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Antifeedant, larvicidal and pupicidal activities of *Madhuca longifolia* (Family: Sapotaceae) plant leaf ethyl acetate fractions against *Spodoptera litura* insect pest

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Abstract
The antifeedant and larvicidal activities of *Madhuca longifolia* leaf (ethyl acetate) extract fractions were evaluated at different concentrations against larvae of *Spodoptera litura.* Antifeedant activity values for fraction I at concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm were 41.10%, 30.54%, 23.92% and 14.14%; for fraction II is 55.47%, 32.46%, 23.64%, and 14.67%; for fraction III is 78.23%, 48.54%, 33.28%, and 21.04%; for fraction IV is 56.10%, 38.69%, 25.97%, and 16.27%; for fraction V is 47.78%, 30.74%, 21.27%, and 14.12% respectively. The larvicidal activity of *Madhuca longifolia* fractions against larvae of *Spodoptera litura* were values recorded in fraction I at concentrations 500ppm, 250ppm, 125ppm, and 62.5ppm were 36.52%, 23.73%, 17.56%, 13.17%, for fraction II is 50.68%, 31.37%, 20.44%, 15.54%, for fraction III is 71.13%, 51.57%, 39.71%, 23.54%; for fraction IV is 51.55%, 37.41%, 23.14%, 14.67%; for fraction V is 31.48%, 26.51%, 19.21%, 11.12%. The antifeedant and larvicidal activities for maximum values recorded in *Madhuca longifolia* leaf (ethyl acetate) extract fraction III against larvae of *Spodoptera litura* respectively.

Keywords: Antifeedant activity, larvicidal activity, pupicidal activity, *Spodoptera litura*, *Madhuca longifolia*

Introduction
Agriculture is the backbone of the Indian economy nearly 80% of the rural areas of Indian villages is depending on agriculture. However, India is the largest producer, consumer and exporter of several agricultural products. It is third ranks in farm and agriculture outputs in globally. In addition to 10 percent of agricultural constitutes export and the fourth largest exporter of principal commodity in the world. Outlook of agricultural and rural development is to a large extent influenced by the rapidly increasing food demand of 2.5 billion people expected to swell the world population by 2020 respectively [1]. Even though insect pest's causes agricultural crop loss of 120 billion US$ dollars worldwide and reduce the yield by 10-30. These insect pests have been controlled with the help of synthetic insecticides over the past fifty years [2].

Synthetic insecticides are used widely for the control of various insect pests because they can be applied whenever and wherever needed, economical and most important thing is the reliability of control methods Many neurotoxic insecticides are damaging the environment and pose a hazard to public health via food residues, ground water contamination and accidental exposure Frequent exposure of *Spodoptera litura* to synthetic pesticides resulted in the development of resistance, as a consequence of which an unexpected population outbreak was observed in the major cotton and groundnut growing regions [3, 4]. Furthermore, *S. litura* has been reported to develop high resistance against some variety of chemical pesticides, including organophosphate, carbamate, pyrethroids and few newer synthetic insecticides Dreadful facts of chemical pesticides demand for Eco friendly and environmentally safer alternate methods for crop protection [4].

Chemical control is an effective strategy used extensively in daily life activity against insects. At the present investigation known about a variety of new insect control agents have been developed, or are being developed, which may fit a variety of insect pest management needs Integrated Pest Management (IPM) has revived the interest in plant-based insecticides which offer an ecologically and economically viable alternative in managing pests below the economic injury levels respectively [5, 6]. Hence, more studies pertaining to the use of plant based phytopesticides should be emphasized, especially those related to the control of insect pests. The aim of this research was to evaluate the potentiality of leaf extracts and their fractions of *Madhuca longifolia* owards the control of selected agricultural field pest, *Spodoptera litura*.

Materials and Mathods
Plant collection and preparation of ethyl acetate crude extractions
The leaves of *Madhuca longifolia* were collected and Plant specimen was identified by from arignar Anna Government arts College campus, Namakkal District, Tamil Nadu, the plant materials were thoroughly washed with tap water and shade dried under room temperature (27.0± 2^0C and 75 ± 5% RH). After complete drying the plant materials were powdered using electric blender and sieved through a kitchen strainer. 1000g of plant powder was extracted by Soxhlet extraction methods with ethyl acetate and filtered through Whatman's No. 1 filter paper. The solvent from the crude extract were evaporated to air dried at room temperature. The crude extracts were collected in clean borosil vials and stored in the refrigerator at 4^0C for subsequent bioassay against *Spodoptera litura*.

Field collections and rearing of *Spodoptera litura*
Different larval stages of egg masses and larvae of *S. litura* were collected from caster fields at ladhuvadi near Arignar Anna Govt. Arts College Namakkal, Tamil Nadu, India. The collected eggs mass and larvae of *S. litura* were reared on

leaves of castor (*Ricinus communis*) till they attain the pupal stage under laboratory conditions (27 ± 2ºC and 75 ± 5% RH). Sterilized soil will be provided for pupation. After pupation, the pupae were collected from soil and placed inside the oviposition samplers. After adult emergence, cotton soaked with 10% sugar solution with a few drops of multivitamins was kept inside oviposition cage for adult feeding. After hatching newly emerged larvae will be providing for feeding of castor leaf for *S. litura*. Generally, healthy and uniform sized fourth instar larvae, pupae, meat and eggs were used for the experiments and the cultures were maintained throughout the study period.

Antifeedant activity

Antifeedant activity of castor leaf (for *S. litura*) discs of 3 cm diameter was punched using a cork borer and dipped in 62.5, 125, 250 and 500ppm for *Madhuca longifolia* plant ethyl acetate fractions separately and air dried for 5 minutes. After air drying, treated leaf discs were kept inside the Petri dishes separately containing wet filter paper to avoid drying of the leaf disc and single 2hrs post starved fourth instar larva of *S. litura* was introduced on each treated leaf disc. One treatment with respective solvent alone was used as positive control and one treatment with Neema Zal was considered as negative control. Five replications were maintained for each treatment. A progressive consumption of leaf area by the larva in 24 hrs [10]. period was recorded in control and treatments using a leaf area meter. Leaf area consumed in plant fraction treatments was corrected from the control. The percentage of antifeedant index was calculated using the formula of (Ben Jannet).

$$\text{Percentage of antifeedant activity} = \frac{\text{Control} - \text{Treated}}{\text{Control} + \text{Treated}} \text{ X100}$$

Larvicidal activity

For the evaluation of larvicidal activity of the *Madhuca longifolia* plant ethyl acetate extract and fractions were preparation of different concentrations of 250, 500, 750 and 1000 ppm concentrations for fractions were tested against the freshly moulted (0-6h) fourth instar larvae of *S. litura*. The branches bearing cotton leaves were tied with wet cotton plug to avoid early drying and placed in a plastic trough. In each concentration 10 pre-starved (2hrs) fourth instar larvae were introduced individually and covered with muslin cloth. One treatment with respective solvent alone was used as positive control and one treatment with Neemazal was considered as negative control. Five replicates were maintained for each concentration, each replicate comprised of 25 numbers of larvae. After 24h of the exposure period, the number of dead larvae was recorded from each replicate at all the concentrations and the percentage of larval mortality was calculated using Abbott's formula (Abbott 1925). The larvae with no symptom of a movement or shake while touching with soft camel brush were considered as dead.

$$\text{Percentage of larval mortality} = \frac{\%\text{MT} - \%\text{MC}}{\%\text{MT} - \%\text{MC}} \text{ X100}$$

The LC_{50} and LC_{95} values were calculated by probit analysis using Microsoft Excel 2007 software (Finney, 1971).

Pupalcidal activity

Two days old pupae were selected for pupalcidal activity. Ten newly pupated *S. litura* 2days old were separated and dipped in various concentrations (as mentioned in antifeedant activity) for 5 minutes. Five replicates were maintained (n=50). Control was used as acetone. They were transferred to Petri plates provided with cotton bed to avoid any physical damage at the time of pupal movement. Then the Petri plates were kept undisturbed until the end of pupal period. After the exposure period the number of adults emerged from each concentration was noted in each replication. Pupicidal activity was calculated method prescribed by Abbott (1925). During the developmental period, pupal duration, fecundity and hatchability were recorded.

$$\text{Percentage of pupal mortality} = \frac{\%\text{MT} - \%\text{MC}}{\%\text{MT} - \%\text{MC}} \text{ X100}$$

The LC_{50} and LC_{95} values were calculated by probit analysis using Microsoft Excel 2007 software (Finney, 1971).

Thin Layer Chromatography (TLC)

Thin layer chromatography was done on pre-coated plates Silica Gel G (0.2mm thick; Merck, India) were trimmed with strips and the position of the origin marked by a straight line. Ethyl acetate extracts of *Madhuca longifolia* and their fractions were dissolved and were spotted on the plate with fine capillary tube at the height of 0.8-1.0cm from the base. In the present study, different solvent mixtures, petroleum ether and ethyl acetate, were used for developing the TLC plates to get better results. After putting the plates in solvent system, the appropriate distance moved by the solvent was measured to find the retention factor. The Retention Factor (Rf) values of all compounds isolated were calculated.

$$\text{Rf} = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the slvent front}}$$

Column Chromatography (CC)

The ethyl acetate extracts of *Madhuca longifolia* for the isolation of fraction used by Column chromatography. Silica gel (100 - 200 mesh) was packed in a column with petroleum ether solvent using the wet slurry method. This involves preparing a solution of silica gel, with petroleum ether in this case, in a beaker and subsequently adding this unto the column till it is about three fourths filled. The solution was stirred for dispersal and quickly added to the column before the gel settles. This method was used to prevent the trapping of air bubbles. A ball of wool was pushed into the column to settle atop the packed silica gel. For the elucidation of components, the polarity of the solvent (mobile phase) was increased using a combination of petroleum ether, petroleum ether: ethyl acetate (7:3, 5:5, 3:7, 2:8 totally collected by different fractions. TLC plate was run on every column fraction and to calculate Rf values. Fractions in which similar spots appeared were collected in one pool. The fractions with similar Rf values were pooled and isolates designated as 5 fractions for *Madhuca longifolia* were obtained. All the isolated fractions were stored in solid form for further experimentation.

Determination of lethal concentration

Lethal concentration (LC_{50} and LC_{95}) represents the concentration of the test material that caused 50% mortality of the test organisms within the specified period of exposure, and it was determined by exposing various development stages of the *S. litura* to different concentration of the extract. Based on the mortality of the test organisms recorded in the bioassays, LC_{50} and LC_{95} was calculated along with their fiducial limits at 95% confidence level by probit analysis using Microsoft Excel 2007 software (Finney, 1971).

Results and Discussions

The view of result of Antifeedant, larvicidal and pupicidal activities for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

In the present study *Madhuca longifolia* was tested against the fourth instar larvae of *Spodoptera litura*. Botanicals are a rich source of organic chemicals on earth. Already 10,000 secondary metabolites have been chemically identified. In nature many plants have unpalatable substances like high content of phenols, alkaloids, flavonoids, terpenes, quinine, coumarin etc., which play a defensive role against particularly agricultural insect pests [6, 7, 8]. maximum antifeedant activity in Fraction I values recorded in *Spodoptera litura* at 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of antifeedant values in 41.10±6.24, 30.54±5.14, 23.92±4.80, 14.14±4.60 % and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 607.49 (476.25-936.78), 1375.71(1012-2367.52)0.339 respectively.

Following antifeedant activity in fraction II of *Madhuca longifolia* was tested against the fourth instar larvae of *Spodoptera litura* and the data pertaining to the experiments are shown in table 2 and figure 2. Perusal of the data clearly indicated that maximum antifeedant activity in fraction II was recorded in *Spodoptera litura at* 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality in 55.47±2.99, 32.46±3.87, 23.64±2.56, 14.67±5.23 and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values 438.53 (374.47-541.06), 943.96(775.36-1256.88)0.684 respectively.

Furthermore, another possible action would be filling of gaps and micropylar region on the surface of the egg shell by the application of plant extracts. In this investigation an attempt was made to study the effect of different concentration of the extracts [8, 9, 10]. Results clearly showed that the ovicidal activity was recorded significantly with the highest concentrations, because, the more the concentration, the more phytochemicals present in it. Nair and Thomas (2000) reported that the methanol extract from *Acorus calamus* exhibited ovicidal activity against *Bactrocera cucurbitae*. Different solvent extracts of *A. monophylla* leaf showed ovicidal activity against *H. armigera*.

Larvicidal activity for different concentration of various solvent fraction of medicinal plant leaf against larvae of *Spodoptera litura* recorded. These findings are in agreement with the earlier reports. Following fraction III values in 78.23±2.14, 48.54±1.13, 33.28±2.90 and 21.04±5.36% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 268.53 (230.97-310.53), 636.40 (552.26-767.48)0.692 and fraction IV larval mortality values recorded in 56.10±2.41, 38.69±2.78, 25.97±3.47 and 16.27±4.18 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 414.65 (351.40-515.65), 947.29 (771.44-1283.29)0.394. Following fraction V values in 47.78±5.24, 30.74±4.12, 21.27±6.10, 14.12±2.12 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values in 511.41 (425.25-672.86), 1099.52 (870.75-1577.73)0.670 respectively.

Larvicidal activity for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura* and percentage of larval mortality values in 36.52±2.17, 23.73±4.72, 17.56±1.76, 13.17±3.71 % and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 694.46 (538.34-1106.61), 1453.13(1061.87-2547.47) 0.246 respectively. Fraction II of *Madhuca longifolia* was tested against the fourth instar larvae of *Spodoptera litura* and shown in table 2 and figure 2. different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality in 50.68±5.49, 31.37±4.89, 20.44±1.76, 15.54±3.27 and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values 483.28 (406.55-618.49), 1036.62(833.30-1441.49)0.297 respectively. Following fraction III values in 71.13±9.44, 51.57±2.27, 39.71±3.17 23.54±1.76 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 268.39 (220.48-322.92), 748.71(624.50-969.76)3.292 and fraction IV larval mortality values recorded in 51.55±6.74, 37.41±2.34, 23.14±2.71, 14.67±2.27 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 455.03 (383.29-578.20), 1009.68 (812.92-1400.05)2.693. Following fraction V values in 31.48±2.34, 26.51±3.22, 19.21±3.29, 11.12±2.45 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values in 798.91(584.80-1560.48)1713.21(1173.27-3724.44)2.929 respectively. Larvicidal activity for different concentration of ethyl acetate fraction plant leaf against larvae of *Spodoptera litura* and presented in sixth fraction against fourth instar larvae of *S. litura* at 1000ppm concentration compared with other fractions and positive control [11, 12, 13].

Pupiciadal assays for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura* and different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of pupal mortality values in 31.42±3.45, 24.43±3.76, 15.37±1.85, 10.11±3.65% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 763.08 (580.13-1290.59), 1550.61(111.97-2875.77) 0.382respectively. Following pupicidal activity in fraction II, experiments are shown in table 2 and figure 2. Perusal of the data clearly indicated that maximum pupae in fraction II was recorded in *Spodoptera litura at* 24 hrs the percentage of pupal mortality in 52.45±3.45, 30.13±6.53, 21.42±3.75, 15.43±6.36 and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values 471.37 (398.65-595.50), 107.58 (816.25-1378.59)0.931 respectively. Following fraction III values in 78.41±3.76, 57.45±4.32, 31.32±6.31, 20.14±2.34 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 254.72 (115.57-449.66), 605.44(424.68-1569.88)0.125 and fraction IV pupal mortality values recorded in 55.43±5.23, 38.71±6.10, 24.65±4.31, 18.31±2.47 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values 421.37 (354.81-531.01) 977.50 (788.82-1348.81)0.569. Following fraction V values in 37.43±6.55, 20.37±3.21, 17.74±3.45, 10.47±4.65 and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values in 666.27 (531.28-977.65), 1334.28 (1009.67-2128.66)0.614 respectively. Methanol extract, its fraction and isolated compound furocoumarin and quinolone alkaloid from *Ruta chalepensis* exhibited maximum larvicidal activity with the LC_{50} values of 2.42, 0.89, 1.59 and 1.21 mg/ml for larvicidal activity against *S. littoralis* [14, 15, 17].

Table 1: Antifeedant assays for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

Fractions	Con. (ppm)	Percentage of antifeedant (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2
Fraction- 1	500	41.10±6.24	607.49 (476.25-936.78)	1375.71 (1012-2367.52)	0.339
	250	30.54±5.14			
	125	23.92±4.80			
	62.5	14.14±4.60			
Fraction- 1I	500	55.47±2.99	438.53 (374.47-541.06)	943.96 (775.36-1256.88)	0.684
	250	32.46±3.87			
	125	23.64±2.56			
	62.5	14.67±5.23			
Fraction- 1II	500	78.23±2.14	268.53 (230.97-310.53)	636.40 (552.26-767.48)	0.692
	250	48.54±1.13			
	125	33.28±2.90			
	62.5	21.04±5.36			
Fraction- 1V	500	56.10±2.41	414.65 (351.40-515.65)	947.29 (771.44-1283.29)	0.394
	250	38.69±2.78			
	125	25.97±3.47			
	62.5	16.27±4.18			
Fraction- V	500	47.78±5.24	511.41 (425.25-672.86)	1099.52 (870.75-1577.73)	0.670
	250	30.74±4.12			
	125	21.27±6.10			
	62.5	14.12±2.12			

The value represents mean ± SD of five replications. LC_{50} = Lethal Concentration brings out 50% Mortality and LC90 = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit

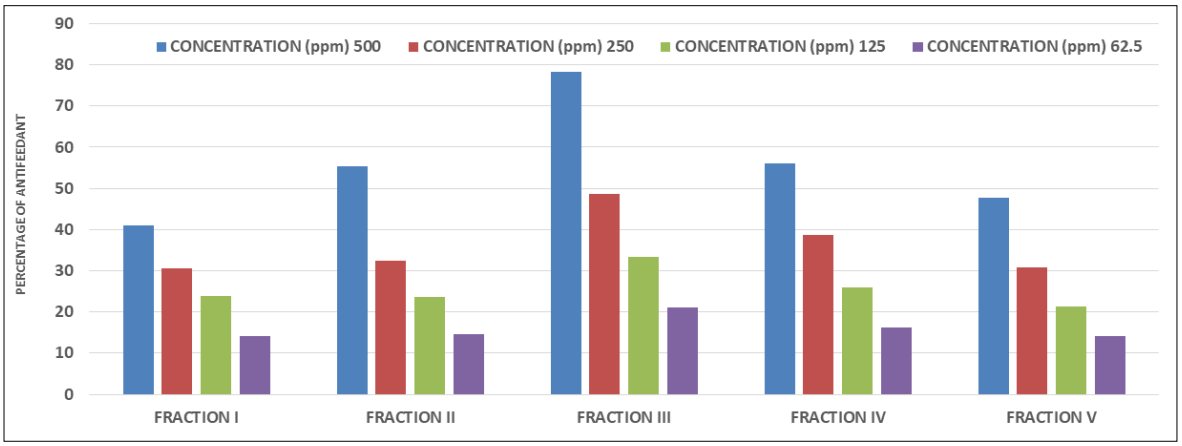

Fig 1: Antifeedant assays for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

Table 2: Larviciadal assays for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

Fractions	Con. (ppm)	Percentage of larval mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2
Fraction- 1	500	36.52±2.17	694.46 (538.34-1106.61)	1453.13 (1061.87-2547.47)	0.246
	250	23.73±4.72			
	125	17.56±1.76			
	62.5	13.17±3.71			
Fraction- 1I	500	50.68±5.49	483.28 (406.55-618.49)	1036.62 (833.30-1441.49)	0.297
	250	31.37±4.89			
	125	20.44±1.76			
	62.5	15.54±3.27			
Fraction- 1II	500	71.13±9.44	268.39 (220.48-322.92)	748.71 (624.50-969.76)	3.292
	250	51.57±2.27			
	125	39.71±3.17			
	62.5	23.54±1.76			
Fraction- 1V	500	51.55±6.74	455.03 (383.29-578.20)	1009.68 (812.92-1400.05)	2.693
	250	37.41±2.34			
	125	23.14±2.71			
	62.5	14.67±2.27			

Fraction- V	500	31.48±2.34	798.91 (584.80-1560.48)	1713.21 (1173.27-3724.44)	2.929
	250	26.51±3.22			
	125	19.21±3.29			
	62.5	11.12±2.45			

The value represents mean ± SD of five replications. LC_{50} = Lethal Concentration brings out 50% Mortality and LC90 = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit

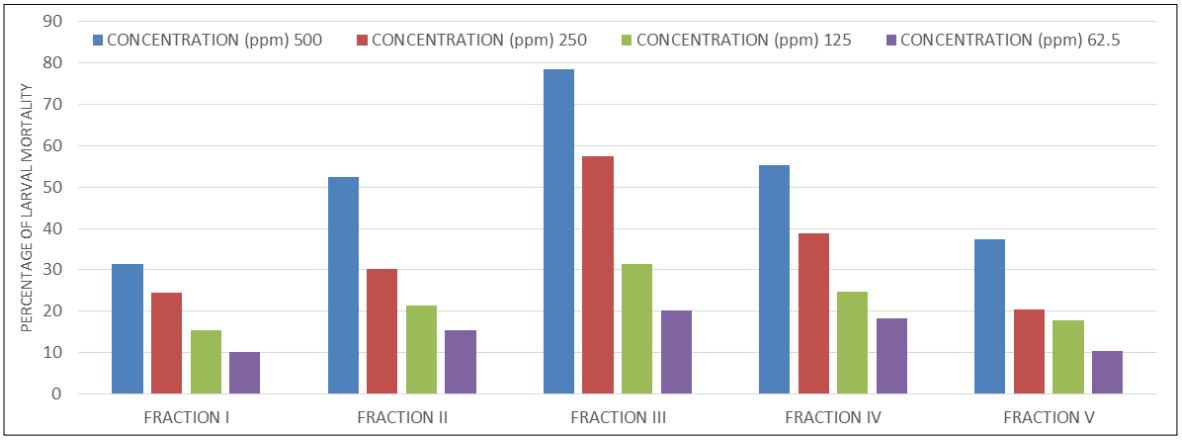

Fig 2: Larviciadal assays for different concentration of ethyl acetate fraction of *madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

Table 3: Pupiciadal assays for different concentration of ethyl acetate fraction of *Madhuca Longifolia* plant leaf against larvae of *Spodoptera litura*

Fractions	Con. (ppm)	Percentage of larval mortality (%)	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2
Fraction- 1	500	31.42±3.45	763.08 (580.13-1290.59)	1550.61 (111.97-2875.77)	0.382
	250	24.43±3.76			
	125	15.37±1.85			
	62.5	10.11±3.65			
Fraction- 1I	500	52.45±3.45	471.37 (398.65-595.50)	107.58 (816.25-1378.59)	0.931
	250	30.13±6.53			
	125	21.42±3.75			
	62.5	15.43±6.36			
Fraction- 1II	500	78.41±3.76	254.72 (115.57-449.66)	605.44 (424.68-1569.88)	0.125
	250	57.45±4.32			
	125	31.32±6.31			
	62.5	20.14±2.34			
Fraction- 1V	500	55.43±5.23	421.37 (354.81-531.01)	977.50 (788.82-1348.81)	0.569
	250	38.71±6.10			
	125	24.65±4.31			
	62.5	18.31±2.47			
Fraction- V	500	37.43±6.55	666.27 (531.28-977.65)	1334.28 (1009.67-2128.66)	0.614
	250	20.37±3.21			
	125	17.74±3.45			
	62.5	10.47±4.65			

The value represents mean ± SD of five replications. LC_{50} = Lethal Concentration brings out 50% Mortality and LC90 = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit

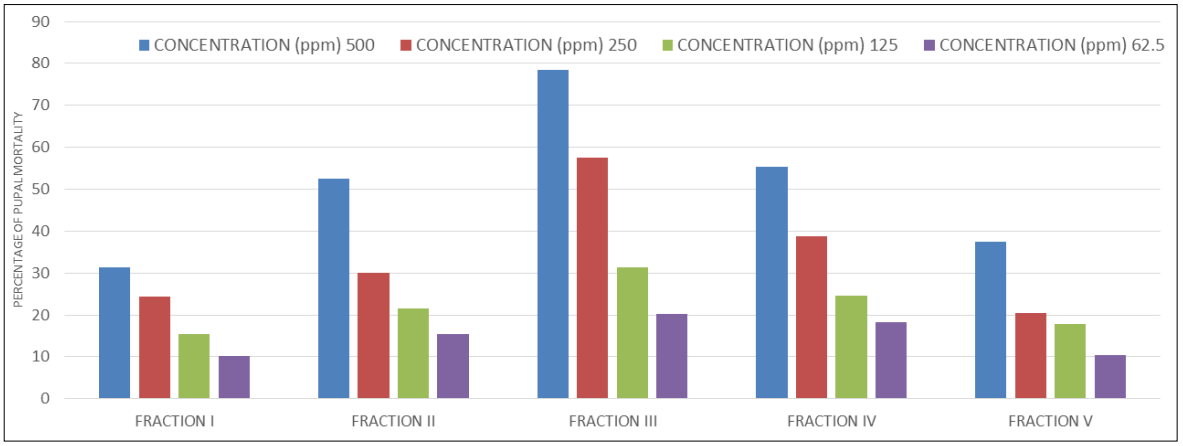

Fig 3: Pupiciadal assays for different concentration of ethyl acetate fraction of *Madhuca longifolia* plant leaf against larvae of *Spodoptera litura*

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