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BIOASSAY OF *FICUS SYCOMORUS* LEAF EXTRACT ON FALL ARMY WORM, *SPODOPTERA FRUGIPERDA* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

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SUMMARY

Maize (*Zea mays* L.) is an important annual cereal crop of the world belonging to the order Poales, family Poaceae, genus *Zea* and species *Zea mays*. Pests and diseases are the major constrains of agriculture which cause great loss of produce both at field and storage leading to food shortage. The fall armyworm (FAW) (*Spodoptera frugiperda*) is an insect from the Order Lepidoptera Family Noctuidae, Genus *Spodoptera* and Species *frugiperda.* The insect poses a great threat to food production due its devastating nature leading to loss of yield and quality of crops especially maize. The aim of the research was to determine the lethal effect of *Ficus sycomorus* leaf extracts on *Spodoptera frugiperda* larvae. The experiments were conducted at the Pathology Laboratory, Department of Plant Biology, Bayero University Kano. A complete randomized design was used to carry out the bioassay with three different fractions from N- Hexane, N- Butanol and Ethyl acetate at three concentrations, 200 mg/ml, 300 mg/ml and 500 mg/ml, a positive and negative controls that is Lambda cyhalothrin 2 ml/L and distilled water respectively, with three replications. It was found that highest activities of 70 to 100% mortalities were recorded on larvae treated with N- hexane fractions within four days after treatment, followed by N-Butanol fractions, while ethyl acetated fractions had the least activities. *Ficus sycomorus* was observed to be a good candidate for use as organic pesticide. Isolation and testing different active compounds of *Ficus sycomorus* on the insect is therefore recommended.

Key words: Bioassay, *Ficus sycomorus*, maize, Leaf extract, *Spodoptera frugiperda.*

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), the fall army worm is one of the economically important insect pests that seriously damage maize. Is an indigenous pest to North and South America and an invasive to Africa (6). The insect prefers maize and impact most of its damage to the crop, although it attacks over 80 crops including sorghum, millet and rice. In Africa it has caused huge losses to staple cereals, especially maize and sorghum, affecting food security and trade. It has the potential to cause 100% yield loss in the absence of control measures. Damage to maize alone was estimated to be between USD$ 2.5 - 6.2 billion per year in twelve countries (1). In view of the human health and environmental hazard posed by chemical pesticide and the ability of the pest to develop resistance to synthetic pesticides, therefore, it is imperative to resort to botanical and biological controls which are safer to human, animals, natural enemies and environment. *Ficus sycomorus* locally known as Baure in Hausa, is a tree that belongs to the family

Moraceae. Is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m, occasionally buttressed. Bark on young stems pale green with a soft powdery covering; on older stems, grey-green, fairly smooth, with scattered grey scales and pale brown patches where scales have fallen off. Slash pale pink with heavy latex flow. Leaves are broadly obovate or elliptic, base (sub)-cordate, apex rounded or obtuse, margin entire or slightly repand -dentate, 2.5-13 (max. 21) x 2-10 (max. 16) cm, scabrous above, petiole 1-5 cm, 5-7 pairs of yellow lateral veins, lowest pair originating at the leaf base. Flowers are unisexual, cyclic and greenish (7). *Ficus sycomorus* is used as food, fodder**,** medicine, timber, fuel, fibre, shade or shelter, erosion control, soil reclamation, intercropping and as an ornamental tree (7).The Objective of the study was to extract the leaves of *Ficus sycomorus* in order to determine its effects *in vitro* on the larvae of *Spodoptera frugiferda*.

MATERIALS AND METHODS

Plant samples collection

The *Ficus sycomorus* leaves were obtained from different farms in Ungoggo local government area of Kano and Qafur in Katsina states, during the months of November, December 2018 and January 2019. The plant materials were authenticated at the herbarium of Plant Biology Department, Bayero University Kano.

Identification and collection of *Spodoptera frugiperda* larvae

The larvae were identified by its features such as inverted Y shape mark on its head, four trapezoid dots on body segments and four dots forming a square shape on second to the last body segment as described by (4). Healthy larvae were collected by hand picking from different maize farms in Kano which were not spread with chemical, for rearing in the laboratory.

Extraction of plant leaves using tissue maceration

The extraction of the leaves was carried out after thoroughly washing, sterilizing with 70% alcohol and shade dried. The leaves were grounded to powder in sterilized mortar and pestle. The plant materials were sieved using a fine sieve of 1mm. The powdered plant materials were soaked in solvents and left for forty-eight hours then filtered and the supernatant dried in a water bath to obtain the extract (3).

Aqueous extract

500g of powdered leaf were added to 5000 ml of distilled water (10% w/v) and soaked for 48 hours under shaking condition, the supernatant was collected and the solvent were evaporated to make the crude extract and stored at 4℃ (3).

Methanol extract

From powdered leaf 500g were added to 5000 ml of methanol water (10% w/v) and soaked for 48 hours under shaking condition, the supernatant was collected and the solvent were evaporated to make the crude extract and stored at 4℃ (3).

Fractionation of plant extracts

The crude extract obtained from the methanol extract was fractionated as described by (10) with modifications, in order of increasing polarity of the solvents. The crude extract was dissolved in 200 ml of water and sieved. The filtrate (aqueous portion) was first mixed with 300 ml of n-hexane in a fractionating funnel; the n-hexane fraction was collected in a clean beaker. The procedure was repeated with ethyl acetate and lastly with N-butanol. The fractions were completely dried on a water bath.

Thin Layer chromatography (TLC)

TLC aluminum sheet of 20×20cm silica gel pre-coated plate using one-way ascending

techniques was employed. The plates were cut into sizes of 5×10cm. The extracts were dissolved in the initial extraction solvents, using capillary tubes spots were made on the plates, the plates were allowed to dry and developed into different solvents ratios in the chromatographic tank. Developed plates were sprayed using general visualizing reagent (*p*-anisldehyde/ 10% sulphuric acid in methanol) and specific detecting reagents: Bontragers, Ferricchloride, Dragendroff, Libermann-buchards and aluminum chloride (it was viewed under UV365nm) and heated at 110 for 2 minutes where applicable. Number of spots and retention factors for each of the spots were determined and recorded while the chromatogram was scanned accordingly (5,11).

Preparation of extracts fractions concentration

Three concentrations were prepared by weighing 0.2g, 0.3g and 0.5g of each of the fractions and dissolved in 1ml of distilled water for *in vitro* assay (10).

In vitro* assay using *F. sycomorus* fractions on larvae of *S. frugiperda

A Completely randomized experimental design was employed. Three different fractions of *F. sycomorus* leaf extract from (N- Hexane, Ethyl acetate and N- Butanol) at three different concentrations 200mg/ml, 300mg/ml and 500mg/ml as the treatments, a negative control (distilled water) and a positive control (lambda cyhalothrin 2ml/L), all with three replications. Ten 4th instar larvae were placed in each petri dish containing filter paper disc, the larvae were then separately sprayed with 1ml from the assigned treatments. Larvae were fed daily with natural diet, fresh maize leaves, at ambient temperature, throughout the period. After 24 hours the filter paper discs were removed. Mortality was recorded every 24 hours for four days and percentage mortality was calculated. The result was subjected to one factor analysis of variance (ANOVA) using Microsoft excel. The means were separated using Fishers least significant difference LSD at 5% probability level. The data was transformed using $\log_{10}$ and probit. LC_{50} was determined using regression analysis (2).

RESULTS

Result for thin layer chromatography:

The *Ficus sycomorus* methanol leaf extract produced seven spots, N- hexane fraction 11 spots, ethyl acetate 11 spots, N- butanol 6 spots were formed. The retention factor (RF) values were calculated and presented on Table 1.

Table 1: Result for thin layer chromatography of *Ficus sycomorus* leaf extracts of N- Hexane, Ethyl acetate N- Butanol and methanol fractions.

Fraction	Solvent system	NOS	Retention factors (Rf values) (cm)	Colour	Inference
F.sycomorus ethyl acetate	N-hexane:Ethyl acetate(8:2)	11	0.2,0.26,0.29,0.35,0.4,0.44,0.5,0.55,0.6,0.64,0.69	Green	Phenolic compounds
F.sycomorus N- butanol	Methanol:Ethyl acetate(8:2)	6	0.21,0.35,0.39,0,5,0.57,0.8	Brown	Cardiac glycosides
F.sycomorus ethyl acetate	N-hexane:Ethyl acetate(8:2)	11	0.2,0.26,0.29,0.35,0.4,0.44,0.5,0.55,0.6,0.64,0.69	Green	Phenolic compounds
F. sycomorus methanol	N-hexane:Ethyl acetate(8:2)	7	0.13,0.18,0.25,0.31,0.5,0.59,0.95	Brown	Cardiac glycosides

NOS = number of spots

Effect of *Ficus sycomorus* leaf extracts fractions on *Spodoptera frugiperda* larval mortality

Result on the effect of *Ficus sycomorus* extracts on *Spodoptera frugiperda* larval mortality is presented on Table 2, Significant effect was observed at P≤ 95% throughout one to four days after treatment. At 1 day after treatment (DAT) *Spodoptera frugiperda* larvae treated with *F. sycomorus* N-butanol at 200mg/ml and *F. sycomorus* N-hexane at 500mg/ml recorded higher larval mortality. *F. sycomorus* N-hexane at 200mg/ml, *F. sycomorus* ethyl acetate at 200, *F. sycomorus* N-butanol at 300, *F. sycomorus* N-butanol at 300, *F. sycomorus* N-butanol at 500 and lambda cyhalothrin all recorded the same number of dead larvae. *F. sycomorus* ethyl acetate at 500mg/ml was the least. At 2DAT *F. sycomorus* N-hexane at 500mg/ml (53.3%), Lambda cyhalothrin (50%), *F. sycomorus* N-hexane at 300 were observed to be the most effective treatments with regards to larval mortality. The least was *F. sycomorus* ethyl acetate at 500mg. At 3DAT *F. sycomorus* N-hexane at 300mg (83%) and lambda cyhalothrin (80%) were observed to yield the highest result followed by *F. sycomorus* N-butanol at 200mg and *F. sycomorus* N-hexane at 500mg (66%). All the remaining treatments were significant compared to control. At 4DAT, *F. sycomorus* N-hexane at 300mg (100%) and Lambda cyhalothrin (100%) recorded the highest mortality rate of larvae.

The LC_{50} values of the treatments were also presented in table 3.

Table 2: Cumulative daily and percentage mortality on the effect of *F. Sycomorus* extract fractions in (mg/ml) on *Spodoptera frugiperda* larvae, Means ± standard errors.

Solv/conc	1 DAT	%Mort	2 DAT	%Mort	3 DAT	%Mort	4 DAT	%Mort
N-hex200	2.00±0.58^{b}	20.00	4.00±0.00^{c}	40.00	6.00±0.00^{c}	60.00	7.00±0.58^{bc}	70.00
etac 200	2.00±0.00^{b}	20.00	2.67±0.33^{ef}	26.70	4.00±0.33^{fg}	40.00	4.00±0.00^{d}	40.00
N-but200	3.00±0.58^{a}	30.00	4.33±0.33^{ac}	43.30	6.66±0.33^{c}	66.60	6.93±0.33^{c}	69.30
N-hex300	2.00±0.00^{b}	20.00	5.00±0.58^{ac}	50.00	8.33±0.33^{a}	83.30	10.00±0.3^{a}	100.0
N-hex 500	3.00±0.58^{a}	30.00	5.33±0.33^{a}	53.33	6.66±0.33^{c}	66.60	6.93±0.33^{c}	69.30
N-but300	2.00±0.00^{b}	20.00	3.33±0.33^{de}	33.30	3.66±0.33^{g}	36.60	3.99±0.33^{e}	39.90
N-but500	2.00±0.00^{b}	20.00	3.33±0.67^{de}	33.30	4.66±0.33^{ef}	46.60	5.99±0.33^{d}	59.90
Etac300	0.67±0.33^{cd}	6.70	3.34±0.33^{de}	33.40	5.01±0.88^{d}	50.10	5.01±0.00^{d}	50.10
Etac500	0.00±0.00^{d}	0.00	2.00±1.15^{fg}	20.00	2.00±0.00^{h}	20.00	2.00±0.00^{f}	20.00
L/cyhalot	2.00±0.58^{b}	20.00	5.00±0.00^{ac}	50.00	8.00±0.58^{a}	80.00	10.00±0.0^{a}	100.0
D/ water	0.00±0.00^{d}	0.00	1.00±0.58^{g}	10.00	1.00±0.00^{i}	10.00	1.00±0.00^{g}	10.00
LSD 5%	0.97		1.23		0.97		0.87	

Means in the same column with different superscripts (letters) are significantly different at p=0.05 using Fishers LSD.

Key: Solv./conc. = solvents/ concentration, DAT = day after treatment. Mort = mortality. Etac = ethyl acetate, Hex = hexane and But = butanol. Concentrations are in mg/ml.

Table 3: LC_{50} values of the concentrations in mg/ml of *F. sycomorus*.

Treatmens	Concentrations	LC_{50}
F. sycomorus N-Hexane	200mg, 300mg, 500mg/ml	389.37mg/ml
N-butanol	200mg, 300mg, 500mg/ml	389.37mg/ml
Ethyl acetate	200mg, 300mg, 500mg/ml	465.57mg/ml

LC = lethal concentration and mg/ml = milligram/millilitre

DISCUSSION

The bioassay experiment of *Ficus sycomorus* leaf extract on the Fall armyworm showed that the plant can be used as a pesticide based on the activities recorded as larval mortality in N-Hexane fraction were 70 to100% mortality, up to 69% in N-butanol fraction and 20 to 50% in ethyl acetate portion within four days after treatment. This indicated the variability in efficacy of extracts from different solvents as reported by Osama and Awdelkarim (2015) (8), in their work on Phytochemical screening of *Ficus sycomorus* L. bark and *Cleome gynandra* L. aerial parts, in which the acetone extract of *F. sycomorus* (bark and stems) led to a mortality of 86.7% while the ethanol extract had low mortality (14.4%) on adult ticks *Rhipicephalus turanicus* (Acari: Ixodidae). Romeh, (2013) (9), also found that, the bioactive phyto-chemicals effects of different concentrations of *F. sycomorus* leaves on the mortality of adults *Sitophilus oryzae*, *Aphis craccivora* and adult females of *Tetranychus urticae* showed a highly toxic effect to all the tested insects. Volatile parts were found to cause a gradual increase in mortality with an increase in the concentration between 0.018 and 0.3% H. However, 100% adult mortality of *S. oryzae, A. craccivora* and *T. urticae* were recorded at 0.4% and above levels at 24 h of treatment.

CONCLUSION

The *in vitro* assay revealed the effectiveness of the treatments on *Spodoptera frugiperda* larvae. The most effective treatments at day 1 after treatment were; *F. sycomorus* N-Hexane 200mg/ml and *F. sycomorus* N-butanol 200mg/ml all were the same as the lambda cyhalothrin used as a check. Those that were not active in day 1 were *F. sycomorus* Ethylacetate 200mg, was the same as the control. In day 2, the treatments that showed the most significant activity were; *F. sycomorus* N-Hexane 300mg and *F. sycomorus* N-Hexane 500mg also have the same as the chemical. The least effective was *F. sycomorus* Ethylacetate 500, with the same effect as control. At day 3, *F. sycomorus* N-Hexane 300, exhibited similar effect as lambda cyhalothrin. *F. sycomorus* N-butanol 300, *F. sycomorus* Ethylacetate 200 were not observed to be active. At day 4 lambda cyhalothrin was the most effective while control was the least.

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