Study of the larvicidal effect of *Eupatorium cannabinum* Linn (Family Asteraceae) leaf extract on *Callosobruchus chinensis* Linn (Family Bruchidae)

Manisha Singh, Arti Saxena, Pankaj Kumar Neeraj
Department of Zoology Govt. Science College Rewa (M.P.) India

Abstract

Different chemicals are used to kill pests. These pesticides often work well, but since they are designed to kill living things they may cause serious problems to human or his pets. Pesticides contaminate the environment and the food that we eat, and they may enter our bodies when we are applying them to our plants or animals. They sometimes harm other organisms in addition to their target pest. Another problem with using chemicals to control pests is that a pest may become resistant to a pesticide. Biological control is one method of preventing pests from causing economic or environmental damage. Biological control methods employ the use of living organisms such as predators, parasites and pathogens to control the populations of pests on agricultural crops. Biological control agents can be bred and reared in large numbers and then released into infected crops to reduce the populations of pests (augmentation) or simple land conservation measures can be implemented on agricultural lands that maintain healthy populations of native predators (conservation). The methanol and chloroform fraction of *Eupatorium cannabinum* proved effective for the control of *Callosobruchus chinensis*. The findings suggest that plant extracted bio-pesticides may play a major role in insect pests control program in near future.

KEYWORDS: *Eupatorium cannabinum, Callosobruchus chinensis,* Biological control, Bio-pesticides.

INTRODUCTION

Pulses are one of the important segments of Indian Agriculture after cereals and oilseeds. These pulses constitute chickpea, pigeon pea, lentil, mungbean, urdbean and field pea. The split grains of these pulses called dal are excellent source of high quality protein, essential amino and fatty acids, fibers, minerals and vitamins. These crops improve soil health by enriching nitrogen status, long-term fertility and sustainability of the cropping systems. They meet up to 80% of the nitrogen requirement by symbiotic nitrogen fixation from air and leaves behind substantial amount of residual nitrogen and organic matter for subsequent crops.

Grain crops are commonly kept on-farm due to the ease in storage when they are dried after harvest **(Duke, 1985)**. However, storage is one of the most crucial post-harvest operations because insects infest grains all the year round under favorable conditions.

India is the largest producer and importer of pulses. The Arhar (Pigeon pea), Gram (Chickpea), Urad (Black gram), Moong (Green gram), Masoor (Lentil) and Peas are major pulses. Production of pulses increased significantly in 2010-11 to 18.2 million

MT as compared to 14.7 million MT in the previous year. But it has stagnated at that level (in fact a little lower) in 2011-12 and 2012-13. Targeted production of pulses in 2012-13 was 17.52 million MT. Export of pulses in 2011-12 was 1.75 lakh MT as compared to the export 2.06 lakh MT in 2010-11 and 1.29 lakh MT in 2009-10.

From the total agricultural products the loss is near about 35% of annual crop due to the feeding by insect larvae or adults because of which we lose billions of rupees every year. The insects damage that much amount of food, which would have been sufficient for millions of people for a year. Thus the insects are in close association and competition with man for food, fiber and shelter. So, it is important for the survival of man to control the agricultural as well as food grain pests.

Though a number of insect pests of different groups cause varying degrees of losses to pulses, bruchids with cosmopolitan distribution affect both qualitative and quantitative losses to pulse right from field to storage. Among bruchids the genus *Callosobruchus,* includes a number of economically important species of which *C. chinensis* causes considerable damage to the green gram, *Vigna radiata* (L.).

Farmers use bush dryers, solar dryers or light fire underneath the crop, to reduce the water contents and to deter or kill the different insect stages. In Cameroon, for example, the use of a 50 kg capacity solar heater eradicated infestations of *Callosobruchus maculatus* from cowpea seeds. It was also demonstrated that temperatures of up to 85^0C did not adversely affect seed germination **(Prakash & Rao, 1997)**.

Other traditional methods include mechanical removal of insects, infested grains or cobs. Winnowing, shaking and restacking the grains lad to the disturbance of insects and a reduction of their activity. Researches reveal that extracts prepared from plants have a variety of properties including insecticidal activity, repellency to pests, anti-feedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests and also antifungal, antiviral and antibacterial properties against pathogens **(Aslam *et al*, 2002)**.

Even currently, pest control measures in storage rely on the use of synthetic insecticides and fumigants, which is the quickest and surest method of pest control **(Shaheen and Khaliq, 2005)**. However, the persistent use of these insecticides in granaries of small-scale farmers has led to a number of problems such as killing of non-target species, user hazards, toxic residues in food, development of genetic resistance in the treated pests, increased cost of application and the destruction of the balance of the ecosystem **(Shaheen and Khaliq, 2005; Boateng and Kusi, 2008)**.

Today more than 2000 species of plants have been reported having insecticidal properties **(Caius, 1986)**. There are a number of plants like *Sapindus trifolisatus* which have been reported to have saponins an ingredient which is a common foaming agent having insecticidal properties (**Joshi & Usher, 1986)**.

Yang and Tang (1988) reviewed the plants used for insect pest control and found that there is a strong connection between medicinal and pesticidal plants.

Higher plants are a rich source of novel natural substances that can be used to develop environmentally safe methods for insect control **(Arnason *et al.*, 1989)**.

MATERIALS AND METHODS

Insect (*Callosobruchus chinensis*)

Callosobruchus chinensis is a major pest of pulses in India. It is a holometabolic insect with the egg and adult stage found on grains and the larval and pupal stages living inside the grain. As the larva eats up the endosperm it is the most damaging stage of the life cycle. Adult beetles are 3-4mm in length, oval in shape, chocolate / reddish brown in colour, and have long, erected antennae. The female lays between 1-8 oval shaped and scale – like eggs/grain. Each larva completes the life cycle in a separate chamber. In India the insect breeds freely from March to November and hibernates in the larval stage during the winter. The adult emergence takes place from January to April. The pest causes maximum damage during February to August, when all its developmental stages exist simultaneously.

Selection of plant

Eupatorium cannabinum was selected for studying its pesticidal properties because of its medicinal properties and its abundance in the study area.

Collection of plant material

The indigenous plant *Eupatorium cannabinum* of Family Asteraceae was collected from Jayanti kunj, Rewa. Collection was done in winter season.

Taxonomic position of the plant

Identification of the collected plant was carried out in the Department of Botany of the study center.

Phylum – Angiosperm

Subphylum - Dicotyledones

Division - Magnoliophyta

Class - Magnoliopsida

Order - Asterales

Family - Asteraceae

Genus - *Eupatorium*

Species – *cannabinum* (Linn.)

Sharma (1989) stated that extracts of *Eupatorium cannabinum* have been used for spleen, liver and biliary diseases, diarrhoea, snakebites, ulcers, wound healing, fever, as a diuretic, anthelmintic and as a repellent against poisonous animals. Extracts of leaves and roots have choleretic, laxative and appetising actions. Aqueous extracts of *Eupatorium cannabinum* had choleretic and hepatoprotective activity in mice against carbon tetrachloride induced hepatotoxicity. The aerial parts of *Eupatorium cannabinum* are used as immuno-stimulating agents in case of influenza, as a remedy against constipation, for decreasing the level of cholesterol and as a diuretic.

For phytochemical analysis of plant the collected material after identification was used and a voucher specimen was procured in the herbarium sheet. The shade dried and powdered leaves were soxhlated in 90% alcohol, rectified spirit and water respectively.

The extract thus obtained was kept in a glass vial and stored in the refrigerator; percentage yield was recorded in the tabular form **(Table No.1)**. Percentage loss in weight after drying of the plant material was also recorded which showed 96.2% loss in weight.

The cold percolation method as mentioned by **Abbott (1925)** was followed for fresh plant leaves. The air dried plant material was powdered using electrical blender. 500 gm of powder was mixed with 300ml of n-hexane and kept for 72 hrs then filtered and it was stored in a reagent bottle. The powder was allowed to dry for 2 hrs before pouring the other solvents-methanol and chloroform and kept for 72 hrs. The crude extracts thus obtained were filtered through Whatman's filter paper No. 1 and were evaporated to dryness in a rotary vacuum evaporator or water bath at the room temperature (40^0C) and pressure (25-30mm hg). Such dried and semisolid crude extract was stored in refrigerator the use i.e for bioassay test against *C. chinensis.*

Chemical analysis and identification of the compounds

First the crude extract of plant was defatted in n-hexane and extracted with methanol and chloroform. The concentrated solution was allowed to stand when a green yellow deposit was obtained.

For further identification and structural elucidation of plant extract, the purified sample was sent to SAIF, CDRI Lucknow for spectral analysis: where IR spectrum, UV spectrum, NMR and Mass spectrum were done.

On the basis of spectral data obtained from SAIF CDRI Lucknow and on comparing the data with authentic markers available finally, sesquiterpene lactone was identified.

Study of *Eupatorium cannabinum* leaf extract on *Callosobruchus chinensis*-

The collection of insects was done from the infested grains.

20 beakers were taken. They were washed with distilled water and then dried.

In each beaker 100 seeds of pulse (e.g. Uradbean) were taken.

In each beaker two drops of chemical: methanol and chloroform fractions of different concentrations e. g. 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm were poured.

In each beaker 3 pairs of insects (3 males and 3 females) were introduced.

The mouth of each beaker was covered with muslin cloth and tied with a rubber band and a control was kept.

After 7 days, the beakers were opened by removing the muslin cloth and the insects were taken out.

The eggs laid on the grains were counted and the beakers were covered with muslin cloths as before.

After 7 days, the beakers were opened for calculating the number of larva emerged from the eggs and then beakers were once again closed as before.

After another 7 days, once again the beakers were opened to calculate the number of pupae. Then beakers were once again closed with the muslin cloth.

After 7 days, again the beakers were opened to see the adult insects emerged from the pupae. They are counted.

The seeds of each beaker were taken in a petri dish and were wetted with water to observe their germination.

The seeds which germinated were counted.

The same experiment was repeated with the wrinkled seeds.

Result and Discussion

Ho *et al.* (1997) and Huang & Ho (1998) found that the extract of *Cinnamon* was also highly repellent to the stored products' beetles *T. castaneum* and *S. zeamais*.

In the present investigation the repellent activity of *Eupatorium cannabinum* leaf extract in five concentrations against *C. chinensis* at one hour showed maximum repellent activity i.e. 90% in 250ppm conc. of methanol and chloroform & minimum in control.

According to **Rahman and Talukdar (2006)** larval mortality at 24, 48 and 72 hours after treatment, due to direct toxicity of acetone extracts of nishinda, eucalyptus and bankalmi leaves on *C. maculatus*, was evaluated at three different rates 2, 4 and 6%. The order of toxicity of the three extracts on pulse beetle was nishinda > eucalyptus >

bankalmi. Mortality percentages were directly proportional to the extract concentrations and also the time after treatment.

In the present study of research work larval mortality was observed maximum in 250 ppm conc. of chloroform and methanol extract of *Eupatorium cannabinum* leaf while it was minimum in the control.

Mulatu and Gebremedhin (2000) reported that *Eucalyptus* seed powder treatment caused the death of emerging adult of *Callosobruchus chinensis* (L.).

T. procumbens and *W. somnifera* leaf powders proved to be better control methods for bruchids than essential oils. Products derived from plants are used as pharmaceuticals worldwide and could therefore be considered less harmful to humans than most conventional insecticides (**Shukla *et al.*, 2007**).

In the present study the leaf extract of experimental plant inhibited adult emergence. Minimum adults emerged in methanol and chloroform 250 ppm conc. & maximum in the control.

References

Aslam, M., **Khan, K.A**. and **Bajwa, M.J.H**. (**2002**). Potency of some species against *C. chinensis* L. *Online J. Biol. Science*, 2:499-452.

Arnason J.T., **Philogene B.J.R**, **Morand P (1989)**. Insecticides of plants origin. American Chemical Society Symposium Series Vol. 387 Washington.

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Eco. Entomol*., 18: 265-267.

Boateng B, Kusi F (2008). Toxicity of Jatropha Seed Oil to *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its Parasitoid, *Dinarmus bassli* (Hymenoptera: Pteromalidae). *J. Appl. Sci. Res*. 4: 945 – 951.

Caius J.F. (1986). The medicinal and poisonous plants of India, Scientific publishers, Jodhpur, India.

Duke, J.A., (1985). Hand Book of Medicinal Plants. CRC Press Inc, Boca Raton, USA.

Ho, S. H. Y. Ma., H. T. W. Tan and H. Halid (1997). Repellency of some plant extracts to the stored products beetles *Tribolium castaneum* Herbst and *Sitophilus zeamais* Motsch. Proceedings of the symposium on pest management for stored food and feed, BIOTROP (Special Publication), 59: 209-215.

Huang, Y. and S. H. Ho (1998). Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects *Tribolium castaneum* Herrbst and *Sitophilus zeamais* Mostsch. *J. of Stored Prod. Res*., 34: 11-17.

Joshi P & Usher (1986). Fish stupefying plants employed by tribal of Southern Rajasthan, A. Pub. Current Science, 55 (**14**): 697-699.

Mulatu B., Gebremedhin T (2000). Oviposition – deterrent and toxic effect of various botanicals on the Adzukibeen beetle, *Callosobruchus chinensis* L. Insect Science and its Application; 20 (**1**): 33 – 38.

Prakesh and Rao, (1997). Leaves of Beguina (*Vitex negundo*) Grain protectant Pulse grains *C. chinensis* India.

Rahman, A. and F.A. Talukder (2006). Bio efficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus. J. Insect Sci*., 6: 19 – 25.

Shaheen F.A., Khaliq A (2005). Management of pulse Beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) in stored chickpea using ashes, red soll powder and turpentine oil. Pak. Entomol. 27: 19 – 24.

Shukla R, Srivastava B, Kumar R, Dubey N (2007). Potential of some Botanical powders in reducing infestation of chickpea by *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *J. Agric. Technol*. 3: 11 – 19.

Sharma, S.S. (1889). Review of Literature of the losses caused by *Callosobruchus* species (Bruchidea: Coleopetera) during storage of pulses Bull. Grain Technol., 22: 62 – 68.

Yang R.Z., Tang C.S. (1988). Plants used for pest control in China: a literature review. Econ. Bot.42: 376 – 406.

Table No. 1
Percentage loss in weigh of the plant material

S. No.	Name of the plant	Wet weight of plant material in (gms)	Weight on drying of the plant material (gms)	Loss in weight on drying (gms)	Percentage loss in weight
1.	*Eupatorium cannabinum* (Linn)	2000	180	1920	96.2%

TABLE No. 2
Statistical data of purified fraction of *Eupatorium cannabinum* (EMf_5) methanol extract against Pulse beetle (*Callosobruchus chinensis*) on fresh Uradbean seeds

Concentration (ppm)	24 hr. larval mortality	Regression equation (y=a±bx)	Chi-square $\chi^2(n-1)$	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	59	y=4.048±1.923x	6.382	127.544	0.025281	0.159	L=100.951 U=159.033
100	94						
150	122						
200	154						
250	182						
Control	5						

60 larvae of *Callosobruchus chinensis* were taken in each of the four replicates.
Values are significantly different than the control ($p<0.05$).

TABLE No. 3
Statistical data of purified fraction of *Eupatorium cannabinum* (ECf_5) chloroform extract against Pulse beetle (*Callosobruchus chinensis*) on fresh Uradbean seeds

Concentration (ppm)	24 hr. larval mortality	Regression equation (y=a±bx)	Chi-square $\chi^2(n-1)$	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	74	y=3.468±1.678x	9.072	116.465	0.024025	0.155	L=79.749 U=157.078
100	98						
150	127						
200	156						
250	183						
Control	5						

60 larvae of *Callosobruchus chinensis* were taken in each of the four replicates.
Values are significantly different than the control ($p<0.05$).

TABLE No. 4
Statistical data of purified fraction of *Eupatorium cannabinum* (EMf_5) methanol extract against Pulse beetle (*Callosobruchus chinensis*) on wrinkled Uradbean seeds

Concentration (ppm)	24 hr. larval mortality	Regression equation (y=a±bx)	Chi-square χ^2 (n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	69	y=3.556±1.708x	7.804	120.766	0.024336	0.156	L=87.731 U=158.787
100	99						
150	127						
200	149						
250	183						
Control	5						

60 larvae of *Callosobruchus chinensis* were taken in each of the four replicates.

Values are significantly different than the control ($p<0.05$).

TABLE No. 5
Statistical data of purified fraction of *Eupatorium cannabinum* (ECf_5) chloroform extract against Pulse beetle (*Callosobruchus chinensis*) on wrinkled Uradbean seeds

Concentration (ppm)	24 hr. larval mortality	Regression equation (y=a±bx)	Chi-square χ^2 (n-1)	LC_{50} (ppm)	Variance (V)	S.E.	Fiducial limits (ppm)
50	74	y=3.508±1.729x	3.558	107.058	0.024025	0.155	L=95.785 U=118.226
100	108						
150	138						
200	162						
250	185						
Control	5						

60 larvae of *Callosobruchus chinensis* were taken in each of the four replicates.

Values are significantly different than the control ($p<0.05$).