Plant Health 03 (01) 2024. 59-70 DOI: 10.33687/planthealth.03.01.4942

Check for updates

Available Online at EScience Press
Plant Health
ISSN: 2305-6835
https://esciencepress.net/journals/planthealth

Allelopathic Potential of Neem and Darek Plant Parts on Barley and Spinach Growth

[a]Naveed Ul Hassan, [a]Madiha Rashid*, [b]Muhammad Aslam, [a]Aqsa Perveen, [a]Maliha Nawaz Mokal, [a]Sanaye Siddique, [a]Muhammad Iftikhar

[a] *Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan.*
[b] *Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan.*

ARTICLE INFO

Article History
Received: November 22, 2023
Revised: March 13, 2024
Accepted: March 25, 2024

Keywords
Allelopathy
Darek
Germination
Growth
Neem
Phytochemicals

ABSTRACT

The present study was designed to investigate the comparative allelopathic evaluation of different parts of *Azadirachta indica* A. Juss. and *Melia azedarach* L. on the seed germination and seedling growth of *Hordeum vulgare* (barley) and *Spinacia oleracea* (spinach). For aqueous extract preparation different parts i.e., leaf, bark and stem of both plant species were collected. After shade drying they were ground to fine powder by using pestle and mortar. The extracts were prepared in various concentrations (0.5%, 1% and 2%) by overnight soaking the ground powder in distilled water and then filtration of the solutions was performed. The filter paper method was use in this study for allelopathic screening. Results revealed that leaf extract of *Azadirachta indica* significantly inhibited seed germination of spinach (81%) at 1% concentration. Similarly, the leaf extract of *Melia azedarach* also retarded seed germination of spinach up to 48% at higher concentration (2%). But its bark extracts slightly stimulated barley seed germination. For seedling growth, the bark extract of *Melia azedarach* strongly suppressed spinach growth. On the other hand, the growth of spinach was exponentially stimulated under the influence of bark extract of *Azadirachta indica*. The stem extracts of both donor species stimulated seedling growth of barley. It was concluded that the allelopathic effects of both tree species are crop dependent. However, there is a possibility of presence of negativelly affecting allelochemcials in different plant parts of tree species. These allelochemicals can further be characterized in order to use these species as border crops around various agricultural lands.

Corresponding Author: Madiha Rashid
Email: botanistpk@yahoo.com; madiha.rashid@ue.edu.pk

INTRODUCTION

Azadirachta indica L. (Neem) and *Melia azedarach* L. (Darek, China tree) are famous remedial herbs and belongs to the Meliaceae. The origin of *A. indica* is India and now it is cultivated all over the world (Alzohairy, 2016; Hemdan *et al.*, 2023). Neem leaves are the source of many phytoconstituents like phenolics, carotenoids, triterpenoids, steroids and flavonoids and these play an important role in biological fields, most commonly in antifungal, antibacterial, antiparasitic, antiviral and many chronic diseases (Srivastava *et al.*, 2020). *A. indica* is valuable medicinally and have great usage history in the treatment of stomach ulcer and jaundice along with its importance to control different infection disease such as malaria (Habluetzel *et al.*, 2019). While *M. azedarach* originated from Africa, Asia and Australia and is used as the best antiseptic and antifungal with many free radicals and scavenging activity (Farag *et al.*, 2011). Both these medicinal herbs show bioactivity which is recognized through the presence of major limonoids compounds, phenolic acids and flavonoids such as rutin (Dougnon and Ito, 2022). *M. azedarach* leaves containing a high amount of liminoids which is the type of nimbolinin and have major therapeutic effects such as anticancer, antimicrobial and antioxidant (Kanwal *et al*., 2011). This plant is also the major source of phytochemicals with significant medicinal effects (Sivaraj *et al*., 2018). Extract and powder coming from the leaves of *M. azedarach* play a major role in protecting against influenza A and B viruses (Nerome *et al*., 2018). While stems and barks of *A. indica* showed significant effects in the curing of leucorrhoea and roasted flowers help to cure jaundice (Lekha and Menakashree, 2018).

Allelopathy is the process of impact of one plant on related plants and microbes by releasing chemical compounds (Schandry and Becker, 2020). Allelochemicals are involved in the activation of allelopathy. Allelochemicals are main elements commonly released as secondary metabolites by living things and exert the negative physiological and morphological impact on other plant and microbial species (Jabran, 2017). Plants commonly release Allelochemicals as byproducts in different physiological and biochemical processes (Bhadoria, 2011; Ashraf *et al*., 2017; Dahiya *et al*., 2017). These allelochemicals are released via different plant parts such as seeds, stem, bark, roots, flowers, leaves and fruits. These are the combination of different compounds such as ketones, fatty acids, amino acids, aldehydes, flavonoids, purines, phenolics and others (Novak and Novak, 2017). These mainly impact the content, functioning, production and activities of different enzymes in many ways. The old research showed that catechol caffeic acid and chlorogenic acids are involved in the suppression of main enzyme such as phophorylase which is involved in seed germination (Latif *et al*., 2017). These affect plant development by changing different stages of respiration such as electron transfer, carbon dioxide production, ATP activity and oxidative phosphorylation. These are also involved in changing cell permeability and membrane functioning after exposure at high concentration which then results in cell leakage and cell death through apoptosis and necrosis. These also affect activity of Na^+/K^+ pumps which help membranes to absorb ions in living roots and restrict the development of targeted plant (Motmainna *et al*., 2023).

The earlier findings reported that leaf extract of darek at various concentrations presented prominant inhibition in the growth and germination of *Fagopyrum esculentum* Moench and *Cyperus iria* L. (Stefanello *et al*., 2023). Similarly results also described phytochemical effects of darek on growth of soyabean crop (Gulimar *et al*., 2017) and *Brassica campestris* L. (Gul *et al*., 2022). Allelopathic potential of leaf extract of neem were also studied in two cereal crops (wheat and maize) and two weed (*Portulaca oleracea* L. and *Bidens pilosa* L.) (El–Hamid *et al*., 2017). Further, literature also found for neem tree where aqueous extract of leaf reduced the growth parameters of maize and rice (Kaur and Leela, 2023). Most research has concentrated on the essential oil fraction, or the activity of the non-volatile fraction that contains phenolic compounds and flavonoids present in different parts of dharek and neem. On the other hand, nothing is known about these trees' phytochemical potential in spinach and barley crops. Thus, the purpose of this study was to determine the allelopathic potential of aqueous extract of dharek and neem plant parts on the germination and growth of spinach and barley.

MATERIALS AND METHODS

The experiments were conducted to investigate the allelopathic potential of different parts of *Melia azedarach* L. and *Azadirachta indica* A. Juss., on seed germination and seedling growth of barley and spinach. Fresh and well-developed plant parts including leaves, stem and bark of *Azadirachta indica* and *Melia azedarach* were collected from different regions of Punjab i.e., Okara and Chunian. The plant material was properly cleaned with water and allowed to air dry for a period of 15 to 20 days. The dried material was then ground with the help of electronic grinder to fine powder and stored in zipper bags till the whole experimentation.

Test plants

The two important crops one from monocot (barley) and other from dicot (spinach) were selected as test plants. The seeds of these crops were taken from Punjab Seed Corporation. Following application of aqueous extracts, the seeds of these two species demonstrated clear effects and readily germinated with a high fecundity rate (Umer *et al.,* 2010). The seeds were germinated during January to March for the comparative allelopathic study of donor plant parts (Neem and Dharek) against them.

Preparation of aqueous extracts

The aqueous extracts of different plant parts were prepared in different concentrations i.e., 0.5%, 1% and 2%. To prepare these concentrations the dried powder of each part was weighed accordingly. The stock extracts were prepared by immersing the powders of different plant parts in distilled water for 24 hours at room temperature (Djanaguiraman *et al*., 2002). The stock solutions of different concentrations were prepared as follows; 0.5% (0.5 g of dried powder was soiked in 100 ml of distilled water), 1% (1 g of dried powder was soiked in 100 ml of distilled water) and 2% (2 g of dried powder was soiked in 100 ml of distilled water). The extracts were then filtered with the help of muslin cloth followed by Whatman filter paper No.1. The filtering was done to prevent any plant material from being dropped in to the extract. Each concentration was placed in small, labeled glass flasks and stored in a fridge till used.

Preparation of medium for germination

For germination, filter paper served as the growth medium. Since filter paper can withstand the laboratory's mild incubation temperature of 25°C, it is a more appropriate approach. For a longer period of time, the aqueous extracts stayed fresh (Hao *et al.,* 2007). The ease of availability and lack of contamination are the main reasons to use filter paper in these procedures. It has a high flow rate for extract movement, is readily handled, and is a suitable germination medium and it also possesses porosity (Gray and Trigiano, 2004). Aqueous extracts of 0.5%, 1% and 2% were applied on test plants by soaking double layers of filter papers. Cotton dipped in 70% ethanol was used to clean petri plates and sterilize the medium of dust particles and microorganisms. Every petri dish included ten seeds of each test plant, sandwiched between two layers of filter paper. Aqueous extracts of different plant parts were applied on the test seeds. Distilled water was used in controlled plates. The experiment with each treatment was replicated thrice.

Germination and growth records

For duration of one week, the petri dishes were kept at room temperature. Equal volume of distilled water was used in place of extract in controlled plates. During the experiment, the number of germinated seeds was observed on daily basis. The germination of barley plant was started on 3rd day after start of experiment while spinach plants germinated on 4th day after start of experiment. Following a week, the lengths of the roots and shoots were measured, and the total number of seeds that germinated was counted. Non-germinated seeds were considered as mortal.

Formulae used

$$Germination\ \% = \frac{\text{Total no. of germinated seeds under various conc.} \times 100}{\text{Total no. of seeds sown}}$$

$$\%\ \text{Inhibition/Stimulation} = 1 - \frac{\text{Length of shoot or root with treatment } \times 100}{\text{Length of shoot or root with control}}$$

Data analysis

Data were analyzed by computerized software SPSS (Statistical Package for the Social Sciences) and Microsoft Excel. Single-factor ANOVA was performed to investigate the significance of activity. Level of significance was 5%. Percentage germination, mortality and growths of hypocotyl and radicle were represented by line graph (Steel *et al*., 1997).

RESULTS

Effect of aqueous extracts on seed germination of test plants

The aqueous extracts of *Melia azedarach and Azadirachta indica* showed both stimulatory and inhibitory effect on the seed germination of barley and spinach. Seed germination of spinach was strongly inhibited (81%) by the leaf extract of *Azadirachta* at 1% concentration (Figure 1d). Similarly, the leaf extract of *Melia azedarach* also reduced the seed germination of spinach at its higher concentration (2%), the seed germination of spinach was reduced up to 48% (Figure 1b). But the bark and stem extracts of both species showed comparatively less inhibitory effect on germination of spinach seeds.

In case of barley, all the extracts of both donor plant species showed variable effects on seed germination. The leaf extracts of both species stimulated the germination of barley. But the bark and stem extracts showed inhibitory effect. Among them, stem extract of *M. azedarach* showed 6% of seed mortality at its lower concentration while same extract of *A. indica* at same concentration showed only 3% inhibitory effect. At 0.5%, the bark extract of *M. azedarach* stimulated the barley germination by 4% (Figure 1a). On the other hand, 10% inhibitory effect was observed at the same concentration of *Azadirachta indica* (Figure 1c).

Effect of *Melia azedarach* and *Azadirachta indica* leaf extract on seedling growth of barley and spinach

The aqueous extract of *Melia azedarach* leaf inhibited growth of hypocotyl and radicl of barley and spinach. The hypocotyl growth of barley was inhibited (p =0.01) by 31%, 32% and 56% at 0.5%, 1% and 2% concentrations respectively (Table 1). There was also inhibition (p=0.001) of radicle growth by 35% and 56% at 0.5% and 2% concentrations respectively. But at 1% concentration the inhibition was slightly higher (60%) (Figure 2a). Similarly, maximum inhibitory effect (60%) was observed on the radicle growth (p=0.049) of spinach at 1% concentration, followed by 56% and 33% at 2% and 0.5% concentrations, respectively. On the other hand, the extract also showed inhibitory effect (p=0.04) on the growth of hypocotyl that was increased by increasing the concentration of the extract. At its highest concentration the maximum growth retardation (56%) was recorded (Figure 2c). The extract of *Azadirachta indica* leaf reduced the growth of both hypocotyl and radicle of the barley while showed diverse effects on the seedling growth of spinach. The inhibitory effect was increased by increasing the concentration of the leaf extract. The hypocotyl growth inhibition (p=0.00) of barley was found to be 15%, 31% and 39% at 0.5%, 1% and 2% concentrations respectively (Table 1). The maximum radicle growth inhibition (p=0.00) (53%) was observed at 2% concentration (Figure 2b). The hypocotyl growth of spinach was highly inhibited up to 56% and 75% at 0.5% and 1% concentrations respectively. But it stimulated 20% hypocotyl growth at 2% concentration (p=0.01). The radicle growth (p=0.01) was also retarded by 66% and 73% at 0.5% and 1% concentrations respectively. While slight retardation (9%) was observed at 2% of extract (Figure 2d).

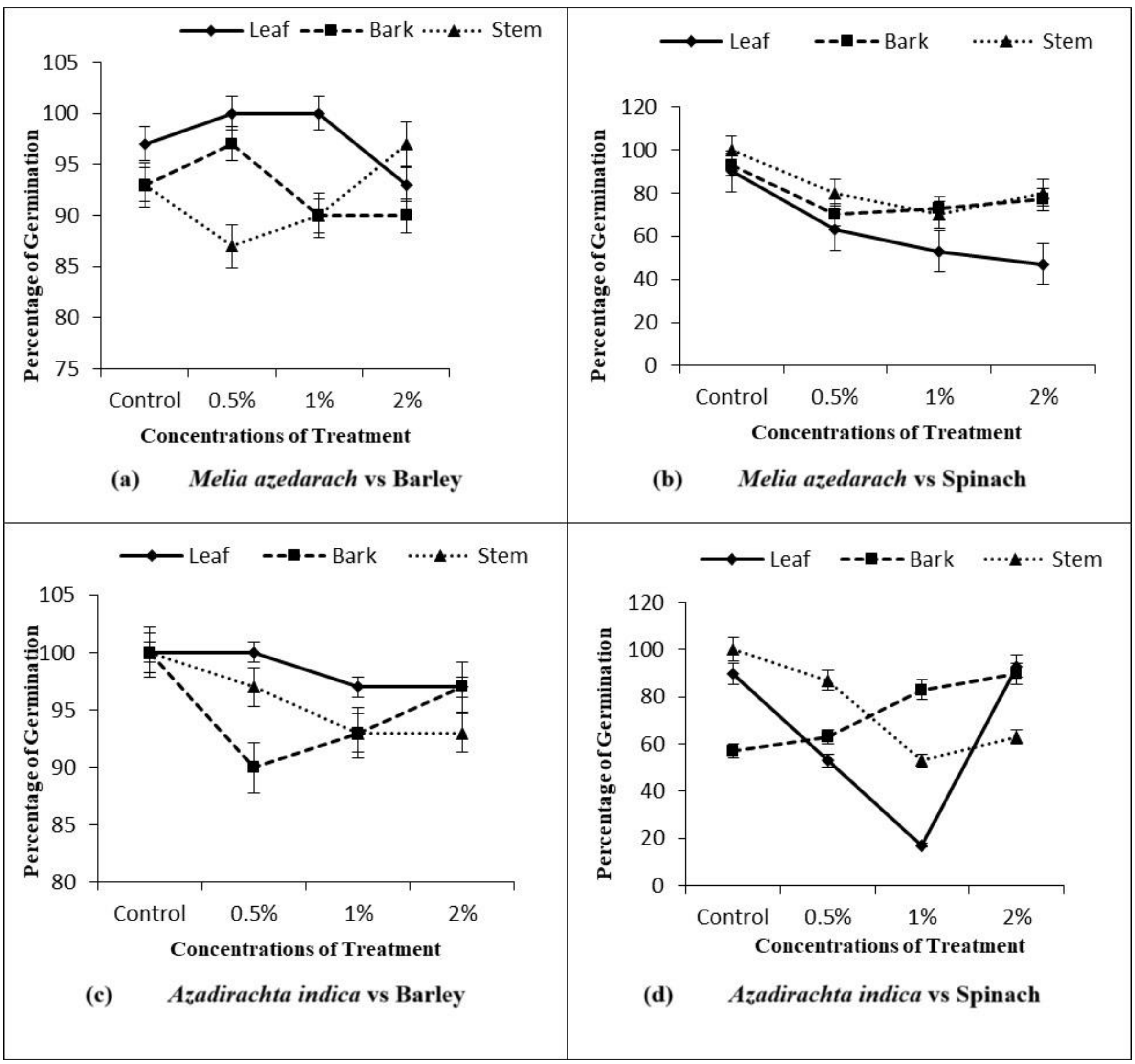

Figure 1. Effect of different parts of *Melia azedarach* and *Azadirachta indica* on germination of barley and spinach.

Table 1. Allelopathic effect of *Melia azedarach* and *Azadirachta indica* leaf on the growth of test plant.

	Test	Control		0.5%		1%		2%		F Value		P Value	
	Species	H	R	H	R	H	R	H	R	H	R	H	R
Melia azedarach	Barley	4.76	7.24	4.71	5.72	4.63	4.95	4.63	5.29	0.02	14.04	0.01*	0.001*
	Spinach	2.62	3.25	1.79	2.10	1.78	1.31	1.15	1.42	3.52	4.18	0.04**	0.049*
Azadirachta indica	Barley	5.88	8.43	4.99	6.15	4.01	4.99	3.58	3.91	49.52	64.40	0.00*	0.00*
	Spinach	2.62	3.25	1.14	1.09	0.64	0.74	3.13	2.94	8.70	8.20	0.01*	0.01*

H= Hypocotyl, R=Radicle, * Significant Effect, ** Non-significant Effect, Alpha Value: 0.05

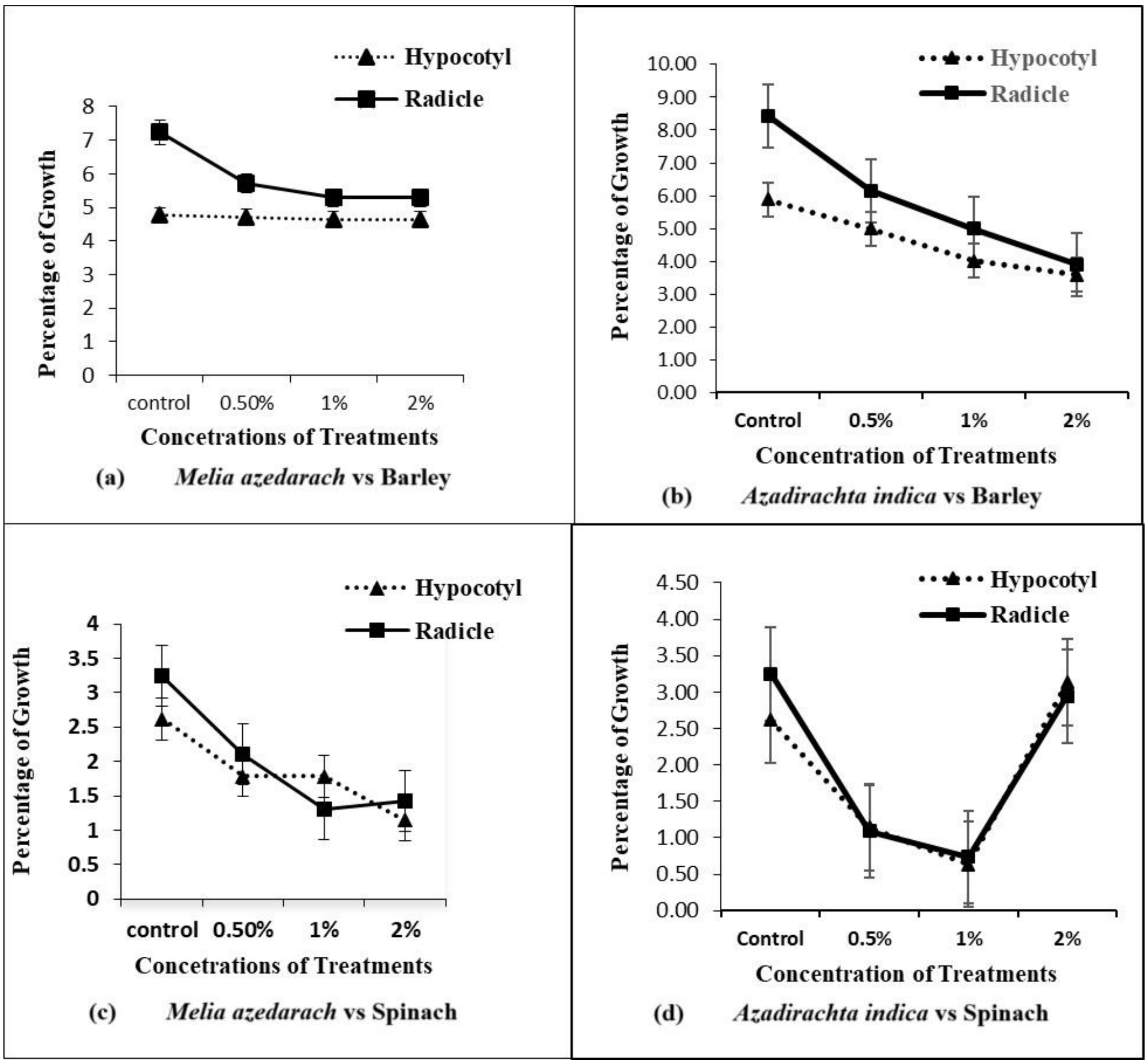

Figure 2. Effect of aqueous leaf extract of *Melia azedarach* (Darek) and *Azadirachta indica* (Neem) on growth of barley and spinach.

Effect of bark extract of *Melia azedarach* and *Azadirachta indica* on seedling growth of barley and spinach

The extract of *Melia azedarach* bark stimulated the growth of hypocotyl (p=0.03) of barley up to 7% at its lower concentration (0.5%) but the growth was inhibited by 13% at higher concentrations (1% and 2%). The bark extract also inhibited radicle growth at all level of concentrations (p=0.003). The growth was retarded by 9%, 52% and 53% at 0.5%, 1% and 2% concentrations respectively (Figure 3a). However, bark extract inhibited the growth of both hypocotyl and radicle of spinach. The inhibitory effect was concentration dependent. The maximum growth retardation (p=0.00) of hypocotyl was shown to be 80% at 2% concentration. The inhibition (p=0.001) of radicle growth was 46%, 73% and 67% at 0.5%, 1% and 2% concentrations respectively (Table 2; Figure 3c).

The extract of *Azadirachta indica* declined (p=0.02) radicle growth in barley (Table 2). The reduction in growth was found to be 21%, 36% and 40% at 0.5%, 1% and 2% concentrations respectively. But it showed a minor inhibitory effect (p=0.04) on the hypocotyl growth of barley. The inhibitory effect seemed to be only 4% at 1% concentration of the extract (Figure 3b). In contrast, bark extract stimulated both hypocotyl and radicle growth of spinach. The hypocotyl growth was enhanced (p=0.02) by 38%, 93% and 96% at 0.5%, 1% and 2% concentrations of the extract respectively. In the same way, the radicle growth was strongly stimulated (p=0.031) up to 21% and 19% at 1% and 2% concentrations respectively (Figure 3d).

Table 2. Allelopathic effect of *Melia azedarach* and *Azadirachta indica* bark on the growth of test plant.

	Test	Control		0.5%		1%		2%		F Value		P Value	
	Species	H	R	H	R	H	R	H	R	H	R	H	R
Melia azedarach	Barley	3.61	6.18	3.87	5.46	3.12	2.92	3.10	2.86	0.80	10.85	0.03*	0.003*
	Spinach	2.99	4.95	1.26	2.67	0.60	1.32	0.58	1.65	21.4	14.8	0.00*	0.001*
Azadirachta indica	Barley	4.00	7.01	3.97	5.53	3.82	4.69	4.00	4.18	0.06	6.09	0.04*	0.02*
	Spinach	1.54	2.73	2.13	2.27	2.97	3.31	3.84	3.16	5.65	1.42	0.02*	0.031*

H= Hypocotyl, R=Radicle, * Significant Effect, ** Non-significant Effect, Alpha Value: 0.05

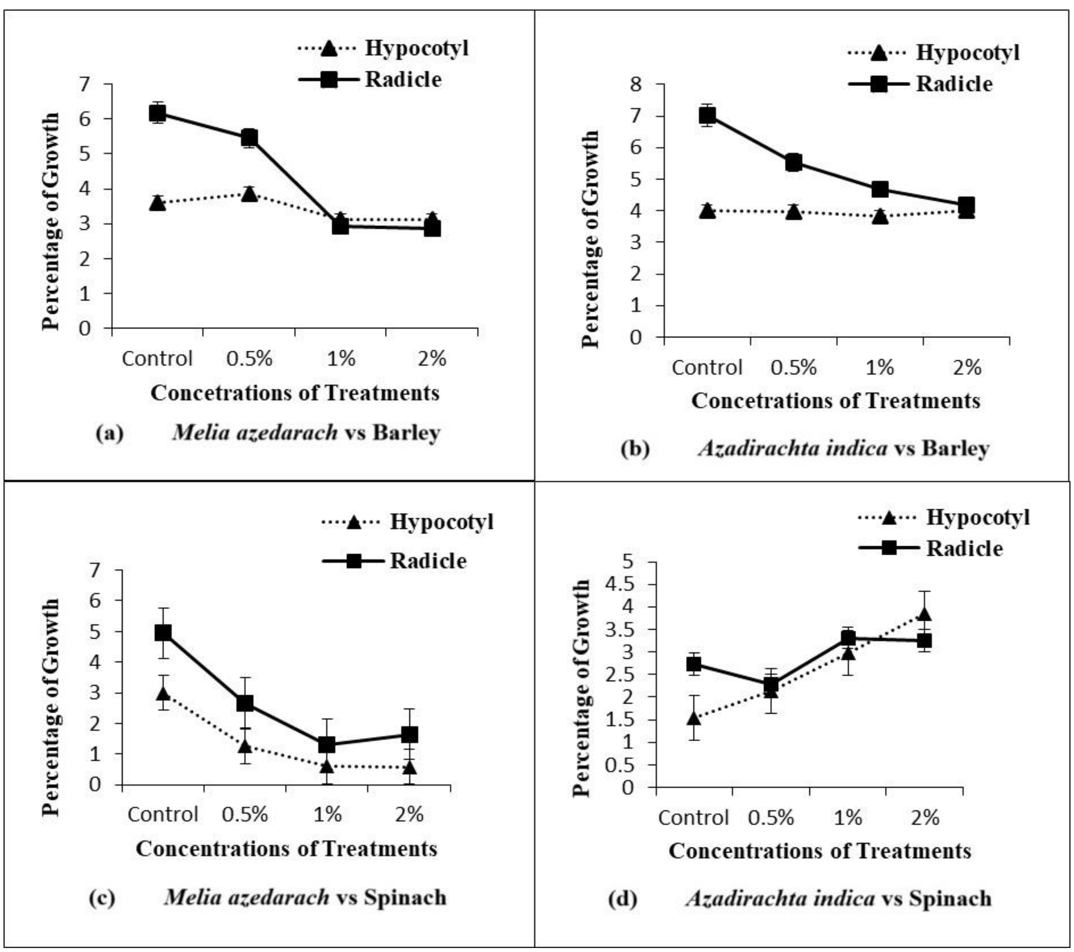

Figure 3. Effect of aqueous bark extract of *Melia azedarach* (Darek) and *Azadirachta indica* (Neem) on growth of barley and spinach.

Effect of stem extract of *Melia azedarach* and *Azadirachta indica* on seedling growth of barley and spinach

The stem extract of *Melia azedarach* stimulated the hypocotyl growth of barley. The increase in the stimulation (p=0.005) of hypocotyl growth was concentration dependent (Table 3). The growth stimulation was found to be 15%, 37% and 56% at 0.5%, 1% and 2% concentrations of the extract respectively. But the growth of radicle was inhibited at all concentrations. The radicle growth retardation (0.04) was observed to be 19%, 31% and 30% at 0.5%, 1% and 2% concentrations respectively (Figure 4a). However, the stem extract of *Melia azedarach* showed both stimulatory and inhibitory effect on the seedling growth of spinach. The extract stimulated (p=0.04) the hypocotyl growth up to 4% at higher concentrations (1% and 2%). At 0.5% extract concentration, hypocotyl and radicle growth was suppressed by 17% and 9% respectively while 17% growth inhibition (0.002) was observed at both 1% and 2% concentration (Figure 4c).

The extract of *Azadirachta indica* stem promoted seedling growth of barley. The stimulatory effect (p=0.019) on the growth of hypocotyl varied but the maximum growth stimulation was 47% at 2% concentration of the extract (Table 3). In case of radicle growth, the overall stimulation (p=0.046) was observed but the effect was decreased with increasing concentration. The maximum stimulation was 46% at lowest concentration (0.5%) and lower value of stimulation was found to be only 2% at high concentration of extract (Figure 4b). Although, the aqueous extract of *Azadirachta indica* stem inhibited both hypocotyl and radicle growth of spinach. The hypocotyl growth inhibition (p=0.04) was observed to be 72% at higher concentrations (1% and 2%) followed by 63% at lowest concentration (0.5%). On the other hand, the inhibitory effect (p=0.022) of radicle growth increased by increasing the concentration. It was observed to be 32% at 0.5% concentration and 53% for both 1% and 2% concentrations of stem extract (Figure 4d).

Table 3. Allelopathic effect of *Melia azedarach* and *Azadirachta indica* stem on the growth of test plant.

	Test	Control		0.5%		1%		2%		F Value		P Value	
	Species	H	R	H	R	H	R	H	R	H	R	H	R
Melia azedarach	Barley	5.24	6.13	7.03	8.96	6.34	7.67	7.70	6.23	2.0	0.96	0.019*	0.046*
	Spinach	2.79	3.58	1.75	2.41	2.00	1.66	2.00	1.66	0.96	1.83	0.04*	0.022*
Azadirachta indica	Barley	4.83	9.72	5.52	7.84	6.60	6.66	7.50	6.76	9.9	4.32	0.005*	0.04*
	Spinach	2.36	2.75	1.94	2.49	2.45	2.33	2.45	2.33	0.18	0.09	0.04*	0.002*

H= Hypocotyl, R=Radicle, * Significant Effect, ** Non-significant Effect, Alpha Value: 0.05

DISCUSSION

Allelochemicals are significant compounds produced mainly by various plants that are associated with process of allelopathy (Ain *et al*., 2023). They are present in varying amounts in practically all plant components, including leaves, stems, bark, flowers and rhizomes (Khatun *et al*., 2023). They can lessen phytotoxic residues in soil and water, promote or inhibit seed germination, facilitate development, and permit agricultural plant growth, which simplifies recycling and wastewater treatment. (Zeng *et al*., 2008; Abouziena and Haggiag, 2016). The concentration of these allelochemicals influences the germination and growth of test plant as it may enhance seed germination at lower concentration and found to reduce seed germination when higher concentration was applied (Farooq *et al*., 2020; Subtain *et al*., 2014). The current findings demonstrated that different plant extracts from both species had different effects on test plant seed germination and seedling growth. Most of the treatments showed reduction in the seedling growth. The inhibitory effect may be associated with the presence of allelochemicals that may modify the photosynthetic apparatus by reducing chlorophyll contents and damaging the PSII (Hussain and Reigosa, 2017; Rehman *et al*., 2019; Yuliyani *et al*., 2019).

In the present findings, the aqueous extracts of *M. azedarach* significantly inhibited the seed germination of spinach. Among different parts, the leaf extract extremely inhibited the germination of spinach. The inhibitory effect was concentration dependent. These findings agreed with the results of Phuwiwat *et al.* (2012). According to their observation the aqueous leaf extract of *Melia azedarach* inhibited germination of *E.*

crus-galli. This data also aligns with findings from studies on *Brassica campestris* L., which showed that aqueous extracts of *M. azedarach* at different doses (10, 15 and 20 g 100 mL^{-1}) inhibited seed germination and seedling growth (Gul *et al*., 2022). Furthermore, it was confirmed in a different study (with extract concentrations ranging from 10 to 100%) that *Melia* negatively impacts the germination, growth, biomass, and production of *Abelmoschus esculentus* L. (Moench) and *Raphanus sativus* L. (Pratap *et al*., 2022). The observed effect might be associated with the presence of secondary metabolites including alkaloids, flavonoids and phenolic contents as these compounds presented negatively affected the seedling development (Patanè *et al*., 2023).

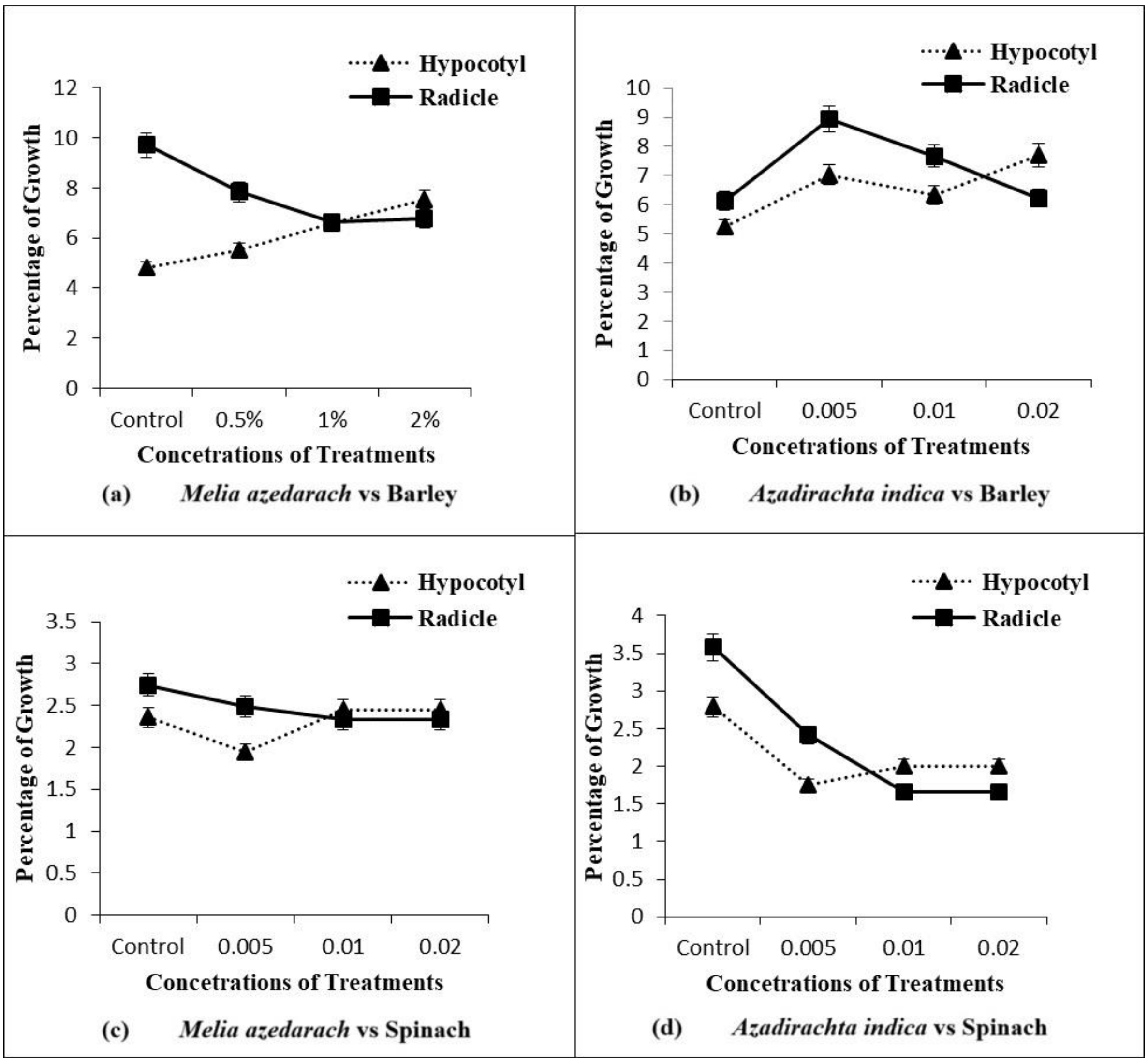

Figure 4. Effect of aqueous stem extract of *Melia azedarach* (Darek) and *Azadirachta indica* (Neem) on growth of barley and spinach.

According to the present results, seed germination of spinach and barley was suppressed by various extracts of *Azadirachta indica*. Among them the leaf extract of *A. indica* significantly retarded the germination of spinach at 1% concentration. Same extract also strongly inhibited the hypocotyl and radicle growth of spinach. These observations were related to the analysis of Lawan *et al*. (2011). They found that various doses of *A. indica* leaf extract suppressed the growth of the shoots, roots, and seeds of various cowpea cultivars. Under laboratory conditions, the seedlings' mortality and decreased vigor demonstrated the buildup of hazardous chemicals from the donor plants, which may have an adverse effect on the development of receptor plant seedlings. (Lawan *et al*., 2011). According to Lungu *et al.* (2011), the inhibitory effect might be due to the presence of phytotoxic compounds in the *Melia azedarach*.

In the present study, the negative effects were also observed under the influence of higher concentration of bark extract of *Melia azedarach*. The results were supported by the observations of Khan *et al.* (2016). They observed that the bark extracts of *Populus nigra* showed inhibitory effect on maize. Many agronomic crop and weed species were reported to be inhibited in growth by the seed and bark extract of *Tamarindus indica*, according to Parvez *et al*. (2004*).* Several investigations have demonstrated that the aqueous extracts of M. azedarach have showed inhibitory action at various concentrations on sesame seed and vigna radiate germination and seedling growth in comparison to control (Soleymani and Shahrajabian, 2012; Shahid *et al*., 2017).

The inhibitory effect might be due to the presence of monoterpenoids and terpenoids i.e., camphor, 1,8-cineole, betapinene, alpha-pinene, and camphene in the *Melia azedarach* (Sultana *et al.,* 2014).

The present findings also showed that the stem extracts of both species stimulated the growth of barley. The higher concentration of stem extracts of *Melia azedarach* stimulated more hypocotyl growth of barley than at the same concentration of *Azadirachta indica*. The results agreed with the report of Sisodia and Siddiqui (2010). It was discovered that the aqueous stem extract of *Croton bonplandianum* stimulated seed germination and seedling growth in weed plants (*Medicago hispida* Gaertn, *Melilotus alba* Medik., *Vicia sat*iva L., and *Brassica rapa* L.) as well as crop plants (*Triticum aestivum* L*.,* *Brassica oleracea* var. botrytis L., and *Brassica rapa* L.). The stimulatory influence might be due to the synthesis of phenolic and flavonoids contents that may cause the activation of antioxidants system (Thiebaut *et al*., 2019; Masum *et al*., 2018).

CONCLUSION

The present results inferred that the leaf extracts of both donor species possessed strong inhibitory effects on the germination and growth of spinach. Moreover, the barley crop showed highest tolerance against different extracts with reference to its germination and growth. It was concluded from results that the leaves of both species exhibited strong allelopathic potential. Hence, these are not suitable in border cropping around the crops of barley and spinach. However, it seems to be crucial to check the allelopathic compatibility of these two species with other crops before introducing them to agricultural practices as cover crops.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES

Abd El–Hamid, H.A., L.M.N. Ibrahim, M.Y. Ammar and M.A. Helmy. 2017. Allelopathic effect of neem (*Azadirachta indica* A. Juss) aqueous leaf extract on the germination and growth of some selected crops and weeds. Biolife, 5, 428-436.

Abouziena, H.F. and W.M. Haggag. 2016. Weed control in clean agriculture: A review. Planta Daninha, 34, 377-392.

Ain, Q., W. Mushtaq, M. Shadab and M.B. Siddiqui. 2023. Allelopathy: An alternative tool for sustainable agriculture. Physiology and Molecular Biology of Plants, 1-17.

Alzohairy, M.A. 2016. Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in disease prevention and treatment. Evidence-Based Complementary and Alternative Medicine, 2016.

Ashraf, R., B. Sultana, S. Yaqoob and M. Iqbal. 2017. Allelochemicals and crop management: A review. Current Science, 3(1): 1-13.

Bhadoria, P.B.S. 2010. Allelopathy: A natural way towards weed management. American Journal of Experimental Agriculture, 1(1): 7-20.

Dahiya S, S. Kumar, R.S. Khedwal and S.R. Jakhar. 2017. Allelopathy for sustainable weed management. Journal of Pharmacognosy and Phytochemistry, 6, 832–837.

Djanaguiraman, M., P. Ravishankar and U. Bangarusamy. 2002. Effect of *Eucalyptus globulus* on green gram, black gram and cowpea. Allelopathy Journal, 10(2): 157-161.

Dougnon, G. and M. Ito. 2022. Essential oils from *Melia azedarach* L. (Meliaceae) leaves: chemical variability upon environmental factors. Journal of Natural Medicines, 1-11.

Farag, M., M.H. Ahmed, H. Yousef and A.H. Abdel-

Rahman. 2011. Repellent and insecticidal activities of *Melia azedarach* L. against cotton leafworm, *Spodoptera littoralis* (Boisd.). Zeitschrift für Naturforschung C, 66(3-4): 129-135.

Farooq, N., T. Abbas, A. Tanveer and K. Jabran. 2020. Allelopathy for weed management. Co-evolution of Secondary Metabolites, 505-519.

Gray, D.J. and R.N. Trigiano. 2004. Getting started with tissue culture media preparation, sterile technique, and laboratory equipment. In Plant Tissue Culture Development and Biotechnology, 15-37.

Gul, S., F. Hussain, S. Abidullah, A. Gul and M. Shuaib. 2022. Allelopathic effect of *Melia azedarach* L. and *Populus nigra* L. on germination and growth of *Brassica campestris* L. Catrina. The International Journal of Environmental Sciences, 25(1): 11-17.

Gumilar R.A., N. Wijayanto and A.S. Wulandar, 2017. Effect of *Azadirachta excelsa* and *Melia azedarach* extracts on soybean germination. Nusantara Bioscience 9(4): 346-351.

Habluetzel, A., B. Pinto, S. Tapanelli, J. Nkouangang, M. Saviozzi, G. Chianese, A. Lopatriello, A.R. Tenoh, R.S. Yerbanga, O. Taglialatela-Scafati and F. Esposito. 2019. Effects of *Azadirachta indica* seed kernel extracts on early erythrocytic schizogony of *Plasmodium berghei* and pro-inflammatory response in inbred mice. Malaria Journal, 18, 1-9.

Hao, Z.P., Q. Wang, P. Christie and X.L. Li. 2007. Allelopathic potential of watermelon tissues and root exudates. Scientia Horticulturae, 112(3): 315-320.

Hemdan, B.A., A. Mostafa, M.M. Elbatanony, A.M. El-Feky, T. Paunova-Krasteva, S. Stoitsova, M.A. El-Liethy, G.E. El-Taweel and M.A. Mraheil. 2023. Bioactive *Azadirachta indica* and *Melia azedarach* leaves extracts with anti-SARS-CoV-2 and antibacterial activities. Plos one, 18(3): p.e0282729.

Hussain, M.I. and M.J. Reigosa. 2017. Evaluation of Photosynthetic Performance And Carbon Isotope Discrimination In Perennial Ryegrass (*Lolium Perenne* L.) Under Allelochemicals Stress. Ecotoxicology, 26, 613-624.

Jabran, K. and B.S. Chauhan. 2015. Weed management in aerobic rice systems. Crop Protection, 78, 151-163.

Kanwal, Q., I. Hussain, L.H. Siddiqui and A. Javaid. 2011. Antimicrobial activity screening of isolated flavonoids from *Azadirachta indica* leaves. Journal of the Serbian Chemical Society, 76(3): 375-384.

Kato-Noguchi, H. and F.A. Macías. 2005. Effects of 6-methoxy-2-benzoxazolinone on the germination and α-amylase activity in lettuce seeds. Journal of Plant Physiology, 162(12): 1304-1307.

Kaur, P. and P. Leela. 2023. Effects of *Azadirachta indica* leaf extract on growth and development of *Oryza sativa* (L) and *Zea mays* (L). Allelopathy Journal, 60(2): 147.

Khan, M., M. Zakaria, F. Ali, F. Hussain and I.S. Musharaf. 2016. Allelopathic effect of *Populus nigra* bark on *Zea mays* in agroforestry ecosystems. Global Journal of Science Frontier Research, 16, 21-27.

Khatun, M.R., S. Tojo, T. Teruya and H. Kato-Noguchi. 2023. *Trewia nudiflora* Linn, a medicinal plant: Allelopathic potential and characterization of bioactive compounds from its leaf extracts. Horticulturae, 9(8): 897.

Latif, S., G. Chiapusio and L.A. Weston. 2017. Allelopathy and the role of allelochemicals in plant defense. In Advances in Botanical Research (Vol. 82, pp. 19-54). Academic Press.

Lawan, S.A., M. Suleiman and S.U. Yahaya. 2011. Inhibition of germination and growth behavior of some cowpea varieties using neem (*Azadiracta indica*) leaf water extracts. Bayero Journal of Pure and Applied Sciences, 4(2); 169-172.

Lekha, K. and P. Menakashree. 2018. Ethno medicinal value of plants in Tiruchengode area of Namakkal district, Tamil Nadu, India. Journal of Medicinal Plants, 6(1): 257-260.

Lungu, L., C.V. Popa, J. Morris and M. Savoiu. 2011. Evaluation of phytotoxic activity of *Melia azedarach* L. extracts on *Lactuca sativa* L. Romanian Biotechnological Letters, 16(2): 6089-6095.

Masum, S.M., M.A. Hossain, H. Akamine, J.I. Sakagami, T. Ishii, S. Gima, T. Kensaku and P.C. Bhowmik. 2018. Isolation and characterization of allelopathic compounds from the indigenous rice variety 'Boterswar' and their biological activity against *Echinochloa crus-galli* L. Allelopathy Journal, 43(1): 31-42.

Motmainna, M., A.S. Juraimi, M.S. Ahmad-Hamdani, M. Hasan, S. Yasmin, M.P. Anwar and A.M. Islam. 2023. Allelopathic potential of tropical plants—a review. Agronomy, 13(8); 2063.

Nerome, K., K. Shimizu, S. Zukeran, Y. Igarashi, K. Kuroda, S. Sugita, T. Shibata, Y. Ito and R. Nerome. 2018. Functional growth inhibition of influenza A and B viruses by liquid and powder components of leaves from the subtropical plant *Melia azedarach* L. Archives of Virology, 163, 2099-2109.

Novak, M. and N. Novak. 2019. Allelopathic effect of tree of heaven (*Ailanthus altissima* (Mill.) Swingle) on initial growth of the barnyard grass (*Echinochloa crusgalli* (L.) P. Beauv.). Fragmenta phytomedica, 33(4): 58-72.

Parvez, S.S., M.M. Parvez, Y. Fujii and H. Gemma. 2004. Differential allelopathic expression of bark and seed of *Tamarindus indica* L. Plant Growth Regulation, 42(3): 245-252.

Patanè, C., A. Pellegrino, S.L. Cosentino and G. Testa. 2023. Allelopathic effects of *Cannabis sativa* L. aqueous leaf extracts on seed germination and seedling growth in durum wheat and barley. Agronomy, 13(2): 454.

Phuwiwat, W., W. Wichittrakarn, C. Laosinwattana and M. Teerarak M. 2012. Inhibitory effects of *Melia azedarach* L. leaf extracts on seed germination and seedling growth of two weed species. Pakistan Journal of Weed Scientific Research, 18 (special issue), 485-492.

Pratap U.B., K.S. Pant, D. Nirala and P. Tiwari. 2022. Allelopathic influence of *Melia composita* Wild. on the performance of vegetable crops. The Pharma Innovation Journal, 11(12): 3737-3742.

Rehman, S., B. Shahzad, A.A. Bajwa, S. Hussain, A. Rehman, S.A. Cheema, T. Abbas, A. Ali, L. Shah, S. Adkins and P. Li. 2019. Utilizing the allelopathic potential of *Brassica* species for sustainable crop production: A review. Journal of Plant Growth Regulation, 38, 343-356.

Schandry, N. and C. Becker. 2020. Allelopathic plants: Models for studying plant–interkingdom interactions. Trends in Plant Science, 25(2): 176-185.

Shahid, M. and M.S. Khan. 2017. Assessment of glyphosate and quizalofop mediated toxicity to green gram [*Vigna radiata* (L.) Wilczek], stress abatement and growth promotion by herbicide tolerant *Bradyrhizobium* and *Pseudomonas* species. International Journal of Current Microbiology and Applied Sciences, 6(12): 3001-3016.

Sisodia, S. and M.B. Siddiqui. 2010. Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crops and weed plants. Journal of Agricultural Extension and Rural Development, 2(1): 22-28.

Sivaraj, I., S. Nithaniyal, V. Bhooma, U. Senthilkumar and M. Parani. 2018. Species delimitation of *Melia dubia* Cav. from *Melia azedarach* L. complex based on DNA barcoding. Botany, 96(5): 329-336.

Soleymani, A. and M.H. Shahrajabian. 2012. Study of allelopathic effects of sesame (*Sesamum indicum*) on canola (*Brassica napus*) growth and germination. International Journal of Agriculture and Crop Sciences, 4(4): 183-186.

Srivastava, S.K., B. Agrawal, A. Kumar and A. Pandey. 2020. Phytochemicals of *Azadirachta indica* source of active medicinal constituent used for cure of various diseases: A Review. Journal of Scientific Research, 64(1): 385-90.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. (1997). Principles and Procedures of Statistics: A Biometrical Approach, 3rd edition, pp: 352-358. McGraw Hill Book Co. Inc., New York, USA.

Stefanello, R., R.A.M. Barreto, F.W. Cervo and A.L. da Silva. 2024. Contribution to the allelopathic study of *Melia azedarach* L. on the germination and initial growth of *Fagopyrum esculentum* Moench and *Cyperus iria* L. seeds. Gaia Scientia, 17(3): 108-117.

Subtain, M.U., M. Hussain, M. Tabassam, M.A.R. Ali, M.A. Ali, M. Mohsin and M. Mubushar. 2014. Role of allelopathy in the growth promotion of plants. Scientia Agriculturae, 2(3): 141-145.

Sultana, S., H.M. Asif, N. Akhtar, M. Waqas and S.U. Rehman. 2014. A Comprehensive review on ethnobotanical uses, phytochemistry and pharmacological properties of *Melia azedarach* L. Asian Journal of Pharmaceutical Research and Health Care, 6(1): 26-32.

Thiébaut, G., M. Tarayre and H. Rodríguez-Pérez. 2019. Allelopathic effects of native versus invasive plants on one major invader. Frontiers in Plant Science, 10, 854.

Umer, A., Z. Yousaf, F. Khan, U. Hussain, A. Anjum, Q. Nayyab and A. Younas. 2010. Evaluation of allelopathic potential of some selected medicinal species. African Journal of Biotechnology, 9(37): 6194-6206.

Yuliyani, E.D., S. Darmanti and E.D. Hastuti. 2019. Allelochemical effects of *Chromolaena odorata* L. against photosynthetic pigments and stomata of *Ageratum conyzoides* L. leaves. In Journal of Physics, Conference Series (Vol. 1217, No. 1, p. 012149). IOP Publishing.

Zeng, R.S. 2008. Allelopathy in Chinese ancient and modern agriculture. Allelopathy in Sustainable Agriculture and Forestry, 39-59.