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Growth Response of Two Cowpea Varieties to the Allelopathic Effects of *Azadirachta indica* (A. Juss) Bark Extract

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Abstract
The aim of this study was to evaluate the growth response of two varieties of Cowpea – *Vigna unguiculata* (SAMPEA 7 and IT97K-499-35) to the bark extract of *Azadirachta indica*. The crop plant was exposed to varying concentrations of 100, 75, 50, and 25 g/L of the aqueous stem bark extract using a Complete Randomised Block Design. There were three replications per treatment, of which three were randomly selected for further analysis. The study lasted 10 days. Data was collected on Germination percentage, root length, shoot length and fresh weight. Data were analysed using ANOVA at the 5% significance level, and DMRT was used to rank the means. The results revealed the susceptibility of the two cowpea varieties to the stem bark extract of *A. indica*, in a concentration-dependent manner, with respect to germination percentage, root and shoot length, and seedling fresh weight. The overall germination and growth rate of seedlings were reduced with the 100 g/L neem stem bark extract treatment, followed by 75 g/L, then 50 g/L; the least was 25 g/L. Variety IT97K-499-35 showed more susceptibility to the treatments. This suggests that A. indica exhibits an allelopathic effect on the examined types and can therefore serve as a biological means of weed control, as natural substances are regarded as more environmentally benign than most synthetic herbicides.

Keywords: *Azadirachta indica*, *Vigna unguiculata*, Allelopathy, Bark extracts, Cowpea

Introduction

Allelopathy refers to the ability of a plant to promote or restrict the growth of other surrounding plants by the production of allelochemicals (Javed, 2020; Popola et al., 2020). Due to the presence of certain allelochemicals, *Azadirachta indica* has allelopathic properties (Jagtap et al., 2016; Kajidu et al., 2023). These compounds can get into the environment through volatilisation, root exudation, photodecomposition, and biodegradation, and can affect surrounding food crops either positively or negatively (Gross & Parthier, 1994; Seligler, 1996; Ferguson et al., 2013; Kajidu et al., 2023). The inhibitory effects of these chemicals are dependent upon the concentration to which impacted plants are exposed and the susceptibility of the recipient plants (Popola et al., 2020).

Cowpea (Vigna unguiculata), like other staple crops, encounters numerous restrictions, including biotic and abiotic variables such as pests, weeds, and drought, along with the allelopathic influences of some plants on its growth and yield (Awosanya, 2024). It is a legume belonging to the genus Vigna. The Latin term Unguiculata translates to "with a small claw," signifying the diminutive stalks on the flower petals. It is a dicotyledonous plant classified under the Fabaceae family, subfamily Faboideae. It is frequently cultivated in mid-altitude areas of Africa, particularly in the arid savanna, occasionally as a monocrop but more typically intercropped with cereals like sorghum or millet. It is an economical source of protein, fatty acids, vital amino acids, vitamins, and minerals (Agbogodi, 2010; Muranaka et al., 2016; Awosanya, 2024). The aim of this study was to investigate the growth response of two varieties of cowpea to the allelopathic effect of *Azadirachta indica* bark extract.

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Material and Methods

***Plant materials*:** Stem bark of Neem (*Azadirachta indica* A. Juss.) was collected from around the Ahmadu Bello University premises (11.1512ºN, 7.6546ºE) during the flowering stage, as the plant releases more allelochemicals at this stage than in the vegetative stage (Oraon & Mondal, 2021). The plant material was transported to the laboratory, thoroughly rinsed with running water, air-dried in the shade at room temperature, and subsequently ground using a mortar and pestle. Plant specimens were preserved in sterilised plastic bags at a temperature of 4 °C until utilised in the bioassay.

***Seed of Test Plants*:** *Vigna unguiculata* seeds that are consistent and devoid of disease. Using the technique outlined by Li et al. (2021) and Oraon & Mondal (2021), SAMPEA7 and IT97K-499-35 were acquired from the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria, with a few minor modifications. To get rid of any remaining contaminants, the seeds were repeatedly rinsed with distilled water after being surface-sterilized for three minutes with 1% sodium hypochlorite. In order to avoid pathogen infection, this was done.

Preparation of Aqueous Stem Bark Extracts

The powdered stem bark (10 g) was soaked in 100 mL of double-distilled water for 24 hours at room temperature, and then gently stirred with a mechanical shaker. To remove debris, the extract was filtered with a double layer of filter (Whatman No. 1 filter paper) to obtain the crude extract. This stock solution was used to prepare concentrations of 25, 50, 75, and 100 g/L by adding the appropriate amounts of double-distilled water. 1(N) NaOH or 1(N) HCl was added as needed to adjust the pH of the various concentrations to 6.5 (Oraon & Mondal, 2021). This was done to avoid unwanted results arising from a pH imbalance

Seed Germination Assay

Ten seeds were arranged at random on two filter paper layers in each 90mm sterile Petri dish. As a control treatment, 5 mL of distilled water was utilised, and the filter sheets were moistened with 5 mL of 25%, 50%, 75%, and 100% (w/v) aqueous stem bark extract. Each treatment had 10 replicates and was set up in a completely randomised design. Mutlu & Atici (2008) recommended that Petri dishes be stored at 25 °C in a growth chamber and 70% relative humidity. Two millilitres of corresponding extract were added every 48 h to maintain the humidity of the filter paper in the petri dish. The germinated seeds were counted from the second day after treatment and continued for 8 days. In addition, the root length, shoot length, and seedling fresh weight for each treatment were measured. The germination percentage was calculated by recording the number of seeds that germinated and by monitoring the setup daily. According to Oraon & Mondal (2021), seeds were deemed to have germinated after the radicle appeared.

***Data Collection*:** The following parameters were used to assess allelopathic effects during the experiment. The germination percentage of seeds was calculated using the following formula (Li *et al* 2021 and Oraon & Mondal, 2021):

$$\frac{Number\ of\ Seeds\ germinated\ \times 100}{Total\ Number\ of\ Seeds}$$

After 8 days, 3 plants from each treatment were selected at random; the length of shoots and roots were measured using a centimeter ruler.

***Statistical Analysis*:** The significance of the treatment effects on the seed was tested using one-way analysis of variance (ANOVA) on all experimental data. Duncan's Multiple Range Test was then used to identify significant differences between mean values at a probability level of 0.05.

The Percentage Inhibition (P.I) for each concentration relative to its corresponding mean was also calculated to normalize measures of sensitivity independent of the initial vigor of the test varieties.

Results

Seed Germination

Two days after sowing, germination was first noticed, and six days later, the total germination was achieved. The two cowpea varieties' seed germination is inhibited by the aqueous neem stem bark extract, as seen in Table 1. Table 1 show that the control treatments had the highest mean seed germination % in both types (SAMPEA 7 and IT97K-499-35). The extract gradually reduced the germination percentage as its concentration rose. The lowest seed germination was observed in both varieties when 100g/L neem leaf extract was used (Table 1).

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Table 1: Effect of *A. indica* bark extracts on seed germination of cowpea varieties.

Variety	**Days (After Planting)**	**Seed Germination Percentage %**				
		Extract Concentration (g/L)				
		0	**25**	**50**	**75**	**100**
SAMPEA 7	1	-	-	-	-	-
	2	99	92	90	76	63
	3	99	93	93	91	95
	4	99	94	94	92	97
	5	99	98	96	93	98
	6	99	98	96	93	98
	7	99	98	96	93	98
	Mean	99	95.5	94.2	89.7	91.5
IT97K-499-35	1	-	-	-	-	-
	2	91	59	45	49	35
	3	95	67	63	49	36
	4	95	72	66	57	46
	5	95	75	70	58	47
	6	95	75	70	58	47
	7	95	75	70	58	49
	Mean	94.3	70.5	64	54.8	43.3

Root Length

Table 2**:** Effect of *A. indica* bark extracts on root length of cowpea varieties**.**

Treatment	**Variety**			
	SAMPEA 7(cm)	**P.I (%)**	**IT97K-499-35(cm)**	**P.I (%)**
0	5.34±0.26^{a}	0.00	4.08±0.10^{a}	0.00
25	2.38±0.26^{b}	55.43	2.89±0.10^{b}	29.17
50	1.96±0.26^{b}	63.30	2.21±0.10^{c}	45.83
75	2.06±0.26^{b}	61.42	1.95±0.10^{cd}	52.19
100	1.95±0.26^{b}	63.50	1.72±0.10^{d}	57.84

Means with same letter(s) in a column are not significantly different at P= 0.05 using DMRT.

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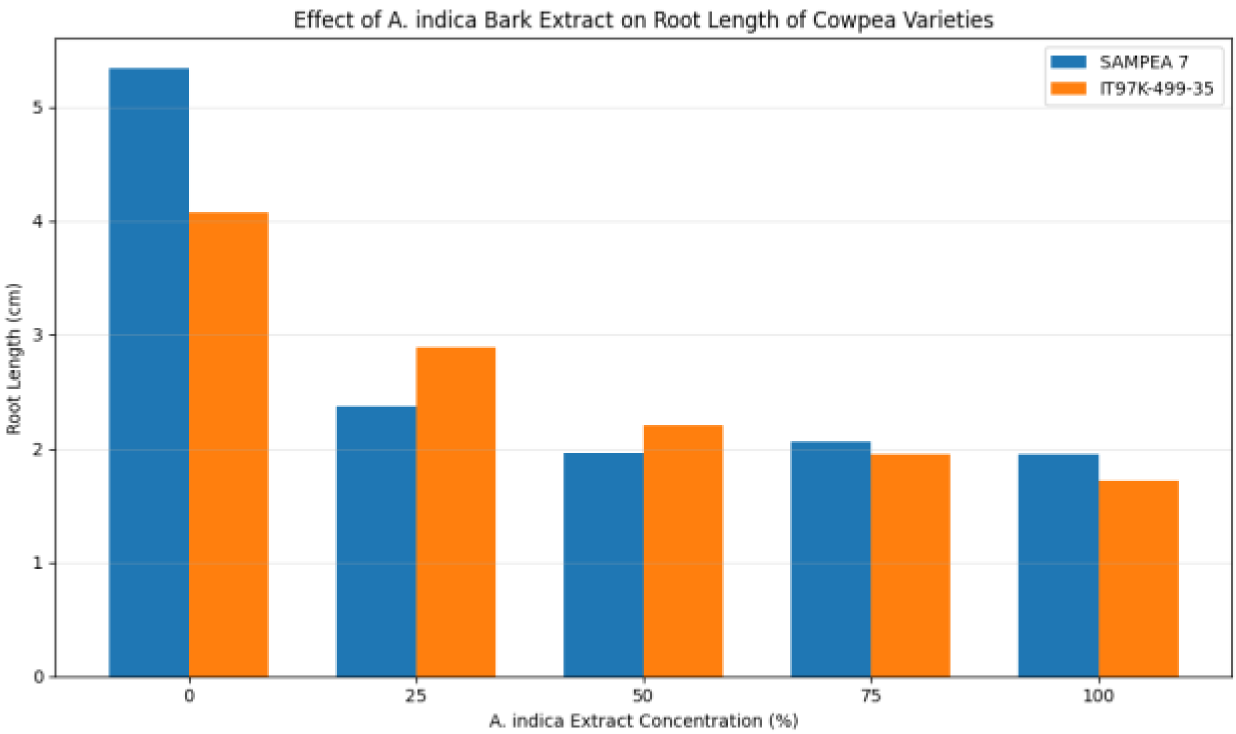

Figure 1: Effect of *A. indica* bark extracts on root length of cowpea varieties**.**

Shoot Length

Table 3**:** Effect of *A. indica* bark extracts on shoot length of cowpea varieties**.**

Treatment	**Variety**			
	SAMPEA 7	**P.I (%)**	**IT97K-499-35**	**P.I (%)**
0	11.13±0.37[a]	0.00	10.91±0.10[a]	0.00
25	5.87±0.37[b]	47.26	10.49±0.10[b]	3.85
50	5.38±0.37[b]	51.66	9.94±0.10[c]	8.90
75	5.01±0.37[b]	55.00	8.35±0.10[d]	23.46
100	5.98±0.37[b]	46.27	7.93±0.10[e]	27.31

Means with same letter(s) in a column are not significantly different at P= 0.05 using DMRT.

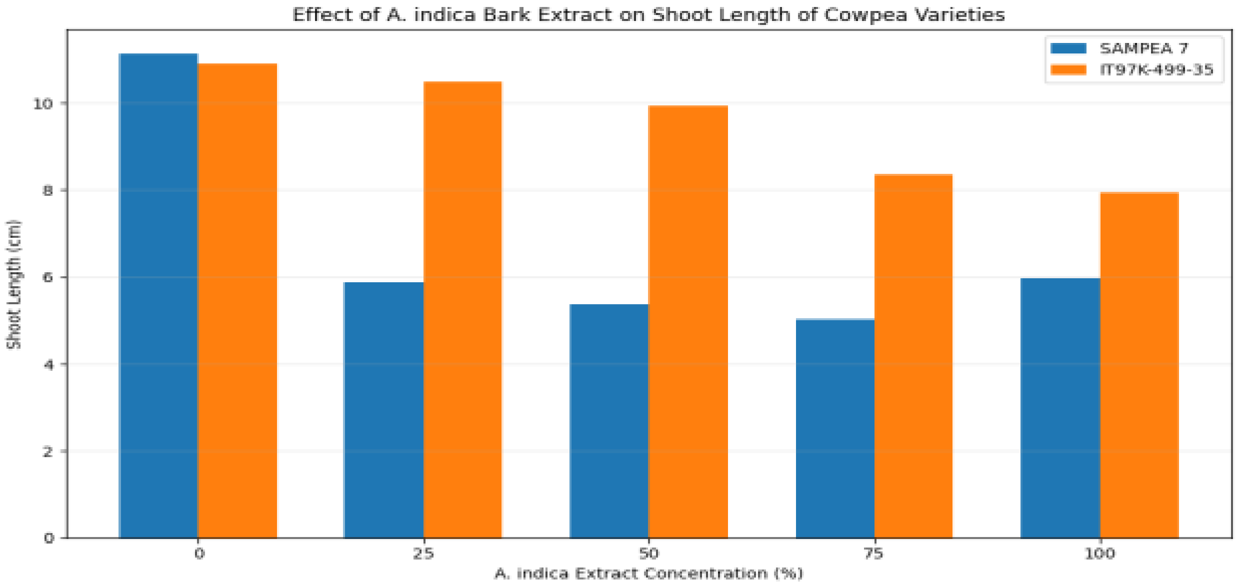

Figure 2: Effect of A. indica bark extracts on shoot length of cowpea varieties**.**

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Seedling fresh weight

Table 4**:** Effect of *A. indica* bark extracts on seedlings fresh weight of cowpea varieties**.**

Treatment	**Variety**			
	SAMPEA 7	**P.I %**	**IT97K-499-35**	**P.I %**
0	4.64±0.17[a]	0.00	4.23±0.32[a]	0.00
25	3.75±0.17[b]	19.18	3.01±0.32[b]	28.84
50	3.29±0.17[bc]	29.09	2.75±0.32[b]	34.99
75	2.95±0.17[c]	36.42	2.60±0.32[b]	38.53
100	2.95±0.17[c]	36.42	1.53±0.32[c]	63.88

Means with same letter(s) in a column are not significantly different at P= 0.05 using DMRT.

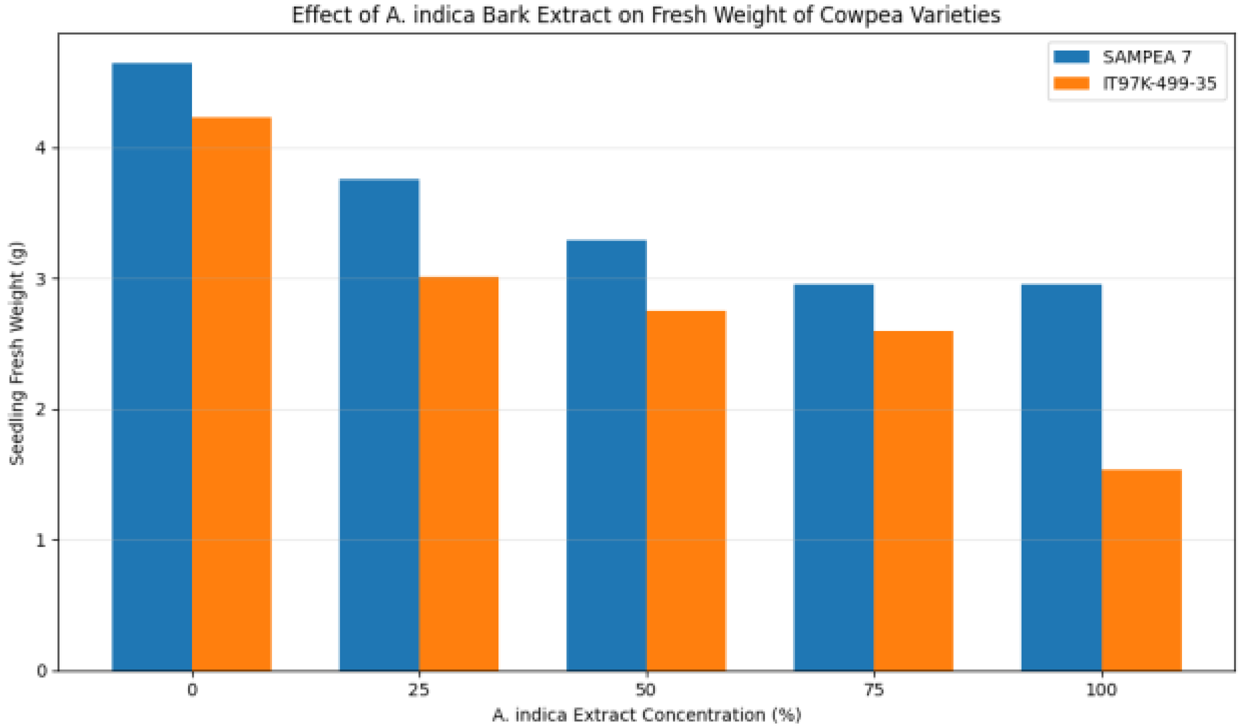

Figure 3: Effect of A. indica Bark Extracts on Seedling Fresh Weight of Cowpea Varieties

Table 5**:** Effect of *A. indica* bark extracts on root length, shoot length and seedling fresh weight when comparing the two cowpea varieties**.**

Treatment (%)	**Root Length (cm)**	**Shoot Length (cm)**	**Seedling Fresh Weight (g)**
0	4.71 ± 0.21[a]	11.02 ± 0.29[a]	4.44 ± 0.28[a]
25	2.63 ± 0.21[b]	8.18 ± 0.29[b]	3.29 ± 0.28[b]
50	2.09 ± 0.21[c]	7.66 ± 0.29[b]	3.02 ± 0.28[b]
75	2.01 ± 0.21[c]	6.69 ± 0.29[c]	2.78 ± 0.28[c]
100	1.83 ± 0.21[c]	6.69 ± 0.29[c]	2.24 ± 0.28[c]

Means with same letter(s) in a column are not significantly different at P= 0.05 using DMRT.

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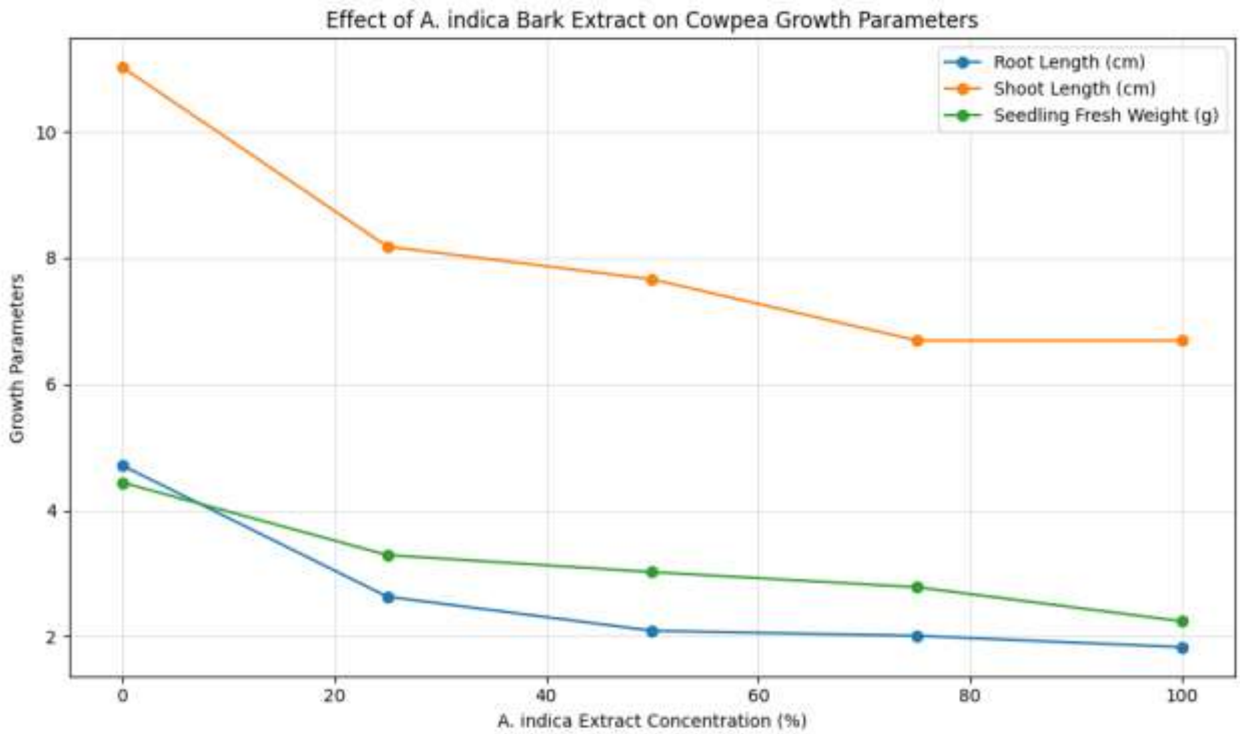

Discussion

Germination Percentage

The results from the seedling germination assay in response to the aqueous stem bark extract revealed a difference in allelopathic tolerance between the two test varieties of cowpea. SAMPEA 7 showed greater tolerance, with higher mean germination percentages across all concentrations, whereas IT97K-499-35 was very susceptible, showing concentration-dependent inhibition. The inhibitory effect is probably related to allelochemicals like Azadirachtin, Nimbidin, Mibolides, Gedunin, Salanin, Nimbin, and Valassin, which disrupt the germination process (Krishnaveni et al., 2025). The results obtained are in agreement with those of Liu et al. (2021) and Awosanya (2024), who observed that Neem extracts (leaves and roots) adversely impact the germination and seedling growth of some cowpea varieties.

Root Length

Despite the initial vigour of SAMPEA 7 observed in the control treatment, IT97K-499-35 proves to be a more tolerant variety in terms of root length. The SAMPEA 7 profile showed rapid saturation at the lowest dose, indicating extreme vulnerability and a lack of an effective defence response mechanism against the allelochemicals present in neem stem bark. In the control treatment, SAMPEA 7 showed an inherent root length of 5.34 cm, demonstrating 30.88% greater vigour (root length) than IT97K-499-35, which measured 4.08cm. At the highest concentration (100 g/L), SAMPEA 7 reached a maximum inhibition of 63.50%, surpassing the 57.84% observed in IT97K-499-35. SAMPEA 7's root grows faster, so it probably reacts more quickly and strongly to the toxic extract than the slower IT97K-499-35. The findings in this study are similar to those of Godwin & Avers 1950 and Krishnaveni et al. (2025).

Shoot Length

The inhibition percentage (P.I.) shows that SAMPEA 7 was more susceptible to the allelochemicals present in Neem than IT97K-499-35. At the lowest concentration of 25g/L, the root length was almost halved with an inhibition of 47.26%. On the other hand, IT97K-499-35 showed a remarkable tolerance to the stem bark extract. At 25 g/L, the inhibition was minimal (3.85%). At the concentration increased, the P. I rose progressively, attaining the peak at P. I was at 27.31 at the highest concentration of 100g/L—the maximum P. The result for IT97K-499-35 was lower than the minimum significant inhibition observed in SAMPEA 7. Therefore, this result shows that SAMPEA 7 was more sensitive to the allelochemicals present in the plant, whereas IT97K-499-35 showed a moderately tolerant response. This may be because IT97K-499-35 possesses a physical barrier at the root (which is the primary site of absorption) that reduces the uptake of the allelochemicals from the medium, or the variety has an enhanced detoxification mechanism which detoxifies

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the allelochemicals before they reach a detrimental concentration (Belz et al., 2005; Kato-Noguchi, 2024). In addition, Kato-Noguchi (2024) found that as roots contact allelochemicals earlier than shoots, this means that many times significant amounts of root inhibition occur before there is also substantial shoot inhibition. Several allelopathic compounds have also been shown to disrupt plant hormones, particularly gibberellins and indole acetic acid, resulting in decreased shoot length due to decreased cell division (Krishnaveni et al., 2025).

Fresh Weight

Despite the excellent tolerances shown by IT97K-499-35 in shoot and root length analysis, IT97K-499-35 showed a significantly greater susceptibility to neem bark allelochemicals when measured by fresh weight. The maximum inhibition for IT97K-499-35 was almost twice that for SAMPEA 7 (36.42%). Thus, while SAMPEA 7 is more sensitive to morphological damage (root and shoot length inhibition), IT97K-499-35 is substantially more susceptible to biomass loss under maximum allelopathic stress. This result was affirmed by Kato-Noguchi (2024), who stated that the disruption of metabolism and cellular processes by allelochemicals may inhibit growth and regeneration processes in the recipient plant species, consequently leading to reduced biomass accumulation.

Conclusion

According to this study, the aqueous extract of neem stem bark includes allelochemicals that, in proportion to the extract's concentration, impede the germination, root length, shoot length, and fresh weight of two cowpea kinds. This could be recommended for weed management, as allelochemicals can serve as suitable substitutes for harmful synthetic chemicals. These chemicals can serve as suitable substitutes because they are cost-effective and environmentally friendly. Farmers are also advised to avoid planting cowpea varieties in areas with a high density of neem plants to maximise yields and gains.

Recommendations

The following recommendations are made in light of the study's findings in order to direct further investigation and enhance useful applications:

1. To identify and measure the particular allelochemicals causing growth inhibition, chemical profiling of the neem bark extract is advised. The potential development of natural herbicidal products will be improved as a result.
2. Research initiatives should concentrate on creating cowpea varieties with increased resistance to allelopathic stress because varietal variations in susceptibility were noted, particularly with IT97K-499-35 being more impacted.
3. The phytotoxic qualities of neem bark extract shown here point to its possible application as a bioherbicide that is both safe and environmentally beneficial. More research should be done on its composition, rate of application, and soil persistence.
4. Future research should assess the effects of neem-derived allelochemicals on long-term crop yield, nitrogen cycling, and soil microbes.

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