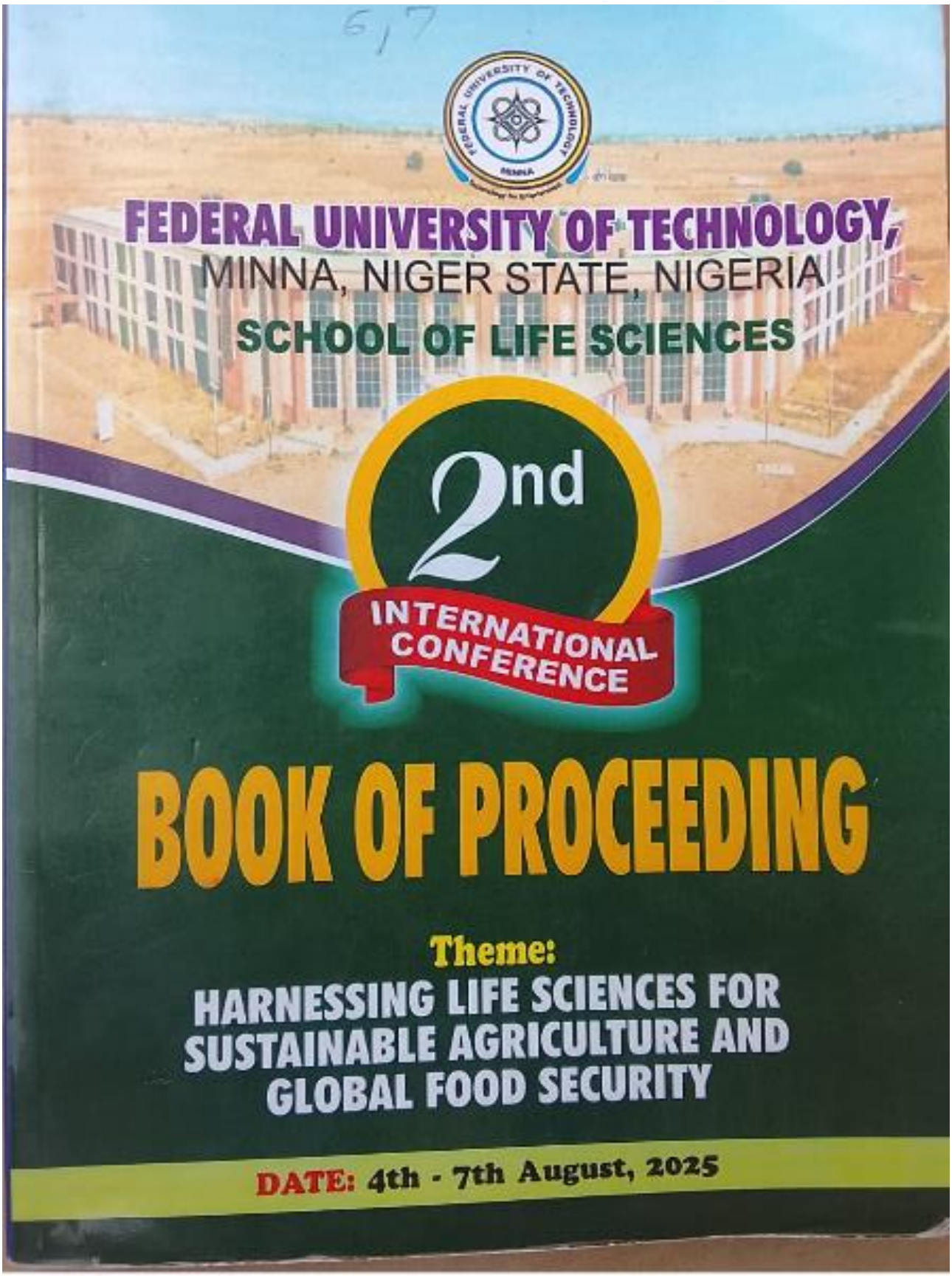
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Phytochemical constituents and allelopathy activities of *Azadirachta Indica* on germination of Maize seed

*[1,2]Hamzah, R. U., [1,3]Busari, M. B., [1,4]Daudu, O. A. Y., [1,3]Eneji'yon, S. O., [1,3]Kuta, F. A., [1,3]Adebara, K. U., [2]Muhammad, T., [2]Dickson, J., [2]Abdullahi, M. O. and [2]Agada, J. B.

[1]Biotechnology for Self-reliance Research Group, Centre for Genetic Engineering and Biotechnology, Federal University of Technology Minna, Nigeria
[2]Department of Biochemistry, Federal University of Technology Minna, Nigeria
[3]Department of Microbiology, Federal University of Technology Minna, Nigeria
[4]Department of Plant Biology, Federal University of Technology Minna, Nigeria

ABSTRACT

Introduction: *Azadirachta indica* leaf (*AIL*) is natural pesticides that has been used on several pests affecting many crops. Nevertheless, most of these pesticidal plants affect the growth of plant negatively. Aim: This study aims at determining the phytoconstituents and the allelopathic effects of *Azadirachta indica* leaf extracts (*AILE*) on germination and growth of maize seed and seedling. Methods: The allelopathic activities of n-hexane, ethylacetate and methanol extract of *AIL* were determined on germination and growth of maize seed and seedling 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. Results: The *AILE* revealed the presence of B-Caryophyllene, Trans-B-Farnes, Alpha Ionone Phytol, Myricetin, Quercetin, Azadiradiol, Azadirachtol, Azadirachta A, Phytene while Azadirachtin was not revealed in methanol extract of *Azadirachta Indica* leaf extracts. The inhibition of plumule and radicle growth were observed to be concentration-dependent with the highest percentage of inhibition in 10% of n-hexane extract of *Azadirachta Indica*. Germination percentage also decreased with higher concentration, with a maximum of 100% germination in the control group when compared with 50, 60, and 60% of n-hexane, ethylacetate and methanol extract of *AIL* at 2.5% concentration respectively. Similarly, Seedling vigour index was drastically reduced at higher concentration of n-hexane, ethylacetate and methanol extract of *AIL* when compared with 423 in the control group. Conclusion: This finding suggests that high concentrations (5 and 10%) of *AILE* could be avoided when using as insecticide during germination stage of maize to prevent growth inhibition.

Keywords: Allelopathy, *Azadirachta indica*, insecticides, Phytochemicals

*Corresponding author: busarihub@futminna.edu.ng (+2347036710704; ORCID: 0009-0001-1608)

INTRODUCTION

Agricultural sustainability is increasingly gaining attention as farmers seek environmentally friendly alternatives to chemical inputs. The use of natural plant-based products such as *Azadirachta indica* (*A. indica*) is emerging as a promising solution in sustainable farming. It has been widely studied for its potential insecticidal and antifeedant properties, making it a versatile tool in agriculture

(Biswas, 2020). Allelopathy refers to the biochemical interactions between plants such as *Azadirachta indica* where compounds released by it affect the growth and development of nearby plants such as Maize, either positively or negatively (Kaur et al. 2020).

Azadirachta indica, commonly known as neem tree is a member of the *Meliaceae* family, which comprises various tropical and subtropical trees. This evergreen tree is characterised by its distinct morphological features, making it easily identifiable. Neem trees can grow to a height of 15 to 20 meters (approximately 50 to 65 feet) and possess a wide, rounded crown that provides significant shade (Michael, 2019). *A. indica* possess strong antimicrobial and antifungal properties, making it valuable in the treatment of skin infections, acne, and various other ailments caused by pathogens (Pan, 2019).

Maize (*Zea mays*) is a major staple crop, essential for food security in many parts of the world, especially in sub-Saharan Africa. The success of maize cultivation largely depends on the germination and early growth stages, which are sensitive to environmental factors and soil conditions. Understanding how *Azadirachta indica* affects maize during these critical stages is vital, as any inhibitory effects could lead to reduced yields and impact food production (Rana et al, 2023). Maize is highly sensitive to environmental conditions during its germination and early growth stages, which are crucial for determining final crop yield (Kheem et al, 2022). Thus, the use of synthetic pesticides or herbicides is being discouraged due to their pollution, residual toxicities, and health effects in both humans and animals (Kheem et al, 2022). As such, phytochemicals from various plants are being used as alternatives to poisonous synthetic agrochemicals.

This study seeks to unravel the phytochemical constituents and allelopathic activity of *Azadirachta indica* leaf extract on maize germination and seedling growth. Understanding these effects will provide valuable insights into the potential integration of plant-based pesticide in maize farming, ensuring that their use does not compromise crop yields. Additionally, it will help determine whether can be used as a natural herbicide or growth enhancer in maize cultivation systems.

MATERIALS AND METHODS

Sample collection

Fresh *Azadirachta indica* leaves were collected within the farm of Federal University of Technology, Minna, Gidan Kwano Campus in July 2025 and authenticated by Dr. O.A.Y. Danlu with identification numbers: FUT/PLB/HEL/CCL. The plant sample was deposited in the Herbarium of the department of Plant Biology, Federal University of Technology, Minna. The *Zea mays* used were sourced from Kasso Market in Minna, Niger state, Nigeria.

Preparation of *Azadirachta indica* Leaves

Azadirachta indica leaves were washed with tap water and dried at 30°C. Thereafter, the plant leaves were reduced to powder using Silver Crest 2L Industrial 850W electric blender, and the powder form was kept inside a tight covered plastic container until further use.

Extraction of *Azadirachta indica* Extracts

Extraction of *Azadirachta indica leaf* was done as reported by Busari *et al.* (2025). Pulverized sample of *Azadirachta indica* leaves were subjected to cold extraction using n-hexane, ethylacetate and methanol using separate volumetric flask. Exactly 250 grams of *Azadirachta indica* leaf powder was weighed into 1000 mL volumetric flask. Afterwards, 750 mL of each solvent was separately poured into flask and mixture was properly agitated and left for 72 hours with occasional vortexing each day. After the 72 hours, the solvent was evaporated using a water bath under reduced pressure before finally lyophilized with freeze drier. The freeze-dried sample was stored in tight covered glass containers and refrigerated at 10°C.

Percentage 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:

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

Germination of Maize Seed and Germination Test

Ten maize seeds were placed evenly on a filter paper in duplicate glass petri dishes (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, ethylacetate and methanol leaf extracts of *Azadirachta indica* 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 Vigour Index

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

SVI = Seedling length (cm) x Germination percentage

High-Performance Liquid Chromatography Analysis of Extracts of *Azadirachta indica*

The extract was analyzed using high-performance 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 in reference standards which were further confirmed by spiking samples with small amounts of these standards.

Data Analysis

Data obtained are 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 statistically significance.

RESULTS

Percentage Yield of N-Hexane, Ethylacetate and Methanol Extracts of *Azadirachta indica* leaf

The percentage yield of *Azadirachta indica* extract from different solvents is shown in Table 1. The percentage yields of extracts are in the following orders: methanol > ethylacetate > n-hexane with 27.18, 26.90 and 4.03% respectively.

Table 1: Percentage Yield of N-Hexane, Ethylacetate and Methanol Extracts of *Azadirachta indica* Leaf

Sample	% Yield
NHEAI	4.03 ± 0.06[c]
EAAI	26.90 ± 1.31[b]
MEAI	27.18 ± 1.76[a]

Values are mean of triplicate ± standard error mean. Values with different superscript (alphabet in a column) are significantly different at $P \leq 0.05$.
NHEAI: N-hexane extract of *Azadirachta indica* leaf, EAAI: Ethylacetate extract of *A. indica* leaf.
MEAI: Methanol extract of *A. indica* leaf, 0.9% NaCl: Sodium chloride.

Effects of N-hexane, Ethylacetate and Methanol Leaf Extracts of *Azadirachta indica* Leaf on Root Length (Radicle) of Maize Seedlings

The effects of n-hexane, ethylacetate and methanol leaf extracts of *Azadirachta indica* leaf on root length (radicle) of maize seedlings is presented in Table 2. Treatments of maize seed with leaf extract at all concentrations decreased the radicle length as compared to the control. Maximum root length was found in 2.5% of NHEAI (1.33 cm) when compared to other treatments, although significantly ($P \leq 0.05$) lower when compared with control (1.88 cm). The minimum root length was found at the highest concentrations 10% (0.23 cm) for both N-hexane and ethylacetate extracts respectively. As the concentrations of the extracts decreased, there was an increment in radicle length.

Table 2: Effects of N-hexane, Ethylacetate and Methanol Leaf Extracts of *Azadirachta indica* on Maize Seedlings' Root Length (cm) at 3rd, 6th, 9th and 12th day

Concentration	Root Length (cm) at				
	3rd day	6th day	9th day	12th day	
2.5% of NHEAI	1	0	1.90 ± 1.1	1.20 ± 0.21	1.33 ± 0.65[a]
2.5% of EAAI	0	0.13 ± 1.10	0.23 ± 1.07	0.29 ± 0.03	0.33 ± 0.17
2.5% of MEAI	1	0.63 ± 1.15	0.50 ± 0.15	0.69 ± 0.03	0.90 ± 0.14
5.0% of NHEAI	0	0			
5.0% of EAAI	1	0	1.05 ± 0.20		
5.0% of MEAI	0	0.30 ± 1.11	0.43 ± 0.17	1.80 ± 0.20	0.96 ± 0.13
10% of NHEAI	0	0	0.23 ± 0.13	0.23 ± 0.09	0.50 ± 0.25
10% of EAAI	1	0	0.10 ± 0.01	0.43 ± 0.21	0.80 ± 0.14
10% of MEAI	0	1	0.25 ± 1.03	0.21 ± 0.04	0.23 ± 1.07
			0.75 ± 1.05	0.13 ± 1.05	0.21 ± 1.07
				0.50 ± 0.15	0.63 ± 1.05

Control (10ml DW)	0	2.65 ± 1.05	0.80 ± 0.20	2.25 ± 1.25	1.83 ± 2.63

Values are mean of triplicate ± standard error mean. Values with the same superscript are not significantly different at $P \leq 0.05$

NHEAl: N-hexane extract of *A. indica* leaf, EAAl: Ethylacetate extract of *A. indica* leaf, MEAl: Methanol extract of *A. indica* leaf, DW: Distilled water.

Effects of N-hexane, Ethylacetate and Methanol Leaf Extracts of *Azadirachta indica* Leaf on Plumule Lengths of Maize Seedlings

The effects of n-hexane, ethylacetate and methanol extracts of *Azadirachta indica* leaf on the lengths of the plumule of maize seedlings is presented in Table 3. Treatments of maize seed with leaf extract at all concentrations decreased the length of the plumule when compared with control. Whereas 2.5% of MEAl showed the maximum shoot length (1.62 cm) out of all treatments but not comparable with the control (2.33 cm). However, the minimum root length was found at the highest concentrations 10% (0.30 cm) of N-hexane and ethylacetate extracts. Therefore, as the concentration of the extracts decreased, there was an increment in plumule length for all extracts.

Table 3: Effect of N-hexane, Ethylacetate and Methanol Leaf Extracts of *Azadirachta indica* Leaf on Plumule Lengths (cm) of Maize Seedlings

Concentration	Plumule Length (cm)/Days				
	3rd day	4th day	5th day	6th day	7th day
2.5% of NHEAl	0	0	0.31 ± 0.01	1.45 ± 0.15	1.55 ± 0.15
2.5% of EAAl	0	0	0.25 ± 0.03	1.35 ± 1.35	1.43 ± 0.03
2.5% of MEAl	0	1.62 ± 0.21	0.32 ± 0.32	2.03 ± 2.03	1.83 ± 1.05
5.0% of NHEAl	0	0	1.27 ± 1.27	1.33 ± 1.27	0.40 ± 2.10
5.0% of EAAl	0	0	1.25 ± 1.25	1.25 ± 1.25	1.25 ± 1.25
5.0% of MEAl	0	0.20 ± 1.01	1.40 ± 1.13	1.67 ± 1.03	1.30 ± 1.32
5% of NHEAl	0	0	1.21 ± 1.12	0.12 ± 0.07	1.25 ± 0.03
10% of NHEAl	0	0	1.10 ± 1.13	0.10 ± 0.06	0.30 ± 0.10
10% of EAAl	0	0	0.95 ± 1.1	0.15 ± 0.05	0.35 ± 0.05
10% of MEAl	0	0.15 ± 1.005	1.20 ± 1.15	1.20 ± 1.23	1.20 ± 1.25
Control (10ml DW)	0	0.15 ± 1.005	1.20 ± 1.15	1.20 ± 1.23	1.20 ± 1.25

Values were expressed as mean ± standard error. Values with the same superscript letters in a column have no significant difference at $P \leq 0.05$

NHEAl: N-hexane extract of *A. indica* leaf, EAAl: Ethylacetate extract of *A. indica* leaf, MEAl: Methanol extract of *A. indica* leaf, DW: Distilled Water, Effects of *Azadirachta indica* Leaf on the Germination Percentage of Maize Seedlings

The effects of n-hexane, ethylacetate and methanol extracts of *Azadirachta indica* leaf on the germination percentage (GP) of maize seed was represented in Table 4. Treatments of maize seed with leaf extract at all concentrations decreased the percentage of germination of the maize seed. The 2.5% of NHEAl, EAAl and MEAl showed maximum percentage of germination when compared with other treatment but not comparable with the control, which was 100%. The minimum percentage of germination (Maximum inhibition) was found at the highest concentrations (10%), showing only 20% and 20% germination of N-hexane and ethylacetate extracts respectively.

Table 4: Effect of N-hexane, Ethylacetate and Methanol Extracts of *Azadirachta indica* Leaf on the Germination Percentage of Maize Seedlings

% Germination/days Concentration	1st day	3rd day	6th day	9th day	12th day
2.5% of NHEAI	0	0	20	20	50[a]
2.5% of EAAI	0	10	10	60	60[a]
2.5% of MEAI	0	30	50	60	60[a]
5.0% of NHEAI	0	0	20	20	30
5.0% of EAAI	0	0	20	20	30
5% of MEAI	0	10	30	40	40
10% of NHEAI	0	0	10	10	10
10% of EAAI	0	0	10	10	20
10% of MEAI	0	0	10	10	20
Control of 10ml of DW	0	40	90	90	100[b]

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 $P \leq 0.05$
NHEAI: N-hexane extract of *A. indica* leaf, EAAI: Ethylacetate extract of *A. indica* leaf
MEAI: Methanol extract of *A. indica* leaf, DW: Distilled Water

Effects of N-hexane, Ethylacetate and Methanol Extracts of *Azadirachta indica* Leaf on Vigour Index of Maize Seedlings

The effects of N-hexane, ethylacetate and methanol extracts of *Azadirachta indica* leaf on the vigour index of Maize seeds was presented in Table 5. The treatment showed significant ($P \leq 0.05$) decrease in all the concentration when compared with control. As concentration of the extracts increased, the vigour index (VI) decreased accordingly. The 2.5% of NHEAI, EAAI and MEAI showed high VI of 93, 69.6 and 150 respectively. Moreover, 5% and 10% of MEAI also produced high vigour index of 72 and 100 respectively, although not significantly compared with the control (423). However, the minimum value was recorded in the highest concentrations10% (3.6) and (10.6) of N-hexane and ethylacetate extracts.

Table 5: Effect of different concentrations of N-hexane, ethylacetate and Methanol Extracts of *Azadirachta indica* Leaf on seedling vigour index of germinated Maize seeds.

Concentration	Seedling Vigour Index (SVI)/days 1st day	3rd day	6th day	9th day	12th day
2.5% of NHEAI	0	0	24	33	93[c]
2.5% of EAAI	0	1	4.8	39.6	69.6[c]
2.5% of MEAI	0	25.5	55	102	150[d]
5.0% of NHEAI	0	0	12.4	20.6	37.8
5.0% of EAAI	0	0	7.6	11.5	27.9
5% of MEAI	0	2	18.9	40	72[c]
10% of NHEAI	0	0	2	3.3	3.6
10% of EAAI	0	0	1.5	3	10.6
10% of MEAI	0	0	2	6.5	100[c]
Control of 10ml DW	0	39.6	202.5	283.5	423[e]

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 $P \leq 0.05$
NHEAI: N-hexane extract of *A. indica* leaf, EAAI: Ethylacetate extract of *A. indica* leaf
MEAI: Methanol extract of *A. indica* leaf, DW: Distilled Water

HPLC Fingerprint of N-hexane, Ethylacetate and Methanol Extracts of *Azadirachta indica* Leaf

The HPLC Fingerprints of N-hexane extract shownin Table6 revealed the presence of several compounds such as B-Caryophyllene, Trans-B-Farnes, Alpha lonone, Phytol, Myricetin, Quarcetin, Azadirachtol, Azadirachnol, Azadirachta A, Phyllene. However, Ascaridol was present in both NHEAI, EAAI but not in MEAI.

Table 6: HPLC Fingerprint of N-hexane, Ethylacetate and Methanol of *Azadirachta indica* Leaf

Concentration (%)			
Components	NHEAI	EAAI	MEAI
B-Caryophyllene	17.92	17.35	18.36
Trans-B-Farnes	7.38	7.31	7.26
Alpha lonone	5.89	5.60	6.43
Phytol	4.95	5.65	4.89
Myricetin	9.54	11.78	8.89
Quercetin	5.81	6.46	4.94
Azadirachtol	35.07	32.71	38.11
Azadirachnol	5.01	4.56	4.91
Azadirachta A	2.26	2.04	2.24
Phyllene	1.67	1.90	1.95
Alpha Funebren	2.16	1.94	2.01
Ascaridol	2.34	2.68	-

NHEAI: N-hexane extract of *A. indica* leaf, EAAI: Ethylacetate extract of *A. indica* leaf
MEAI: Methanol extract of *A. indica* leaf

DISCUSSION

The results of this study highlight the significant allelopathic effects of *Azadirachta indica* extracts on maize (*Zea mays*) germination and early seedling growth. The demonstration of inhibitory effects on germination percentage, root length, shoot length, and vigour index of maize seedlingsby*A.Indica*leaf extract are aligned with previous work. This effect has been linked with the presence allelochemicals such as Azadirachtin and nimbinwhich also produced same suppressive effects on various other crops (Kaur and Bhardwaj, 2020). The study also found that the inhibitory effects were concentration-dependent which could be linked with the highest quantities of allelochemicals present in the *A. indica* which could produce greater allelopathic interactions with the maize seedlings (Saha and Dutta, 2021).

Azadirachta indica is known for its bioactive compounds, such as azadirachtin, nimbin, quercetin, and salannin, which contribute to its allelopathic properties (Mahmood *et al.*, 2021). In the present study, the presence of these compounds in both N-hexane and ethylacetate extracts was confirmed through HPLC analysis, which detected a range of phytochemicals known for their growth-inhibiting effects. These findings are in tandem with that of Pandey *et al.*

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