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ALLELOPATHIC EVALUATION OF *ACACIA* SPECIES AGAINST SEED GERMINATION AND SEEDLING GROWTH OF FOUR ECONOMICALLY IMPORTANT CROPS

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Abstract

Some plants use allelopathy to compete against neighboring plants, allowing them to conserve resources in the absence of competitors. Several *Acacia* species have shown allelopathic effects that could potentially affect crop production. This study aimed to evaluate the allelopathic potential of 3 *Acacia* species (*Acacia nilotica*, *Acacia catechu*, and *Acacia modesta*) leaf extracts on seed germination and seedling growth of 4 economic crops (wheat, millet, spinach, and sorghum). The leaf extract of *A. modesta* inhibited the seed germination of millet and wheat, while *A. catechu* showed inhibition in seed germination of millet at 3 concentrations (1%, 3%, and 5%). *A. nilotica* showed stimulatory as well as inhibitory effects on the seed germination of all test crops. On the other hand, the leaf extract of *A. catechu* showed comparatively higher inhibitory effects against the seedling growth of all test species. *A. modesta* showed inhibition on the seedling growth of hypocotyl (8%, 51%, and 59%) and radicle (14%, 74%, and 94%) of spinach at all concentrations while showing various stimulatory and inhibitory effects on hypocotyl and radicle of all crop species. All three concentrations of *A. nilotica* extract showed stimulation on the seedling growth for hypocotyl of sorghum and millet while reduction for hypocotyl of wheat and inhibition for radical of sorghum, millet, and wheat have been observed. The results indicate that *A. catechu* has more inhibitory effects on seed germination and seedling growth, so it should be kept away from crops due to its adverse effects.

Key words: Radicle, Inhibition, Leaf extract, Hypocotyl, Stimulation.

Introduction

Plants can release chemicals that affect the growth of the same and different species. Allelopathy is an important mediator among plant species in natural and managed ecosystems (Xu *et al*., 2023). Allelochemicals are released into the surrounding environment through different processes such as leaching, volatilization, and root exudation (Sangeetha & Baskar, 2015) and affect the bordering plants seed germination and seedling growth. These compounds mainly affect physiological processes such as cell division, membrane permeability, plant hormone production, photosynthesis, respiration, and enzymatic activity (Gulzar & Siddiqui, 2017). Phytochemicals are found in different parts of the plant, such as leaves, stems, roots, rhizomes, flowers, fruits, and seeds, and also in pollen grains excreted from the plants by different ways such as volatilization, leaf leachate, root transude, and decomposition from plant residues (Zhang *et al*., 2021). These phytochemicals are classified as secondary metabolites in invasive plants, which have multiple functions such as anti-herbivore, anti-microbial, anti-fungal, and allelopathy activity (Kato-Noguchi, 2022). These allelochemicals directly or indirectly affect the neighboring plants by altering the soil microbial activity and nutrient availability (Inderjit *et al*., 2011). When sensitive plants are exposed to allelopathic chemicals, germination and growth are decreased, and allelopathic effects on different parts of plants vary with region, season, and plant to plant (Mango *et al*., 2022). There are different plant species that release more than 100 allelopathic compounds (Kato-Noguchi, 2024), and it has been estimated that only a limited number of these chemicals have been studied (Wink, 2010). Hence it is essential to check the allelopathic compatibility of crops with their surrounding plants before introducing them to agriculture practice.

Acacia belongs to *the Fabaceae family,* with about 1350 species around the world and 89 species in Asia. These species are distributed throughout all the habitats; approximately 800 are found in Australia, 130 in Africa, and 20 in India (Jelassi *et al*., 2014). This is a rapidly growing tree and is becoming invasive, spreading in open areas and distributed in forestland and replacing many invasive species (Le *et al*., 2018). *Acacia* plants contain a variety of bioactive compounds such as phenolic acid, amines, alkaloids, di- and triterpenes, tannins, flavonoids, phenolic acid, saponins, gums, fatty acids, and seed oil (Abdallah *et al*., 2020; Malviya *et al*., 2011). A third of Australian species are exotic and 24 are invasive. *Acacia* species which spread outside their native environment can be considered as invasive species (Suarez-Ronay *et al*., 2024).

Invasive *Acacia* species distress plant growth by competing with various environmental resources, and their litter interferes with the growth and reproduction of other species (Aguilera *et al*., 2015) and releases many other phenolic compounds in their litter. Riveiro *et al*., (2024) described that plant extract of many *Acacia* and *Eucalyptus* species contained the allelopathic compounds, which showed the inhibitory effects on the germination of other species. *Acacia* species were introduced for industrial, forestry, and ornamental uses for the rehabilitation of degraded land, but successive invasions have largely reduced the economic and environmental impacts (Wit *et al*., 2001).

In recent years, a number of researchers have determined the allelopathic potential of various plant species against different crops and weeds. Muzzo *et al*., (2023) determined the allelopathic potential of *Chromolaena odorata on* the seed germination and seedling growth of important crops (beans, cowpea, and finger millet) and grassland species. The leaf extract of *Chromolaena odorata* inhibits seed germination and seedling growth of crops and grassland species. The allelopathic effect of the aqueous extract of *Ageratum conyzoides* on seedling growth of *Sesanum indicum* was examined by Idu & Oghale, (2013). Negi *et al*., (2016) studied the allelopathic effect of invasive tree *Broussonetia papyrifera* against germination and seedling growth on *Triticum aestivum* and *Oryza sativa.* Shahzad *et al*., (2022*)* studied the allelopathic potential of *Acacia*, *Brassica*, *Eucalyptus*, and Sorghum on two weed species. Results showed that a 1% concentration of leaf extract of the 4 test species has significant stimulation in the growth of two weed species, while retardation was observed at a 5% concentration of plant extract. Dar *et al*., (2023) also determined the allelopathic effect of *Euphorbia hirta* extracts and powder on seedling growth of *Cicer arietinum* in Pakistan.

With these perspectives and research in mind, this study was initiated to investigate the allelopathic potential of leaf extracts from *A. nilotica*, *A. catechu*, and *A. modesta* on the germination and seedling growth of wheat, millet, spinach, and sorghum. This research aims to contribute to the management of crop planting and the control of invasive *Acacia* species in agricultural settings.

Material and Methods

Laboratory experiments were performed to examine the effects of leaf extracts for 3 *Acacia* species (*A. nilotica*, *A. catechu*, and A. modesta) on seed germination and seedling growth of test plants, including *Triticum aestivum* (wheat), *Pennisetum glaucum* (millet), *Spinacia oleracea* (spinach) and *Sorghum bicolor* (sorghum). Leaf samples were chosen because these parts are the most common source of allelochemicals.

Collection of *Acacia* species samples and test crop plants: Leaf samples were collected in the morning from three healthy *Acacia* species in Jinnah Garden located in Lahore, Pakistan. Jinnah Garden is the biggest garden in Lahore city and was established in 1860. It has a total area of 121 acres and contains 150 different types of trees, 140 types of shrubs, 30 types of palms, 100 types of succulents, and more than 1000 indoor plants, which contain all varieties of annual flowers (Fazal *et al*., 2014). The 3 *Acacia* species were commonly found in the Jinnah Garden. Leaf samples were stored in polythene bags and were taken to the laboratory for further analysis. Leaves were washed thoroughly with distilled water and air-dried for 25–30 days. The dried samples were ground to a fine powder with the help of a pestle and mortar and stored in dry condition until used.

The seeds of 4 crops (wheat, millet, spinach and sorghum) were used to investigate the allelopathic potential of *Acacia* plants against them. Seeds were obtained from Seed Corporation. The test plants were grown in February and April; the average temperature of those months was 23-35°C.

Preparation of *Acacia* species aqueous extracts: The fine powder of each *Acacia* species was divided into three parts (1, 3, and 5 g) to prepare 3 different concentrations (1%, 3%, and 5%, respectively). Before starting the experiment, all personnel should wear lab coats, gloves, and safety glasses to protect against chemical exposure and dust in the laboratory. The maceration technique was used to prepare the aqueous extracts of different concentrations (Siddiqui, 2009). Weighed fine powder of leaves 1, 3, and 5 g with the help of electronic balance, then soaked in 100 mL of distilled water for each concentration and kept overnight in the beakers. After 24 hours, the leaf extracts were filtered with cheesecloth firstly to eliminate leaf remains and then filtered with the help of Whatman filter paper. The filtrate was obtained in the flasks and stored in the refrigerator for further use.

Seed bioassay: Filter paper is the best medium for germination of seeds as it provides porosity for airflow and absorbance of plant extract (Wang *et al*., 2024). Aqueous extracts of 1%, 3%, and 5% concentrations were applied to test crops by soaking a double layer of filter papers. Petri dishes were cleaned with ethanol dipped cotton to prevent bacteria fungal attack. Ten seeds of each test crop, wheat, millet, spinach, and sorghum, were placed between two folds of filter paper in each Petri dish. For the control treatment, distilled water was replaced with plant extract. The experiment with each treatment was replicated three times.

The experiment petri dishes were set at room temperature. The results were determined by counting germinated seeds and measuring the length of the root and shoot after seven days. The length of the hypocotyl and radicle of seedlings was measured on a centimeter scale. Seed germination percentage was calculated using the following formula (Maurya *et al*., 2024).

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated}}{\text{Number of seeds}} \times 100$$

Data analysis: Data was analyzed by SPSS 13.0 (Statistical Package for the Social Sciences) and Microsoft Excel. Single-factor ANOVA was performed to investigate the significance of the activity. The level of significance was 0.05.

Results

Effect on seed germination of test crops

Effect of *A. nilotica*: Millet and sorghum showed 70% and 90% seed germination at all 3 extract concentrations, but in the case of wheat, 90% seed germination at 1% concentration and 100% at 1% and 5% concentrations. For spinach, seed germination percentages were 80%, 100%, and 40% at 1%, 3%, and 5% concentrations. For the control sample, seed germination was 90% for spinach (Fig. 1a).

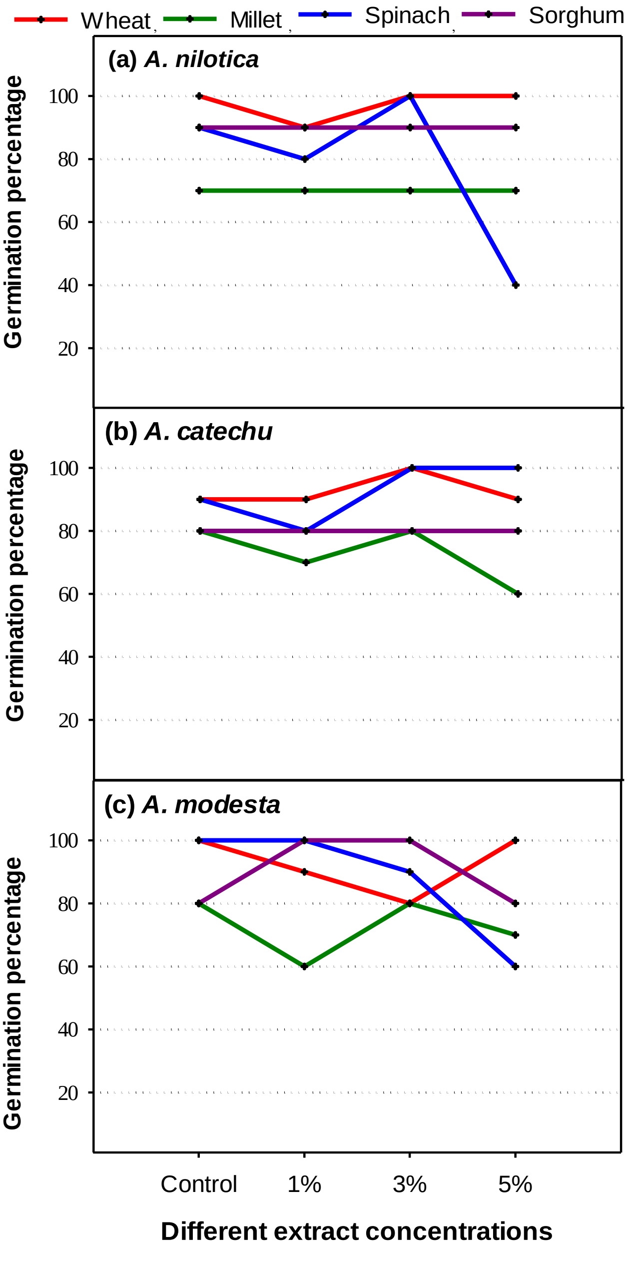

Fig. 1. Effects of (a) *A. nilotica* (b) *A. Catechu* (c) *A. modesta* species on seed germination of test crops.

Effect of *A. catechu*: Wheat showed 90%, 100%, and 90% seed germination at all 3 extract concentrations. For spinach, seed germination percentages were 80% at 1%, 100% at 3%, and 5% extract concentrations. For the control sample, seed germination was 90%. Sorghum showed seed germination was 100% at all 3 extract concentrations. In the case of millet, seed germinations were 70%, 60%, and 80% at 1%, 3%, and 5% concentrations (Fig. 1b).

Effect of *A. modesta*: Wheat showed 90%, 80%, and 100% seed germination at all 3 extract concentrations. In the case of spinach, seed germinations were 100%, 90%, and 60% at all 3 extract concentrations. For sorghum, seed germinations were 100% at 1% and 3% extract concentrations and 80% at 5% concentration. For millet, seed germination was 60%, 80%, and 70% at all 3 extract concentrations (Fig. 1c).

Effect on seedling growth of test crops

Effect of *A. nilotica:* Different leaf extract concentrations of *A. nilotica* showed diverse effects against the growth of test crops. In the case of wheat, the hypocotyl lengths were 5.3, 5.4, and 4.8 cm under 1%, 3%, and 5% extract concentrations, which were 15%, 13%, and 23% reduced as compared to the control sample of 6.2 cm. Radicle lengths of wheat (10.2, 8.7, and 7.3 cm at 1%, 3%, and 5% extract concentrations) were significantly reduced (16%, 29%, and 40%) as compared to control sample of 12.2 cm. *A. nilotica* showed non-significant effect on hypocotyl growth ($P = 0.59$) and significant effect on radicle growth ($P = 0.01$) of wheat at all concentrations (Table 1).

Extract of *A. nilotica* significantly stimulated hypocotyl growth of millet. The hypocotyl lengths were 4, 4.7, and 4 cm at 1%, 3%, and 5% extract concentrations. The growth reductions were 67%, 56%, and 61% in comparison with the control sample with a hypocotyl length of 3.7 cm. Millet showed stimulation (9 and 9.4 cm) effect for radicle growth at 1% and 3% extract concentrations and reduction effect (5.6 cm) as compared to the control sample 7.5 cm. *A. nilotica* showed non-significant effects on hypocotyl growth ($P = 0.26$) and significant effects on radicle growth ($P = 0.009$) of millet at all concentrations (Table 1).

For spinach, hypocotyl lengths significantly increased (3 and 2.8 cm) at 1% and 3% extract concentration but reduced (0.7 cm) at 5% concentration as compared to the control sample (1.8 cm). A high stimulation effect (radicle length 1.7 cm) was observed for radicle growth of spinach at 1% and 3% concentrations as compared to the control sample (0.9 cm). *A. nilotica* showed significant effects on the hypocotyl (p=0.008) and radicle growth (p=0.025) of spinach at all concentrations (Table 1).

A. nilotica extract showed stimulation effects on the hypocotyl growth of sorghum. The hypocotyl lengths were 9.7, 8.9, and 9 cm under the 3 extract concentrations, and the increase percentages are 26%, 16%, and 17% as compared to the control sample (7.7 cm). Plant extract increased inhibition in radicle growth of sorghum by increasing concentration. *A. nilotica* showed non-significant effects on hypocotyl (p=0.48) and radicle growth (p=0.66) of sorghum at all concentrations (Table 1).

Effect of *A. catechu*: For wheat, radicle lengths were 2.9, 3.1, and 3.3 cm at 1%, 3%, and 5% extract concentrations which were significantly reduced (74%, 72%, and 70%) as compared to the control sample of 11.1 cm. Wheat hypocotyl lengths were 2.5, 3.4, and 2.5 cm at all extract concentrations. The percentages are 44%, 24%, and 44% reduced as compared to the control sample of 4.5 cm. *A. catechu* showed non-significant effects on the growth of hypocotyl (p=0.13) and significant effects on the growth of radicle (p=0.00) of wheat at all concentrations (Table 2).

A. catechu plant extract showed inhibitory effects on the hypocotyl growth of millet. Millet hypocotyl lengths were 3.2, 3.5, and 2.8 cm under all 3 extract concentrations. Hypocotyl lengths were 31%, 25%, and 40% reduced as compared to the control sample of 4.7 cm. Millet radicle lengths were 2.5, 2.5, and 2.4 cm reduced. The percentages of reduction were 65%, 65%, and 66% as compared to the control sample of 7.2 cm. *A catechu* showed significant effects on the growth of hypocotyl (*p*=0.02) and radicle (*p*=0.02) of millet at all concentrations (Table 2).

Spinach hypocotyl lengths were significantly reduced (1.9 and 3 cm at 1% and 3% extract concentrations) but stimulated (3.9 cm at 5% plant extract) as compared to the control sample (3.3 cm). Spinach radical lengths were 1.1, 1.4, and 2.4 cm, which were significantly reduced (75%, 65%, and 40% at all extract concentrations) as compared to a control sample of 4 cm. *A. catechu* showed non-significant effects on the growth of hypocotyl (*p*=0.11) and significant effects on the growth of radicle (*p*=0.00) of spinach at all three concentrations (Table 2).

In the case of Sorghum, hypocotyl lengths were 6.8, 6.7, and 6.3 cm. The percentages of reduction were 15%, 16%, and 21% at all 3 extract concentrations as compared to the control sample of 8 cm. Radicle lengths were 6.2, 4.7, and 3.9 cm, which were significantly reduced at all 3 extract concentrations. The reduction percentages were 29%, 46%, and 56% as compared to the control sample of 8 cm. *A. catechu* showed non-significant effects on the growth of hypocotyl (*P* = 0.31) and significant effects on the growth of radicle (*p*=0.00) of sorghum at all concentrations (Table 2).

Effect of *A. modesta:* *A. modesta* extract showed a significant reduction in the hypocotyl lengths of wheat. Hypocotyl lengths of wheat were 3.1 and 3.6 cm under 1% and 5% extract concentrations while 4.8 cm at 3% extract concentration. Hypocotyl lengths were 34%, 31% reduced, and 2% stimulated in comparison with a control sample of 4.7 cm. Radicle lengths were 4.9, 5.8, and 4.5 cm under 1%, 3%, and 5% extract concentrations, which are 24%, 10%, and 31% reduced as compared to the control sample of 6.5 cm. *A. modesta* showed non-significant effects on the growth of hypocotyl (*P* = 0.69) and radical (*p*=0.48) of wheat at all concentrations (Table 3).

A. modesta showed stimulatory as well as inhibitory effects on the hypocotyl and radicle lengths of millet. Hypocotyl lengths were 8 cm under 1% and 3% extract concentrations and 5.4 cm at 5% extract concentration, which were 33% reduced and 10% stimulated in comparison to the control sample of 6 cm. Radicle lengths were 9.8 and 9.6 cm under 1% and 3% extract concentrations and 4 cm at 5% extract concentration. Radicle lengths were 11% and 9% stimulated, and 54% reduced as compared to the control sample of 8.8 cm. *A.modesta* showed non-significant effects on the growth of hypocotyl (*p*=0.13) and significant effects on the growth of radicle (*p*=0.008) of millet at all concentrations (Table 3).

In the case of spinach, hypocotyl lengths were 3.6, 1.9, and 1.6 cm under 1%, 3%, and 5% extract concentrations, which were 8%, 51%, and 59% reduced as compared to the control sample of 3.9 cm. Radicle lengths were 3, 0.9, and 0.2 cm under 1%, 3%, and 5% extract concentrations, and the inhibition percentages were 14%, 74%, and 94% as compared to a control sample of 3.5 cm. *A. modesta* showed significant effects on the growth of hypocotyl (*p*=0.001) and radicle (*p*=0.00) of spinach at all concentrations (Table 3).

Table 1. *Allelopathic* effects of *A. nilotica* on the growth of test crops.

Test crops	Length (cm) at different concentrations								P value	
	Control		1%		3%		5%			
	H	R	H	R	H	R	H	R	H	R
Wheat	6.2	12.2	5.3	10.2	5.4	8.7	4.8	7.3	0.590**	0.015*
Millet	3.7	7.5	4.0	9.0	4.7	9.4	4.0	5.6	0.260**	0.009*
Spinach	1.8	0.9	3.0	1.7	2.8	1.7	0.7	0.7	0.008*	0.025*
Sorghum	7.7	8.2	9.7	6.6	8.9	5.0	9.0	4.4	0.480**	0.660**

H = Hypocotyl, R = Radicle, * = Significant effect, ** = Non-significant effect

Table 2. Allelopathy effects of *A. catechu* on the growth of test plant.

Test crops	Length (cm) at different concentrations								P value	
	Control		1%		3%		5%			
	H	R	H	R	H	R	H	R	H	R
Wheat	4.5	11.1	2.5	2.9	3.4	3.1	2.5	3.3	0.13**	0.00*
Millet	4.7	7.2	3.2	2.5	3.5	2.5	2.8	2.4	0.02*	0.02*
Spinach	3.3	4	1.9	1	3	1.4	3.9	2.4	0.11**	0.00*
Sorghum	8	8.8	6.8	6.2	6.7	4.7	6.3	3.9	0.31**	0.00*

Key: H = Hypocotyl, R = Radicle, * = Significant effect, ** = Non-significant effect

Table 3. Allelopathy effects of *A. modesta* on the growth of test plant.

Test crops	Length (cm) at different concentrations								P value	
	Control		1%		3%		5%			
	H	R	H	R	H	R	H	R	H	R
Wheat	4.7	6.5	3.1	4.9	4.8	5.8	3.6	4.5	0.69**	0.48**
Millet	6	8.8	8	9.8	8	9.6	5.4	4	0.13*	0.008*
Spinach	3.9	3.5	3.6	3	1.9	0.9	1.6	0.2	0.001*	0.00*
Sorghum	9.6	6.7	10	10.4	8.2	7.3	5.6	3.4	0.17**	0.026*

H = Hypocotyl, R = Radicle, * = Significant effect, ** = Non-significant effect

In the case of sorghum, hypocotyl lengths were 8.2 and 5.6 cm at 3% and 5% extract concentrations and 10 cm at 1% concentration. Hypocotyl lengths were 40%, 41% inhibited, and 4% stimulated as compared to the control sample of 9.6 cm. Radicle lengths were 10.4 and 7.3 cm at 1% and 3% extract concentrations and 3.4 cm at 5% extract concentration. Radicle lengths were 55% and 9% stimulated, and 49% inhibited as compared to a control sample of 6.7 cm. *A. modesta* showed non-significant effects on the growth of hypocotyl (p=0.17) and significant effects on the growth of radicle (p=0.02) at all concentrations of sorghum (Table 3).

Discussion

Allelopathy is a phenomenon through which organisms produce biochemicals that may affect the growth, survival, and reproduction of other organisms. These biochemicals (also known as allelochemicals) may cause stimulatory and inhibitory effects on other organisms. Arora *et al*., *(2024)* reported that allelochemicals have important and significant effects on cell division, cell differentiation, respiration, photosynthesis, enzyme function, ion and water uptake, water status, phytochrome metabolism, signal transduction as well, and gene expression. Some plants suppress the competitors by releasing chemicals into the environment, so their production would be beneficial when plants experience competition (Yuan *et al*., 2022).

In the present findings, leaf extracts of *A. nilotica* showed stimulation in the seedling growth of hypocotyl of millet and sorghum at all of its concentrations and also showed stimulation in seedling growth of both hypocotyl and radical of spinach at lower concentrations (1% and 3%) and inhibition at its highest concentration (5%). Ullah *et al*., (2023) reported that plant extract of *A. modesta* had a stimulatory effect on the growth and seed germination of test crops. Al-Wakeel *et al*., (2007) reported that the lower concentrations of *A. nilotica* leaf residue stimulated the growth of pea shoots and roots, but the higher concentrations were inhibitory to the seedling growth. In the case of wheat and sorghum, *A. nilotica* extract showed inhibition in the seedling growth of radicles at all concentrations. Similarly, Jandova *et al*., (2015) described that the germination and root length of *Plantago lanceolata* were significantly inhibited by the root exudates of *Heracleum mantegazzianum.* Shao *et al.,* (2019) described that weed extract *Vicia sativa* and Wild oat have strong inhibitory effects on the growth of wheat.

Results of this study showed the leaf extract of *A. catechu* had an inhibition effect on the seedling growth of hypocotyl and radicles of all test crops except spinach hypocotyl, which showed stimulation (18%) at a 5% concentration of plant extract. Alamdari *et al*., (2013) reported that water extracts of *Fallopia convolvulus* inhibited the germination and growth of wheat, indicating that wheat is a more sensitive crop. These results might be due to the large number of compounds released from different species, including complex phenolic compounds (Hapani *et al*., 2024). Haddadchi & Gerivani, (2009) also explained the negative effects of phenolic extracts of rapeseed (*Brassica napus*) on soybeans, showing the toxicity of phenolic compounds towards crops.

In these findings, the leaf extract of *A. modesta* showed inhibition in seedling growth of spinach radicles and hypocotyl and showed inhibitory effects on the radicle of wheat at all of its concentrations. In the case of sorghum, millet (hypocotyl and radicle) and hypocotyl of wheat showed stimulatory and inhibitory effects on the seedling growth at all concentrations of plant extract. Al-Wakeel *et al*., (2007) investigated that leaf extract of *A. nilotica* showed stimulation in the growth of pea at its lower concentration. Abdelmalik *et al*., (2023) found that some *Acacia* species have affected other crops through allelopathy due to the presence of numerous phenolic compounds in their litter.

In the present findings, the aqueous extract of *A. modesta* showed inhibition in seed germination of wheat and millet at all of its treatments while sorghum seed germination showed stimulation at 1% and 3% concentrations. *A. catechu* showed inhibition in seed germination of millet at all of its concentrations while in case of sorghum, wheat and spinach showed stimulatory and inhibitory effects on seed germination. *A. nilotica* showed stimulatory as well as inhibitory effects on the seed germination of all test crops. Similarly, Rigon *et al*., (2012) reported that plant extract of rapeseed showed inhibition on the germination of *Phaseolus vulgaris* at its higher concentrations. Barabasz-Krasny *et al*., (2024) reported that seedling growth was inhibited in different crops due to the presence of tannins, wax, flavonoids, and phenolic acids as the major allelochemicals.

Conclusion

Present observations indicated that *A. catechu* exhibited a stronger inhibitory effect on the growth of all test crops across all concentrations, while *A. nilotica* and *A. modesta* showed variable effects on crop growth. This study demonstrates that *A. catechu* has a significant negative allelopathic impact on various crops, suggesting that it may not be suitable for use in cover cropping systems due to its detrimental effects on crop growth. Moreover, it is crucial to assess the allelopathic compatibility of crops with their surrounding plants before incorporating them into agricultural practices to avoid potential negative interactions.

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