Published by The College of Science & Information Technology (COSIT), TASUED, Vol. 18, No. 2, pp. 193-201.

COSIT, TASUED
Journal of Science and Information Technology (JOSIT)

Allelopathic Effect of Neem (*Azadirachta indica*) Extracts on the Germination and Viability of Cowpea Seedlings

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Abstract

Allelopathic effect of Neem extracts on the germination and viability of Cowpea seedlings was investigated. The experimental design adopted was complete randomized design (CRD) with three replicates. The three treatments were; (1) Control (distilled water), (2) neem root extract and (3) neem leaf extract. Leaves and root of the *Azadirachta indica* were extracted using distilled water. Distilled water was used as the control. The result of this study showed that the root extract has greater inhibiting effect on the germination and growth of the selected cowpea. Neem extract have been proved effective. It was suggested that the root extract of the neem has greater phenolic compounds than the leaves, this result in the wide significant differences among the treatments (root, leaves and the control).

Keywords: Cowpea, Allochemicals, Alleloparthy, Viability

INTRODUCTION

Cowpea (*Vigna unguiculata [L.]* Walp), is a legume from the genus Vigna. Cowpea is part of the group Vigna. The Latin word *Unguiculata* means “with a small claw”, which indicates the small stalks on the flower petals. It is a dicot plant belonging to the family Fabaceae, sub-family Faboideae. It is commonly grown in the regions of Africa with mid-altitudes (especially in the dry savanna) sometimes as sole but more often intercropped with cereals such as sorghum or millet (Agbogodi, 2010). Due to its high value in protein content, cowpea is referred to as poor man’s food (Saima, Sabeera, Ahmed, Ahmad and Munaf, 2016).

It is one of the most important grain legumes in most developing countries and the sub-Saharan Africa. Cowpea contains 21–33 % protein, a rich source of calcium and iron, 57 % carbohydrate, the leaves’ protein value ranged 27-34% (Boukar *et al*., 2011; Alidu *et al*., 2020). Cowpea grain is also made of some amounts of zinc, thiamine, β-carotene, riboflavin and folic acid (Gonçalves *et al*., 2016 and Owade *et al*., 2020).

Cowpea genotypes are drought and heat tolerant and also adapted to warm season. Determinate bush type cowpea reaches about 24 inches, but cowpea generally reaches a height of 30 to 36 inches. The seed pods are usually visible and are borne above the leaf axil. Their pods are 6 to 12 seeds per pod and seed pod is typically 3 to 6 inches long. Cowpea thrives in sandy and well drained soils but not poorly drained soils.

Ability to produce nitrogenous compounds that are of great importance to plants from atmospheric nitrogen is the main attribute of. The presence of root nodules with the aid of *Rhizobium* made the production of this nitrogenous compounds possible. A symbiotic relationship is established between these bacteria and the legumes that are fixing free nitrogen for the plants’ benefit. The bacteria is being supplied with a source of carbon that fixed by the legumes as a result of photosynthesis. This allowed many legumes to

Cite as:

Awosanya, A. O. (2024). Allelopathic Effect of Neem (*Azadirachta indica*) Extracts on the Germination and Viability of Cowpea Seedlings. *Journal of Science and Information Technology (JOSIT),* Vol. 18 No. 2, pp. 193-201.

survive and be able to thrive effectively in poor nitrogen conditions.

The growth of the embryonic plants enclosed in a seed is known as germination which results in the formation of a new plant. Empty seeds are sometimes produced by some plants. These are seeds with no embryo. Most seeds undergo a period of quiescence, though they are ripe but do not germinate (Susan *et al*., 2005). Most seeds go through a period of quiescence where there is no active growth because they are subject to external environmental conditions that which affect the establishment of metabolic processes and cell growth.

Cowpea and other staple crops are faced with many constraints including biotic and abiotic constraints like pests, weeds, drought and also the allelopathic influence of some plants on the growth and yield of cowpea.

The International Allelopathy Society (IAS), defined allelopathy "as any process involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of agricultural and biological systems" (Duke, 2010). One of the most controversial points in defining allelopathy is to differentiate it with the phenomenon of competition. In the environment, it is not possible to differentiate these two mechanisms, therefore, scientists refer to allelopathy as part of the competition for resources. This can simply be solved by Muller's suggested term "interference" as a general influence between plants, which include both allelopathy and competition. Chaïb *et al*., (2021) differentiate allelopathy from the competition for the fact that it involves the removal or reduction of some factor that is required by some other plants sharing the habitat from the environment. Competition is related to the acquisition of various resources: water, light, pollinators, minerals, food, shoot and root space.

The allelopathic effect is largely dependent on stress. In reality, a plant for instance, that is short of mineral elements would suffer both the stress of nutrient deficiency and phytotoxic effect of allelopathic compounds, therefore, its growth would be inhibited. This can be proved by the fact that competition is often related with 43–57% of interference (An *et al*. 2008).

Volatilization under dry and semi-arid conditions causes release of allelochemicals, which are subsequently absorbed as vapours by angiosperm plants, or can be absorbed in the form of dew from the condensate of these vapours, or the condensate can be absorbed by the roots when it reached the soil (Kassam *et al*. 2019).

Selective activity of tree's allelochemicals on crops and other plant has also been reported. For example, *Leucaena leucocelpha*, also known as the miracle tree is well known for animal improvement, water and soil conservation and vegetation in India. It also contains a non-protein amino acid that is toxic, in leaves and foliage that inhibits the growth of other trees but not its own seedlings. *Leucaena* species have also been shown to increase the yield of rice but reduce the yield of wheat (John and Narwal, 2003).

At plant cellular and molecular levels, the important effects of allelopathy have also been identified. For models simulating biochemical processes, such as photosynthesis, respiration or factors such as interference with enzymes, hormones, or nucleic acids should be taken in account. (Kumar *et al*. 2020).

The inhibitory effect phenolic acids on seedlings' growth and germination depend more on the type of corn hybrid than the type of acid. The mixture of the investigated acids (vanillic, ferulic, p-coumaric, and p-hydroxybenzoic acids) produces a stronger inhibitory effect than these acids alone (Parthasarathy *et al*. 2021). The inhibition of elongational growth of the aerial parts and the root are the main indicator revealing the presence of allele-inhibitors in the environment. The result is accompanying inhibition of embryonic root elongation and delayed germination, which can cause plant death (Liu *et al*. 2021).

Neem is considered as one of the allelopathic plants having some of these allelochemical properties. Neem (*Azandirachta indica*), is a tree in the mahogany family Meliaceae. The meliaceae plant family is known to contain a variety of compounds that show insecticidals, antifeedant, growth regulating and development-modifying properties (Nugroho *et al*., 1999.

Residue of neem seeds after oil extraction and other extraction derived from the plants of neem when used for soil amendment or added to the soil, do not only enrich the soil with organic matter but also lower the rate of seed germination by inhibiting the proper growth and development of its neighbouring plants, due to the fact that neem possesses some allelopathic properties. The objective of this research work is to determine the allelopathic effect of neem extract (leaves and roots) on the germination and viability of some selected cowpea species and also to determine the most potent extracts between the neem leaves and the root.

METHODOLOGY

Materials

In the course of this study, three varieties of cowpea were collected from Bodija market, Ibadan, Oyo State, Nigeria. The varieties used are 'Sokoto white', 'Oloyin' and 'Malla'. These are local varieties cultivated by farmers in the western region of Nigeria for human consumption and for livestock feeds.

The materials used for this study includes; experimental bottles, cotton wool (as substrate), cowpea seeds, blending machine, pestle and mortar, sieve, distilled water, ruler, thread, neem leaves and root, buckets, 50ml measuring cylinder and knife.

Table 1. Analysis of variance for germination viability, epicotyl length, leaf length, leaf length, hypocotyl length, number of lateral roots and radicle length.

Parameters	Source of Variation	df	SS	MSS	F	F-Prob.	Remark
Germination Viability	Treatment	2	5138.90	2569.45	14.80	<0.001	**
	Variety	2	2916.70	1458.35	8.40	0.003	*
	Treatment x Variety	4	694.40	173.60	0.99	0.436	ns
	Residual	16	2777.80	173.61			
	Total	26	11527.00				
Epicotyl Length	Treatment	2	10.74	5.37	67.13	<0.001	**
	Variety	2	1.78	0.89	11.13	<0.001	**
	Treatment x Variety	4	1.33	0.33	4.13	0.015	**
	Residual	16	1.25	0.08			
	Total	26	15.13				
Leaf Length	Treatment	2	11.82	5.91	53.73	<0.001	**
	Variety	2	0.69	0.35	3.18	0.070	ns
	Treatment x Variety	4	1.05	0.26	2.36	0.100	ns
	Residual	16	1.79	0.11			
	Total	26	15.35				
Hypocotyl Length	Treatment	2	60.92	30.46	87.03	<0.001	**
	Variety	2	12.70	6.35	18.14	<0.001	**
	Treatment x Variety	4	14.58	3.65	10.43	<0.001	**
	Residual	16	5.56	0.35			
	Total	26	93.76				
Number of Lateral Roots	Treatment	2	204.44	102.22	15.87	<0.001	**
	Variety	2	5.64	2.82	0.44	0.654	ns
	Treatment x Variety	4	17.48	4.37	0.68	0.619	ns
	Residual	16	103.46	6.44			
	Total	26	331.02				
Radicle length	Treatment	2	2.41	1.21	24.20	<0.001	**
	Variety	2	0.07	0.04	0.80	0.487	ns
	Treatment x Variety	4	0.10	0.03	0.60	0.721	ns
	Residual	16	0.76	0.05			
	Total	26	3.34				
Total Length of the Seedlings	Treatment	2	506.59	253.30	102.97	<0.001	**
	Variety	2	49.21	24.61	10.00	0.002	**
	Treatment x Variety	4	55.44	13.86	5.63	0.005	ns
	Residual	16	39.38	2.46			
	Total	26	650.62				

Methods

Two main parts (leaf and root) of a neem tree (*Azadirachta indica*) were collected within the University premises. The leaves parts were blended, squeezed and filtered using a sieve while the root parts were pounded and soaked to collect the extract. The experiment was conducted in the Biology Laboratory, Tai Solarin University of Education, Ijagun, Ogun State, Nigeria on 13th May, 2023. The plants were germinated in vitro (in bottles). The experimental design adopted was the complete randomized block design with three replicates serving as the blocks. The cowpea seeds were exposed to three treatments; (1) Control (distilled water), (2) neem root extract and (3) neem leaf extract.
The treatments were applied by measuring 50ml (using measuring cylinder) of the treatments and poured in the already sterilized cotton wool and bottle and labeled accordingly. Four hand-picked viable seeds were planted in each bottle. The trait studied and measured include: hypocotyl length of the germinated seedlings; epicotyl length of the germinated seedlings; number of lateral roots; radical length at day 2; leaf length; total length of the whole plant; and percentage germination (viability). Most measurement were done using ruler or thread where necessary while visual observation was used to observed the viability of the seedlings.

Data Analysis

The mean, standard deviation, coefficient of variation and analyses of variances (ANOVA) were done to test the effect of the neem extracts on the cowpea's viability using Social Package for Social Science (SPSS) version 23

RESULTS AND DISCUSSION

Results

After carrying out the experiment on the allelopathic effect of neem (Azandirachta indica) extracts on the germination and viability of some selected cowpea cultivars. Radicle length was first observed in day 2 after germination and others were obtained on the fifth day of the experiment. The data collected from the experiment were subjected to descriptive statistical analysis like mean and variance. The analysis of variance (ANOVA) was used to determine the significance of the effect of neem (Azandirachta indica) extracts on cowpea germination.

In table 2, it was observed that the mean values of Sokoto white in leaf extract was 75%, 92% in control and 50% in root extract for percentage germination viability. Sokoto white performed well in control with the mean value of 91.7%. The mean percent viability was also determined, Oloyin was observed to have mean value of 100% in leaf extract treatment, 91.7% viability in control and 75% in the root extract and Oloyin performed well in leaf treatment with 100% viability. In Malla, the mean value was 75% in leaf extract, 75% viability was observed in the control. The lowest viability was observed in the treatment with root extract with malla having the lowest viability (41.7%).

The Analysis of variance (ANONA) for percentage germination for all the treatment gave F-probability value of $p=<0.001$ indicating that there is a highly significant difference among the three treatments. For varietal differences, Analysis of Variation gave a value of $p=0.003$ which shows that there is a significant difference in the viability rate among the varieties. In table 2 above, it was observed that the mean values of Sokoto White in leaf extract was 0.25cm, in control was 1.15cm and in root treatment was 0.10cm for epicotyls length of cowpea. Sokoto White performed very well in control treatment with a mean value of 1.15cm. Oloyin was observed to have mean value of 0.467cm in leaf extract treatment, 2.27cm in control treatment and 0.12cm in root extract. Oloyin performed well in control treatment with the mean value of 2.27cm and poorly in the root with the mean value of 0.12cm. Figures 1-6 shows data in charts.

Table 2. Mean value for germination viability, epicotyl length, leaf length, leaf length, hypocotyl length, number of lateral roots and radicle length.

Parameters	Treatment	Sokoto White	Oloyin	Malla
Germination Viability (%)	Control	91.70	91.70	75.00
	Leaf extract	75.00	100.00	75.00
	Root Extract	50.00	75.00	41.70
Epicotyl Length (cm)	Control	1.15	2.27	1.04
	Leaf extract	0.25	0.47	0.00
	Root Extract	0.10	0.12	0.00
Leaf Length (cm)	Control	1.21	1.90	1.82
	Leaf extract	0.45	0.73	0.00
	Root Extract	0.17	0.21	0.00
Hypocotyl Length (cm)	Control	3.19	7.08	4.32
	Leaf extract	2.27	2.19	1.21
	Root Extract	1.63	1.90	1.02
Number of Lateral Roots	Control	13.92	13.19	13.33
	Leaf extract	9.17	9.33	9.22
	Root Extract	5.08	8.89	6.50
Radicle length (cm)	Control	0.79	1.10	0.84
	Leaf extract	0.36	0.35	0.29
	Root Extract	0.24	0.24	0.23
Total Length of The Seedlings	Control	8.89	16.90	11.76
	Leaf extract	4.39	4.99	2.91
	Root Extract	2.54	3.19	2.55

For malla, the mean value was 0cm in leaf extract, 1.04cm in the control and 0cm in root extract treatment. Malla performed well in the control treatment with mean value of 1.04cm while it was observed to have 0.00cm mean value in both leaf and root extract.

Analysis of variance (ANOVA) for epicotyl length in this experiment gave F value of 69.03 and 11.4 for treatment and varietal differences with p-value of <0.001 and <0.001 for the treatment and varietal differences respectively indicating a highly significant differences for both the treatment and varieties. It was observed that there is a significant interaction effect ($p=0.015$) of treatment and variety for the epicotyl length.

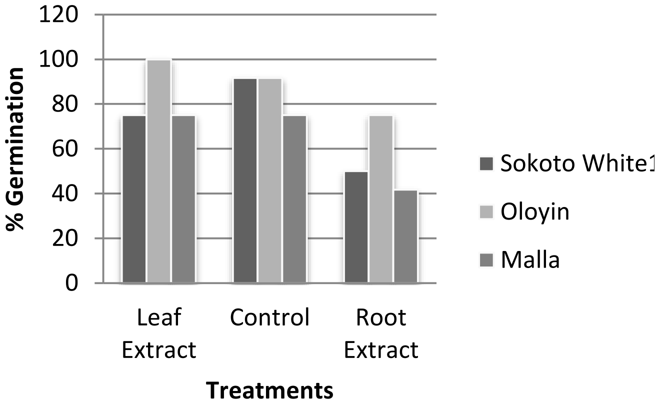

Figure 1. Germination Viability.

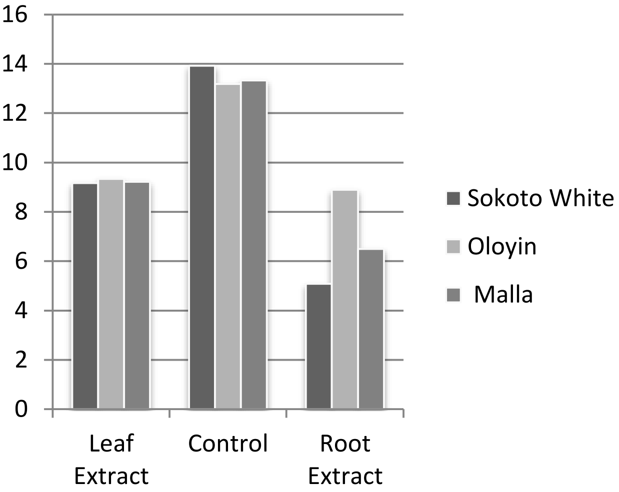

Figure 4. Hypocotyl Length.

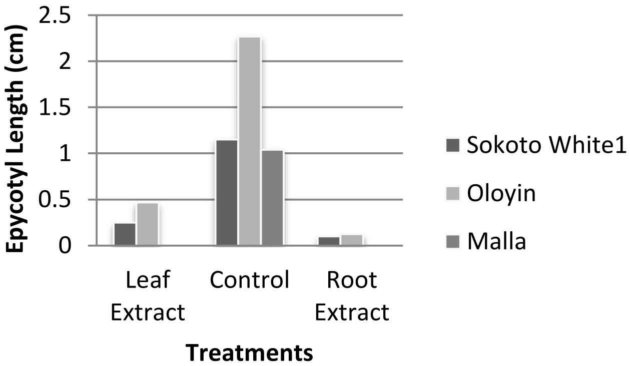

Figure 2. Epicotyl Length

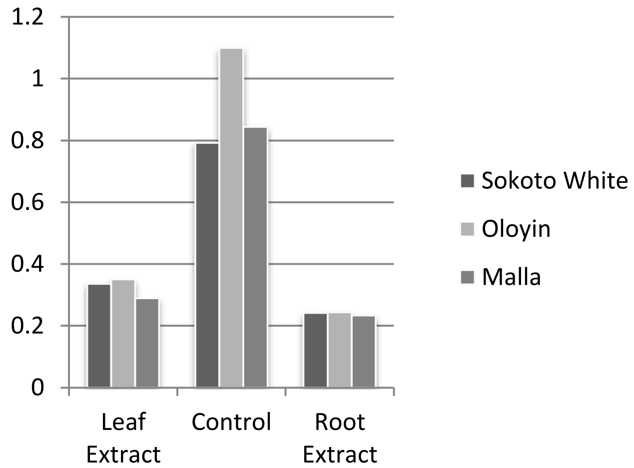

Figure 5. Number of Lateral Roots.

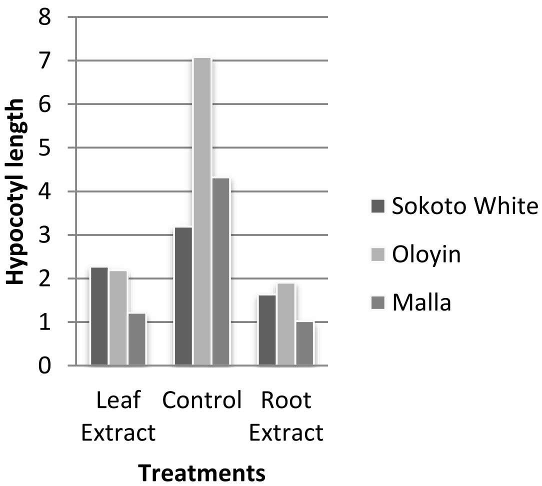

Figure 3. Leaf Length.

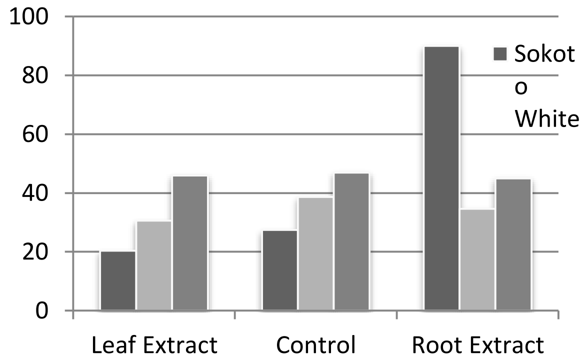

Figure 6. Radicle length.

The observed mean value of Sokoto White in leaf extract was 0.45cm. In control treatment, it was 1.21cm and in root extract was 0.17cm for the leaf length of cowpea seedlings. Sokoto White performed well in control treatment with the mean value of 1.21cm. Mean value observed in Oloyin in leaves treatment was 0.73cm it was observed to be 1.90cm in control treatment and 0.21cm in root extract. Oloyin performed well in control treatment with the mean value of 1.90cm. Also in malla, mean value was observed to be 0.0cm in leaf extract, 1.82cm in control and in root extract, the mean value was also 0.0cm. malla performed poorly in both root and leaves treatment with mean value of 0.00cm. The grand mean value was observed to be 0.72cm. Variance and co-efficient of variation was 0.34 and 9.0% respectively. Analysis of variance (ANOVA) for leaf length of cowpea in treatment with F-probability of p=<0.001 which was observed to be highly significant but in variety p=0.073 as well as in treatment and variety p=0.099 indicated that they are both not significant.

The mean value of Sokoto White in leaf extract was 2.27cm, in control treatment, it was 3.19cm and in root extract, it was 1.63cm. The Sokoto White performed well in control with mean value of 3.19cm. For Oloyin, the mean values in leaf, control and root extract were observed to be 2.19cm, 7.08cm and 1.90cm respectively. Oloyin has the highest mean value (7.08cm) in control treatment therefore; it performed well in control. Also malla was observed to have the mean value of 1.21cm in leaf extract, 4.32cm in control and 1.02cm in root extract. The control also observed to have the highest mean value of 4.32cm. This shows that it performed well in control group.

Analysis of variance (ANOVA) for hypocotyl length of cowpea in treatment, variety and both treatment and variety gave the F-probability value of p = <0.001 which showed that they are all highly significant. It was observed that the mean value of Sokoto White in leaf extract was 9.17cm. In control group, it was 13.92cm and in root extract, it was 5.08cm for the number of lateral roots of cowpea. Sokoto White performed well in control treatment with mean value of 13.92cm. Also, Oloyin observed to have mean value of 9.33cm in leaf extract, 13.19cm in control and 8.89cm in root extract. Oloyin performed well in control treatment with mean value of 13.19cm. As well as, in malla the mean value was 9.22cm in leaf extract. It was 13.33cm in control and 6.50cm in root extract. It was observed that the mean value of malla in control group have the highest value of 13.33cm.

Analysis of variance (ANOVA) for number of lateral roots in treatment with F-probability value of p = <0.001 which was shown to be highly significant. In variety and both in treatment and variety, the value of p=0.654 and 0.619 respectively. This shows that both have no significant difference. Sokoto White was observed to have mean value of 0.336cm in leaf extract, 0.792cm in control treatment and 0.242cm in root treatment. Sokoto White performed well in the control treatment with mean value of 0.792cm. Likewise in Oloyin, the mean value in leaf treatment is 0.350cm, 1.10cm in control, and in root extract it is 0.244cm. Oloyin also performed well in control treatment with mean value of 1.100 followed by the leaf extract with mean value of 0.350cm. Root extract performed very poor with mean value of 0.244cm. Also, malla was observed to have mean value of 0.289cm in leaf extract treatment, 0.844cm in the control treatment and 0.233cm in the root extract. malla also performed well in the control with mean value of 0.844cm.

Analysis of variance (ANOVA) for radical length of cowpea seedlings in treatment with F-probability value of p=<0.001 which was observed to be highly significant but in variety as well as in treatment and variety the value of p=0.4487 and 0.721 respectively. This shows that both have no significant difference. The mean value of Sokoto White in leaf extract was 4.39cm, in control, it was 8.98cm and in root treatment it was 2.54cm. For Oloyin, the mean values in leaves, control and root extract treatment were observed to be 4.99cm, 16.9cm and 3.19cm respectively. Oloyin has the highest mean value (16.9cm) in control treatment therefore it performed well in control treatment. Also, malla was observed to have the mean value of 2.55cm in root extract treatment. The control was observed to have the highest mean value of 11.76cm. This shows that it performed well in the control. Also malla was observed to have the mean value of 2.91cm in leaf extract. Analysis of variance (ANOVA) for the total length of the seedlings in treatment with F-probability value of p=<0.001 showed that it was highly significant. Likewise in variety as well as in treatment and variety the level of significance, p=0.002 and 0.005 respectively were also shown but slightly significant.

DISCUSSION

The result showed that neem extracts (leaves and roots) affects the germination and growth of the cowpea seedlings. However, there is a wide significant difference between the three treatments (control, leaves and root extracts) used during the experiment. Virtually in all traits studied, treatment has F-probability value of p=<0.001 which showed that they are highly significant. Meanwhile, the control has the highest mean values in the traits studied, the varieties performed excellently in the control (distilled water). In the leaf extract, the mean value is an average one, the varieties performed fairly but the root extract has the lowest mean values, this indicates that the varieties performed poorly in the root extract substrate.

A significant difference was recorded for both the treatments and the varieties with both having F-value <0.01. There was no interaction effect between the treatments and the varieties for viability of the cowpea seedlings. There was a great treatment effect for all the parameters studied. Varietal effect was observed for total length of the seedlings, length of radicle, number of lateral roots, hypocotyl length, leaf length and viability. This study is in line with work of Parthasary *et al*., (2021) and Kumar (2020). No anatomic distortion was observed as against the report of Liu *et al*., (2021). Neem (*Azadirachta indica*) extract is bio-degradable, that is, they have no side effect nor leaving any chemical (herbicides) which remains non-biodegradable in the soil and eventually accumulated in food product which can later endanger our internal system through the chemical residues that remain in the body system after consumption.

CONCLUSION

Based on the findings of this study, farmers are advised to avoid planting their cowpea where there is larger population of neem plant to acquire higher yield and gain. It could be recommended that *Azadirachta indica* may be used to control weeds and unwanted plants biologically as herbicide. The use of this wonderful tree extract can be recommended to all stakeholders in cowpea production, based on its high effectiveness, non-toxic and bio-degradable nature. The government should organize enlightenment campaign programme to enlighten the farmer and concern people on the effective use of *Azadirachta indica* to control the prevalence of weeds or unwanted plants.

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