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Studies on the effect of aqueous leaf extract of *Parthenium hysterophrous* on the control of *Helicoverpa armigera*

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Abstract: *Herbivorous insects have achieved remarkable success on suitable host plants. Helicoverpa armigera is a polyphagous caterpillar and is having high mortality and fecundity to develop resistance to synthetic insecticides. The study was focused on the effect of leaf extract of Parthenium hysterophorus (Congress grass) on the control of Helicoverpa armigera. Aqueous extracts were prepared and sprayed on Helicoverpa armigera. Significiant behavioural changes were not noticed when H. armigera were exposed to aqueous leaf extracts of Parthenium hysterophorus.*

Key words**:** *Helicoverpa armigera, Parthenium hysterophorus, aqueous extract.*

1. INTRODUCTION:

It has been a major problem to protect vegetables and fruits in agriculture field. Plant products are exploited using synthetic insecticides, insect repellents and insect antifeedants [1,2,3].The losses caused due to pests and diseases are as old as plants themselves. Increase in pest problems resulted in indiscriminate use of pesticides and that is a main concern of environmental problems and ecological imbalances[4].

The pesticide element contains "dynamic" fixings and "inactive" fixings. The dynamic fixings are the synthetic compounds used to control the objective pest.The latent fixings are utilized as transporters for the dynamic fixings, to help disintegrate them and make them simpler to apply or to safeguard them. The synthetics utilized as idle mixes incorporate a portion of the presumed cancer-causing agents and have been connected to other long haul medical conditions like focal sensory system problems, liver and kidney harm and birth absconds. They can likewise cause transient wellbeing impacts like eye and skin bothering, sickness, unsteadiness and respiratory troubles. A portion of the dormant pesticide fixings are carbon tetrachloride, chlorobenzene, cresols, dibutylphthalate, dimethylphalate, ethylbenzene, isophrone, o-dichlorobenzene, phenol, toluene and so forth [5]. Variety of synthetic insecticides are efficiently used to kill the insect pests in all agricultural fields. The profuse use of pesticides has created hazardous effects on human health and an addition to insecticide resistance development in target organisms[6]. The hazardous effects of chemical pesticides have provided the impetus for the development of alternative control measures, including botanical insecticides. Agricultural pests can also be controlled by natural mechanisms. The nature maintains the balance in insect population by some factor like climate, topograph, biotic components in the biosphere and natural diseases. Artificial control includes physical or mechanical control, biological control and chemical control[7]. Over past couple of years, concerted efforts have been made to reduce the application of chemical pesticides and emphasis has been given on the discovery and application of safer biopesticides[8]. For the work, as an attempt to develop new plant based insecticide and to understand its mode of action on larvae, *Parthenium hysterophorus* was been selected to check its potentialities as biopesticides on *Helicoverpa armigera*. *Parthenium hysterophorus* (family :Asteraceae) is an aggressive herbaceous weed and it has spread and become widely distributed even in India. It threatens human and animal health, the environment, agriculture and natural biodiversity through its prolific growth, copious pollen and seed production and constitute bioactive and toxic allelochemicals[9]. Parthenin, a sesquiterpene lactone of *Parthenium hysterophorus* L. is a high toxicity clastogen with allergenic and irritant action[10].

Helicoverpa armigera(family : Noctuidae, class : Insecta, order : Lepidoptera) is a pest for successful cultivation of several economically important crops. This notorious pest has a very high migration capacity coupled with enormous fecundity.This vermin species is very polyphagous, it is one of the vital foes of cotton and maize, yet in addition often assaults vegetable plants: tomato, bean, onions and other line crops: sunflower, soybean.It is a significant bugs in a few pieces of the world, specifically in Asia-Pacific, Africa, South-Europe, Eastern Europe and all the more as of late in Latin America. Adults show up in April-May and can be seen until October. Females lay a few hundred eggs (up to 3 000) on all pieces of the plant, blossoms and organic products included. Contingent upon the atmosphere 2 to 7 ages can be noticed. The existence cycle is finished in around 30 days.The most extreme harm is brought about by the assault on conceptive parts (bloom buds and heads, products of the soil inflorescence. *H.*

armigera has established resistance to various pesticides from majority of the recent chemical modules in all sectionswherever it has established[11].

2. MATERIALS AND METHODS:

To evaluate the insecticidal properties of *Parthenium hysterophorus*, the plant were screened and selected on the basis of the literature survey, *Parthenium hysterophorus*(family : Asteraceae), locally known as Ragweed have been selected and leaf extracts were tested for their biological effects against agriculture pest *Helicoverpa armigera* (class : Insecta ; order : Lepidoptera ; family : Noctuidae)

2.1. Extraction of leaves:

Preparation of aqueous extract (By Soxhlet extraction method)

To evaluate the insecticidal properties of the hydrophilic molecules of the leaves of the plant were washed with tap water, weighed (200 gm) and crushed into paste using domestic grinder. For extraction of active ingredients the paste was mixed with distilled water (1:10) and extracted in a soxhlet apparatus for 8 hours over a heating mantle (Sintex) at 90°C. Extracts were cooled and filtered with Whatmann filter paper No. 1. The filterate was lyophilized to get dry powder for further investigations. For lyophilization, filterate was freezed at -70°C and lyophilized at -20°C at 200 vaccum for 3 hours. Fresh extracts in powdered form were stored in tight screwed bottle under refrigeration.

2.2. Collection of instar:

3^{rd} and 4^{th} instar larvae of *Helicoverpa armigera* (Hubner), agricultural pest have been selected as test larvae to check biocidial properties of the leaves of *Parthenium hysterophorus*. Larvae was collected from the control agricultural plots of cabbage and gram from Gujarat Agricultural University, Anand. Collected larvae were maintained in the laboratory. Larvae were maintained separatedly in plastic jars with proper aeration. As a feed, everyday morning fresh tomato leaves, collected from the control tomato field of Gujarat Agricultural University, Anand were provided. Before feeding all the jars were cleaned thoroughly.

2.3. Experimental set up:

Experiments were carried out separatedly to understand the insecticidal properties of aqueous extract of *Parthenium hysterophorus* on 3^{rd} and 4^{th} instar larvae of *Helicoverpa armigera*.

For aqueous extracts of *Parthenium hysterophorus*:

From the fresh extracts (in powder forms) of *Parthenium hysterophorus*, required test solutions were prepared with different strengths viz. 5%, 10%, 15% and 20% (w/v) in distilled water and to check the effects of these test solutions, larvae were divided in 1^{st} , 2^{nd} , $3^{rd}$and 4^{th} experimental groups respectively. A common control group has been maintained for the plant extracts. In each group, 10 healthy larvae have been maintained separatedly in 100 ml plastic jars to prevent viral infections. All the jars were covered with thin nylon cloth. To check the effects of plant extracts on the surface structures of the larvae, aqueous test solutions with 5%, 10%, 15% and 20% strength have been sprayed on the surface of the larvae. Surface of the control larvae were sprayed with distilled water only. Both the control and experimental set up larvae of *Helicoverpa armigera* were feed with tomato leaves. For spraying on larvae, baby sprayer have been used. During experiments, spraying on larvae was done once in a day in the morning. Bottles were cleaned everyday in the morning. During experiment, larvae were maintained at room temperature. To understand the insecticidal effects of *Parthenium hysterophorus*, extracts of *Parthenium hysterophorus*, during the experiment and at the end of the experiment, different parameters viz., mortality rate of larvae, their behavioual changes, fees avoidance mechanisms have been noted and LC_{50} value of extracts for *Helicoverpa armigera* have been calculated. Effect of plant extract on the growth and metamorphosis have also been studied. Closed up photographs with SLR camera have been taken for both experimental and control larvae as well as pupa.

3. RESULTS:

During the present experiment, insecticidal properties of aqueous extracts of *Parthenium hysterophorus* was investigated on 3^{rd} and 4^{th} instar larvae of *Helicoverpa armigera* (Hubner). Aqueous extracts of different concentrations were sprayed directly on the larvae. Immediately after spraying the leaf extracts, in the initial period, movement of larvae were highly irritative as compared to control. No antifeedent behavior was noticed in the experimental group. In control group (with distilled water , larvae were observed to be continuously feeding on tomato leaves and regular movement was noticed during the experiment. No antifeedent behavior of larvae was noticed. Larvae of *Helicoverpa armigera* were exposed to various concentrations of formulations, exhibited larval mortality. When exposed to aqueous extract of *Parthenium hysterophorus* 15% and 20% concentrations have shown 100% mortality within 10 days and for 5% and 10% concentration 60% mortality was noticed within 12 days.

No mortality of larvae was registered in control group. In control group, all the larvae entered into pupation in the period of 10 to 12 days. From experimental group also, remaining larvae entered pupation almost in the same time period as control. Toxicity studies revealed that aqueous leaf extracts of *Parthenium hysterophorus* seem to be toxic to larvae of *Helicoverpa armigera*. Mortality rate was nearly 60% with 5% and 10%. Results suggests that scanning electron micrographs of the surface structures of control larvae revealed presence of intact setae with clear rim. The surface was also covered by densely and uniformly packed papillae like cuticular structures (Fig. 1). Damage to the setae, rim and cuticular papillary out growths were clearly noticed on the surface of the larvae sprayed with *Parthenium hysterophorus* aqueous (20%) (Fig. 2)

Table 1: Average time taken for mortality and percent mortality rate of *Parthenium hysterophorus* aqueous extracts which were sprayed directly on the surface of the larvae

% Concentration	Average time taken for mortality	% mortality
5%	2.02	60%
10%	1.97	60%
15%	4.25	100%
20%	4.07	100%

4. DISCUSSION:

Control of insect pests is becoming increasingly difficult because of several species becoming physiologically resistant to the conventional insecticides and increasing contamination of the environment. Alternative control methods and materials that are both effective against the target pests and that have minimal detrimental effect on the environment are needed. In the view of the hazards involved in the use of insecticides and due to other problems, attempts have been made in recent years to develop such chemicals that interfere with insect growth hormone system, control moulting, diapauses, egg maturation, Chitin synthesis and certain reproductive activites[7]. Some biochemical pesticides like microbial pesticides can control many different kind of pests. Most widely used microbial pesticides prepared from *Bacillus thuringiensis* known as B*t*. They produce a mixture of proteins which specifically kills one or few related species of insect larvae. Some fungi control certain weeds and kill specific insects[12]. The susceptibility of the major pest of cotton, *Helicoverpa armigera* and *Helicoverpa punctigera*, to some insecticidal proteins from *Bacillus thuringiensis* were tested by bioassay. A commercial formulation, Dipel and other individual purified insecticidal proteins were tested against both. *Helicoverpa armigera* was consistently becoming more tolerant to *B. thuringiensis* insecticidal proteins than *H. punctigera*, although both were susceptible to only a limited range of these proteins[13].

Biological control never gives immediate results like insecticide, but it has been proved to be economical and provides permanent control. Botanical pesticides are relatively safe and biodegradable so plant pesticides are more preferred [14]. During the investigations, the effect of leaf extracts of plants on the surface sensory structures like setae and other cuticular projections by taking scanning electron micrographs of surface structures of control and experimental larvae were observed. Scanning electron micrograph photographs of control larvae (fig. 1) clearly shows the presence of intact setae, their circular rim and other cuticular papillary structures. A clear damage to the setae, rim and papillary structures were observed in the photographs (fig. 2) of the larvae treated with aqueous extracts of *Parthenium hysterophorus*. Damage to surface structures could be because of the contact toxicity of these plants to *Helicoverpa armigera*, as leaf extracts have been directly sprayed on the larvae. The exact reason for these damages were not known but it could be because of the penetration of extracts to the interior of skin through the setae and other surfaces.

5. CONCLUSION:

The experimental findings showed that the aqueous leaf extract of *Parthenium hysterophorus* exhibited significant lethal and antifeedant effects on the 3rd and 4th instar larvae of *Helicoverpa armigera* in laboratory.

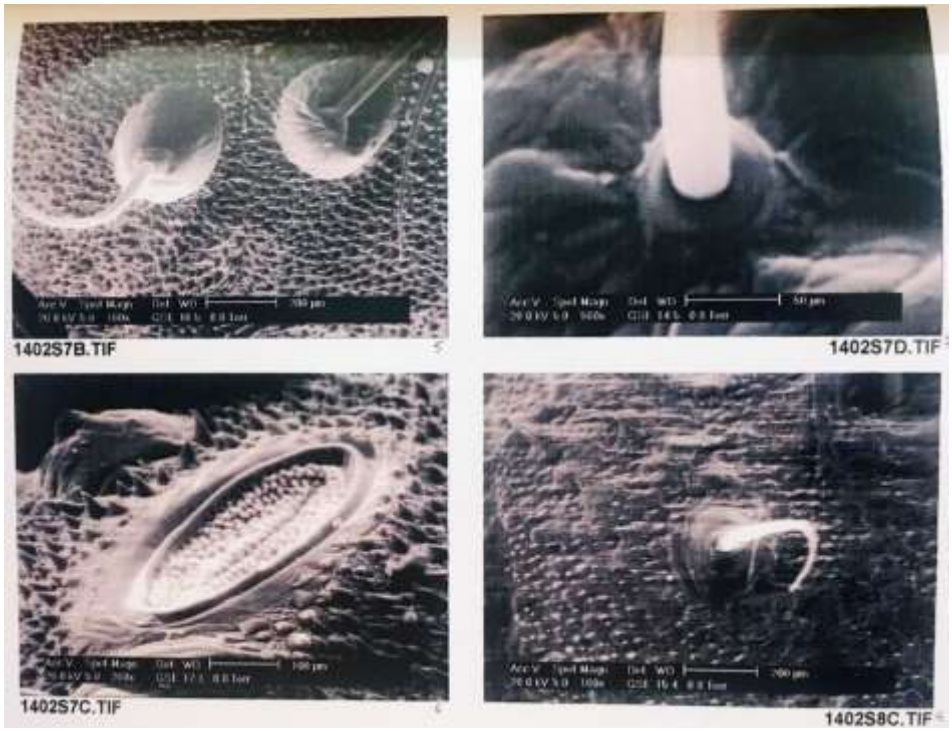

Figure 1:Photographs of control larvae of *Helicoverpa armigera* (SEM)

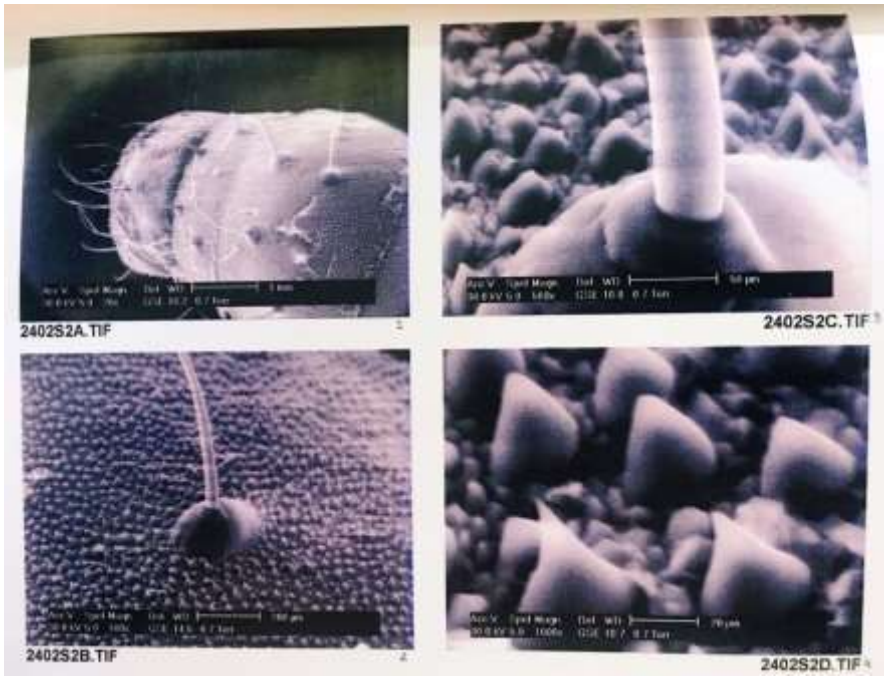

Figure 2- Photographs of larvae of *Helicoverpaarmigera* after spraying leaf extract (SEM)

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