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The efficacy of *Datura ferox* (fierce thorn apple) leaf extracts against the Yellow Sugarcane Aphid (*Sipha flava*) in the Zimbabwe Sugar Industry †

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Abstract: The study was carried out to test the efficacy of *Datura ferox* (fierce thorn apple) leaf extracts against Yellow Sugarcane Aphid (*Sipha flava*) in the laboratory. Sequential exhaustive extraction was performed using three solvents of varied polarities and these included ethanol, hexane and ethylacetate. This was done to obtain crude extracts with components of a wide range of polarity. The most polar solvent yielded the highest followed by an intermediate polar and then non polar. This showed an increase trend in yield with solvent polarity. The laboratory experiment had three treatments which were extracts from different solvents. Different concentrations were prepared using the N1V1= N2V2 method. Acetamprid (Allice) was used as the standard/ positive control and pure solvents were also used as negative controls. To test for the toxicity of the extracts, the filter paper method was adopted and petri dishes were used to confine the aphids. Aphid numbers between 50 and 70 were chosen. Mortality was recorded after 3, 24 and 36 hours. LD50 and LD90 were calculated in excel. Ethanol extracts showed the highest efficacy against the aphids. Alkaloids which are the main active ingredients in *Datura ferox* are polar and they tend to be extracted by more polar solvents which explain why ethanol extracts showed the highest efficacy against the aphids. The extracts that showed the highest insecticidal activity were taken for phytochemical screening. Alkaloids were phytochemicals of interest in this survey. However, screening was also done for flavonoids, tannins, phenols, coumarins, saponins anthraquinones and terpenoids. Alkaloids and flavonoids were also confirmed using Thin Layer Chromatography.

Keywords: Sequential Exhaustive Extraction; insecticidal activity; phytochemical screening; Thin Layer Chromatography

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1. Introduction

Pre-harvest damages due to YSA (Yellow Sugarcane Aphid) feeding are one of the most important restraining factors of sugarcane production. Sugarcane quality and quantity is affected as a result of Yellow Sugarcane Aphid feeding. [11], reported that YSA feeding can trim down sugarcane yield by 6% following death of few as two leaves within the first three month of growth and death of three pairs of leaves due to aphid feeding results in 19% yield loss.

To prevent losses due to Yellow Sugarcane Aphid, dimethoate and Acetamprid (Allice) are currently used in the Zimbabwe Industry against the Yellow Sugarcane Aphid (Bandit). Acetamprid belong to a group of insecticides called neonicotinoids and they are widespread contaminants of surface and ground water [16]. Repetitive exposure to synthetic insecticides builds resistance in insects until there is slight or veto effects on those insects. According to [5], each insect populace has got some individual genetic

composition that allows them to resist insecticide's effect. These individuals survive synthetic insecticide spraying and pass the protective genetic composition to their young ones. Furthermore, a large amount of synthetic insecticides do not degrade in the environment easily thereby causing pollution to the soil, underground water and depletion of the ozone layer [6]. As a result, the consequences associated with the misuse of synthetic insecticides have stimulated the need for alternative insect pest management such as natural insecticides and development of these has gained considerable weight [4]. The intricacy and lack of uniformity in the compounds and the concentrations of the active ingredient helps in reducing the risks of insects building resistance. [7], mentioned that, botanical or natural insecticides are naturally occurring chemicals (insect toxins) extracted or derived from plants or minerals. This has also stirred the need to assess a botanical plant known as *Datura ferox* (fierce thorn apple) as a potential botanical insecticide against Yellow Sugarcane Aphid in Zimbabwe. *Datura ferox* is a weed with both medicinal and poisonous properties and is believed to have great pharmalogical properties. Researches shows that it is enriched with tropane alkaloids scopolamine and atropine [10].A study by[19], in India showed that *Datura* species like *Datura stramonium* have been used in the control of mosquitos and the ethanolic leaves extract of *Datura stramonium* provided complete protection time (Mosquito repellency) against *Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus*. [13], also assessed the insecticidal properties of *Datura stramonium* against *Sitophilus oryzae* in stored wheat grains. The effects of crude aqueous extracts of indigenous pesticidal plants on the ladybird beetle, *Hippodamia variegata* (Goeze) were also done in Zimbabwe [15].

In the Zimbabwe Sugar Industry, Datura ferox is found along railway lines and in dumping sites making them accessible by most farmers. *Datura ferox* is also easy to propagate since it does well in most soils. The main objective of this study was to test the efficacy of Datura ferox against Yellow Sugarcane Aphid.

The specific objectives of the study were to:

- To determine the optimum concentration of *Datura ferox* leaf extracts to effectively control Yellow Sugarcane Aphid under laboratory conditions.
- To determine the LD_{50} and LD_{90} of extracts from solvents of different polarities.
- To carry out phytochemical characterization on the extracts with highest insecticidal efficacy.

2. Materials and methods

2.1. Study site

The laboratory experiment was carried out at the Zimbabwe Sugar Association Experiment Station which is located south –east Lowveld of Zimbabwe on a 99km peg along the Ngundu –Tanganda road. It is located 430m above the sea level at latitude of 20°01′ S and longitude 28° 38′ E.

2.2. Plant materials and extraction

Plant samples used in this study were taken from 8weeks old plants from ZSA-ES and were taken for identification at National Herbarium and Botanical Gardens in Harare, Zimbabwe. The fresh leaves were dried for seven days under shade on laboratory bench tops to avoid direct sunlight and grounded into a powder using a Wiley mill and a ball mill.

The leaf powder (25g each) was macerated in 250 ml of ethanol, ethylacetate and hexane separately to obtain crude extracts of a wide range of polarity and the process was completed in three cycles on a laboratory shaker at room temperature. The samples were concentrated to dryness using a rotary evaporator under reduced pressure and temper-

ature of 40oC. The leaf extracts were obtained in a solid form and were dissolved in the respective solvents. The stock solution was 50mg/20ml followed by (40mg/20ml, 30mg/20ml, 20mg/20ml, 10mg/20ml and 5mg/2ml) which were prepared following the N1 V1=N2V2 method from the basic solution. Pure solvents were used as controls and Acetamprid was used as a standard.

2.3. Toxicity tests

The leaf extracts of Datura ferox from different solvents were tested for toxicity by exposing filter papers to the above mentioned concentration for twenty minutes and removed for the solvent to dry for ten minutes. The filter paper method by [1] was adopted where Whatman No1 filter papers were cut to be 6cm in diameter and were attached at the bottom of the petri dishes (6cm) in diameter and the petri dishes were used to confine the aphids. Infested (with 50-70 yellow sugarcane aphids) sugarcane leaves were exposed to the above mentioned concentration and each concentration was replicated three times. Mortality (number of dead aphids) was counted after 3, 24 and 36 hours of exposure. Estimation of LD50 and LD90 was analyzed using excel

2.4. Phytochemical screening

Ethanol leaf extracts showed the highest efficacy against Yellow Sugarcane Aphids and were taken for phytochemical analysis. The phytochemicals of interest in this research were alkaloids however screening was also done for flavonoids, tannins, phenols, terpenoids, saponins, coumarins and anthraquinones

2.5. Testing for Alkaloids

It was done using the dragendorff reagent test where I ml of the ethanol extract solution was placed in a test tube and mixed with 5ml of distilled water A few drops of 2 M Hydrochloric acid was added followed by 1ml of the dragendorff reagent. Formation of orange or orange reddish brown precipitate indicated the presence of alkaloids [20].

2.6. Testing for flavonoids

The Sodium hydroxide (NaOH) test was used to test for flavonoids in which1 mL of the stock solution was taken in a test tube and a few drops of 1M NaOH solution were added. Formation of an intense yellow color that disappears after the addition of a few drops of 1 M Hydrochloric acids shows the presence of flavonoids [12]

2.7. Testing for tannins

It was performed using the Ferric chloride test.1ml of the stock solution was taken into a test tube and a few drops of 5 % ferric chloride were added. Presence of a greenish or blue- black or blue -green color indicates the presence of tannins [21]

2.8. Testing for saponins

2 ml of distilled water was added to 2ml of the extract solution and shaken in a test tube for 10s Formation of a foam indicated the presence of saponins [14]

2.9. Testing for coumarins

1 ml of 10% NaOH (sodium hydroxide) was added to 1ml of the extract in a test tube. Formation of yellow color shows the presence of coumarins [20].

2.10. Testing for terpenoids

2. ml of chloroform and a few drops of concentrated sulphuric acid were added to 0.5 ml of the extract.. Presence of a red brown color indicates the presence of terpenoids [17].

2.11. Testing for Phenols

2 ml of distilled water and a few drops of 10% ferric chloride were added to 1ml of the extract. Presence of blue or green color shows the presence of phenols [9].

2.12. Testing for anthraquinones

A few drops of 10% ammonia solution were added to 1ml of the plant extract, formation of a pink color indicates the presence of anthraquinones [8].

Qualitative analysis of alkaloids from ethanol extracts using TLC (Thin Layer Chromatography) plates.

Alkaloids were some of the phytochemicals found in the ethanol extracts of the plant species as shown by the Drangendorff test.

2mg of the plant extract was dissolved in 1ml of ethanol .TLC plates were prepared according to [2], with minor modifications. About 1cm from the edge of the plates, a line was drawn using a pencil. A small amount of the dilute mixture was transferred to the center of the plate with the help of a spotting capillary. Development of the plates was done with hexane: water (7/3) (v/v) in a developing chamber. The developing chamber which was used was a beaker which was large enough to house the TLC plate. The plates were removed from the developing chamber and the solvent front (the furthest point moved by the solvent) was drawn using a pencil. The plates were left to dry. After drying, the plates were sprayed using Drangendorff reagent and visualized under normal light. An orange color under normal light indicated the presence of alkaloids [22].

2.13. Anti-oxidant activity: DPPH Assay

The anti- oxidant activity of the ethanol extract was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay [18]. It was carried out on TLC plates which were developed with hexane: water (7/3) (v/v).After separation on TLC plates, the compounds with free radical scavenging activity were determined in situ with DPPH reagent. The TLC plate was observed under visible light. Areas producing yellowish bands against the purple background were considered as antioxidants [3].

3. Results and Discussion

3.1. Plant extracts yields

An increasing trend in extract yields from hexane to ethanol was observed showing a greater extractive potential with solvent polarity. Ethanol extracted13.06% ethylacetate 6.34% and hexane 4.09%. Plant yields as a percentage of dry plant material are shown in Fig 1 below.

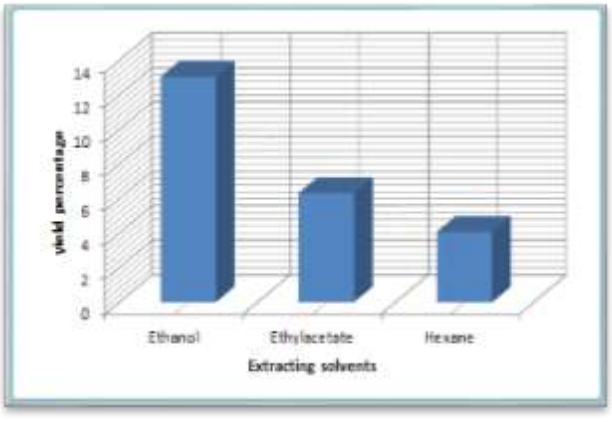

Fig 1. Shows Average percentages of plant extract yields per solvent.

3.2. Toxicity tests of the extracts.

Long exposure times are needed to reach the satisfactory level of mortality. The results from the toxicity tests showed that aphids were susceptible to *Datura ferox* extracts. For all the extracts from ethanol to ethylacetate to hexane extracts, mortality increased with an increase in time (from 3 hours to 36hours) and concentration (from 5 to 50mg/20ml) and this is in agreement with a study by [1], who reported that with an increase in concentration, mortality also increases The LD_{50} and LD_{90} calculated for ethanol extracts was 5.81mg and 14.68mg/20ml respectively. For ethylacetate extracts the LD_{50}

and LD_{90} calculated was 14.11 and 39.54mg/20ml respectively and lastly the LD_{50} and LD_{90} for hexane was 23.33 and 50.15mg/20ml respectively. A comparison of LD_{50} and LD_{90} for the different solvents and regression equations is shown in Table 1 below.

Table 1. Shows a Comparison of LD 50 and LD90 of the different solvents and regression equations [1].

Plant name	Solvent	R	LD_{50}	LD_{90}
Datura ferox leaf extracts	Ethanol	Y=4.531x +23.662	5.81mg/20ml	14.68mg/20ml
	Ethylacetate	Y=1.573x +27.806	14.11mg/20ml	39.54mg/20ml
	Hexane	Y=1.4915x +15.201	23.33mg/20ml	50.15mg/20ml

3.3. Phytochemical screening

The results from phytochemical screening showed that the ethanol extracts contained seven phytochemicals out of the eight that were screened. Results on qualitative tests performed on ethanol extracts are shown in table 2 below

Table 2. Shows results on qualitative tests performed on ethanol extracts

Ethanol extracts	Alkaloids	Flavonoids	Saponins	Tapins	coumarins	Phenols	Terpenoids	Anthraquinones	Antioxidants
	+++	+++	+++	+++	+++	+++	+++	-	+++

Key

+ Means present

- Means absent.

4. Conclusion

The study showed that *Datura ferox* leaf extracts has got insecticidal effects against the Yellow Sugarcane Aphid under laboratory conditions . As a result, this study suggests that Datura ferox leaf extracts maybe used a potential botanical insecticide against the Yellow Suagrcane Aphid which is cheaper and affordable for most farmers in the Industry. However further research on this plant is utmost needed.

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