was observed in *A. annua* methanol leaf extract. Maximum normal adult emergence was found on average 80 % in methanol seed extract as compared to untreated control.

However 20 and 15 % abnormal adults were recorded in leaf and essential oil, respectively. The formation of larva pupa intermediate and pupal mortality ranged between 5 to 10 % in treatments (Table 2). In relation to fecundity and fertility of *H. armigera*, the methanol leaf extract and essential oil of *A. annua* showed considerable lower no of egg laid by per female 780.0 and 745.0 as compared to control 1240.00 no eggs and substantially reduced hatching of eggs (70 to 71%) as compared to control 95% (Table 3).

Table 2: Insect growth inhibitory activity of different parts of *A. annua* on development of *H. armigera*

Treatments	Mean insect growth regulator (IGR) activity (%)					Total IGR
	Lm	Lpi	Pd	Adult	Ad	
Methanol leaf extract	20	10	-	50	20	50
Methanol stem extract	20	05	05	65	05	35
Methanol seed extract	10	05	-	80	05	20
Essential oil	25	10	10	40	15	60
Control	-	-	-	100	-	NIL

*Lm-Larval mortality, Lpi-larva pupa intermediates, Pd-pupal death, Ad-adultoid (Abnormal adults)

Table 3: Effect of *A. annua* various parts on fecundity and fertility of *H. armigera*

Treatments	No. of eggs laid/ female	% hatching of eggs
Methanol leaf extract	0780.0 ^a	71.88 ^a
Methanol stem extract	0890.0 ^b	82.00 ^b
Methanol seed extract	0985.0 ^c	85.00 ^b
Essential oil	0745.0 ^d	70.45 ^a
Control	1240.0 ^e	95.00 ^c

*All values are mean of ten replicates, same letters within a column are significantly different at $P < 0.05$, and different letters shows significantly different at $P < 0.05$ (Tukey's test).

4. Discussion

During present investigation, the dietary utilization experiment showed that *A. annua* methanol leaf extract and essential oil significantly reduced the larval weight at 2% concentration. The pupal weight also showed the significantly reduction in methanol leaf extract and essential oil as compared to control. The results indicated that *A. annua* methanol leaf extract and essential oil have growth inhibitory activity against *H. armigera*. Several authors reported plant extracts possess similar type of activity against lepidopteran pests [11, 12]. Morphological differences were also observed in treated insect. The treated larvae and pupae were smaller in size as comparison to control. The larval period was prolonged to 18.1 and 19.5 days with treatments of *A. annua* methanol leaf extract and essential oil, respectively while decreasing their feeding potential as compared to control. Moreover some treated larvae developed into permanent larvae leading to larval mortality, and larva-pupa intermediates which were not able to develop into the subsequent developmental stages and hence died ultimately. These defects observed in the development of treated larvae may be due to interference by *A. annua* extract. It is possible that the insecticidal property present in the selected plant compound might have arrested the various metabolic activities of the larvae during the

development and ultimately the larvae failed to moult and finally died. Similar work has also been reported by earlier researchers; Jeyabalan *et al.* [13] reported that the extract of *Pelargonium citrosa* delayed larval and total stage duration of *Anopheles stephensi*. The extracts of *Rhododendron molle* promoted growth duration in *Pieris rapae* [14] and *Peganum harmala* extract delayed larval growth of *Tribolium castaneum* [15]. Methanolic extract of *A. annua* has been found to be responsible for larvicidal activity against lesser mulberry pyralid and *Xanthogaleruca luteola* [16, 17]. In the present study, very low number of eggs was found to be laid by female adults in treated insects. Moreover, the majority of these eggs were also found infertile. Neem leaf extract and neem seed kernel extract have been reported to adversely affect the gonadal weight, fecundity weight and fertility of *H. armigera* eggs [18, 19]. The fecundity and fertility of adults from *A. annua* treated essential oil on *Plodia interpunctella* was found inhibited [11]. The tentative answer for such a result may be the reduction in biochemical parameters (Protein and lipid) which have been considerably decreased after larval treatment. Similar results concluding that changes in fecundity and fertility may be the result of changes in protein have earlier been reported [20]. Though the degree of the effect varied among different treatments, *A. annua* demonstrated its potential against *Helicoverpa armigera* by negatively influencing both the survival and feeding of larvae. Therefore the finding of present study suggested that methanol leaf extract and essential oil of *A. annua* may be potentially used as eco- friendly pest control agents against insect pest of agriculture in integrated pest management.

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