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Allelopathic Effect of *Alternanthera paronychioides* L. Roots and Leaves Extracts on Seedling Growth of *Raphanus sativa* L.

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ABSTRACT

Allelopathy is a biological process whereby plants secrete certain chemicals through their roots, shoots or leaves, called allelochemicals. These chemicals directly cause some biological responses among nearby plants, microorganisms, or even small animals. The allelochemicals represent a large group of compounds, including leachate, root exudates, volatiles or even debris decomposed from the parts of the plant, which have appreciable allelopathic effects ranging from germination, seedling, and overall productivity drawbacks to nearby species. The present study includes the evaluation of allelopathy of the plant *Alternanthera paronychioides* using its aqueous extracts on the seed germination and early growth of *Raphanus sativus* (radish) seedlings. The dried plant parts (particularly the *A. paronychioides* leaves and roots) have undergone aqueous extraction and tested on the *R. sativus* seeds under controlled experimental conditions. Seedling development was found to be severely suppressed in terms of germination; both root and leaf extracts showed highly significant declines in their germination percentages, radicle, and plumule (shoot) growth. The effect was more pronounced with the increasing concentration on the inhibition effects; it showed that *R. sativus* seedlings were inhibited in the root and shoot lengths as the concentration of *A. paronychioides* extracts was increased, showing very aggressive phytotoxic effect. This means that with greater concentrations of allelochemicals available within these extracts, greater physiological stress would be on the seedlings affecting growth processes in the normal ways. The results indicate that *A. paronychioides* has strong allelopathic action on R. sativus, thus supporting the view that secondary metabolites from this species can be concerning to other species in their early developmental stages. Such interactions have implications such as competitive interactions in plants and community dynamics, while in application, they must be subjected to current theory of sustainable weed management.

INTRODUCTION

The term "allelopathy" explains the chemical interactions in which one plant releases chemicals (allelochemicals) that adversely affect the growth, survival, and reproduction of neighboring organisms. This phenomenon has earned greater consideration in plant ecology and physiology (Seigler, 1996). These allelochemicals are defined by allelopathy-that is, reactions that affect the biological functioning of nearby plants (Scavo *et al.,* 2018). There is variability among the concentration or amount of allelochemicals of the plant causing an allelopathic effect, other species will be affected, and this could again interact with environmental factors to either inhibit or promote growth (Abideen *et al.,* 2024). Various such ecochemical interactions are responsible for the assembly of plant communities through inhibition and facilitation of neighboring species' growth (Losapio *et al.,* 2023). Therefore, allelopathy is an important ecological mechanism that decides plant behavior in succession, dominance, and competition interaction within the ecosystem (Hierro *et al.,* 2021).

The concept of allelopathy was officially ushered in during the early 1970s and has since developed rapidly, accelerated during the mid-1990s; it has thus emerged as an active area of research in many fields, ranging from botany to agronomy or horticulture, and even soil science (Huang *et al.,* 2021). These recent developments in research have shifted the focus toward understanding the role of allelopathy in species distribution, biodiversity, and the success of some plants as invaders. Clearly, allelopathy could be an important factor controlling plant species richness in a given ecological community, while many exotic plants could owe their invasive success partly to allelopathy (Rice, 2012). The allelochemicals involved in these processes are generally derived from leaves, roots, bark tissues, and seeds that are released into the environment by different means, including leaching, root exudation, and volatilization (Bachheti *et al*., 2019). Therefore, they can act selectively against neighboring plant species, thus serving as a strong natural mechanism for competition among plants to establish their own territory for resource use (Schenk *et al*.,2006).

From the biochemical level, it can be said that allelochemicals act on all growth stages, from seed germination through photosynthesis and respiration to cell division and later absorption of nutrients by target plants (Bachheti *et al*., 2019). These allelochemicals perturb the recipient plant's cellular machinery by modifying membrane permeability, influencing enzyme action, and altering hormonal balance (Staszek *et al*., 2021). The resultant effect, among others, would include oxidative stress and chlorosis and impairment of water or nutrient transport to the plant, with the consequences of generalized growth inhibition or the direct death of the plant (Hajiboland *et al*., 2011). So, the concept of allelopathy provides opportunities to understand ecological interactions and opens avenues for developing environmentally friendly strategies for weed management and agricultural sustainability (Sigh *et al*., 2003).

The research presented here is intended to study the allelopathy of a low-growing herbaceous plant, *Alternanthera paronychioides*, which is one of the most important plants from the family Amaranthaceae (Tanveer *et al*., 2018). This plant succumbs to become an invasive plant and grows in tropical and sub-tropical regions around the world. It has tiny ovate leaves with clusters of white-to-greenish flowers *A. paronychioides* is expected to have allelochemicals inhibiting other species' growth (Abbas *at al*., 2022). This aims to study the effect of aqueous extracts of leaves and roots of *A. paronychioides* on root and shoot growth and total chlorophyll content of the crop Raphanus sativus, which is widely cultivated and has an important economically valued vegetable crop. Further, it evaluated different concentrations of plant extracts that affect early seedling development with a view toward understanding plant-plant interactions in the ecological effect of invasive species.

MATERIAL AND METHODS

Sample Collection and Preparation

During the summer of May 2023, plant material of *Alternanthera paronychioides* was collected inside Green Acre Town, a place very close to Abdul Wali Khan University, Mardan. Fresh samples were put into plastic bags to avoid desiccation and transported to the laboratory for processing. Thereafter, the plant parts were separated for roots, stems and leaves and dried under shaded conditions at room temperature for about two weeks to retain the phytochemical integrity of materials. After drying, each part was ground into fine powder with a mechanical grinder. The powders were collected separately and stored in clean, airtight, labeled bottles for further experimental procedures.

Aqueous Extracts Preparation

The extracts were prepared by placing known quantities (2.5 g, 5 g, and 7.5 g) of the powdered stem and root materials in beakers filled with distilled water (100 mL). Aluminum foil was then used to cover the tops to prevent any fungal or bacterial contamination for 24 h at 35°C in a shaker (Moosavi *et al*., 2011). The extracts were filtered through Whatman No. 1 filter paper to remove impurities (Krumsri *et al*., 2024). Then the filtered extracts were returned to the shaker for eight days to evaporate water from them and make concentrated forms (Borghetti *et al*., 2013). Once evaporation was done, the pure extracts were sealed again with aluminum foil and stored in a refrigerator for preservation; a similar procedure was followed for extracts with a 72-h incubation period to study their allelopathic activity for the temporal effects (Jabeen *et al*., 2023).

Viability Test and Seed Selection

Conducting viability testing on seeds of Raphanus sativus (radish) confirmed the seeds as viable for experimentation. Soaking seeds in water for 30 min for buoyancy measurement made 87 seeds sink, giving a viability indication. The other 13 floated and were discarded. Only these viable seeds were used in the bioassays as seed viability has a minimal effect in assessing the toxicity of plant samples, and only viable seeds will be more susceptible to any stimuli acting on them. In another case, the plant powder samples weighing 2.5 g, 5 g, 7 g, and 7.5 g were separately weighed using an accurate balance to within 0.0001 g and placed into 50 mL of methanol in flasks. "The mixtures were prepared and shaken for 24 to 48 hours, then filtered and covered with aluminum foil to prevent microbial contamination, and finally stored in a refrigerator for later use (Richards *et al*., 2019).

Experimental Design and Data Analysis

The prepared aqueous extracts were tested with respect to their allelopathic effects by conducting laboratory bioassays that primarily sought to evaluate the growth measurements of seed germination and seedling development in terms of radical and plumule lengths and chlorophyll levels of Raphanus sativus. The experimental design considered extract concentration, time of exposure, and the origin of the part used (root versus shoot) as factors. The data drawn from these bioassays were analyzed through four-way analysis of variance (ANOVA) for treatment effect significance. A level of significance defined at a P-value of ≤ 0.05 was considered for testing the treatment differences in results obtained to maintain scientific rigor (Lau *et al*., 2008).

RESULTS

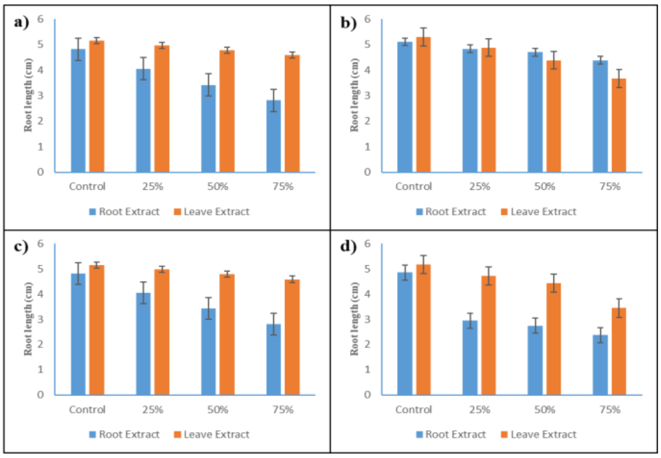
Effect of Root and Shoot Extract of *Alternanthera paronychioides* on Growth Attributes of *Raphanus sativa*

The application of the aqueous extract obtained from *Alternanthera paronychioides* notably reduces the root and shoot length as well as the Total chlorophyll content of the plants, as shown in the Figures (1, 2 and 3). The adverse Impact exhibited a linear relationship with increasing *A. paronychioides* extraction concentration and prolonged incubation time. The root and shoot length growth was remarkably inhibited with the increase in incubation time and concentration of *A. paronychioides* leaf aqueous extract. The inhibitory effect of *A. paronychioides* can be attributed to the existence of bioactive compounds that are known for their allelopathic properties.

Data regarding root length shows that the existence of 2.5g root extract and shoot of *A. paronychioides* with an incubation time of 24 hours (Fig. 1 a) declined root length to resulted in a decrease in root length 4.2 and 3.7 cm and 75% treatment while in control unit the root length was observed 5.6 cm and 5.1 cm. Similarly, Root and shoot extract of *A. paronychioides* at 2.5 and incubation time 72 hours (fig. 1 b) showed a maximum decrease in root length 4.3 and 3.6 cm in 75% treatment, whereas in control experimental unit root length was recorded at 5.1 and 5.2 cm. 5-gram root and shoot extract of *A. paronychioides* with incubation period 24 hours (Fig. 1 c) caused decrease in root length 2.8 and 4.5 cm at 75% treatment, respectively in comparison with control where root length was observed 4.8 and 5.1 cm. Similarly, at an incubation time of 72 hours, root and shoot extract of 5 g *A. paronychioides* (Fig. 1 d) dropped root length to 2.3 and 3.4 cm at 75%, while root length in the control was recorded at 4.8 and 5.1 cm. Moreover, a significant reduction was observed when root and shoot extract derived from 7.5 g of *A. paronychioides* was applied in the case of both 24 hours and 72 hours' incubation time compared with control experimental units. In the case of 24 hours (Fig. 2 a) incubation, root and shoot extracts resulted in the minimum root length of 1.4 and 2.1 cm, respectively, while in the control, root length was recorded as 5.1 and 4.9 cm. Extending the incubation time causes even more reduction in root length. Data shows that at 72 hours (Fig. 2 b) incubation time, root and shoot extract extensively minimized root and shoot lengths 0.84 and 0.80 cm, respectively, whereas, in the control group, the root length was determined to be 5.1 and 4.9 cm.

Figure 1

Root Length Response of Alternanthera paronychioides under Various Treatments.

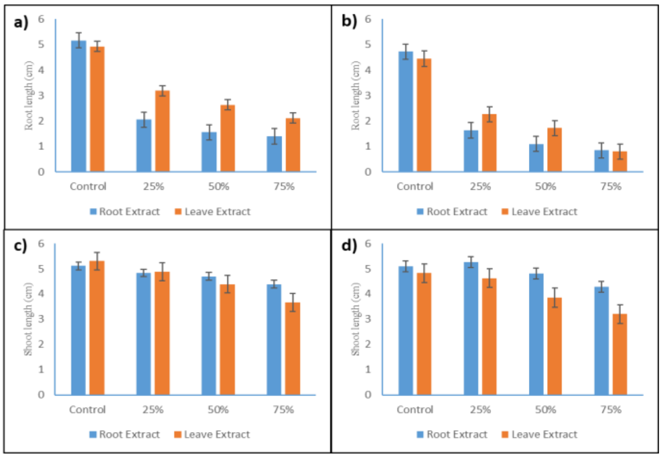


a), root length is shown after 24 hours of exposure to the treatment at 2.5 g; subfigure (b) illustrates root length after 72 hours of exposure to the same dosage. Subfigure (c) represents root length after 24 hours with 5 g treatment, while subfigure (d) refers to the 72-hour measurement with 5 g. Such comparisons emphasize the interaction of time and dosage on root elongation; the differences point toward treatment duration and concentration affecting early root establishment.

Data about shoot length indicates a negative impact on the shoot length of the plants. The resulting data shows that the presence of 2.5g root and shoot extract of *A. paronychioides* indicated for 24 hours resulted (Fig. 2 e) shows reduction in shoot length 4.2 and 3.2 cm in 75% treatment in comparison with control unit where shoot length was recorded 5.1 and 4.8 cm. in a comparable manner, the root and shoot extract of *A. paronychioides* at a concentration of 2.5 and an incubation time of 72 hours (Fig. 2 d) demonstrated the most significant decrease in shoot length, measuring 4.3 and 3.6 cm. in the 75% treatment, whereas in control, shoot length was recorded 5.1 and 5.2 cm. A 5g extract of the root and shoot of *A. paronychioides,* incubated for 24 hours (Fig. 3 a), declined shoot length by 1.9 and 3.9 cm in 75% treatment, respectively, compared to the control, where shoot length was recorded at 4.1 and 4.8 cm. Likewise, after 72 hours of incubation time, the root and shoot extract of 5 g *A. paronychioides* (Fig. 3 b) tested at 75% inhibited shoot length to 3.5 cm and 2.3 cm in comparison to control shoots, which had lengths of 4.9 and 5.1 cm. Root and shoot extracts from 7.5 g of the plant *A. paronychioides* showed a significant decrease from the control experimental units when applied at 24 h and 72 h incubation periods. After 24 hours of incubation (Fig. 3 c), the root and shoot extracts developed the shortest shoot lengths of 1.4 and 2.1 cm, while the control had shoot lengths of 4.9 and 4.9 cm. The shoot length is further reduced when the incubation period is extended. That is why analysis of the data obtained now, when plants were incubated for 72 hours (Fig. 2 d) shows that root and shoot extract significantly reduced the shoot length by 0.7 and 0.9 cm, whereas in the control group, the shoot length was measured at 5.1 cm and 4.9 cm.

Figure 2

Growth Response of Alternanthera paronychioides Concerning Root Length and Shoot Length under Variable Treatments.

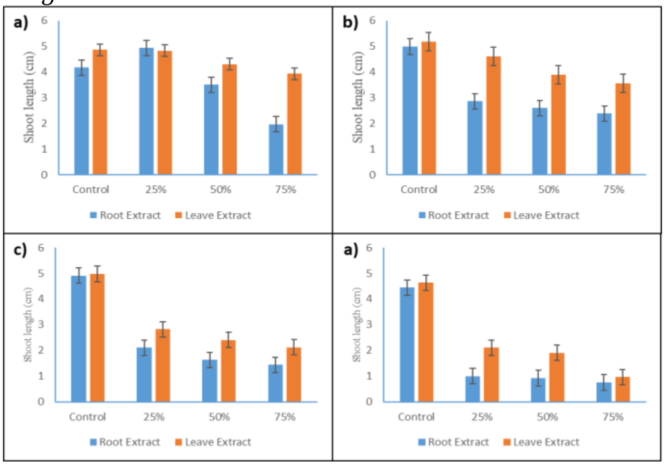


a) Root length observed after 24 hours under 5 g treatment, (b) shows root length after 72 hours with this treatment, c) Shoot length observed at 24 hours under the treatment of 5 g, (d) shows shoot length after 72 hours under the 5 g treatment. This figure shows that time duration and treatment concentration affect root and shoot development.

Results suggest that growth response varies in accordance with the plant part, exposure time, and treatment concentration.

Figure 3

Response of Alternanthera paronychioides in Terms of Shoot Length under Several Treatments.

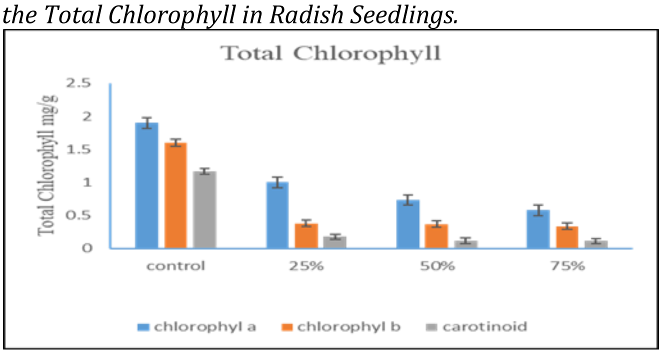


a) shows shoot length at 24 hours after treatment with 7.5 g, b) shows shoot length at 72 hours with 7.5 g, c) shows shoot length at 24 hours at 7.5 g, d) indicates 72 hours at 7.5 g. These comparisons demonstrate the effects of treatment time and treatment dose on the shoot elongation. The data point to a differential effect of exposure time and concentration on the shoot development.

Root and shoot extract of *A. paronychioides* showed a negative effect on total chlorophyll content in the plantlets of radish seedlings (Fig. 4). The present study reveals that there was an inhibitory effect with the increase of extract concentration. At lower concentrations, the chlorophyll level was lower than the control but not significantly, and at moderate to higher concentrations, the chlorophyll level was significantly reduced, showing the dose-dependent inhibitory effects. Furthermore, the findings of the current study showed that the maximum decline in Chlorophyll a, b, and carotenoids was observed 0.5, 0.3, and 0.1 mg/g when radish seeds were exposed to 75% treatment, this observation was in comparison with control experimental units where chlorophyll a, b, and carotenoids content was recorded 1.8, 1.5 and 1.1 mg/g respectively. This decline implies that some of the Allelopathic compounds in the extracts may inhibit chlorophyll synthesis or promote chlorophyll breakdown, reducing the photosynthetic potential of radish seedlings.

Figure 4

Root and Shoot Extracts of Alternanthera paronychioides on the Total Chlorophyll in Radish Seedlings.



Treatments consisted of various concentrations and periods of exposure to the root and shoot extracts, the chlorophyll content being measured to assess the physiological effects of the extracts on the health and vigor of the seedlings. Results indicate differential responses depending on the extract type and treatment conditions. The figure thus highlights the potential of bioextracts from A. paronychioides in the photosynthetic pigment accumulation of radish seedlings.

Analysis of Variance (ANOVA) for Root and Shoot Growth

The analysis of variance (ANOVA) for shoot and root growth shows both significant and non-significant impacts of different factors and their interactions (Figs. 1 & 2). In terms of root growth, the interaction between concentration and time exhibited a significant ($P \leq 0.05$) effect, indicating a strong allelopathic effect on root growth. However, the other factors, such as plant material, weight, concentrations, and time separately, as well as their interactions, were non-significant ($p \geq 0.05$). For shoot growth, plant material ($P \leq 0.05$), plant material ($P \leq 0.05$), concentrations ($P \leq 0.05$), and time ($P \leq 0.05$) revealed significant influence, highlighting their strong allelopathic effect on shoot growth. At the same time, the influence of weight and all other interactions was non-significant. Conclusively, ANOVA demonstrates that concentration and time are the key factors that negatively affect the root and shoot parameters.

Table 1

Analysis of Variance Table for Root

Source of variance	DF	SS	MS	F	P
Replication	2	194.268	97.1338		
Plant material	1	0.127	0.1267	0.17	0.6807
Weight	2	0.843	0.4214	0.57	0.5692
Concentration	2	3.937	1.9683	2.65	0.0764
Time	1	2.398	2.3982	3.23	0.0759
PlantMate*Weight	2	0.363	0.1811	0.24	0.7842
PlantMate*Concentration	2	0.390	0.1948	0.26	0.7700
PlantMate*Time	1	2.547	2.5474	3.43	0.0675
Weight*Concentra	4	0.061	0.0151	0.02	0.9992
Weight*Time	2	0.142	0.0708	0.10	0.9091
Concentra*Time	2	13.475	6.7375	9.07	0.0003
Error	86	63.880	0.7428		
Total	107				

Table 2

Analysis of Variance Table for Shoot

Source of variance	DF	SS	MS	F	P
Replication	2	144.850	72.4250		
Plant material	1	6.626	6.6263	15.33	0.0002
Weight	2	0.070	0.0352	0.08	0.9219
Concentration	2	11.795	5.8977	13.64	0.0000
Time	1	3.169	3.1694	7.33	0.0082
PlantMate*Weight	2	1.692	0.8462	1.96	0.1477
PlantMate*Concentration	2	0.128	0.0639	0.15	0.8629
PlantMate*Time	1	0.068	0.0684	0.16	0.6919
Weight*Concentra	4	0.608	0.1519	0.35	0.8424
Weight*Time	2	0.334	0.1668	0.39	0.6811
Concentra*Time	2	1.003	0.5016	1.16	0.3185
Error	86	35.888	0.4324		
Total	107				

DISCUSSION

The *Alternanthera paronychioides* extracts have been found to significantly inhibit the seedling growth development of Raphanus sativus as proved with changes in roots and shoot lengths in different treatment conditions Figures. With increasing concentrations and durations of extract exposure, the same growth reduction trend was extremely evident compared to root elongation. Comparing 72 h exposure, the 24 h exposure at 25% using 2.5% g of extract showed significant reduction in root length. This trend had reflected in 50% and 75% extract treatments, as they recorded shorter root lengths in 24 hours than their respective 72 h counterparts. This observation signifies that allelopathic action is evident at minimum exposure durations possibly because of the early release of phytotoxic components.

The suppressive effect became stronger at increased concentrations of the extract. In the case of treatment with 5 g of extract, the growth of both root and shoot lengths was reduced significantly with radicle growth being inhibited even more. The treatment with extracts of 7.5 g saw the growth of seedlings more greatly reduced in which the lengths of both shoot and root would decrease approximately 2.7 cm in between concentrations from 25% to 75% as compared with the untreated control. Those observations are in correspondence with earlier reports showing that the species of Alternanthera have a phythotoxic effect because such species are known to have allelochemicals such as phenolics, flavonoids, and other secondary metabolites which affect cellular processes in plants.

The inhibitory effect of *A. paronychioides* extracts would usually be because of the disturbance in the activities of enzymes, hormonal imbalances, or oxidative stress evoked in the seedlings. These mechanisms bring about a decrease in the cell division and elongation of tissues, especially in roots, which are often more sensitive to the action of allelopathic compounds. The difference observed in inhibition between roots and shoots may be due to the permeability or absorption rate differences of the allelochemical among the tissues of the plant.

Our findings corroborate that allelochemicals present in Solanum xanthocarpum can inhibit considerably both root and shoot development of Triticum aestivum. Previous investigations have also found reports of various parts of S. xanthocarpum, particularly herb and root extracts for their phytotoxic effects on seedling growth. Such allelochemical interactions have also been attributed to other plants; for instance, Ageratum conyzoides extracts have been observed to effect a noteworthy reduction in the germination and seedling growth of radish (Gowri *et al.*,2015).

In the wider context of plant-based cytotoxicity research investigation involving ethanolic extracts from forty-three medicinal plants of Jordan have brought forth Saracha punctata, a member of Solanaceae family hailing from Bolivia, as one of the most potent inhibitors. This member completely inhibited the growth of Leishmania braziliensis, L. donovani, and L. amanuensis in their promastigote forms indicating the diverse bioactivity of allelopathic micromolecules in Solanaceae members (Tran Dang Xuan *et al.*, 2004).

Moreover, aqueous extracts of Alternanthera sessilis roots and *Alternanthera philoxeroides* stems indeed showed a stimulatory effect on T50 (time to 50% germination) and MGT (mean germination time) for rice seed as compared to the control. It is noteworthy that the control has always presented statistically the highest germination index compared to treatments. The significantly lowest GI values were recorded for leaf and whole plant extracts from A. sessilis and A. philoxeroides, respectively. Increasing concentrations of these aqueous leaf extracts resulted in increased reduction of both T50 and MGT which implied a dose-dependent effect of these extracts on inhibition (Mehmood *et al.*, 2014).

Interestingly, root and stem extracts caused more pronounced delays in germination than other plant parts. This implies that different tissues may have different concentrations or compositions of allelochemical impacts. This pattern of inhibition agrees with findings that allelopathic interactions were organ-specific (Kadioglu *et al.*, 2005).

According to our results, it has been shown that the extracts of *Alternanthera paronychioides* interfere with the biosynthesis of chlorophyll in Raphanus sativus, which significantly reduced the total chlorophyll content. Such mix of allelochemicals in *A. paronychioides* seems to inhibit other essential physiological processes like production of pigment, nutrient uptake, and balance of hormones within the plants. Usually, these inhibitory effects have been associated with increased generation of oxidative stress in the plant system, thereby worsening the inhibition on seedling development.

In summary, *A. paronychioides* has high allelopathic potential due to its diverse chemical compounds with the ability to inhibit chlorophyll synthesis, cause oxidative stress, and reduce nutrient absorption. These results show that allelopathy could be widely implicated in plant-plant interactions, providing a perspective into its applications towards sustainable agriculture, especially in developing eco-friendly weed management strategies (Huang *et al.*, 2017).

CONCLUSION

The research findings highlight the significant potential of *Alternanthera paronychioides* as a natural source of potent growth inhibitors against *Raphanus sativa* (radish). The observed dose-dependent relationship underscores the potency of these extracts, where even small amounts can noticeably hinder germination and early development. These results warrant further investigation into the specific compounds within the extracts responsible for this inhibitory effect, as they could lead to the development of new, environmentally friendly bioherbicides.

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