Toxic Effect Of Crude Aqueous Leaf Extracts of Clerodendron inerme, on the Total haemocyte Count of Sixth Instar Larva of Helicoverpa armigera (H)

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Abstract

Crude aqueous leaf extract of C.inerme was tested for its effect on total haemocyte count of sixth instar larva of Helicoverpa armigera (H). After the treatment, the haemocyte count was found to be reduced and resulted in the increased per cent mortality of larvae, pupal and adult deformity, diseased larvae larval-pupal intermittants, hollow pupae ,flight less adults etc. This suggests that the crude aqueous leaf extract of C.inerme is having insecticidal properties and can be used to control the insects.
Keywords: Helicoverpa armigera, Clerodendron inerme

I. INTRODUCTION

Clerodendron inerme is a biennial flowering plant and much branched shrub, 0.9 to 2.1 cm long, branches are screwny.Leaf extract of Clerodendrum inerme has many active compounds which are reported to show pesticidal properties against many yield- threatening pests. Holihosur et.al (2013) has reported the use of C.inerme plant extract against Aedes aegypti mosquito The effect of crude leaf extract of C. inerme has shown insecticidal activity against larvae and pupae of Culex quinquefasciatus and the efficacy of C. inerme plant extract in the management of The tobacco caterpillar, Spodoptera litura has also been studied (Khetagoudar et.al 2012). . Treatments with 1% V. negundo and C. inerme extracts significantly reduced P. xylostella larval density, and the percentage of infested plants, proving to be more effective than a standard insecticide Challenger 10EC (cypermethrin). Treatment with L. camara extract (1%) reduced the percentage of H. armigera infested plants and the intensity of cabbage damage(Shivanand R. Yankanchi* and Sachinkumar R. Patil) In Indian tribal medicine, leaves of Clerodendrum inerme are used for treating fever, cough, skin rashes, chronic pyrexia and boils, and are used in conjunction with other plant leaves.

Fig. 1: Clerodendron inerme G.

Aerial parts of Clerodendrum inerme showed potent anti-viral activity against Hepatitis B virus [5]. Whole plant parts of Clerodendrum inerme are used as to treat coughs, scrofulous infection, venereal infection, skin diseases and Beriberi diseases [Shanthakumar*, M Sathish, and A Jerad Suresh]. It is also used as febrifuge, vermifuge and antioxidant, for scrupulous and venereal infections, the juice of the leaves and root of this plant are considered as the best medicines; a tablespoon full with or without a little castor oil is the effective dose. The boiled root oil is useful in the treatment of rheumatism The plant contains mainly iridoids, flavonoids, sterols and triterpenes.

Furthermore, the quantities of these compounds are likely to be altered by climatic and edaphic factors, grazing herbivores and other environmental factors (Yankanchi, 2003). Even within a single toxin can vary both in space and time.

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Rapid growth, multiplication of insects and their fast resistance developing body physiology to many synthetic insecticides have offered a challenge to man. If using the synthetic insecticides solves the problem of insects, another problem of environmental pollution may arise.

Botanical insecticides are plant derived natural products, which are secondary metabolites. Secondary metabolites include thousands of alkaloids, terpenoids, phenolic compounds and other minor secondary chemicals. Almost every plant species has developed a unique chemical complex that protects it from pest. In the present study the aqueous extract of Clerodendron inerme (C. inerme) plant leaves was evaluated against laboratory strain H.armigera larvae.

The effect of crude leaf extract of C. inerme has shown insecticidal activity against larvae and pupae of Culex quinquefasciatus and the efficacy of C. inerme plant extract in the management of the tobacco caterpillar, Spodoptera litura has also been studied (Khetagoudar et.al 2012).

Recently, new sterols have reported from C.inerme (Pandey et.al, 2003) and three new neo-clerodane diterpenoids, been found in the hexane extract of aerial parts of C.inerme. Arguably these may be the biochemicals responsible for the larvicidal activity at different developmental stages of P.brassicae.

The leaf extract of C.inerme is effective larvicide, economic, easy to prepare and ecofriendly.It can be used against phytophagous pests for their control. C. Inerme at 1% significantly reduced the P. xylostella larval density and proved more effective than Challenge (Shivanand R. Yankanchi* and Sachinkumar R. Patil).

The haemocytes form the major immune system of all insects. It is a well known that most of the insecticides and biocides have toxic effects on the haemocytes where the total haemocyte count and differential haemocyte count are altered because of which the insects become susceptible to the microbes ultimately result in the death of the insects. Thus the control of the insects can successfully be achieved by using insecticides and biocides. In the present study the plant extracts have been used to test their effect on the total and differential haemocyte counts of Helicoverpa armigera.

Phagocytosis by haemocytes is similar to that of mammalian leucocytes or Amoeba (Salt 1970). It is evident that the particulate matter is phagocytosed by haemocytes. They can detoxify certain poisons (Jones in Press).

In the present study, the total haemocyte count in the haemolymph of treated larvae of Helicoverpa armigera was recorded and was compared with the control. It was found that the total haemocyte count was lowered in the treated ones. The adipohaemocytes were not considered as the separate haemocytes but a variant of granulocyte. But in the present study we have reported it as a separate type of haemocyte.

II. MATERIALS AND METHODS

Sixth instar larvae were used; the thoracic leg was pulled to ooze out the haemolymph which was collected by a micropipette of the haemocytometer (Mini and Jesudasasan. 2006). The total haemocyte count was done by using a convention procedure for human WBC counting using Naubaur's chamber. The total haemocyte count was calculated using the formula

$$THC = (N \times D) / (n \times d),$$

where:

N = number of haemocytes in 8 squares each of 1sq.mm area,
D = number of times of dilution,
n = number of haemocytes in 1sq.mm area,
d = depth of the squares.

A. Experimental Design:

There were six treatments each with three replicates and each replication was an average of ten readings of haemocyte counts. There were five treatments of 10, 20, 30, 40 and 50 micro litres of extracts and one control of distilled water.Haemolymph of one larva was used for one reading.

B. Extract Preparation:

Leaves of the plant Clerodendron inerme are opposite elliptic or obovate, obtuse, glabrous, with long petiole with three flowers that are white in colour The leaves are mucilaginous, bitter and fragrant and have medicinal properties. Fresh mature leaves of the the plants were collected , cleaned, washed with distilled water and spread on blotting paper to dry them. Then the known weight of the leaves and volume of the distilled water was maintained in 1:2 ratios. Then these leaves were cooked in a pressure cooker, under 15 lbs pressure for 30 minutes. Then the cooked material was homogenized and squeezed through cheesecloth followed by filtration through filter paper. This solution was known as a 50% stock solution and was stored for further use.

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III. RESULTS AND DISCUSSION

A. The Effect of Crude Aqueous Leaf Extract of Clerodendron Inerme on The Total Haemocyte Count of Sixth Instar Larva of Helicoverpa Armigera (H):

Crude aqueous leaf extract of Clerodendron inerme plants prepared in the laboratory were topically applied to the fifth instar. There were 5 treatments of the aqueous extract of Clerodendron inerme with 10 per cent, 20 per cent, 30 per cent, 40 per cent, and 50 per cent concentrations of the extract.

At 10 per cent concentration of the extract the THC ranged between 11450 and 12100. The average was 11762.50 haemocytes per cc of haemolymph. At the next higher dosage of 20 per cent of the extract, the total haemocytes counted were between 10150-10875 haemocytes per cc of haemolymph and average of 9035.40. When 30 per cent concentration of the extract was tested, the THC was 9900-10600 and the average THC was 10375.00 per cc of haemolymph. The 40 per cent concentration when applied, the THC were 10575-11250 and the average THC being 10956.25 per cc of haemolymph. At the highest concentration of 50 per cent, the THC was 13400-13800 and the average was 13562.50 per cc of haemolymph.

In the distilled water control, the THC range was 23000 - 23050and the average being 23436.25 per cc of haemolymph.

Table – 1

Effect of aqueous leaf extract of Clerodendron inerme,on total haemocyte count of haemolymph of sixth instar larva of Helicoverpa armigera H

Treatment	*Application in microlitres*	*Clerodendron inerme*
		Mean±SE
T_1	*10*	$11743.75^c \pm 65.00$
T_2	*20*	*9035.40± 102.90*
T_3	*30*	*10375.00 ± 76.40*
T_4	*40*	*10956.25 ± 78.10*
T_5	*50*	*13562.50 ± 206.61*
T_6	*Distilled water control*	$23436.25^a \pm 207.56$
One way ANOVA F critical = 2.62		*341.24*

Haemocytes are the cells which perform the functions such as phagocytosis during metamorphosis, healing of wounds, formation of membranes, regulation of growth and development, tanning of cuticle, detoxification of poisons etc. When phagocytosis is blocked, the insect becomes susceptible.

According to Gupta, plasmatocytes and granulocytes are known as immunocytes, as they perform immunological functions such as encapsulation, phagocytosis, secretion of agglutinins coagulation etc. They are playing significant role in insects that can be compared to that of T lymphocytes and B lymphocytes of higher vertebrates.

In the present study, the treatment of the extracts has resulted in bursting of the haemocytes..The spherulocytes have been increased in their size and number of spherules is also increased which is an indication of phagocytic activity of the cells.

Fig. 2: Effect of aqueous leaf extract of Clerodendron inerme G. on different developmental stages of Helicoverpa armigera (H)

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Haemocytic activity can be blocked by injections of various substances (Wigglesworth 1955 and 1959), which causes delay in moulting. The secretion of the prothoracic glands stimulates moulting which may be due to the factor produced by the haemocytes. In the present study, this secretion by prothoracic glands might be prevented by these phytochemicals, as a result of which there is moult inhibition or delayed moulting. In the present study, there is inhibition of moulting at larval, pupal stages; abnormal cuticle of the adults is due to the treatment with the extracts

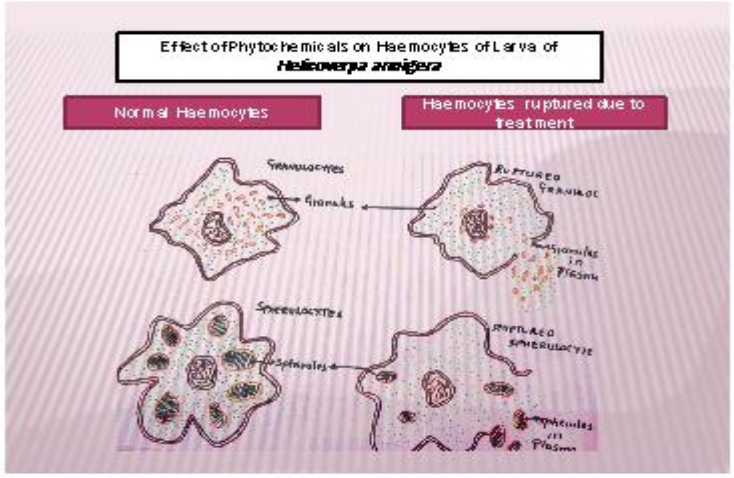

Fig. 3: Effect of Phytochemicals on Haemocytes of Larva

Plasmatocytes became shapeless, losing their pseudopodial processes. They exhibit reduction in number. It is evident that these extracts contain haemolytic compounds resulting in reduction in number due to the treatment with the extracts Due to the decreased number of plasmatocytes and due to the change in their morphology and structure after treatment, the larval haemolymph exhibits altered functions. The physiological activities such as synthesis of antibacterial, antiviral factors, synthesis of agglutinins,phagocytosis, wound healing, coagulation of blood are also affected. The melonization and cuticularization are also altered because of these cells.

IV. CONCLUSION

The results of present study show that the haemocytes being the important inclusions of the haemolymph, performing the life saving function of the insect are unable to perform their functions totally resulting in mortality of larva in any stage of its life cycle, or they may not be able to function well due to their altered morphology and reduction in number. This is due to the treatment of the larvae with crude aqueous leaf extract of *Clerodendron inerme.* The results strongly suggest that they can be used in controlling all kinds of insect pests without causing any kind of pollution.

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