Effects of Plant Extracts on Salivary Gland Chromosomes of House fly (*Musca domestica* L.)

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Abstract: Plant extracts are the best alternatives of chemical insecticides and can save the plant species as well as the world's environments. For releasing the plant materials as a botanical insecticide proper plant species and plant parts screening and identifying the mode of action on insect is essential. For this purpose, different plant parts of *Calotropis procera, Derris indica, Ipomoea quamoclit, Piper longum* and *Polygonum hydropiper* and salivary gland chromosomes of *Musca domestica* L. (Diptera: Muscidae) had selected for investigation. Dose mortality test result showed the intensity of activity in a descending order as *I. quamoclit* (911.83 ppm) > *P. hydropiper* (1205.47 ppm) > *C. procera* (5410.82 ppm) > *D. indica* (5519.30 ppm) > *P. longum* (10737.43 ppm) and in all the cases significant differences were found for dose differences. The test results demonstrated potential effects on salivary gland chromosomes where highly effective plant extracts showed more compact chromosomes than lower effective extracts *i.e.* the compactness of chromosomes depended upon the activities of the plant extracts.
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1. Introduction

Botanical insecticides are an interesting alternative for insect pest control but only a few from more than 2,50,000 plant species on our planet have been properly evaluated for this purpose. Moreover, insecticides from plant extracts were considered as the suitable alternatives of chemical insecticides for its pest specific and biodegradable nature (Periera and wohlgemuth, 1982). They are only alternatives that may be used in integrated pest management programs together with other available control measures. There are a lot of publications with lists of plants which have insecticidal properties. More than 500 plants grows or available in Bangladesh and have been reported to possess medicinal properties and enumerated in the literature of indigenous drugs (Ghani, 1998). In this proposition *Calotropis procera, Derris indica, Ipomoea quamoclit, Piper longum* and *Polygonum hydropiper* have been selected for investigation. The housefly *Musca domestica* was chosen for cyto-genetical studies because it is easy to rear, it has a short development time (about 12 days at 26^0c) and it has economic and medical significance. It was reported that housefly could transmit more than 20 humans and animal diseases (Hicking, 1974). Moreover, the strategy taken for housefly as a model system to investigate genetic materials might also be applied to other species (Wagoner et al., 1973). In addition, salivary gland chromosomes were investigated because it is a special type of chromosomes present in many larval tissue as well as some adult tissues of insects which belong to the family Diptera. These chromosomes are characterized by nuclei with gait chromosomes which are distinct and easily be observed. As a result, the effects of plant extracts were clearly identified. The increasing interest to observe the effects of plant extracts at chromosome level which may lead to identify exact plants part for use as insecticides for other insects were the main objective of this investigation.

2. Material and methods

In order to find useful compounds in the shortest possible time, careful selection of plant materials is obviously very important. By way of illustration, plants used in traditional medicine are more likely to provide pharmacologically active compounds (Huxtable, 1992), similarly folk use of toxic plants would be taken with desirable output. In this investigation, root bark of *C. procera*, seeds of *D. indica*, the whole plants of *I. quamoclit*, *P. longum,* and *P. hydropiper* were collected and only chloroform was selected to extract all the selected and collected plant materials separately. In addition, to carry on insecticidal test we have able to produce a consistent quality insect at an economical cost by rearing the housefly according to the methods described by Morgan (1980, 1981) and Morgan and Patterson (1975). For testing the insecticidal properties of the extracts 3rd instar larvae of the housefly *M. domstica* were selected. To test the efficacy of chloroform extracts of the five selected plants against cultured larvae of *M. domestica* same aged population were used and provided the foods as a unit of volume by

volume measurement. The food was prepared with 6.25gm of wheat bran and 0.5gm of milk powder (Red cow) and 12.75 ml of water as a total of 19.5 gm. For *C. procera* seed extract 500 mg was dissolved in 1 ml solvent (Chloroform) and mixed with the prepared food. To have a dose-effect to calculate toxicity by Probit analysis, 4 others successive doses were prepared and applied with 0.5 serial dilutions. The bioassay experiments were always done in 3 replications and a control treatment was also set simultaneously. So, the doses for *C. procera* were 26,641-, 12,820.5-, 6,410.25-, 3205.12 and 1,602.56 ppm in v/v state. In the same way for *D. Indica* the doses were 120820.5-, 6,410.25-, 3,205.12-, 1,602.56 and 801.28 ppm in v/v state. For *I. quamoclit* the doses in the same manner were 26,64-, 12,820.5-, 6,410.25-, 3,205.12 and 1,602.56 ppm in v/v state. For *P. longum* 300 mg of extract was added to 19.5g of food medium and thus the doses were 15,384.62-, 7,692.31-, 3,846.15-, 1,923.08 and 961.54 ppm in v/v state. For *P. hydropiper* the doses level were 12,820.5-, 6,410.25-, 3,205.12- and 1,602.56 ppm in v/v state. The recorded mortality of the larvae was corrected by using the Abbott's formula (Abbott, 1925):

$$pr = \frac{Po - pc}{100 - Pc} \times 100$$

Where,
Pr = Corrected mortality (%)
Po = Observed mortality (%)
Pc = Control mortality (%)
Then this percentage mortality was subjected to statistical analysis according to Finney (1947) and Busvine (1971).

A detailed protocol for making spread preparations of xenopus GV contents for the study of lampbrush chromosomes and other nuclear organelles is now well known to all. The technique which was used to study salivary gland chromosomes were the modified procedure from a laboratory procedure by Jannet et al., (2000) formerly of queen's university. Kingston, Ontario.

3. Results and discussion

These studies have pointed numerous plant species possessing potentiality for pest controlling properties under laboratory conditions, but the steps from the laboratory to the field elements have many contenders. Unfortunately, efficacy against pests is only one of a number of important criteria that need to be met for a plant extract or derivative to move successfully toward commercialization and use (Isman, 1995). In the present investigation the extracts of *C. procara, D. Indica, I. quamoclit, P. longum and p. hydropiper* were collected and biological activity of them were assessed to find out their potentiality for using in the future health and agriculture sectors. Dose mortality tests and tests for effect on chromosome have been done with the crude extracts. The data of all the five cases were analyzed and the results were illustrated in Table 1. and Table 2. as below:

Table 1. LD_{50} values and 95% confidence limits of dose mortality experiments of *1. quamocit, P. hydropiper, C. procera, D. indica* and *P. longum* crude extracts against *M. domestica* larvae with 24 h. of exposure

Plant organ	Time	LD_{50} value (ppm)	95% confidence limits	
			Upper	Lower
I. quamoclit	24h	911.83	54.02	15389.33
p. hydropiper	24h	1205.47	431.16	3370.36
C. Procera	24h	5410.82	2922.64	10017.29
D. indica	24h	5519.30	2586.41	11778.01
P. longam	24h	10737.43	5362.74	21498.79

Table 2. Regression equation and χ^2 value of dose mortality experiments of *1. quamocit, P. hydropiper, C. procera, D. indica* and *P. longum* crude extracts against *M. domestica* larvae with 24 h. of exposure

Plant organ	Regression equations	χ^2value (df)
I. quamoclit	Y = 3.91+0.36X	4.33 (3df)
p. hydropiper	Y = 1.85+1.02X	0.82(2df)
C. Procera	Y= 2.09+0.77X	5.23 (3df)
D. indica	Y =2.37+0.70X	0.78(3df)
P. longam	Y = 1.07+0.97X	1.00 (3df)

3.1 Dose-mortality assessment

The dose mortality results of crude extracts against larvae of *M. domestica* were found promising. All the chloroform extracts of selected five plant materials had been found strongly effective against the 3rd instar larvae of *M. domestica*.

The LD_{50} value for *C. procera* extract was 5410.82 ppm for 24h experiments, while the regression equations were as Y= 2.09 + 0.77 X; the χ^2 value was 5.23 for 3 degrees of freedom and the 95% confidence limits were 2922.64 to 10017.29 for 24h. The LD_{50} value for *D. indica* extract was 5519.30 ppm for 24h of exposures and the regression equations were Y= 2.37 + 0.70 X; the χ^2value was 0.78 for 3 degrees of freedom and the 95% confidence limits were 2586.41 to 11778.01 for 24h. The LD_{50} value for *I. quamoclit* extract was 911.83 ppm for 24h treatments where the regression equations were Y= 3.91 + 0.36 X; the χ^2 value was 4.33 for 3 degrees of freedom and the 95% confidence limits were 54.02 to 15389.33 for 24h. The LD_{50} value for *P. longum* extract was 10737.43 for 24h of exposures and the regression equation were Y= 1.07 + 0.97 X; the χ^2 value was 1.00 for 3 degree of freedom and the 95% confidence limits were 5362.74 to 21498.79 ppm for 24h. The LD_{50} value for *P. hydropiper* extract was 1205.47 ppm for 24h treatments while the regression equation were Y= 1.85 + 1.02 X; the χ^2 value was

0.82 for 2 degrees of freedom and the 95% confidence limits were 431.16 to 3370.36 ppm for 24h. According to the intensity of activity of the plant extracts against 3rd instar larvae the plant could be arranged in a descending order as *I. quamoclit* (911.83 ppm) > *P. hydropiper* (1205.47 ppm)> *C. procera* (5410.82 ppm) > *D. indica* (5519.30 ppm) > *P. longum* (10737.43 ppm) and in all the observations significant differences were found in case of dose differences. A number of investigators isolated, identified and screened chemical compounds from leaves and seeds of many botanical families for insect feeding deterrence and growth inhibition as toxicant (Jacobson et al., 1975; Bernays and Chapman, 1977; Doskotch et al., 1977; Carpenter et al., 1979; Warthen, 1979; Jurd and Manners, 1980; Menn, 1980; Ho et al., 1995). From the academic point of view, plants were represented as a vast storehouse of potential and useful natural products and many laboratories worldwide have screened thousands of species of higher plants for pharmaceuticals and pest control products (Van Beek and Breteler, 1993; Gonzales-Coloma et al., 1994a, b; Addor, 1995; Cornelius et al., 1995; Assabgui et al., 1997; Blaske and Hertel, 2001). However, potentials of the test plants were being contributed here mainly based on dose mortality and efficacy on insect chromosomes.

3.2 Salivary gland chromosome observation

In this investigation we observed the effects of plant extracts on salivary gland chromosomes of 3rd instar larva of *M. domestica* and tried to identify the changes at chromosome level demonstrated by death and live larva after the treatment. The effects induced by botanicals on the polytene chromosomes of salivary gland of death 3rd instar larva were both physiological and structural in nature. Among the physiological effects, the most common was chromosome stickiness; often the chromosomes completely lost their individuality and appeared clumped. The structural effects were of various kinds. For example, some chromosomes showed with unequal lengths and weak points, some chromosome showed breakage of chromosomal arms. The effects of the test material were also observed in the breakage and fusion of the chromosomes. Occasionally, due to the absence of fusion, the broken pieces of chromosome were stayed independent. The structure of chromosomes treated with different concentrations of effective extracts were dignified the potentials towards their further use for the control of crop pests, since the chromosomes were being destroyed by the biodegradable properties of plant and caused death of the insects.

The 3rd instar larva of *M. domestica* which lived after the treatment with test material showed compact chromosomes then the dead larva. Moreover, the compactness of chromosomes of live 3rd instar larvae depended on the efficacy of plant extracts *i.e.* highly effective plant extracts showed more compact chromosomes than lower effective extract. On the other hand, in case of dead 3rd instar larva opposite scenario were observed *i.e.* highly effective extracts showed less compact chromosomes than the lower effective extracts. The results were illustrated in Figure 2a., 2b., 3a. and Figure 3b. where the chromosomes represented the compactness depending upon the activity of plant extracts on death and lived 3rd intar larva compare to the normal structure presented on Figure 1.

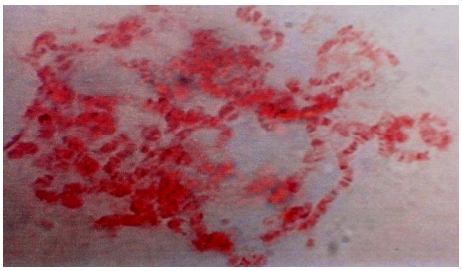
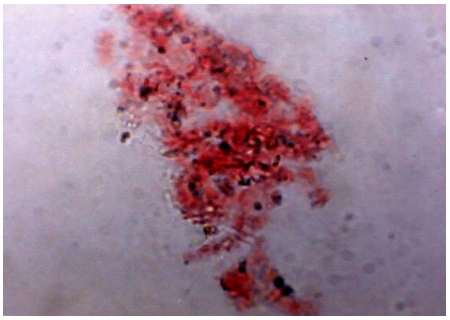
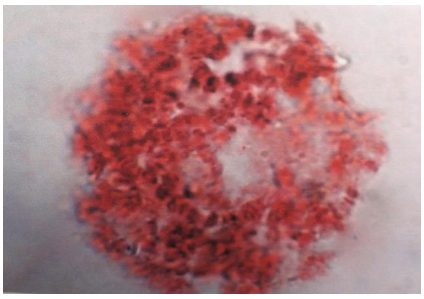


Figure 1. Polytene chromosomes of *Musca domestica* L.

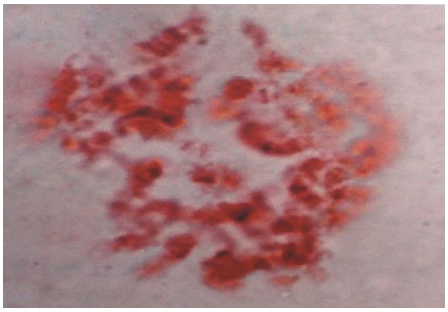
The genus *Musca* possess domestica complex, along with several species. This complex contains several integrating forms which differ according to their bio-geographical distribution, morphological characteristics, chromosomal data and hybridization tests (Milani, 1975). Previously, Milani (1967) reported that several traits such as front width and abdominal color pattern were used for taxonomical purposes but they were modified by developmental conditions and by their genetic back grounds and thus they could not be used as unconditional criteria for species identification. So, he suggested that domestica complex might be an important criterion for species identification. In addition, previous reports on the cytology of *M. domestica* had represented the basic mitotic karyotype (Ramade, 1961; Boyes, 1967; Milani, 1967) and mapping of polytene chromosomes both in the salivary gland of larvae (Sharma et al., 1979) and in the bristle-forming cells of thoracic epidermis of the pupae (Kaur and Kaur, 1982).



a) Effect of *I. quamoclit*, Dose-1602.56 ppm on 3rd instar larvae, alive.



b) Effect of *P. hydropiper,* Dose-1602.56 ppm on 3rd instar larvae, alive.



c) Effect of *C. procera*, Dose-1602.56 ppm on 3rd instar larvae, alive.

Figure 2a. Effect of plant extracts (*I. quamoclit, P. hydropiper, C. procera)* on salivary gland chromosomes on alive 3rd instar lavae of *Musca domestica* L.

Unfortunately, none of the reports had indicated the full potentiality of cytogenetic analysis for these chromosomes. Later on, the main features of polytene chromosome were found in a standard European house fly culture, *M. domestica* by Sacca, 1967.

After this investigation, the polytene chromosomes of *M. domestica* were characterized by a clear banding pattern with high levels of ectopic pairing, break points and fragmentation (Vecchi and Rubini, 1973; Sharma et al., 1979). In the salivary gland chromosomes of *M. domestica* there were no evidence of the presence of a chromocentre binding together with non-homologous chromosomes at their centric hetro-chromatin region like *Drosophila melanogaster* (Leffevre, 1976) and some species of chironomus (Sorsa, 1988); however, some ectopic pairing may exist. These important findings lead us for studying chromosomal changes due to the effects of botanicals. Moreover, the plant extracts were used because they have some phytochemical properties which effected directly to the chromosomes. The present study confirmed the results of the earlier studies (Puttaraju, 1988) which showed that thio-TEPA is one of the best chemicals to induce chromosomal mutation in the mosquito *Culex P. fatigans*. The different types of chromosomal abnormalities noted in this study were almost similar to those of *Aedes aegypti* and *Aedes asbopictus* (Puttaraju, 1988). However, the other aberrations such as the chromosome stickiness, clumping of chromosomes, the occurrence of centric and terminal breaks recorded in the present study had also been reported by Grover et al., (1973) in *Culex P. fatigans* due to effect of Apholate, Metapa and Hempa independently. The occurrence of laggards, stickiness, acentric and dicentric bridges and the formation of ring chromosomes in the present study had also been recorded by Rai (1963), Tadano and Kitzmiller (1969) and Grover et al., (1973) using other chemicals.

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