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Detection of +vely and –vely charged protein and lipid fractions in the ovaries of *Musca domestica treated* with *Annona squamosa* seed extract

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Musca is a house fly transmitting a number of diseases in human beings and their livestock and having high rate of fertility. To control the fertility the flies were topically treated with sub lethal concentrations of *Annona squamosa* seed extract and protein and lipid fractions were detected out from the ovaries of 3, 6, 9, 12 and 15 days control and experimental flies by paper electrophoresis. The results are summarized in electrophoretograms Fig. No. 1- 4 showed the gradual increase in the number of protein and lipid fractions in the ovaries of control flies indicated the phase of vitellogenesis while depletion in the numbers as well as the decrease in the intensity of protein and lipid fractions due to the intoxication effect of the treated extract was correlated with the formation of large number of degenerated pathological oocytes in the ovaries which never attain such maturity which is required for oviposition, so in the treated flies the extract acts as anti-ovipositional agent and it is quite significant after formulation and synthesize in large quantity it should be use to control the other pest insects of our valuable crops and increase the economy of Indian farmers.

Key words: *Musca* control by *Annona squamosa.*

Introduction :

Detection of protein and lipid fractions in the ovarian tissue of control and treated insects is an important aspect of this investigation which evident the continuous increase (in control groups) or regular decline (in experimental groups) in the number and intensities of protein and lipid fractions in the detected bands by electrophoresis and on the basis of these observations one can correlate this increase(in control groups) or decrease(in experimental groups) on molecular level in the ovarian architecture as reported in *Atractomorpha crenulata* by Murugan *et al*., (1996) and Babu *et al.*, (1997) after neem extract treatment, Rathore (1999) in *Poekilocerus pictus* with *Annona squamosa* seed extract treatment, in some species of *Dysdercus* by Mugdam and Banerjee (1999), Yadu *et al*., (1999), Mugdam (2000) after plumbagin and gossypol exposure, Gupta and Rathore (2002)

in *Poekilocerus pictus* after neem extract treatment, Bhide (2003) in *Musca domestica* after *Annona squamosa* seed extract treatment, Kumar *et al*. (2003) in *Periplaneta americana* and *Musca domestica* after *Delonix regia* and *Cassia fistula* seed extract treatment, respectively. So this investigation was done to know more on these significant aspects and co -related the results with the antiovipositional activity of the *Annona squamosa* seed extract used in this investigation.

Materials and methods :

The materials and methods used in this investigation are as follows:

[i] Procurement and rearing of houseflies : Adult flies were collected locally and cultured in the laboratory by the method adapted after Goel [1984] and the adult flies emerged from the pupae were used for experimental purposes.

[ii] Procurement and extraction of seed of *Annona* squamosa : The seeds of *Annona squamosa* belonging to family – *Annonaceae*, were collected locally, washed, shadow dried, mechanically ground in coarse pieces and extracted with petroleum ether (BDH, 60*C) by Soxhlet method. The extract was concentrated by evaporated to dryness in water-bath, 1% stock solution was prepared with distilled water, and further dilution was made to decide the lethal doses and the detected values were 0.175% LC100, 0.076% LC50, 0.035% LC0 and 0.03% sub lethal concentration used during experimentations.

[iii] Preparation of protein and lipid samples of the ovaries : Control and topically treated 3, 6, 9, 12 and 15 day old adult flies were dissected out and the ovaries were processed for the extraction of protein and lipid samples. The samples were applied in the form of streak on the 1cm. width x 39 cm. long Whatman no.1 chromatography paper strips presoaked in 8.6 pH borate buffer.

[iv] Electrophoresis of extracted samples : at +ve or – ve pole for the separation of +vely and –vely charged protein or lipid fractions respectively by VMC (Vertical migration chamber) Systronics model no.

604V using digital power supply Systronics model EPA 610 by the method supplied in the manual of Systronics Co. Ltd. Ahmedabad.

[v] Staining of paper strips for the detection of protein and lipid fractions:

[a] Mercuric bromophenol blue staining method was used for the detection of protein bands on the already electrophoresis strips after clearing the background with 1% acetic acid solution and fix the bands in methanol.

[b] Sudan black B staining method was applied for the detection of lipid bands on the already electrophoresis strips after clearing the background with 40% ethanol.

Results and Discussion :

The number and intensities of protein and lipid fractions detected out by densitometer (Systronics Co. Ltd. Ahmedabad) presented by electrophoretograms and summarized as follows :

Detection of –vely and +vely charged protein fractions in the ovaries of control and treated flies:

Note: where PF = Protein fractions and LF = Lipid fractions.

[1] –vely charged protein fractions (fig. 1) :

[i] In control groups

Minimum 6 PF.

Maximum 8 PF. [1PF added at day 9, 2 PF added on day 12^{tth} and 15th day] Addition of fractions were evident the phase of vitellogenesis.

[ii] In treated groups:

Minimum 5 PF.

Maximum 6 PF.

5th gradually decreases in it’s density from day 6 onwards.

It is a dominating fraction.

PF 1 was constant in its density.

[2] +vely charged protein fractions (fig. 2) :

[i] In control groups:

Minimum 7 PF.

Maximum 8 PF. 1 PF added in vitellogenesis from day 9^{th} to 15^{th}

PF 4 of high density but constant in the ovaries of all control flies.

PF 2 of high density and constant from day 9^{th} to 15^{th} day.

[ii] In treated group:

Minimum 5 PF were constant from day 6^{th} to 15^{th}.

Maximum 7 PF were detected in 3 days treated flies.

PF 1 was dominant and of high intensity. It was a largest band.

Detection of +vely and –vely charged lipid fractions in the ovaries of control and treated flies

[1] +vely charged lipid fractions (fig. 3) :

[i] In control groups

Minimum 5 LF.

Maximum 6 LF (evident the lipid synthesis and lipid deposition).

[ii] In treated groups:

Minimum 2 LF (In 6^{th} to 15^{th} days).

Maximum 6 LF.

3rd LF was a dominating lipid fraction of high intensity in the ovaries of all the treated groups.

1stand 3rd LF in 6 days and 4th and 3rdLF were detected in 15 days.

[2] –vely charged lipid fractions (fig. 4) :

[i] In control groups

6 LF is constant in all the control flies.

Fraction 6th increases in it's density from 12th to 15th day.

Fraction 1st and 3rd LF constant and of high density.

[ii] In treated groups:

5 LF in 3rd day.

4 LF in 6 and 9 day.

3 LF in 12th and 15th day.

1st LF was of very high intensity.

The increase in the number of PF and LF in control groups was evident the phase of vitellogenesis in the ovaries of *Musca* as reported by Kumar *et al.*, (2003), Bhide (2003) and Bhide *et al.*, (2004) in *Periplaneta americana* and *Musca domestica* after *Delonix regia, Annona squamosa* and *Cassia fistula* seed extract treatment while depletion in the number as well as in the intensities of PF and LF showed the dose and duration of treatment dependent effect of *Annona squamosa* in the present investigation as reported in *Atractomorpha crenulata* by Murugan *et al*., (1996) and Babu *et al.*, (1997) after neem extract treatment, in some species of *Dysdercus* by Mugdam and Banerjee (1999), Yadu *et al*., (1999), Mugdam (2000) after plumbagin and gossypol exposure, Rathore (1999) in *Poekilocerus pictus* with *Annona squamosa* seed extract treatment, Gupta and Rathore (2002) in *Poekilocerus pictus* after neem extract treatment, Kumar *et al.*, (2003) and Bhide *et al.*, (2004) in *Periplaneta americana* and *Musca domestica* after *Delonix regia* and *Cassia fistula* seed extract treatment respectively

due to destruction of fat bodies in the haemolymph which synthesizing the vitellogenin protein and other nutrients and their up take by developing oocytes by inter follicular cell spaces, pinocytosis and reverse pinocytosis were made in the phase of vitellogenesis but due to seed extract intoxication the process of vitellogenesis became arrested resulted into the production of large number of pathological oocytes which never attain such maturity which is required for oviposition hence *Annona squamosa* seed extract acts as an antiovipositional agent and after formulation should be synthesize in large amount for spraying in the fields to protect our valuable crops from pest insects.

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Fig. 1 : Electrophoretogram showing detection of negetively charged protein fractions in the ovaries of control and experimental groups of *Musca domestica* after *Annona squamosa* extract treatment

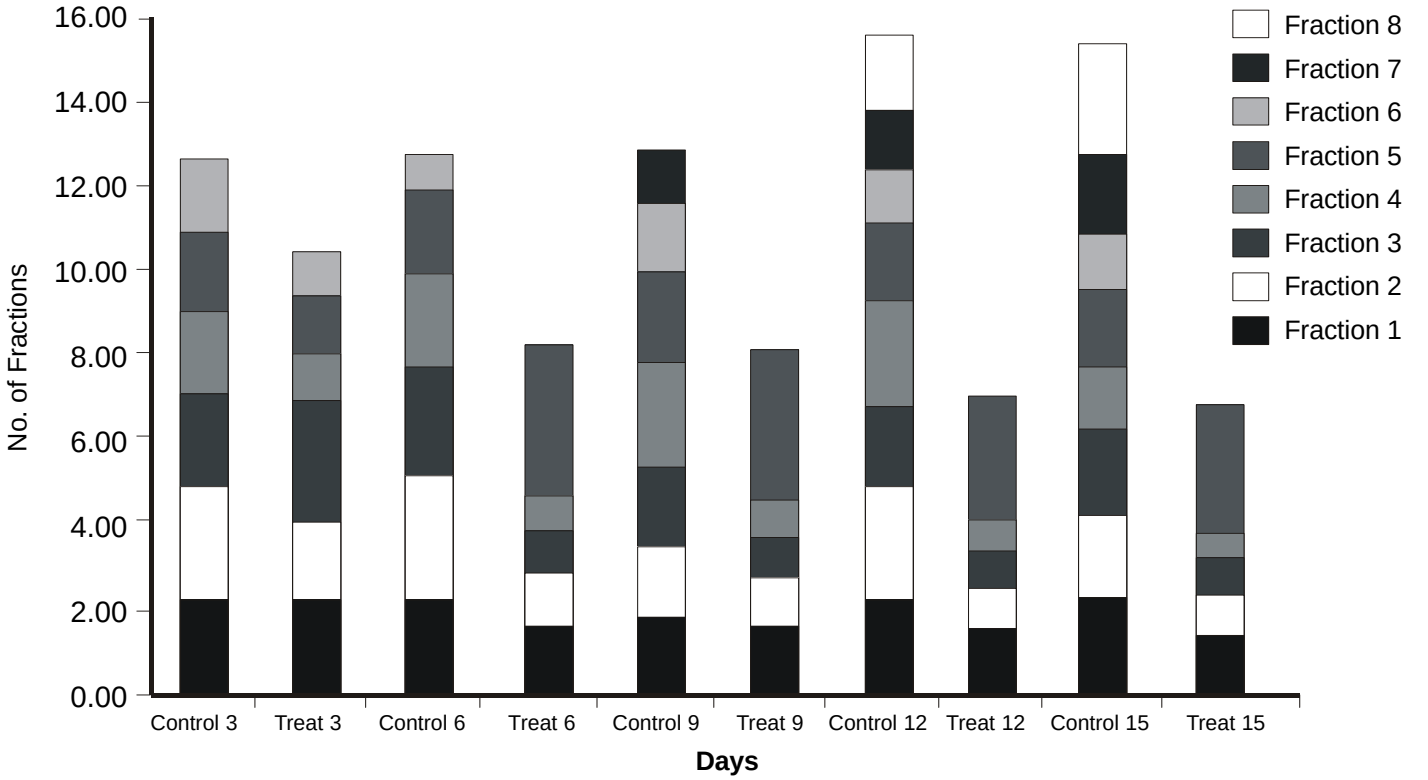

Fig. 2 : Electrophoretogram showing detection of positively charged protein fractions in the ovaries of control and experimental groups of *Musca domestica* after *Annoma squamosa* extract

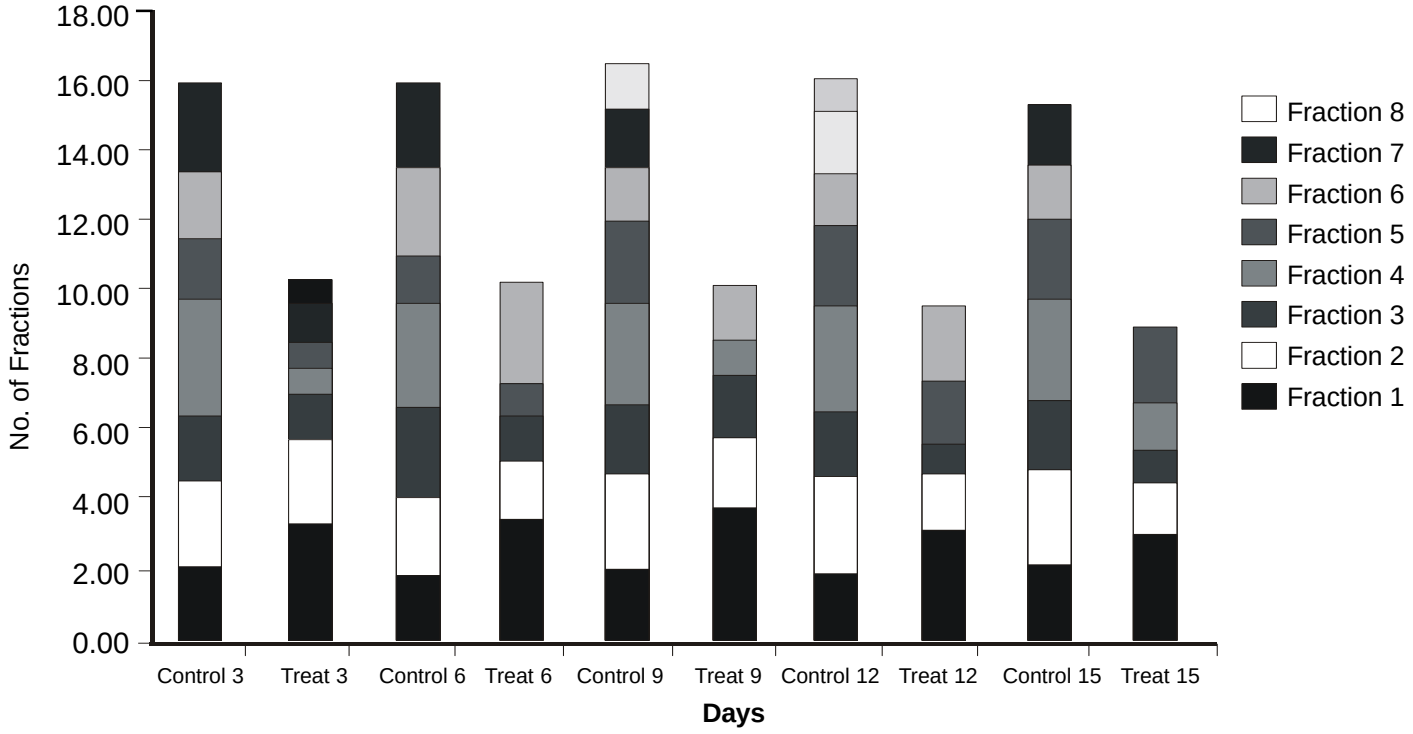

Fig. 3 : Electrophoretogram showing detection of positively charged lipid fractions in the ovaries of control and experimental groups of *Musca domestica* after *Annoma squamosa* extract treatment

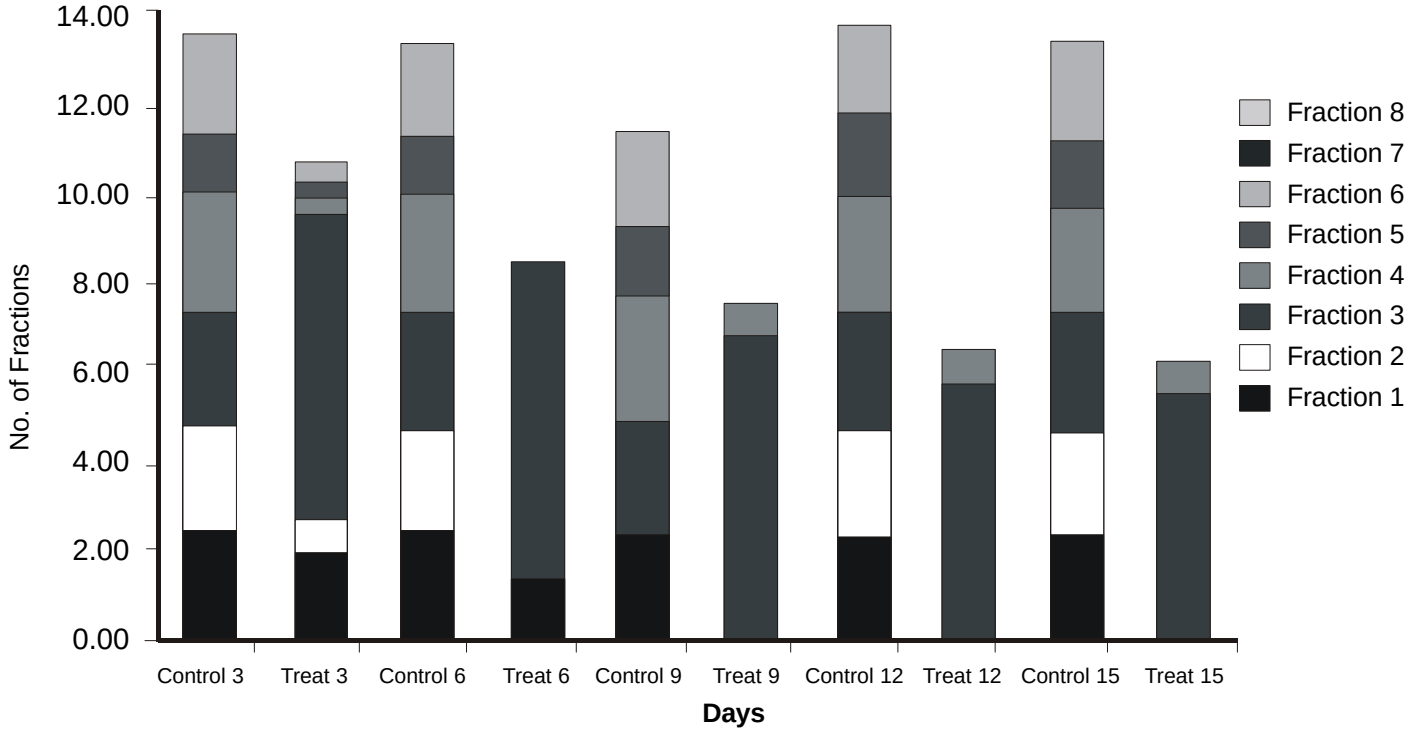

Fig. 4 : Electrophoretogram showing detection of positively charged lipid fractions in the ovaries of control and experimental groups of *Musca domestica* after *Annoma squamosa* extract treatment