



Research Paper

Detection of secondary metabolites contained in aqueous extracts of *Datura stramonium* (F.E. Köhler, 1897) and *Thevetia peruviana* (K. Schum, 1895) with a view to biological control of fall armyworms (*Spodoptera frugiperda* J. E. Smith, 1797) at the laboratory in Kisangani, Democratic Republic of Congo

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Abstract

The objective of this study is to detect secondary metabolites in aqueous extracts of *D. stramonium* and *T. peruviana* to control attacks by fall armyworms in Kisangani, Democratic Republic of Congo. The methodological approach used in this work involves the detection of secondary metabolites in the selected plants. The leaves of *Datura stramonium* and *Thevetia peruviana* contain secondary metabolites including flavonoids, alkaloids, tannins, saponins, sterols, and terpenes. Therefore, the local plants tested contain insecticidal secondary metabolites.

Keywords: extracts, aqueous, metabolites, secondary, *Datura stramonium*, *Thevetia peruviana*, caterpillars, legionnaires, Kisangani

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I. Introduction

Corn (*Zea mays* L.) is one of the most widely grown cereals in the world. It occupies more than 33 million hectares worldwide each year. It accounts for 41% of global cereal production, with production estimated at around 843 million tons for the period 2013 to 2014 (FAO, 2016).

Grown all over the world, mainly for its grains, corn is one of the most important sources of nutrition (rich in starch and carbohydrates, with proteins and minerals) and economic value (easy to produce, harvest, and store), two elements that are essential for sustaining life. Its global cultivated area is smaller than that of wheat and rice, but its average yield of 5.5 t/ha is far higher than that of wheat and rice (Issa et al., 2011).

In terms of human consumption in Mexico and South Africa, for example, figures can reach 50 to over 100 kg/year/per person of corn consumed, hence the importance of corn in global production (Anzala, 2006).

Since 2016, the African continent has been facing threats from the fall armyworm. Native to America, the fall armyworm (*Spodoptera frugiperda*) was first reported on the African continent in January 2016 (Goergen et al., 2016).

In the Democratic Republic of Congo (DRC), maize is the second most important food crop after cassava (Nyembo et al., 2014). Its cultivation is widespread and national production has continued to increase despite climatic and social disruptions, rising from 118,400 tons in 2000 to 2,078,352 tons in 2018, a growth rate of 56.97% (FAO, 2020).

It is a polyphagous pest that attacks more than 80 plant species in its native habitat, causing damage to economically important cereal crops, with a preference for maize, as well as vegetable crops and cotton (FAO,

2019). Currently, more than 30 African countries have identified the pest on their territories, including the Democratic Republic of Congo.

In the DRC, this pest causes significant damage to maize crops and has a negative impact on agricultural production. Losses are estimated at \$2.5 to \$6.2 billion per year (CABI, 2019).

Average national yields remain low (0.7 to 1 t/ha) compared to those of the East African Community (EAC) countries, partly due to the low use of improved varieties and agricultural inputs, as well as the significant damage caused by various pests and diseases (Kalonji et al., 2004; Nyembo et al., 2012).

The overall objective of this study is to detect secondary metabolites in aqueous extracts of *D. stramonium* and *T. peruviana* that can be used as biological insecticides to control fall armyworm attacks on corn in the laboratory. Specifically, the study aims to identify the insecticidal compounds present in *Datura stramonium* and *Thevetia peruviana*.

II. Materials and methods

Environment

Our experiment was conducted in the Entomology Laboratory of the Yangambi Faculty of Agricultural Sciences in Kisangani. Kisangani's climate is classified as Af in the Koppen climate classification system. It is a hot and humid climate. The temperature varies between 25 and 30°C, rainfall exceeds 1,800 mm per year, relative humidity is between 80 and 90%, and insolation is 1,972 hours. The soils are ferralitic, very deep with a predominance of clay and kaolinite, and the pH is between 4 and 5.

The geographical coordinates of the laboratory taken from the study site using GPS are as follows: 0°30'46.99" North Latitude, 25°9'50.53" East Longitude, Altitude 404 m. Balandi et al. (2024).

Materials

To carry out this work, we used entomological materials, plant materials (Figure 1), and some laboratory materials (Figure 2).

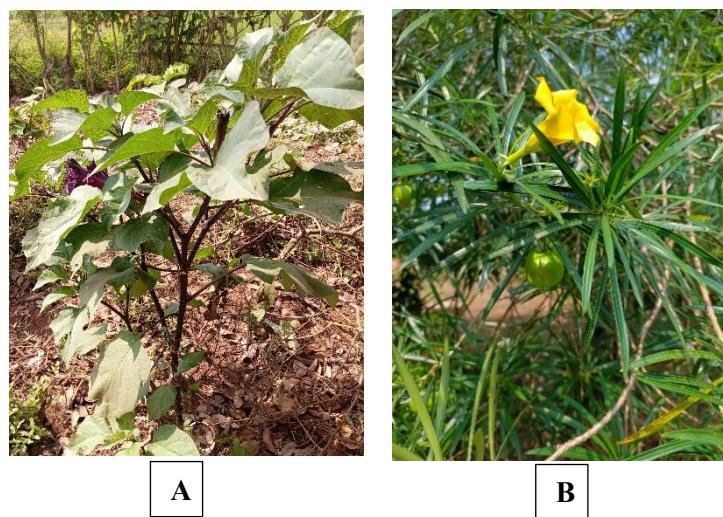


Figure 1: *Datura stramonium* (A) and *Thevetia peruviana* (B)

These include:

- A mortar, pestle, and sieve for grinding and sifting the dried leaves of *D. stramonium* and *T. peruviana*,
- Test tubes used for reactions involving small quantities of reagents,
- A beaker used to store a solution before sampling and also to perform certain measurements,
- An Erlenmeyer flask used to temporarily store volatile chemicals, perform chemical reactions with volatile compounds,
- Measuring cylinder used to measure the volume of a liquid with moderate accuracy,
- Graduated pipette used to measure small volumes of liquid with moderate accuracy. It is used in the preparation of solutions, with a propipette (suction bulb) or pipette, to withdraw the stock solution, according to a specific protocol,
- The funnel is used to pour liquid into a narrow-necked bottle without spillage. It is also used in filtration setups.
- Balance: to measure the quantities of products to be macerated.



Figure 2: Materials used

Methods and techniques

For this study, the research was inspired by the method used by Looli et al. (2021), who adopted a methodological approach consisting of detecting secondary metabolites in selected plants.

The leaves of the plants suspected of being insecticides were harvested and dried in the shade and in the open air at room temperature in the laboratory. They were spread out on workbenches for 20 days to ensure complete drying. After drying, the samples were crushed in a mortar and then sieved using a sieve.

The powders obtained were stored in clean, dry bottles. These metabolites are generally phytotoxic. The main groups are as follows:

A. Flavonoids; Weast & Robert (1970)

- Appetite suppressants: Flavonoids modulate eating behavior, reduce consumption, and delay growth and reproduction,
- Enzyme inhibitors: interfere with digestive and metabolic enzymes.

Reagents Used

The reagents used are:

- Ethanol (95%),
- Concentrated hydrochloric acid,
- Magnesium shavings,
- Isoamyl alcohol.

Procedure :

- 5g of sample powder was infused for 30 minutes in boiling distilled water,
- After filtration, 5 ml of ethyl alcohol, HCl, and 2 ml of distilled water were added,
- 0.5 g of magnesium shavings and a few drops of isoamyl alcohol were added.

The appearance of a pink, red, or purplish color in the isoamyl alcohol supernatant layer indicates the presence of a free flavonoid.

B. Alkaloids, Wome (1985)

These are neurotoxic, blocking nerve transmission, disrupting protein and enzyme synthesis, and interfering with cell division, causing paralysis or death in insects.

Tests are carried out using precipitation reactions with Dragendorff's reagent. Dragendorff's reagent, which is based on iodobismuthate, produces a red precipitate in the presence of an alkaloid. Dragendorff's reagent is a mixture of two solutions, A and B, prepared as follows:

Solution A

- Bismuth nitrate $\text{Bi}(\text{NO}_3)_3$: 1.7 g
- Glacial acetic acid CH_3COOH : 2 ml
- Distilled water: 80 ml.

Solution B

- Potassium iodide: KI : 10 g,
- Distilled water: 40 ml.

Preparation

- Dissolve $\text{Bi}(\text{NO}_3)_3$ in 80 ml of distilled water,

- Add 20 ml of glacial acetic acid. This is solution A,
- Dissolve 10 g of KI in 40 ml of distilled water to make solution B,
- Mix the two solutions together.

Procedure

- Take 1 g of each plant powder and leave to macerate in 10 ml of 1% HCl for 24 hours,
- Filter and take 1 ml of filtrate, which is placed in two test tubes, to which a few drops of detection reagents are added. If present, a red precipitate will form with Dragendorff's reagent.

C. Detection of Saponins

Saponins have the following effects on insects:

- Irritation and damage to the digestive tract: these amphipaths disrupt intestinal membranes, causing damage to epithelial cells and translocation of microorganisms, which leads to mortality.
- Inhibition of digestive enzymes: they bind to digestive proteins, reducing assimilation and growth in the insect.

Procedure:

- Saponins are identified by their foaming power,
- 15 ml of a 10 g decoction is placed in a test tube. Observation is made after vigorous shaking and a 10-minute rest period,
- The formation of persistent foam after this time reveals the presence of saponins.

D. Detection of tannins; Weast & Robert (1970)

- Protein complexation: they form complexes with dietary proteins, reducing their assimilation,
- Enzyme blocking: they also inhibit digestive enzymes, hindering insect nutrition.

Reagents

The reagent used here is a 1% ferric chloride solution.

Procedure:

- Add 5 drops of a 1% FeCl solution to 5 ml of the infusion obtained in the flavonoid test,
- If there is any coloration or precipitation, the test is positive.

E. Detection of sterols and terpenes Weast & Robert (1970).

Direct toxicity: disrupts the cell membrane of insects and acts as a neurotoxin.

Reagents

The reagents used are:

- Diethyl ether,
- Acetic anhydride,
- H₂SO₄. 32%

Procedure:

Take 1 g of plant material powder macerated in a flask containing 20 ml of ether, evaporate a few drops on a watch glass.

The residue is collected with two drops of acetic anhydride. The addition of a drop of concentrated H₂SO₄ produces a purple color that turns green in the presence of sterolic and terpenic compounds.

Using a micropipette, place 5 µl of a solution of known concentration of each extract representing a specific dose of the active substance extracted using the solvent (diethyl alcohol) on each individual: 5 larvae of each stage per jar and 20 eggs per Petri dish.

Each jar used to hold the batches of treated organisms was fed tender corn leaves every 24 hours to prevent them from dying of starvation.

Mortality was determined after 5 days. At the end of this period, the efficacy of each extract at different doses was determined by calculating the mortality rate for each treatment. Each dose was repeated 3 times.

For each type of extract, a control consisting of the solvent (diethyl alcohol) used for extraction was used. The effectiveness of each extract at different doses was determined by calculating the corrected mortality rate for each pest. The Abbott formula is used to calculate corrected mortality.

Where Mc: Corrected mortality as a percentage; M0: Mortality observed in treated individuals; Mt: Mortality observed in controls; and n: Number of individuals tested in each repetition.

III. Results and discussion

The analysis detected secondary metabolites in the selected test plants, namely *Datura stramonium* and *Thevetia peruviana*, in the laboratory.

The following secondary metabolites were obtained:

Flavonoids

Flavonoids were detected in the test plants, namely *Datura stramonium* and *Thevetia peruviana*, which were studied due to the appearance of pink coloration in the isoamyl alcohol supernatant layer.

Effects on insects:

- Appetite-suppressing flavonoids: Flavonoids modulate feeding behavior, reduce consumption, and delay growth and reproduction,
- Enzyme inhibitors: Flavonoids interfere with digestive and metabolic enzymes.

Alkaloids

Alkaloids were also detected in both plants (*Datura stramonium* and *Thevetia peruviana*) because a red precipitate actually formed with Drangendorff's reagent.

Effects on insects:

- They are neurotoxic: they block nerve transmission, disrupt protein and enzyme synthesis, and interfere with cell division, causing paralysis or death in insects.

Saponins

Datura stramonium and *Thevetia peruviana* produced saponins, as evidenced by the formation of persistent foam after this record time during the analysis (10 minutes).

Effects on insects:

- Irritation and damage to the digestive tract: these amphipaths disrupt intestinal membranes, causing damage to epithelial cells and translocation of microorganisms, leading to mortality.
- Inhibition of digestive enzymes: they bind to digestive proteins, reducing assimilation and growth in the insect.

Tannins

Tannins were detected in compounds from *Datura stramonium* and *Thevetia peruviana*, as orange coloring was observed with the 1% ferric chloride solution reagent.

The effects on insects are as follows:

- Protein complexation: they form complexes with dietary proteins, reducing their assimilation,
- Enzyme blocking: they also inhibit digestive enzymes, hindering the insect's nutrition.

Sterols and terpenes

Sterols and terpenes are also detected in the two test plants (*Datura stramonium* and *Thevetia peruviana*) due to a purple color changing to green in the solution.

The effects on insects are as follows:

- Direct toxicity: disrupts the cell membrane of insects and acts as a neurotoxin.

IV. Discussion

The purpose of this study is to analyze two local plants in order to detect secondary metabolites that could be used in the fight against fall armyworms. Laboratory analysis confirmed that the two test plants (*Datura stramonium* and *Thevetia peruviana*) contain metabolites that are useful for controlling fall armyworms on corn crops.

The analysis showed that *Datura stramonium* and *Thevetia peruviana* provide secondary metabolites such as flavonoids, which are considered appetite suppressants, modulating feeding behavior, reducing consumption, delaying growth and reproduction on the one hand, and acting as enzyme inhibitors by interfering with certain digestive and metabolic enzymes on the other. As noted by Koul et al. (2008), who showed that extracts of *Datura stramonium* exert sublethal effects, including reduced food intake and larval development.

Alkaloids were also detected in both plants (*Datura stramonium* and *Thevetia peruviana*) because a red precipitate actually formed with Drangendorff's reagent. These alkaloids are neurotoxic to insects, capable of blocking nerve transmission, disrupting protein and enzyme synthesis, interfering with cell division, and causing paralysis or death in insects. This thesis is supported by Adedire and Akineye (2004), according to which the compounds in *Thevetia peruviana* induce significant mortality in various species of caterpillars.

The local plant species *Datura stramonium* and *Thevetia peruviana* provided saponins due to the presence of persistent foam formation after this record time during the analysis (10 minutes). These metabolites have effects on insects such as irritation and damage to the digestive tract: these amphipaths disrupt intestinal membranes, causing epithelial cell damage and translocation of microorganisms, which leads to mortality. The inhibition of digestive enzymes binds to digestive proteins, reducing assimilation and growth of the insect, as noted by Barary (2020).

Tannins were extracted from *Datura stramonium* and *Thevetia peruviana* because an orange coloration was observed with the 1% ferric chloride solution reagent. These have effects on insects, particularly in protein complexation: they form complexes with dietary proteins, reducing their assimilation; enzyme blocking: they also inhibit digestive enzymes, hindering insect nutrition, as attested by Dida (2021) and Marion (2024).

Sterols and terpenes are also detected in the two test plants (*Datura stramonium* and *Thevetia peruviana*) due to a purple color turning green in the solution. The effects on insects include direct toxicity, which disrupts the insects' cell membranes and acts as a neurotoxin (Bouzerida et al., 2016).

In light of the above, Sexena et al. (1992) show that *Datura stramonium* has significant insecticidal activity against several insect pests. Abdullahi and Mohammed (2011) confirm the sensitivity of armyworm caterpillars to aqueous extracts of *Datura stramonium*. Ogiangbe et al. (2010) state that aqueous and organic extracts of *Thévetia péruviana* induce significant mortality in various species of caterpillars.

V. Conclusion

The purpose of this research is to detect secondary metabolites in the leaves of certain local plants, notably *Datura stramonium* and *Thévetia peruviana*.

The following results were found:

- The aerial parts (leaves) provided secondary metabolites including flavonoids, alkaloids, tannins, saponins, sterols, and terpenes,
- These secondary metabolites have a direct or indirect insecticidal effect on insects, which proves that these plants are biological insecticides and can be used to limit the actions of armyworms, which are pests of corn crops.

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