

Research Article

The Allelopathic Analysis of *Melia azedarach* Leaf and Berries Extracts on Barley

Afroz Rais¹, Alia Achakzai¹, Hina Ali¹, Abdul Qadir³, Salal Chakar⁴

¹Department of Botany, Sardar bahdur khan Women's University, Quetta, Pakistan.

²Department of Zoology, Sardar Bahdur Khan Women's University, Quetta, Pakistan.

³National Agriculture Research Center, Pakistan Agriculture Research Council, Islamabad, Pakistan.

⁴Directorate of Pulses, Agriculture Research Institute (ARI), Quetta, Pakistan.

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*Correspondence: abdulqadirjan@gmail.com

Abstract

Despite allelopathy's significance for agriculture and ecology, not much is understood about its mechanism or the adaptive tactics used by plants to defend themselves against allelochemicals. In a laboratory trial, the extracts of dry plant parts, such as leaves and berries of *Melia azedarach*, were used against barley seed varieties (Sanober). The extracts were formed with 0.5, 1, and 1.5g concentrations at different time periods (12, 48, and 72 h). The data was obtained by germination percentage, seedling growth, seedling weight, chlorophyll content, superoxide dismutase (SOD) activity, and phytochemical analysis. The order of growth inhibition was observed in berries>leaf. Berries extract showed a more inhibitory effect on seed germination. The seedling length, weight, and chlorophyll a+b were reduced, and antioxidant SOD activity was increased in the root and shoot at different concentrations of berries and leaf extract, with a slight difference as compared to the control. The phytochemical qualitative activity showed that the inhibitory effects in Sanober proved that *Melia azedarach* had water-soluble allelochemicals such as amino acids, phenols, flavonoids, tannins, terpenoids, and quinones that were released into the environment and had effects on different attributes of plants.

Keywords: Allelochemical; Barley; Inhibition; *Melia azedarach*



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Introduction

Allelopathy is a normal and eco-friendly phenomenon that involves inhibitory or promotional effects of released phyto-toxins "Allelochemicals" by the plants. Allelopathy occurs when plant release some specific chemicals, which affect other species in its vicinity, usually their detriment Xu *et al.* (2023). Allelochemicals consist of various chemicals and their derivatives. Plant growth regulators, including salicylic acid, gibberellic acid and ethylene, are also considered to be allelochemicals (Cheng and Cheng, 2015; Cotruț, 2018). Allelopathy is a mechanism whereby secondary metabolites synthesized by different microorganisms and plants influence biological and agricultural systems, which may be either stimulatory or inhibitory (Jabran *et al.*, 2013; Kostina-

Bednarz *et al.*, 2023). Secondary metabolites are non-nutritional and can be synthesized in any plant part, i.e. leaves, stems, roots, bark, seeds, etc. Under favorable environmental conditions, allelochemicals are released into the environment through the processes of volatilization, root exudation, decomposition and/or leaching, thereby affecting the growth of adjacent plants (Pedone-Bonfim *et al.*, 2015; Kostina-Bednarz *et al.*, 2023).

Several researchers have suggested that water-soluble secondary metabolites present in the plant tissues are extracted in water to use them for disease management. Water extracts can be used as a medium for the expression of allelochemical activity to suppress the growth of other organisms. Use of allelochemicals extracted in water for disease suppression in the laboratory and also application under field conditions evaluated the potential of wheat, barley, oat, rye, furthermore their versatile activities are involved against abiotic and biotic stress (Ahmed *et al.*, 2012; Bachheti *et al.*, 2020).

Despite the ecological and agronomic importance of allelopathy, relatively little is known concerning the mechanism or the adaptive strategies by plants in defence system against allelochemicals (Jabran *et al.*, 2015; Ladhari *et al.*, 2022). The role and mechanism of antioxidant enzymes not only protect various components of the cells from damages, but also play an important role in plant growth and development by modulating cellular-biology sub-cellular processes such as cell differentiation, cell growth/division, regulation of senescence, regulation of enzymatic activities, synthesis of proteins (Bhaduri and Fulekar, 2012). Plant secondary metabolites, including phenolic compounds or other antioxidative compounds through leaching, are released into organisms and may cause the accumulation of soluble biochemicals and the phenomenon of allelopathy. To test this hypothesis, the *Melia Azadrach* aqueous extracts were assessed for their morphological and ant-oxidative activity.

Methodology

Mature plant parts leaves and the barriers of *Melia azedarach* L were collected from university campus of Sardar Bahdur Khan Women University. The fresh samples of leaves and the barriers were store carefully in paper begs, at room temperature. While barely variety was obtained from Federal seed certification department (FSCRD) Quetta

Aqueous extraction and experimental outline

Aqueous extracts were obtains by soaking the 0.5g, 1g 1.5g of fresh crushed leaves and barriers in 100ml distilled water in 250ml conical flasks for different time periods i.e. 24-72hr. Extracts were filtered by using whatman 's filter paper. Waste products were discarded, aqueous extracts' were stored in capped flasks, and were placed in fridge (Nunes *et al.*, 2021).

Ten seeds of the barley verity were placed on filter paper in 9.0 cm diameter Petri dishes. The Petri dishes were irrigated with extract solutions and distilled water was used as control. Each treatment was replicated five times. Petri dishes were placed in a germinator at ± 20 °C for seven days (Rais *et al.*, 2017).

Several parameters were measured to understand the mechanism of allelopathy effects including; Estimates germination percentage (Nunes *et al.*, 2021). Seedling length (cm) (Beedi *et al.*, 2018). Seedling fresh and dry weight (g) (Almaghrabi, 2012). Chlorophyll content (Wu *et al.*, 2008). For qualitiative phytochemical analysis different test were

followed such as (Amino Acid: Xanthoprotein test), (Phenol: FeCl₃ test), (Flavonoids: Alkaline reagent test), (Tannins: FeCl₃ test), (Saponin: Foam test), (Terpenoids: Liebermann-Burchard test), (Quinones: Hydrochloric acid test), (Coumarin: Sodium hydroxide test: Glycoside), (Anthocyanin: Sulphuric acid test) (Farook *et al.*, 2019). Antioxidant Superoxide dismutase (SOD) content (Rais *et al.*, 2017).

Statistical analysis

Statistical analysis of the results was evaluated by analysis of variance using the Statistical Analysis System software v. 9.1.

Results and Discussion

Fresh leaves and berries extracts of 0.5gm- 1.5gm with different time period 24hr- 72hr were used.

Germination percentages (leaf and berries extracts)

The maximum germination percentage was found in 1.5g of leaves and berries extracts (98%), (100%) respectively. While other concentrations 0.5g and 1g were showed slightly inhibition as comparison to control (Table 1).

Table 1. Effects of aqueous extracts of berries and leaf of *Melia azedarach* on germination percentage of Sanobar each value is a mean of 5 replicates.

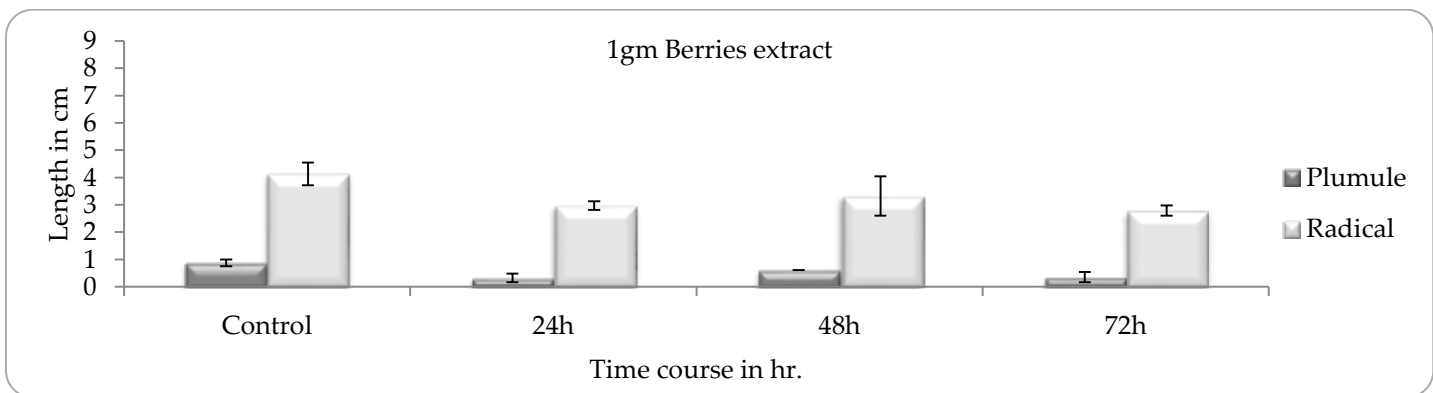
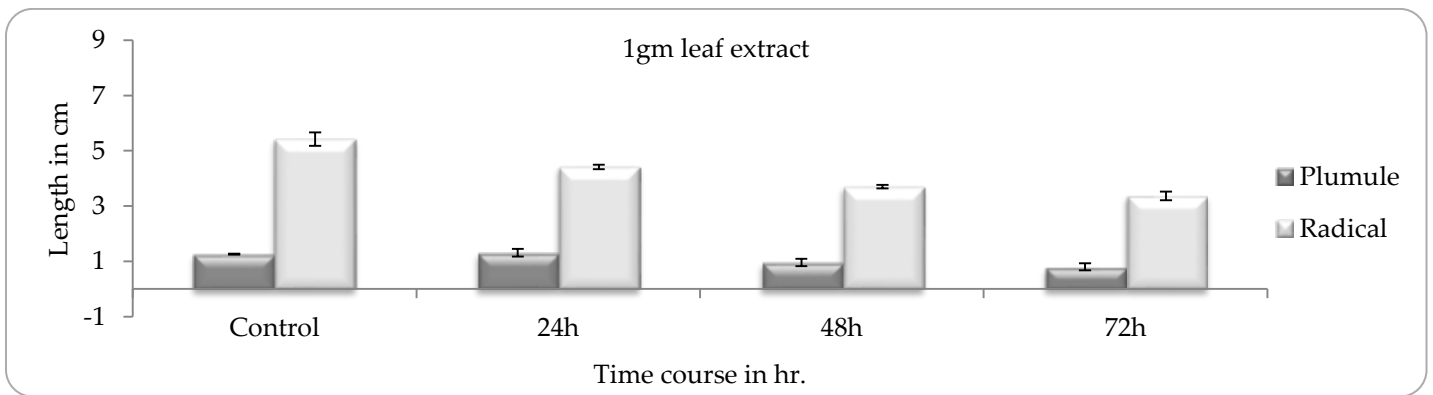
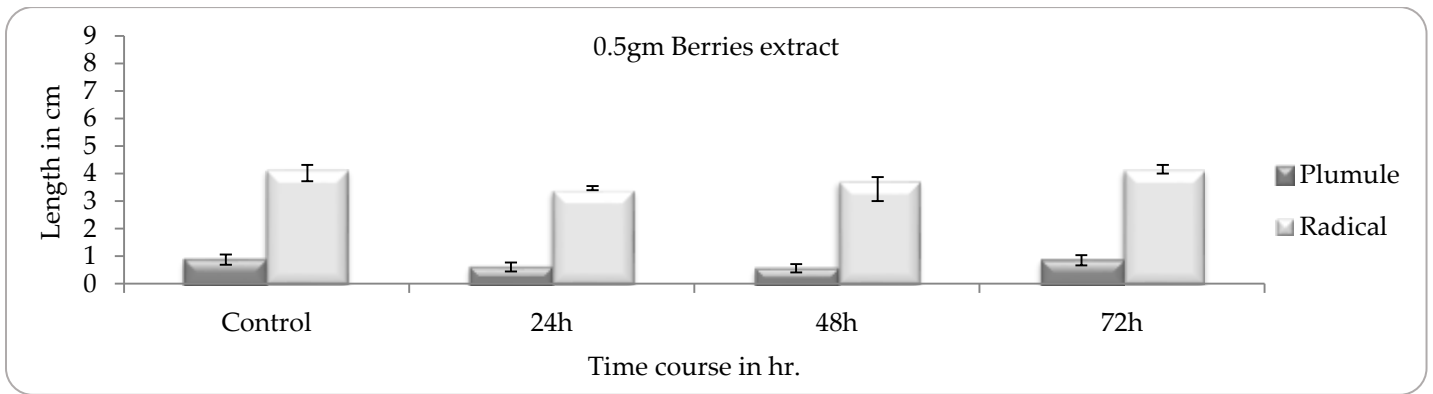
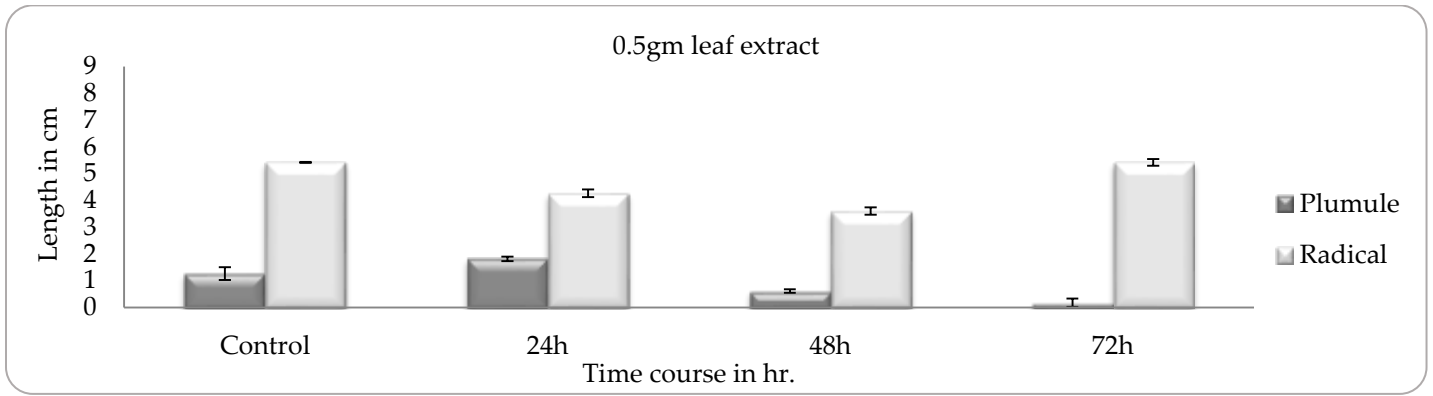
Treatments	Germination %	
	Berries	Leaf
Control	100%	100%
0.5gm		
24 hours	92 ^c %±0.005	98 ^a %±0.004
48 hours	96 ^b %±0.005	98 ^a %±0.003
72 hours	98 ^a %±0.004	94 ^b %±0.004
1.0gm		
24 hours	98 ^a %±0.003	98 ^a %±0.004
48 hours	96 ^b %±0.003	98 ^a %±0.004
72 hours	92 ^c %±0.002	98 ^a %±0.002
1.5gm		
24 hours	94 ^b %±0.002	98 ^a %±0.002
48 hours	100 ^a %±0.003	98 ^a %±0.003
72 hours	94 ^b %±0.002	98 ^a %±0.002

Values are means of three replicates and ± represent the standard error.

Seedling length cm (leaf and berries extracts)

The maximum stimulation takes place in root and shoot length in a leaf extracts. The highest calculated values were found in 1.5gm, root length is (0.28 – 0.73cm) and shoot length (0.06 – 0.32cm) as compare to control root length (0.48cm) and shoot length (0.27cm) respectively. Same observations were showed in 0.5g and 1g with the comparison of control. However in case of berries extracts inhibition take place. The highest length was found in 0.5g, root length (3.39 - 4.13cm) and shoot length is (0.56 -

0.85cm) as compare to control root length (4.13cm) and shoot length (0.87cm) (Figure 1).



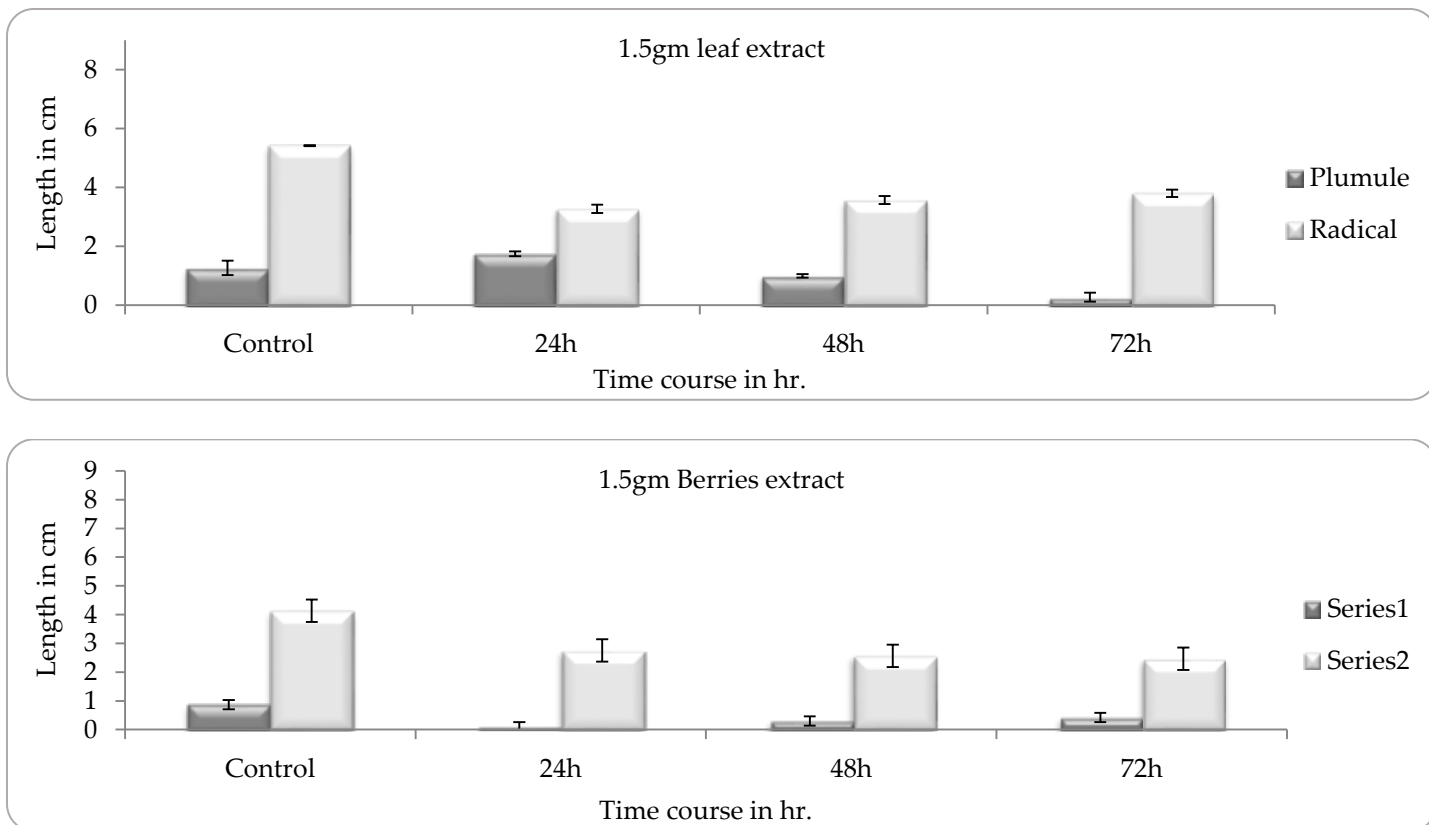


Figure 1. Effects of aqueous extracts of leaf and berries of *Melia azedarach* on seedling length of Sanober.

Seedling weight (leaf and berries extracts)

Maximum inhibition take place in fresh weight of leaf extracts. The highest seedling fresh and dry weight (0.09 – 0.14g), (0.04g) respectively were exhibited in 1.5gm. The fresh weight (0.15g) and dry weight (0.04g) was observed in control. Likewise slightly inhibition take place in fresh weight and dry weight of a berries extract with similar trends (Table 2).

Table 2. Effects of aqueous extracts of leaf and berries of *Melia azedarach* on weight percentage of Sanober.

Treatments	Time	Fresh weight		Dry weight	
		leaf	berries	leaf	berries
Control		0.14	0.13	0.03	0.03
0.5gm	24 hours	0.17 ^a ±0.005	0.11 ^c ±0.004	0.03 ^{ab} ±0.014	0.04 ^a ±0.003
	48 hours	0.13 ^b ±0.006	0.13 ^b ±0.004	0.03 ^{ab} ±0.015	0.03 ^{ab} ±0.003
	72 hours	0.11 ^c ±0.005	0.15 ^a ±0.004	0.04 ^a ±0.017	0.04 ^a ±0.003
1.0gm	24 hours	0.12 ^a ±0.005	0.12 ^a ±0.004	0.03 ^a ±0.019	0.04 ^a ±0.003
	48 hours	0.12 ^a ±0.006	0.12 ^a ±0.004	0.03 ^a ±0.023	0.02 ^{bc} ±0.003
	72 hours	0.11 ^a ±0.005	0.11 ^b ±0.004	0.03 ^a ±0.003	0.03 ^{ab} ±0.003
1.5gm	24 hours	0.11 ^{ab} ±0.005	0.15 ^a ±0.0033	0.03 ^{ab} ±0.003	0.04 ^a ±0.002
	48 hours	0.12 ^a ±0.006	0.12 ^b ±0.005	0.03 ^{ab} ±0.003	0.03 ^{ab} ±0.003
	72 hours	0.07 ^c ±0.007	0.12 ^b ±0.005	0.04 ^a ±0.005	0.03 ^{ab} ±0.003

Values are means of three replicates and ± represent the standard error.

Chlorophyll content (leaf and berries extracts)

In 0.5gm extract the chlorophyll a+b content was observed (6.13 - 6.17) and in 1gm extract chlorophyll a+b content (6.14), followed by 1.5gm extract the chlorophyll a+b content (6.1 - 6.14) were found as compared to control (6.15) (Table 3).

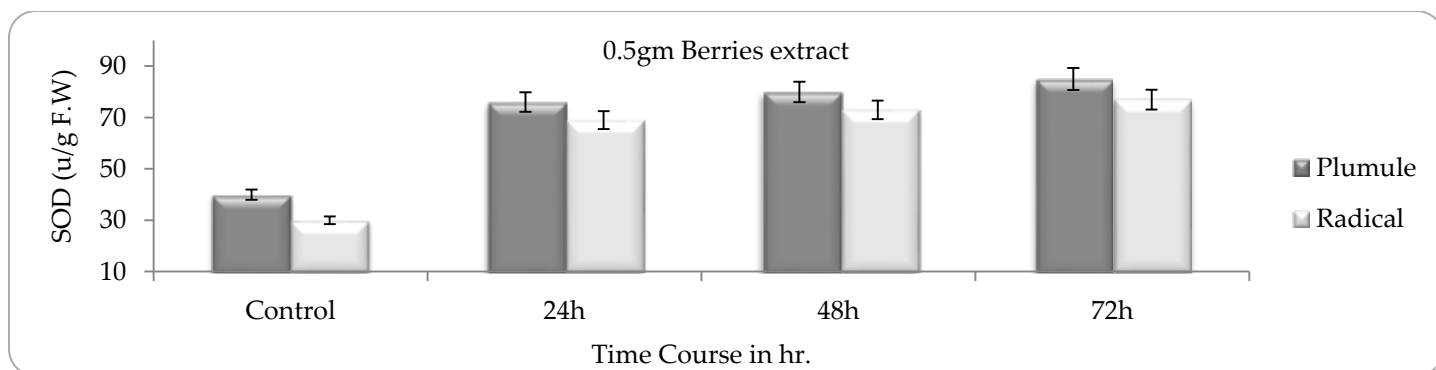
Table 3. Effects of aqueous extracts of leaf and berries of *Melia azedarach* on Chlorophyll concentration of Sanober.

Treatment	Time	Chl a	Chl b	Chl a+b
Control		4.32	1.83	6.15
0.5gm	24 hours	4.33	1.84	6.17 ^a ±0.003
	48 hours	4.32	1.82	6.14 ^a ±0.005
	72 hours	4.32	1.81	6.13 ^b ±0.005
1.0gm	24 hours	4.31	1.83	6.14 ^a ±0.001
	48 hours	4.31	1.83	6.14 ^a ±0.001
	72 hours	4.31	1.83	6.14 ^a ±0.001
1.5gm	24 hours	4.3	1.81	6.11 ^b ±0.002
	48 hours	4.32	1.82	6.14 ^a ±0.002
	72 hours	4.3	1.8	6.11 ^b ±0.002

Values are means of three replicates and ± represent the standard error.

Superoxide dismutase (SOD) activity (leaf and berries extracts)

The maximum stimulation was observed in 1.5gm of leaf and berries extracts. The SOD activity was showed in root (63 - 77 U/g f.w) and shoot (74 - 80 U/g f.w) in leaf extracts The stimulation was observed in root and shoot (30 U/g f.w), (40 U/g f.w) as compare to control SOD. Similar observations were appeared in berries extracts (Figure 2).



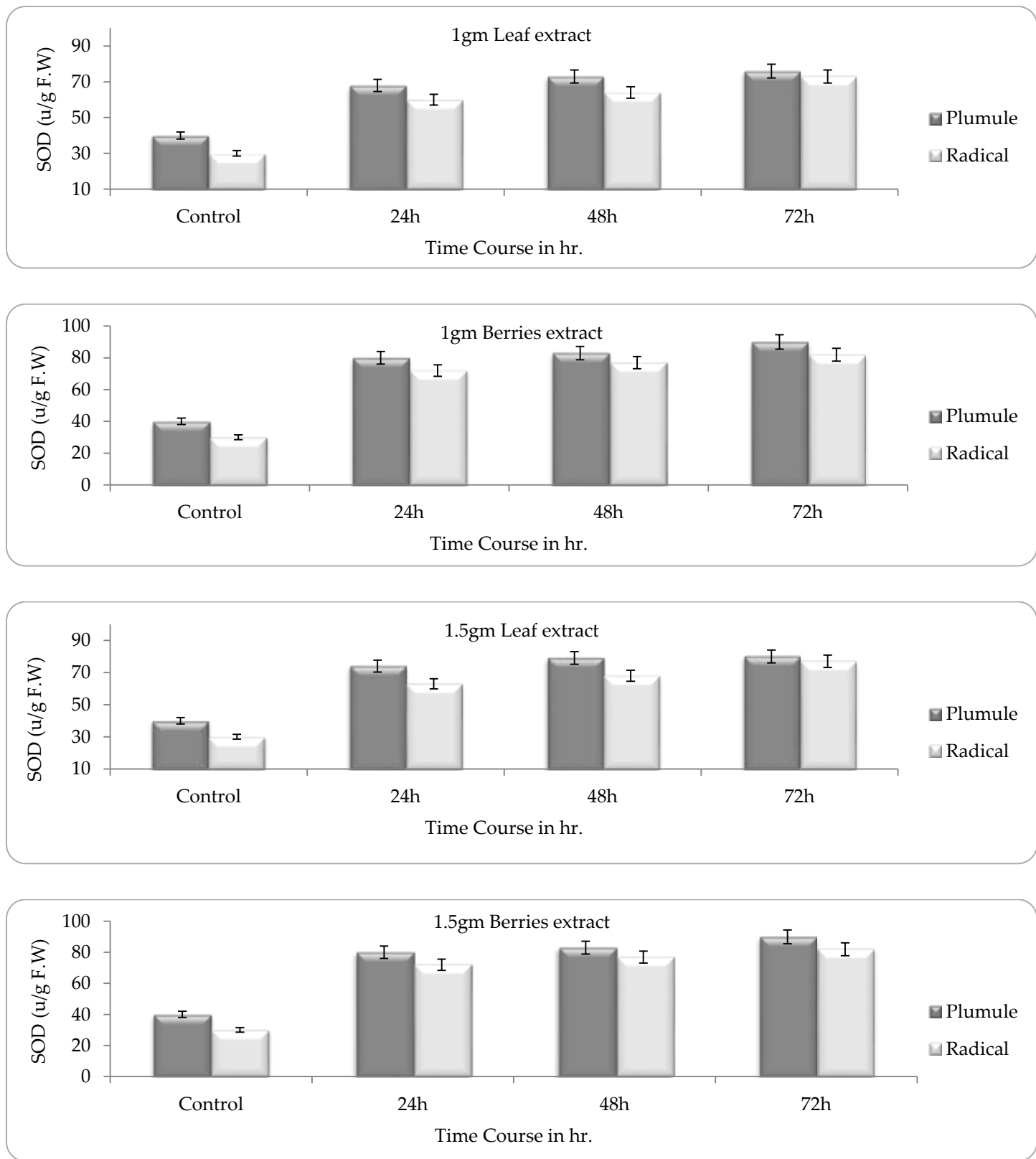


Figure 2. Effects of aqueous extracts of leaf and berries of *Melia azedarach* on Antioxidant (SOD) content of Sanober.

Phytochemical screening of *Melia azedarach*

The plant extracts of *Melia azedarach* were investigated to determine phytochemical

compounds in the leaves extracts. The common phytochemistry content from the plant such as amino acid, phenol, flavonoid, tannin, saponin, terpenoid, quinones, coumarin, glycoside and anthocyanin were checked In which saponin, coumarin, glycoside and anthocyanin were absent (Table 4).

Table 4. Phytochemicals screening test of *Melia azedarach*.

S. No	Phytochemicals	Dictation color	Result
1	Amino Acid	orange color	+
2	Phenol	reddish brown / dark green color / blue / violet / purple	+
3	Flavonoids	yellow fluorescence color	+
4	Tannins	No formation of stable foam	+
5	Saponin	Foam test	-
6	Terpenoids	Blue green color/reddish ring	+
7	Quinones	yellow color	+
8	Coumarin	Yellow color	-
9	Glycoside	Pink color	-
10	Anthocyanin	yellowish orange color	-

Discussion

Allelochemical contamination in soil is one of the foremost problems accountable for a reduction in agricultural yield. The most crucial segments of plant life are seed germination and the growth of seedlings, and these are adversely affected by allelochemicals (Singh *et al.*, 2019). Plants exposed to biotic stress endure plentiful physiological and biochemical alterations because allelochemical exposure involves oxidative stress (Redondo-Gómez, 2013). The successive events intended to reinstate cell function are regarded as the stress response. The allelochemical stress response is activated by a signal from a suitable receptor instantaneously after the commencement of the prevalence of the stress factor. Oxidative damage generated by stress in plant tissue is controlled by a combined operation of both antioxidant enzymes and non-enzymatic antioxidant systems. Apart from those, pigments, polyphenols, organic acids, and amino acids also play a key role in the plant's defence against abiotic stress.

In the present study, the allelopathic effects of fresh leaf and berry extracts of *Melia azedarach* on germination percentage and seedling growth of barley were studied. The inhibitory effect is specific and depends on the concentration of the extract. The order of the growth inhibition was berries > leaf. *Melia azedarach* plant already shows the inhibitory allelopathic effect (Kumar *et al.*, 2017). The extract of berries shows an allelopathic effect on seed germination in all concentrations as compared to the control. So, inhibition in seedling growth was caused by berry extract. The phenolic compounds are major phytotoxins that cause inhibition in seed germination and early seedling development. In addition, some mechanisms of action of allelochemical seem to resemble those of the synthesis of plant hormones. Thus, these compounds probably affect inducible hormones of germination, such as gibberellin, or the activity of specific enzymes, such as amylases and proteinases, which are necessary for seed germination

and seedling growth. Therefore, plant growth regulators are decreasing in treated seed germination and its growth by means of allelochemical. Similar studies were also reported (Lambers *et al.*, 2019).

The berries show a greater inhibitory effect as compared to leaf extract; high inhibition was observed in the radical and plumule. While slight inhibition occurred in dry weight. Ercisli *et al.* (2005) reported that the effect of *juglone* and walnut leaf extracts on yield growth, chemical, and plant nutrient elements of strawberry vegetative and reproductive growth was effected by the extract of walnut leaf. The nutrients analysed in the leaves were also lowered as compared to the control. Oyun (2006) reported the effect of *Gliricidia sepium* and *Acacia accriculiformis* on the seed germination of maize. The germination percentage and seedling vigour index significantly decreased. *Gliricidia* perform more inhibitory effects than *acacia*. Siddiqui *et al.* (2009) reported the effect of *prosothis juliflora* on *triticum astivum*.

In the present study, the content of chlorophyll was reduced with elevation concentrations for allelochemical applications. The chlorophyll content is affected by the allelochemicals (phenolics). The research exhibited that Mg-chelatase may be the major target of the phenolic compounds. Similar studies were reported by Hussain and Reigosa (2011). In our investigation, the SOD activity was enhanced relatively with increased extract concentration. SODs form the front line of defense against reactive oxygen species (ROS)-mediated injury. These proteins catalyze the dismutation of superoxide anion free radical (O_2^-) into molecular oxygen and hydrogen peroxide (H_2O_2) and decrease O_2^- level which damages the cells at excessive concentration. The physiological effects on receptor plants are useful in determining the role of the allelochemicals in the system. Recently, it has been proposed that allelochemicals can cause oxidative stress in target plants and therefore activate the antioxidant mechanism. Currently, we are studying the dynamics of allelochemicals in different physiological contexts. Parameter results have shown inhibitory effects, but enzyme activities are high in response to allelochemical concentrations.

Conclusion

Allelopathy has been known and used in agriculture since ancient times; however, its recognition and use in modern agriculture are very limited. Allelochemicals can act as environmentally friendly herbicides, fungicides, insecticides, and plant growth regulators and can have great value in sustainable agriculture. However, there are a few research investigations testing natural-product herbicides. With increasing emphasis on organic agriculture and environmental protection, increasing attention has been paid to allelopathy research, and the physiological and ecological mechanisms of allelopathy are gradually being elucidated. Moreover, progress has been made in research on the associated molecular mechanisms. It is obvious that allelopathy requires further research for widespread application in agricultural production worldwide.

Conflict of Interest

There is not any conflict of interest to authors.

Authors Contributions

All the authors equally contributed to manuscript.

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