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Original Article

# Metabolomic profile and allelopathy effects of Calotropis procera and Nicotiana tabacum extracts used as pesticidal agents on germination of maize seed

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ABSTRACT

Calotropis procera and Nicotiana tabacum leaves are among the natural pesticides that are generally used on various crops at various stages. However, many of these natural pesticides affect plant growth. This study determined the metabolomic profile and the allelopathic effects of Calotropis procera and Nicotiana tabacum leaf extracts (CPLE and NTLE) on germination of maize seed. The allelopathic activities of n-hexane, ethylacetate and methanol of CPLE and NTLE were determined on maize seed using concentration of 2.5%, 5.0% and 10% for 12 days. On days 3, 6, 9 and 12, the root and shoot development, germination percentage, and seedling vigour of maize seeds were determined. All CPLE contained calotropin, calotoxin, calactin, caffeic acid, thioacetate, alpha-amyrin, beta-sitosterol, stigmasterol, isoquercitrin, quercetin, kaempferol, rhamnetin, luteolin, lineolone, voruscharin, uscharin and uscharidin, while azeleatin, tyranton were present in only the ethylacetate extracts. The NTLE revealed the presence of nicotine, chlorogenic acid, nornicotine, 4-hydroxybenzaldehyde, catechin, catechol, protocatechuic acid, vanillin, vanillic acid, nicotelline, nicotianine, tabacine, tabacinine, harmine and anabasine while ajmalicine, pyridine and cotinine are absent in methanol extracts of NTLE. All seedlings show a reduction in germination percentage when compared to the control from the 3rd to the 12th day of germination. However, only 2.5% of both extracts exhibit ≥ 50% of germination percentage and high values of vigor index when compared with

concentrations 5% and 10%. Hence, high concentrations (5 and 10%) of CPLE and NTLE should be avoided when using as insecticide during germination stage of maize to prevent growth inhibition. of maize.

Keywords: Allelopathy, Calotropis procera, Herbicides, Nicotiana tabacum, Pesticides

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## INTRODUCTION

The growing concern over the adverse effects of synthetic chemicals has shifted attention toward natural, plant-based alternatives for enhancing crop productivity and soil health [1]. As such, phytochemicals in various plants with pesticidal, insecticidal, herbicidal, and medicinal properties are being explored as potential as natural agrochemicals [1]. Calotropis procera and N. tabacum are among the two prominent plants that have been extensively used for pesticidal and insecticidal activities in various crops [2, 3]

Calotropis procera is an evergreen, perennial shrub belonging to the Apocynaceae family, commonly found in arid and semi-arid regions. This versatile plant serves multiple purposes, including use in medicine, animal fodder, and fuel, as well as in timber and fiber production, phytoremediation, and nanoparticle synthesis. It has long been utilized in traditional medicine across regions in North Africa, the Middle East, and Southeast Asia, and is currently being actively researched for its potential pharmacological benefits [3].

Nicotiana tabacum, commonly known as tobacco, belongs to a species of flowering plant in the nightshade family (Solanaceae), originating from South America. It is an essential agricultural crop globally, primarily cultivated for its leaves, which are processed for nicotine production, tobacco smoking products, and industrial applications. Nicotiana tabacum is exclusive from other species in the genus Nicotiana, particularly Nicotiana rustica, which is used less extensively. Tobacco has been cultivated for centuries, and its leaves contain alkaloids, most notably nicotine, which has both pharmacological and ecological effects [3].

Maize is highly sensitive to environmental conditions during its germination and early growth stages, which are crucial for determining final crop yield [4]. Thus, the use of synthetic pesticides or herbicides are being discouraged due to their pollution, residual toxicities, and health effects in both humans and animals [4]. As such, phytochemicals from various plants are being used as alternatives to poisonous synthetic agrochemicals.

Despite the uses of the phytochemicals of these plants as pesticide and insecticide on various crops, there is limited understanding of their allelopathic effects on non-target plants, particularly maize (Zea mays), a major staple crop. This lack of knowledge poses a challenge for farmers who may wish to incorporate Calotropis procera and N. tabacum as pesticides into maize cultivation without negatively impacting crop growth. To address this gap, this research aims to examine the impact o f Calotropis procera and N. tabacum on

maize germination and early growth, providing essential insights into its viability as a sustainable alternative in maize farming systems.

Therefore, the allelopathic properties of C. procera N. tabacum on maize germination will present opportunities for developing integrated pest management strategies, as its bioactive compounds may deter pests or pathogens that affect crop health. Furthermore, the extracts may have potential applications in organic farming practices, where natural plant-based solutions are increasingly sought after to enhance plant resilience and yield [5]

## MATERIALS AND METHODS

### Chemicals and reagents

Chemicals and reagents of analytical grades were used for this research. Reagents and chemicals which include Ethylacetate, Nhexane and Dimethyl sulfoxide (DMSO), were products of Sigma Chemical Co., USA.

Calotropis procera and Nicotiana tabacum leaf powder weighed into 1000 mL volumetric flask. Afterwards, 750 mL of each solvent was separately poured into different flasks and mixtures were properly agitated and left for 72 hours with occasional vortexing each day. After the 72 hours, the solvents were evaporated using a water bath under reduced pressure before finally lyophilized with freezing drier. The freezedried samples were stored in tight covered glass containers and refrigerated at 10°C.



### Experimental Plants

Fresh Calotropis procera and Nicotiana tabacum leaves were collected within the farm of Federal University of Technology, Minna, Gidan Kwano Campus in July, 2024 and authenticated by Dr. O.A.Y. Daudu with identification numbers: FUT/PLB/ASC/004 and FUT/PLB/SOL/008. The plant samples were deposited in the herbarium of the department of Plant Biology, Federal University of Technology, Minna. The maize seeds used were sourced from Bosso Market in Minna, Niger State, Nigeria.

### Preparation of Calotropis procera Leaves

Calotropis procera and Nicotiana tabacum leaves were washed with tap water and dried at 30°C. Thereafter, the plant leaves were reduced to powder using Silver Crest 2L Industrial 8500W electric blender, and the powder form was kept inside a tight covered plastic container until further use.

### Extraction of Calotropis procera and Nicotiana tabacum Extracts

This powdered Calotropis procera and Nicotiana tabacum were subjected to cold extraction using n-hexane, ethyl acetate and methanol as solvents for separate extractions. Exactly 250 grams of each

### Percentage of Yield

The percentage yield of extract is calculated by comparing the weight of the extract obtained to the initial weight of the powdered leaves as depicted in the formula below:

$$\text{Percentage Yield} = \left(\frac{\text{Weight of extract obtained}}{\text{Initial weight of } \textit{powdered leaves}}\right) \times 100$$

## Germination of Maize Seed and Germination Test

Ten maize seeds were placed evenly on filter paper in a duplicate glass petri dish (10 seeds per petri-dish) and with each duplicate petri dish received 10 mL of different concentrations of 2.5%, 5.0% and 10.0% of n-hexane, ethyl acetate and methanol leaf extracts of Calotropis procera and Nicotiana tabacum while 10 mL of distilled water was applied to the control group. The petri dishes were kept at room temperature (30°C) in the laboratory for 12 days, during which the percentage germinated seeds, lengths of the radicle and plumule were measured at three-day intervals as depicted in the formula below:

$$\text{Percentage Germination} = \left(\frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}}\right) \times 100$$

## Seed Vigor Index

The seedling vigor index (SVI) was calculated by multiplying the germination percentage by the total seedling length (the sum of radicle and plumule lengths). The seedling vigor index was determined using the following formula:

SVI = Seedling length (cm) x Germination percentage

## High-Performance Liquid Chromatography Analysis of Extracts of Calotropis procera and Nicotiana tabacum

Each extracts were analyzed using highperformance liquid chromatography

(HPLC), following the method outlined by Naz et al. (2020).

The HPLC analysis of the extracts was conducted using a Shimadzu LC-20AD system (Shimadzu, Japan), which included a binary solvent delivery unit (LC-20AD), a Rheodyne injector with a 20 µL sample loop, and a diode array detector (DAD, SPD-M 20A). For reverse-phase chromatographic separation, a Capcell Pack C-18 column (MGII, 5 µm, 250 mm x 4.6 mm) was used along with an extended guard column. The mobile phase comprised a mixture of methanol, acetonitrile, and water in a ratio of 40:15:45 (V/V/V), with the addition of 1.0% acetic acid, and was run under isocratic elution for 30 minutes. Data acquisition and processing were managed using Shimadzu LC software, with the DAD detection range set between 240 to 800 nm. The flow rate was kept at 1 mL/min, and both sample and standard solution volumes were 20 µL. Peaks were identified by comparing retention times and UV spectra to reference standards, which were further confirmed by spiking samples with small amounts of these standards.

Effects of N-hexane, Ethylacetate and Methanol extracts of C. procera and N. tabacum on Radicle Length (Root) of Maize Seedlings

Data obtained is expressed as mean plus or minus standard error of mean of triplicate or twenty samples. The analysis was done by using analysis of variance (ANOVA) followed by Post Duncan multiple comparisons test, using SPSS 22.00 version. The test results with P values < 0.05 were taken to be statistical significance.

Table 1: Percentage Yield of Different Extracts of C. procera and Nicotiana tabacum

| Sample | Solvent | % Yield |
|---|---|---|
| Calotropis procera | N-Hexane | $12.24 \pm 0.83^{a}$ |
| | Ethylacetate | $23.08 \pm 1.30^{c}$ |
| | Methanol | $33.90 \pm 2.28^{d}$ |
| Nicotiana tabacum | N-hexane | $18.48 \pm 1.82^{b}$ |
| | Ethylacetate | $24.88 \pm 1.30^{c}$ |
| | Methanol | $45.46 \pm 3.26^{e}$ |

Values are mean of triplicate ± standard error mean. Values with the same superscript alphabet in a column have no significant difference at $P<0.05$

RESULTS Percentage Yield

The effect of n-hexane, ethylacetate and methanol extracts of C. procera and N. tabacum on root length (radicle) of maize seedlings is presented in Table 2. Generally, all seedlings treated with the extracts of C. of N-Hexane, Ethyacetate and Methanol Extracts of C. procera and Nicotiana tabacum

The percentage yield of C. procera and Nicotiana tabacum extracts from different solvents is shown in Table 1. In both cases, the percentage yields of extracts are in the following orders: methanol > ethylacetate > n-hexane. Although, Nicotiana tabacum showed higher percentage yield from each solvent.

procera and N. tabacum decreases in plumule length when compared to the

control from the 3rd to the 12th day. The decrease is concentrations dependent with highest reduction in 10% of each extract. Nevertheless, n-hexane and ethylacetate minimum radicle length reduction on the 12th day when compared to other concentrations.

Effects of N-hexane, Ethylacetate and Methanol Extracts of C. procera and N. tabacum on Plumule (Shoot) of Maize Seedlings

The effects of n-hexane, ethylacetate and methanol extracts of C. procera and N. tabacum on shoot length (plumule) of maize seedlings is presented in Table 3. All seedlings treated with the extracts of C. procera and N. tabacum showed reduction in plumule length when compared to the extract of C. procera (0.68 $\pm$ 0.10 and 1.17 $\pm$ 0.17) and N. tabacum (0.87 $\pm$ 0.17 and 1.10 $\pm$ 0.20) at concentrations of 2.5% show

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control from the 3rd to the 12th day the same way as radicle. The decrease is also concentration dependent with highest reduction in 10% of each extract. Although, ethylacetate extract of C. procera (3.20 ± 1.30) and N. tabacum (2.80 ± 0.04) at concentrations of 2.5% show minimum plumule length reduction on the 12th day when compared to other concentrations.

Table 2: Effects of N-hexane, Ethylacetate and Methanol extracts of C. procera on Root Length (Radicle) of Maize Seedlings

| Concentration | Root Length (cm)/days | | | | |
|---|---|---|---|---|---|
| | 1st day | 3rd day | 6th day | 9th day | 12th day |
| 2.5% of NHECP | 0 | 0.10 ± 0.02 | 0.23 ± 0.02 | 0.63 ± 0.03 | 0.68 ± 0.10** |
| 2.5% of NHENT | 0 | 0.15 ± 0.05 | 0.25 ± 0.05 | 0.45 ± 0.05 | 0.87 ± 0.17** |
| 2.5% of EACP | 0 | 0.30 ± 0.07 | 0.63 ± 0.03 | 0.87 ± 0.08 | 1.17 ± 0.17* |
| 2.5% of EAENT | 0 | 0 | 0.33 ± 0.03 | 0.56 ± 0.02 | 1.86 ± 0.03* |
| 2.5% of MECP | 0 | 0.35 ± 0.05 | 0.20 ± 0.02 | 0.87 ± 0.03 | 1.10 ± 0.20* |
| 2.5% of MENT | 0 | 0.45 ± 0.05 | 0.95 ± 0.05 | 1.30 ± 0.10 | 0.93 ± 0.23** |
| 5.0% of NHECP | 0 | 0 | 0.20 ± 0.02 | 0.50 ± 0.02 | 0.53 ± 0.03 |
| 5.0% of NHENT | 0 | 0 | 0.13 ± 0.03 | 0.23± 0.03 | 0.37 ±0.07 |
| 5.0% of EACP | 0 | 0.05 ± 0.01 | 0.20 ± 0.02 | 0.43 ± 0.03 | 0.50 ± 0.10 |
| 5.0% of EAENT | 0 | 0 | 0.13 ± 0.03 | 0.27 ± 0.07 | 0.40 ± 0.04 |
| 5.0% of MECP | 0 | 0.13 ± 0.03 | 0.30 ± 0.03 | 0.70 ± 0.02 | 0.93 ± 0.03** |
| 5.0% of MENT | 0 | 0.20 ± 0.02 | 0.20 ± 0.01 | 0.23 ± 0.13 | 0.37 ± 0.17 |
| 10% of NHECP | 0 | 0 | 0 | 0.35 ± 0.05 | 0.37 ± 0.07 |
| 10% of NHENT | 0 | 0 | 0.10 ± 0.01 | 0.20 ± 0.10 | 0.35 ± 0.15 |
| 10% of EACP | 0 | 0 | 0.05 ± 0.04 | 0.15 ± 0.05 | 0.13 ± 0.07 |
| 10% of EAENT | 0 | 0 | 0.10 ± 0.01 | 0.23 ± 0.03 | 0.47 ± 0.07 |
| 10% of MECP | 0 | 0 | 0.10 ± 0.02 | 0.45 ± 0.05 | 0.65 ± 0.05** |
| 10% of MENT | 0 | 0 | 0.10 ± 0'01 | 0.25 ± 0.05 | 0.45 ± 0.05 |
| 10 mL of DW (Control) | 0 | 0.64 ± 0.06 | 0.80 ± 0.04 | 1.25 ± 0.05 | 1.88 ± 0.08*** |

Values were expressed as mean of twenty samples plus or minus of the error mean. Values with the same superscript hysteric in a column have no significant difference at P<0.05 KEYS: NHECP: N-hexane extract of Calotropis procera EACP: Ethylacetate extract of Calotropis procera MECP: Methanol extract of C. procera NHENT: N-hexane extracts of Nicotiana tabacum EAENT: Ethylacetate extracts of Nicotiana tabacum MENT: Methanol extracts of Nicotiana tabacum DW: Distilled Water

Table 3: Effect of N-hexane, ethylacetate and methanol extracts of C. procera on Plumule lengths Maize seedlings.

| Concentration | Plumule Length (cm)/days | | | | |
|---|---|---|---|---|---|
| | 1st day | 3rd day | 6th day | 9th day | 12th day |
| 2.5% of NHECP | 0 | 0.1 ± 0.10 | 0.17 ± 0.07 | 0.33 ± 0.03 | 0.20 ± 0.10 |
| 2.5% of NHENT | 0 | 0.15 ± 0.05 | 0.25 ± 0.05 | 0.45 ± 0.05 | 0.47 ± 0.17* |
| 2.5% of EACP | 0 | 0.10 ± 0.10 | 0.33 ± 0.03 | 0.68 ± 0.07 | 3.20 ± 1.30** |
| 2.5% of EAENT | 0 | 0 | 0.33 ± 0.03 | 0.56 ± 0.02 | 2.80 ± 0.04** |
| 2.5% of MECP | 0 | 0.20 ± 0.20 | 0.13± 0.03 | 0.27 ± 0.03 | 0.43 ± 0.03 |
| 2.5% of MENT | 0 | 0.45 ± 0.05 | 0.95 ± 0.05 | 1.30 ± 0.10 | 1.93 ± 0.13** |
| 5.0% of NHECP | 0 | 0 | 0.06 ± 0.04 | 0.23 ± 0.17 | 0.50 ± 0.10* |
| 5.0% of NHENT | 0 | 0 | 0.13 ± 0.03 | 0.23 ± 0.03 | 0.37 ± 0.07 |
| 5.0% of EACP | 0 | 0.05 ± 0.04 | 0.23 ± 0.07 | 0.27 ± 0.07 | 0.50 ± 0.02* |
| 5.0% of EAENT | 0 | 0 | 0.13 ± 0.03 | 0.27 ± 0.07 | 0.40 ± 0.02 |
| 5.0% of MECP | 0 | 0 | 0.23 ± 0.03 | 0.20 ± 0.10 | 0.37 ± 0.03 |
| 5.0% of MENT | 0 | 0.20 ± 0.02 | 0.20 ± 0.01 | 0.23 ±0.03 | 0.37 ±0.07 |
| 10% of NHECP | 0 | 0 | 0 | 0.20 ± 0.10 | 0.35 ± 0.05 |
| 10% of NHENT | 0 | 0 | 0.10 ± 0.01 | 0.20 ± 0.02 | 0.35 ± 0.15 |
| 10% of EACP | 0 | 0 | 0.05 ± 0.04 | 0.13 ± 0.03 | 0.27 ± 0.03 |
| 10% of EAENT | 0 | 0 | 0.12 ± 0.02 | 0.23 ± 0.03 | 0.47 ± 0.07* |
| 10% of MECP | 0 | 0 | 0.10 ± 0.01 | 0.13 ± 0.03 | 0.27 ± 0.07 |
| 10% of MENT | 0 | 0 | 0.10 ± 0.01 | 0.25 ± 0.05 | 0.45 ± 0.05* |
| 10 mL of DW (Control) | 0 | 0.35 ± 0.05 | 1.45 ± 0.15 | 1.90 ± 0.20 | 2.35 ± 0.05** |

Values were expressed as mean of twenty samples plus or minus of the error mean. Values with the same superscript hysteric in a column have no significant difference at P<0.05 KEYS: NHECP: N-hexane extract of Calotropis procera EACP: Ethylacetate extract of Calotropis procera MECP: Methanol extract of C. procera NHENT: N -hexane extracts of Nicotiana tabacumEAENT: Ethylacetate extracts of Nicotiana tabacum MENT: Methanol extracts of Nicotiana tabacum DW: Distilled Water

Effects of different concentrations of Nhexane, ethylacetate and methanol extracts of C. procera on the germination percentage of maize seeds. Table 4 shows the effects of n-hexane, ethylacetate and methanol extracts of C. procera and N. tabacum on germination percentage of maize seeds. All seedlings treated with the extracts of C. procera and N. tabacum show reduction in germination percentage when compared to the control from the 3rd to the 12th day of germination. However, n-hexane, ethylacetate and methanol extract of C. procera and N. tabacum at concentrations of 2.5% exhibit $\geq$ 50% of germination percentage on the 12 th day when compared with concentrations 5% and 10%.

Effect of N-hexane, ethylacetate and methanol extracts of Calotropis procera on the seedling vigor index of germinated maize seeds.

Table 4 shows the effects of n-hexane, ethylacetate and methanol extracts of C. procera and N. tabacum on seedling vigor index of germinated Maize seeds. All seedlings treated with the extracts of C. procera and N. tabacum also show reduction in vigor index when compared with the control from the 3rd to the 12th day of germination. However, n-hexane, ethylacetate and methanol extract of C. procera and N. tabacum at concentrations of 2.5% exhibit high values of vigor index on the 12th day when compared with concentrations 5% and 10%.

Table 4: Effects of different concentrations of N-hexane, ethylacetate and methanol extracts of C. procera on the germination percentage of Maize seeds.

| | % Germination/days | | | | |
|---|---|---|---|---|---|
| Concentration | 1st day | 3rd day | 6th day | 9th day | 12th day |
| 2.5% of NHECP | 0 | 10.30 ± 1.20 | 20.25 ± 2.00 | 40.75 ± 3.00 | 50.25 ± 2.15* |
| 2.5% of NHENT | 0 | 0.40 ± 0.10 | 40.40 ± 4.20 | 50.20 ± 3.40 | 50.60 ± 3.20* |
| 2.5% of EACP | 0 | 10.20 ± 1.80 | 30.80 ± 4.20 | 40.15 ± 3.50 | 60.40 ± 3.10* |
| 2.5% of EAENT | 0 | .40.00 ± 0.10 | 40.60 ± 2.60 | 50.10 ± 2.70 | 60.35 ± 2.40* |
| 2.5% of MECP | 0 | 10.28 ± 2.15 | 40.20 ± 2.10 | 50.90 ± 5.10 | 60.20 ± 2.15* |
| 2.5% of MENT | 0 | 20.20 ± 4.30 | 30.30 ± 2.70 | 40.30 ± 2.20 | 50.20 ± 3.10* |
| 5.0% of NHECP | 0 | 0.20 ± 0.10 | 20.25 ± 2.30 | 20.20 ± 3.10 | 30.10 ± 2.30 |
| 5.0% of NHENT | 0 | 0 | 20.10 ± 2.15 | 20.20 ± 2.20 | 30.50 ± 4.10 |
| 5.0% of EACP | 0 | 10.40 ± 2.10 | 30.40 ± 2.60 | 40.20 ± 2.80 | 40.15 ± 2.90 |
| 5.0% of EAENT | 0 | 0 | 40.35 ± 3.10 | 40.15 ± 2.10 | 40.38 ± 2.70 |
| 5.0% of MECP | 0 | 10.20 ± 2.80 | 30.10 ± 2.60 | 40.25 ± 2.15 | 40.35 ± 2.12 |
| 5.0% of MENT | 0 | 20.50 ± 4.10 | 30.15 ± 2.10 | 40.10 ± 2.10 | 40.30 ± 3.10 |
| 10% of NHECP | 0 | 0 | 0 | 20.40 ± 2.10 | 24.60 ± 2.20 |
| 10% of NHENT | 0 | 0 | 10.20 ± 2.10 | 20.26 ± 2.40 | 22.80 ± 2.40 |
| 10% of EACP | 0 | 0 | 10.50 ± 2.10 | 20.20 ± 2.10 | 2200 ± 2.20 |
| 10% of EAENT | 0 | 0 | 10.40 ± 1.20 | 30.20 ± 3.30 | 30.60 ± 3.10 |
| 10% of MECP | 0 | 0 | 10.20 ± 1.60 | 20.00 ± 2.80 | 20.40 ± 2.20 |
| 10% of MENT | 0 | 0 | 20.35 ± 1.70 | 20.10 ± 2.60 | 20.60 ± 2.20 |
| 10 mL of DW (Control) | 0 | 40.20 ± 6.50 | 90.60 ± 4.30 | 90.80 ± 4.10 | 100 ± 0.00** |

Values were expressed as mean of twenty samples plus or minus of the error mean. Values with the same superscript hysteric in a column have no significant difference at $P<0.05$

KEYS: NHECP: N-hexane extract of Calotropis procera EACP: Ethylacetate extract of Calotropis procera
MECP: Methanol extract of C. procera NHENT: N-hexane extracts of Nicotiana tabacum EAENT: Ethylacetate extracts of Nicotiana tabacum MENT: Methanol extracts of Nicotiana tabacum DW: Distilled Water

Table 5: Effect of N-hexane, ethylacetate and methanol extracts of Calotropis procera on the seedling vigor index of germinated Maize seeds.

| Concentration | Seedling Vigor Index (SVI)/days | | | | |
|---|---|---|---|---|---|
| | 1st day | 3rd day | 6th day | 9th day | 12th day |
| 2.5% of NHECP | 0 | 2.00 ± 0.20 | 8.00 ± 1.40 | 28.80 ± 2.60 | 44.00 ± 1.10* |
| 2.5% of NHENT | 0 | 0 | 26.00 ± 3.40 | 52.00 ± 4.40 | 56.00 ± 2.20* |
| 2.5% of EACP | 0 | 1.30 ± 0.40 | 28.80 ± 2.80 | 40.20 ± 6.20 | 131.10 ± 18.20** |
| 2.5% of EAENT | 0 | 0 | 37.20 ± 4.60 | 67.50 ± 3.40 | 84.00 ± 3.30** |
| 2.5% of MECP | 0 | 5.50 ± 0.60 | 13.20 ± 0.62 | 57.00 ± 5.20 | 91.80 ± 6.40** |
| 2.5% of MENT | 0 | 15.00 ± 0.60 | 30.00 ± 3.30 | 76.00 ± 6.10 | 52.00 ± 1.20 |
| 5.0% of NHECP | 0 | 0 | 5.00 ± 1.60 | 14.60 ± 2.40 | 30.90 ± 0.80 |
| 5.0% of NHENT | 0 | 0 | 0 | 14.60 ± 1.40 | 30.60 ± 1.20 |
| 5.0% of EACP | 0 | 1.00 ± 0.10 | 12.90 ± 2.10 | 28.00 ± 2.10 | 40.00 ± 3.40 |
| 5.0% of EAENT | 0 | 0 | 24.00 ± 1.60 | 30.00 ± 4.70 | 33.60 ± 3.40 |
| 5.0% of MECP | 0 | 1.30 ± 0.15 | 15.90 ± 3.60 | 36.00 ± 2.20 | 52.00 ± 3.40 |
| 5.0% of MENT | 0 | 7.00 ± 0.70 | 11.10 ± 1.10 | 16.00 ± 3.40 | 24,00 ± 2.20 |
| 10% of NHECP | 0 | 0 | 0 | 11.00 ± 0.40 | 14.40 ± 0.90 |
| 10% of NHENT | 0 | 0 | 0 | 12.00 ± 2.10 | 19.60 ± 0.40 |
| 10% of EACP | 0 | 0 | 1.00 ± 0.14 | 5.60 ± 1.60 | 8.00 ± 1.20 |
| 10% of EAENT | 0 | 0 | 4.50 ± 0.20 | 18.90 ± 1.40 | 30.00 ± 3.10 |
| 10% of MECP | 0 | 0 | 2.00 ± 0.40 | 11.60 ± 2.20 | 24.60 ± 1.20 |
| 10% of MENT | 0 | 0 | 4.00 ± 0.30 | 8.00 ± 0.80 | 14.00 ± 2.20 |
| 10 mL of DW (Control) | 0 | 39.60 ± 3.10 | 202.50 ± 6.40 | 283.50 ±5.60 | 423.00 ± 4.40*** |

Values were expressed as mean of twenty samples plus or minus of the error mean. Values with the same superscript hysteric in a column have no significant difference at $P<0.05$

KEYS: NHECP: N-hexane extract of Calotropis procera EACP: Ethylacetate extract of Calotropis procera
MECP: Methanol extract of C. procera NHENT: N-hexane extracts of Nicotiana tabacum
EAENT: Ethylacetate extracts of Nicotiana tabacum MENT: Methanol extracts of Nicotiana tabacum
DW: Distilled Water

alpha-amyrin are dominant in ethylacetate extract.

HPLC-Fingerprints of Different Extracts of Different Extracts of Calotropis procera The HPLC-fingerprints of n-hexane, ethylacetate and methanol extracts of C. procera is shown in Table 6. The Calotropis procera extracts revealed the presence of calotropin, calotoxin, calactin, caffeic acid, thioacetate, alpha-amyrin, beta-sitosterol, stigmasterol, isoquercitrin, quercetin, kaempferol, rhamnetin, luteolin, lineolone, voruscharin, uscharin and uscharidin. Likewise, Azeleatin and tyranton were detected in ethylacetate and methanol extracts but not detected in n-hexane extract. In addition, most of calotropin, calotoxin, calactin, caffeic acid, thioacetate,

Table 6: HPLC-Fingerprints of Different Extracts of Different Extracts of Calotropis Procera

| Components | Concentration (ppm) | | |
|---|---|---|---|
| | N-hexane Extracts | Ethylacetate Extracts | Methanol Extracts |
| Calotropin | 61.11 | 139.39 | ND |
| Calotoxin | 124.56 | 293.13 | 211.99 |
| Calactin | 45.32 | 70.90 | 44.55 |
| Caffeic Acid | 8.15 | 20.68 | 8.08 |
| Thioacetate | 16.05 | 7.97 | 9.17 |
| Alpha-Amyrin | 8.63 | 9.83 | 8.15 |
| Beta-Sitosterol | 14.45 | 12.27 | 13.21 |
| Stigmasterol | 11.50 | 9.87 | 11.03 |
| Isoquercitrin | 9.60 | 8.31 | 11.08 |
| Quercetin | 702.87 | 986.46 | 897.43 |
| Kaempferol | 170.24 | 310.82 | 254.85 |
| Rhamnetin | 46.43 | 118.14 | 85.23 |
| Luteolin | 35.08 | 21.02 | 20.70 |
| Lineolone | 7.57 | 19.75 | 17.24 |
| Voruscharin | 48.29 | 52.19 | 39.13 |
| Uscharin | 8.31 | 7.82 | 9.18 |
| Uscharidin | 7.38 | 9.33 | 9.30 |
| Azeleatin | ND | 9.82 | 10.94 |
| Tyranton | ND | 7.39 | 7.72 |

ND: Not Detected

## HPLC-Fingerprints of Different Extracts of Nicotiana tabacum

Table 7 shows the HPLC-fingerprints of nhexane, ethylacetate and methanol extracts of Nicotiana tabacum. The Nicotiana tabacum extracts revealed the presence of nicotine, chlorogenic acid, nornicotine, 4-hydroxybenzaldehyde, catechin, catechol, protocatechuic acid, vanillin, vanillic acid, nicotelline, nicotianine, tabacine, tabacinine, harmine and anabasine. However, ajmalicine, pyridine and cotinine are not present in methanol extracts of Nicotiana tabacum.

Table 7: HPLC-Fingerprints of Different Extracts of Nicotiana tabacum

| Components | Concentration (ppm) | | |
|---|---|---|---|
| | N-hexane Extracts | Ethylacetate Extracts | Methanol Extracts |
| Nicotine | 27.88 | 26.94 | 31.67 |
| Chlorogenic Acid | 7.49 | 7.29 | 7.10 |
| Nornicotine | 11.59 | 11.84 | 11.40 |
| 4-Hydroxybenzaldehyde | 0.92 | 1.09 | 0.97 |
| Catechin | 1.04 | 1.26 | 1.46 |
| Catechol | 0.66 | 0.71 | 1.12 |
| Protocatechuic Acid | 0.58 | 1.62 | 1.43 |
| Vanillin | 0.68 | 0.79 | 0.92 |
| Vanillic Acid | 0.78 | 0.94 | 0.72 |
| Nicotelline | 8.96 | 9.25 | 7.41 |
| Nicotianine | 32.88 | 31.91 | 30.14 |

| | | | |
|---|---|---|---|
| Tabacine | 0.60 | 0.86 | 1.07 |
| Tabacinine | 0.61 | 1.06 | 1.41 |
| Harmine | 0.85 | 1.04 | 1.20 |
| Anabasine | 0.66 | 0.91 | 1.15 |
| Anatabine | 0.77 | 0.75 | 0.83 |
| Ajmalicine | 1.42 | 0.80 | ND |
| Pyridine | 1.00 | 0.92 | ND |
| Cotinine | 0.62 | ND | ND |

ND: Not Detected



## DISCUSSION

The differential extract yield observed among the different solvent could be attributed to the polarity of the solvents used in extraction. More polar solvent such as methanol and ethylacetate were able to extract a broader range of bioactive compounds, including more hydrophilic compounds [6, 7]. This study also reveals the significant allelopathic effects of pesticidal plant extract such as C. procera and N. tabacum extracts on maize seed germination and seedling growth. Both extracts exhibited inhibitory effects on the radicle and plumule lengths of maize seedlings, with the highest concentrations (10%) showing the most pronounced inhibition while the lowest concentration produced the least growth inhibition. The inhibitory effects observed in both extracts could be linked to presence of some secondary metabolites in each extract. Allelochemicals or secondary metabolites such as, catotoxin, quercetin, caffeic acid and calactin as well as nicotine, nornicotine, anabasine, and anatabine are common alkaloids found in tobacco present in C. procera and N. tabacum extracts could have caused negative impacts on the germination, plumule and radicle development in maize seedlings as it occurred in several plants [8, 9]. Furthermore, insecticidal effects of the C. procera have been linked to its strong allelopathic potential.

A few studies also suggested that this plant causes acute toxicity as a weed in cropping systems, with considerable effects on cereal crops such as maize and vegetables [10, 9]. This might be due to the ability of these bioactive compounds to disrupt the essential physiological processes in the maize plants. This result is in consistency with the findings of [11] that affirm the allelopathy effects of alkaloids and some phenolics in several medicinal plants. The actual mechanisms through which these

allelochemicals present in C. procera and N. tabacum use to carry out the inhibitory effects is not yet established in this study. However, process like mineral intake, photosynthetic activity, respiration rate, pigments, hormone production, membrane stability and enzyme activities are examples of plants' morphological, molecular and physiological characteristics that could be affected by the aforementioned allelochemicals intervention [12]. Phenolic acids like caffeic acid, which were detected in the extracts, are known to interfere with cellular respiration and enzyme activity in plants [13]. These compounds can also affect the permeability of plant cell membranes, reducing water and nutrient uptake, which is essential for seed germination and early seedling growth [13]. This explains the reduced seedling vigor and the stunted growth of maize seedlings in the presence of C. procera extracts. Moreover, quercetin and kaempferol which are both the flavonoids as well as various phenolics identified in both extracts through HPLC analysis, are known to inhibit key growth regulators, including auxins, which are crucial for root and shoot elongation [14]. The suppression of auxin transport by flavonoids could explain the reduced root and shoot development in the maize seedlings treated with the extracts. These findings are consistent with previous studies that have reported the allelopathic effects of flavonoids in other plant species, demonstrating their ability to inhibit seed germination and reduce growth rates in competing plants.

It is important to note that the concentration of the extracts had a direct relationship with the level of inhibition. At higher concentrations, the allelopathic effects were more pronounced, with nearcomplete inhibition of germination and a drastic reduction in seedling growth. This dose-dependent response suggests that the allelopathic activity of C. procera is mediated by the concentration and combination of allelochemicals present in the extracts. This aligns with the findings of [15], who reported a similar dosedependent allelopathic effect of phenolic acids and flavonoids on Scindapsus officinalis.

Another key finding in this study is the reduced seedling vigor index (SVI) observed in maize treated with C. procera and N. tabacum extracts. Seedling vigor is a critical indicator of plant health and growth potential and its reduction implies that the extracts not only inhibited germination but also compromised the overall growth capacity of the maize plants [16]. This could have serious implications for crop productivity if such extracts are applied indiscriminately in agricultural settings. The allelopathic effects of Calotropis procera and N. tabacum observed in this study are consistent with previous research on the plant's bioactive compounds. Baig et al. [17] reported that C. procera extract exhibits strong phytotoxicity towards tomato (Solanum lycopersicum), wheat (Triticum aestivum), and cucumber (Cucumis sativus) reducing their growth and competitiveness while N. tabacum exhibits allelopathy effects on Wild Solanaceae Species [18, 19]

## CONCLUSION

The ethyl acetate and N-hexane extracts of Calotropis procera and N. tabacum at lower concentration have weak allelopathic effects on the germination and early growth stages of maize. Therefore, lower concentration of these two plant extracts should be considered for pesticides for the purpose of maize production so as to avoid poor growth while controlling maize pest so as not to fall in unintended consequences during crop productivity.

## Authors' contributions

Authors BMB, ANU and KFA designed and conceptualized the study; DOAY, BMB and HRU participated in the extraction, data

collections and analysis; MMD, JSS and ESO performed the laboratory analysis; BMB, ANU and KFA interpreted the data; BMB, prepared the first draft of the manuscript, reviewed by ANU and KFA. All authors contributed to the development of the final manuscript and approved its submission.

Conflict of interest

None

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