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ALLELOPATHY | ORIGINAL ARTICLE

Assessment of allelopathic potential of selected legume leaf extracts on seedling growth of *Raphanus sativus*

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ABSTRACT

The aim of the present investigation was to assess the allelopathic potential of 25 Bangladeshi legume plants against the seedling development of the allelopathic-sensitive plant *Raphanus sativus*. Aqueous leaf extracts of five different concentrations *viz.*, 1:05, 1:10, 1:15, 1:20, and 1.25 (*w/v*) of these plant were tested. Distilled water (with no extract) was acted as a control, and the bioassay was repeated three times. The inhibitory actions relied on concentration and the shoot growth was less responsive to plant extracts than the root development. The shoot growth of *R. sativus* was less inhibited (70%) by lentil (*Lens culinaris*) leaf extract whilst African dhaincha (*Sesbania rostrata*) at concentration of 1:05 (*w/v*) provided the highest inhibition (100%) and the value was closely followed by Winged bean (*Psophocarpus tetragonolobus*), Faba bean (*Vicia faba*). At a concentration of 1:05 (*w/v*), the root of Tetul (*Tamarindus indica*), Bokful (*Sesbania grandiflora*), Radhachura (*Peltophorum pterocarpum*), Minjiri, (*Cassia siamea*), Polash (*Butea monosperma*), Ipilipil (*Leucaena leucocephala*), Tripatri shak (*Desmodium triflorum*), Faba bean (*Vicia faba*), Soybean (*Glycine max*), Country bean (*Lablab purpureus*), Black gram (*Vigna mungo*), Ground nut (*Arachis hypogae*), Yardlong bean (*Vigna unguiculata*) and African dhaincha (*Sesbania rostrata*) exhibited the highest level of inhibition (100%) while Sada lojjabhoti (*Mimosa invisa*), had the lowest level of inhibitory activity (85%). Comparing root growth inhibition (ranged 56–81%) to shoot growth inhibition (ranged 37–77%), the aqueous leaf extracts of legume plants demonstrated a lower level of inhibition on shoot growth. Lentil (*Lens culinaris*) provided the lowest average inhibition (37%) on the development of *R. sativus* shoots and Faba bean (*Vicia faba*) provided the greatest average inhibition (77%). Meanwhile, Tripatri shak (*Desmodium triflorum*), a herb legume, provided the least average inhibition (56%) on the root development of *R. sativus* and Winged bean (*Psophocarpus tetragonolobus*) offered the greatest (81%) level of inhibition. Compared to the categories of legume species the shrubs had the most limitation on the growth of *R. sativus* shoots (65%), whereas herb species had the least (60%). However, the tree species had the most root growth inhibition (70%) while the herb species had the lowest (68%). According to the findings, African dhaincha (*Sesbania rostrata*), followed by Soybean (*Glycine max*), Faba bean (*Vicia faba*), Blackgram (*Vigna mungo*), and Winged bean (*Psophocarpus tetragonolobus*), are prospective candidates among the examined legume plant species that have substantial allelopathic features and may be used for further allelochemical extraction and characterization.

Keywords: Allelopathy, legume, bioassay, inhibition, root growth

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1 Introduction

Weeds are one of the key biotic constrains to agriculture that fight with crops for major growth resources including light, air, water, nutrients, and space, which substantially limits crop growth, yield, and quality (Bajwa et al., 2015; Khan et al., 2019). While weed control tactics might vary by nation, they today primarily focus on synthetic chemicals (Thill et al., 1991). In numerous developing nations, the labor force has relocated to sectors paying better earnings than agriculture, which has increased the usage of herbicides (Shrestha et al., 2021). Small-holder farms in nations like Bangladesh, where farmers are adopting chemical weed management to attempt and sustain crop productivity in order to ensure adequate food supply, are notably afflicted by this conundrum. Over the past three decennaries, the nation's usage of herbicides has surged around 88-fold, from 90 metric tons (MT) or kiloliters (kL) in 1992 to 7881 MT/kL in 2021 (BBS, 2022). The widespread use of synthetic herbicides over the past fifty years has greatly boosted agricultural output, but at a massive ecological and environmental cost (Aktar et al., 2009; Bajwa et al., 2015; Dass et al., 2017). Furthermore, 514 instances of herbicide-resistant weed biotypes have been detected nationwide, with the majority of these occurrences appearing in industrialized nations (Heap, 2022), and to address this challenge, weed control tactics must adapt (Colbach et al., 2017). As component of integrated weed management, forthcoming weed control must incorporate new approaches in addition to those now in use. In a response, experts are presently centering their efforts on devising groundbreaking alternative weed management techniques that would pose less environmental risks and be more feasible.

Any processes comprising secondary metabolites generated by plants, microbes, viruses, and fungi that have an impact on the development and maturation of biological and agricultural systems are alluded as allelopathy (IAS, 2022). Allelochemicals are the names given to the compounds that are emitted by allelopathic flora. The germination, growth, and permanence of nearby species as well as the secreting plant itself may be hindered by these allelochemicals after they have been released, either actively by changing their physiological properties (Weir et al., 2004) or passively by altering the rhizosphere soil properties (Zhou et al., 2013). Allelopathic species have therefore been proposed as a promising alternative for weed suppression in the context of eco-friendly agriculture (Cheema and Khaliq, 2000; Tabaglio et al., 2008; Islam et al., 2018b; Ullah et al., 2022). Such species might be employed for weed control as cover crops, rotational/companion crops, use of their extracts directly or with reduced herbicide dosages, integration of their residues as mulch, allelochemicals as natural herbicides, or allelopathic crop cultivars created via breeding programs (Cheng and Cheng, 2015).

Leguminosae is the third largest plant family, with 750 genera and over 18,000 species (Faria et al., 1989). It is the second-largest family in terms of human relevance, behind Gramineae. Furthermore, legumes are excellent for insect management and soil enhancement (Khanh et al., 2005). Apart from their pharmaceutical and soil restorative properties (Graham and Vance, 2003; Fustec et al., 2010; Vijayakumar and Haridas, 2021), Leguminous plants have been the subject of very few studies evaluating their allelopathic properties in various nations (Mondal et al., 2015) but no one so far in Bangladesh. In this backdrop, current research is conducted to investigate the allelopathic properties of Bangladeshi legume plant species.

2 Materials and Methods

2.1 Location

From July 2019 to March 2020, the research was carried out at the Agro-Innovation Laboratory, Department of Agronomy, Bangladesh Agricultural University, Mymensingh 2202.

2.2 Plant sample collection and extract preparation

From the Bangladesh Agricultural University campus and the Agronomy Field Laboratory, 25 species of legume plants, including trees, herbs, and shrubs, had their entire plants or fresh leaves were gathered. The identification of plants and authentication of the botanical name was performed. The plants' name has been checked with http://www.theplantlist.org (accessed on December 2019). Voucher specimens are deposited in the corresponding author's laboratory. Table 1 provides a summary of the common and botanical names of such plants.

2.3 Experimental technique

Fresh leaves of 25 leguminous plants (herbs, shrubs, and trees) were collected. Harvested leaves from each plant were washed twice: once with tap water and then with distilled water. Then, 200 mL of distilled water was added to 100 grams of these leaves, which were ground into a paste and homogenized in a waring mixer for five minutes at normal temperature. The extract was then let to rest at room temperature for 24 hours before being filtered throughout one layer of grade 1 filter paper. After that the filtrate was poured in a 500 mL volumetric flask, half of which was filled with distilled water, and homogenized with an orbital shaking device. The obtained solution, which was 1:05 (*w/v*) in strength, was kept in the refrigerator at 4 °C until it was needed.

Table 1. Bangladeshi medicinal herbs investigated in the current study

Sl.	Common name	Scientific name	Voucher specimen
1	Tripatri shak	*Desmodium triflorum* L.	LPS 001/2021
2	Sada lojjabhoti	*Mimosa invisa* Martius ex Colla	LPS 002/2021
3	Black Gram	*Vigna mungo* (L.) Hepper	LPS 003/2021
4	Cow pea	*Vigna unguiculata* (L.) Walp.	LPS 004/2021
5	Faba bean	*Vicia faba* L.	LPS 005/2021
6	Soybean	*Glycine max* (L.) Merr.	LPS 006/2021
7	Chick pea	*Cicer arietinum* L.	LPS 007/2021
8	Country bean	*Lablab purpureus* (L.) Sweet	LPS 008/2021
9	Yard long bean	*Vigna unguiculata* ssp. Sesquipedalis (L.) Verdc.	LPS 009/2021
10	Lentil	*Lens culinaris* Medik.	LPS 010/2021
11	Winged bean	*Psophocarpus tetragonolobus* (L.) D.C.	LPS 011/2021
12	Ground nut	*Arachis hypogaea* L.	LPS 012/2021
13	Grass pea	*Lathyrus sativus* L.	LPS 013/2021
14	African Dhaincha	*Sesbania rostrata* Bremek. & Oberm.	LPS 014/2021
15	Deshi Dhaincha	*Sesbania aculeate* L.	LPS 015/2021
16	Tetul	*Tamarindus indica* L.	LPS 016/2021
17	White series	*Albizia procera* (Roxb.) Benth.	LPS 017/2021
18	Radhachura	*Peltophorum pterocarpum* (DC.) K. Heyne	LPS 018/2021
19	Minjiri	*Cassia siamea* Lam.	LPS 019/2021
20	Polash	*Butea monosperma* (Lam.) Taub.	LPS 020/2021
21	Ipil ipil	*Leucaena leucocephala* (Lam.) de Wit	LPS 021/2021
22	Bokful	*Sesbania grandiflora* (L.) Poiret	LPS 022/2021
23	Sonalo	*Cassia fistula* L.	LPS 023/2021
24	Krishnochura	*Delonix regia* (Boj. Ex Hook.) Raf.	LPS 024/2021
25	Rain tree	*Samanea saman* (Jacq.) Merr.	LPS 025/2021

2.4 Procedure of bioassay

The produced aqueous extracts were subsequently diluted into four more concentrations, namely 1:10, 1:15, 1:20, and 1:25 (*w/v*), and distilled water lacking extract was additionally preserved as a control. The bioassay test was replicated three times using a completely randomized design (CRD). In Petri dishes, thirty radish seeds (*Raphanus sativus* L.) were placed on the filter paper. Due to its high sensitivity to allelochemicals at low doses, radish was chosen as an indicator plant (Islam et al., 2018a). The length of the radish shoot and root were recorded after being incubated for 48 hours. The potential of each extract to limit the growth of indicator plants was then evaluated using a conventional laboratory bioassay technique.

2.5 Calculation of inhibition percentage

According to Islam et al. (2018a) the inhibition (%) was computed. The inhibition percentage was computed as follows:

$$I\ (\%) = \left(1 - \frac{L_A}{L_C}\right) \times 100 \qquad (1)$$

where, I (%) = inhibition (%), and L_A = Shoot or root length of radish with aqueous extract of selected legume plant species and L_C = Shoot or root length of radish in control treatments.

2.6 Statistical analysis

The recorded data were statistically analyzed using open source statistical environment 'R' (R Core Team, 2021). The Analysis of Variance (ANOVA) were conducted using 'agricolae' package of 'R'. The differences among treatment means were adjudged by Tukey's *post hoc* test.

3 Results

3.1 Shoot growth of *R. sativus*

3.1.1 Effect of legume trees

The shoot length of *R. sativus* was significantly influenced by aqueous leaf extract of all legume trees at $p < 0.05$. *R. sativus* shoot length was the highest in control for all species and the lowest in 1:05 (*w/v*) concentration (Fig. 1). With the exception of Raintree (*Samanea saman*), all tree species extracts at a concentration of 1:05 (*w/v*) severely inhibited (>85%) the growth of *R. sativus* shoot. Raintree showed 84% inhibition, on the other hand Tetul (*Tamarindus indica*), Sada koroi (*Albizia procera*), Radhachura (*Peltophorum pterocarpum*), Minjiri (*Cassia siamea*), Polash (*Butea monosperma*), Ipil ipil (*Leucaena leucocephala*), Bokful (*Sesbania grandiflora*), Sonalo (*Cassia fistula*), Krishnochura (*Delonix regia*) inhibited the shoot growth of *R. sativus* by 95, 92, 95, 91, 92, 88, 88, 91, 92% at 1:05 (*w/v*) level of concentration, respectively (Fig. 1).

3.1.2 Effect of legume herbs

R. sativus shoot length was drastically lessened when herbaceous legume plant species' aqueous leaf extracts were administered at $p < 0.05$. As like the other tree species, the maximum length of shoot of *R. sativus* was noticed in the control (no extract) and the minimum in the 1:05 (*w/v*) concentration (Fig. 2). Except for lentil (*Lens culinaris*) and grass-pea (*Lathyrus sativus*), which exhibited about 70% and 85% inhibition, respectively, at the concentration of 1.05 (*w/v*), the majority of the herbaceous legume plants displayed more than 90% inhibition (Fig. 2). The aqueous leaf extracts of Tripatri shak (*Desmodium triflorum*), Sada lojjabhoti (*Mimosa invisa*), Soybean (*Glycine max*), Faba bean (*Vicia faba*), Chickpea (*Cicer arietinum*), Country bean (*Lablab purpureus*), Black gram (*Vigna mungo*), Winged bean (*Psophocarpus tetragonolobus*), Ground nut (*Arachis hypogae*), Cowpea (*Vigna unguiculata*) and Yard long bean (*Vigna unguiculata*) inhibited the shoot growth of *R. sativus* by 92, 93, 97, 99, 98, 97, 98, 99, 93, 97 and 94%, respectively at 1:05 (*w/v*) concentration (Fig. 2).

3.1.3 Effect of legume shrubs

Aqueous leaf extracts of legume shrubs also substantially inhibited the shoot growth of *R. sativus* at 5% level of probability. The control plant had the longest shoots of *R. sativus*, whereas the concentration of 1:05 (*w/v*) had the shortest (Fig. 3). African dhaincha (*Sesbania rostrata*) completely inhibited (100%) the development of *R. sativus* shoots at 1:05 (*w/v*) concentration (Fig. 3). But Deshi dhaincha (*Sesbania aculeata*) demonstrated 97% inhibition at the same dose.

3.2 Root growth of *R. sativus*

3.2.1 Effect of legume trees

All extract concentrations greatly restricted the development of *R. sativus* roots at $p < 0.05$. Similar to the effects on shoot growth, the leaf extracts of legume tree species also showed concentration dependent inhibitory activity on the root growth of *R. sativus* (Fig. 4). *R. sativus* longest roots were consistently shown in control treatments (those without extracts), and they got shorter as the concentration of leaf extracts from different legume tree species increased. In maximum extract concentration (1:05 (*w/v*)), *R. sativus* roots were reported to be the shortest. At the highest concentration 1:05 (*w/v*), the aqueous leaf extracts of Tetul (*Tamarindus indica*), Radhachura (*Peltophorum pterocarpum*), Minjiri (*Cassia siamea*), Polash (*Butea monosperma*), Ipil ipil (*Leucaena leucocephala*), Bokful (*Sesbania grandiflora*), Sada koroi (*Albizia procera*), Sonalo (*Cassia fistula*), Krishnochura (*Delonix regia*) and Raintree (*Samania saman*) slowed the growth of *R. sativus* roots by 100, 100, 100, 100, 100, 100, 98, 87, 98 and 99%, respectively (Fig. 4).

3.2.2 Effect of legume herbs

R. sativus root length was drastically reduced when herbaceous plant species' aqueous leaf extracts were employed at $p < 0.05$. Similar to legume trees, control had the longest roots, whereas 1:05 (*w/v*) concentration had the shortest roots (Fig. 5). Compared to tree species, herbaceous plant extracts had a lower proportion of *R. sativus* shoot length inhibition. At the concentration 1:05 (*w/v*) the aqueous leaf extracts of Tripatri shak (*Desmodium triflorum*), Soybean (*Glycine max*), Faba bean (*Vicia faba*), Country bean (*Lablab purpureus*), Black gram (*Vigna mungo*), Groundnut (*Arachis hypogae*) and Yard long bean (*Vigna unguiculata*), absolutely (100%) suppressed the root growth of *R. sativus* (Fig. 5). On the other hand, Sada lojjabhoti (*Mimosa invisa* Martius), Chick pea (*Cicer arietinum*), Lentil (*Lens culinaris*), Winged bean (*Psophocarpus tetragonolobus*), Grass pea (*Lathyrus sativus*), Cow pea (*Vigna unguiculata*), showed 84, 98, 92, 99, 92 and 98% inhibition, respectively at 1:05 (*w/v*) concentration (Fig. 5).

3.2.3 Effect of legume shrubs

Legume shrub leaf aqueous extracts considerably reduced the length of *R. sativus* roots at the 5% level of probability. The similar trend of root length as like tree and herb species were observed in case of shrubs (Fig. 6). As opposed to that, Deshi dhaincha (*Sesbania aculeata*) showed 98% inhibition at the same level of concentration.

3.3 Root and shoot growth inhibition

Fig. 7 demonstrated that the root growth (ranged 56–81%) was more suppressed by the aqueous leaf extracts of legume plants than the shoot growth (ranged 37–77%). Lentil provided the lowest average obstruction on the growth of *R. sativus* shoots (37%), whereas Faba bean exhibited the greatest (77%). In contrast, the herb legume Tripatri shak (56%) and the Winged bean (81%) were shown to have the minimum and maximum average inhibitions on the root growth of *R. sativus*, respectively (Fig. 7). Compare to the categories of legume species the shrubs showed greater inhibition on the shoot development of *R. sativus* (65%) and that of lowest (60%) was obtained from herb species (Fig. 8). Besides that, herb species had

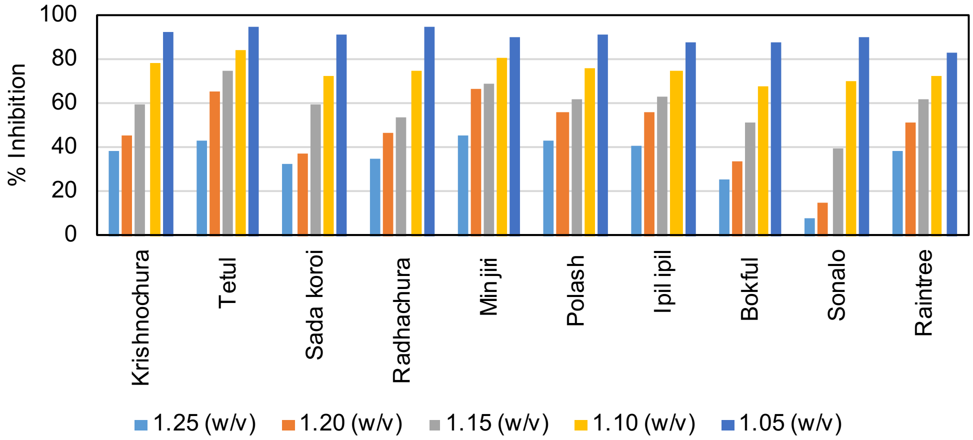

Figure 1. Inhibition percentage of the aqueous leaf extracts of legume tree species on shoot growth of *Raphanus sativus* over control

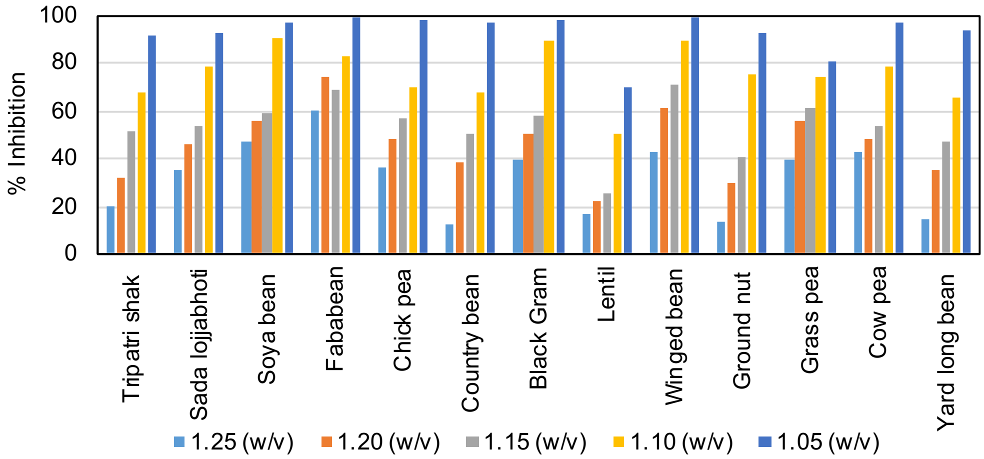

Figure 2. Inhibition percentage of aqueous leaf extracts of legume herb species species on shoot growth of *Raphanus sativus* over control

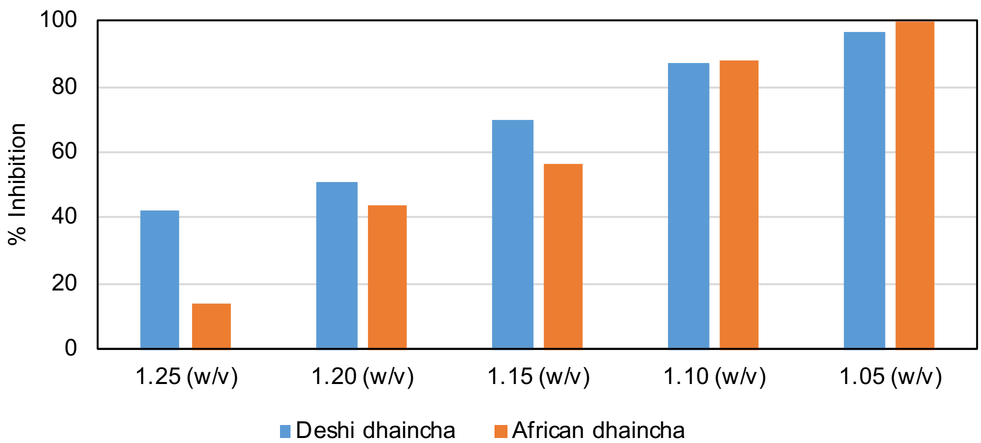

Figure 3. Effect of aqueous leaf extracts of legume shrubs on the shoot growth of *Raphanus sativus* over control

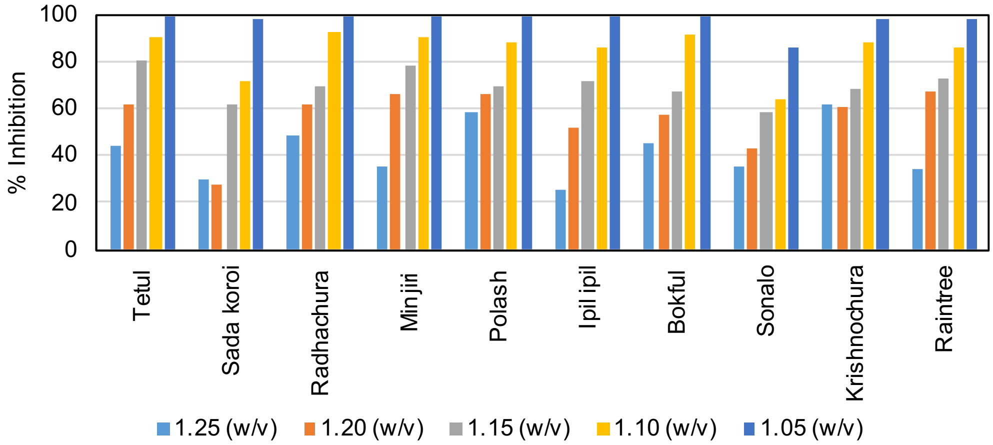

Figure 4. Effect of aqueous leaf extracts of legume trees on the root growth of *Raphanus sativus* over control

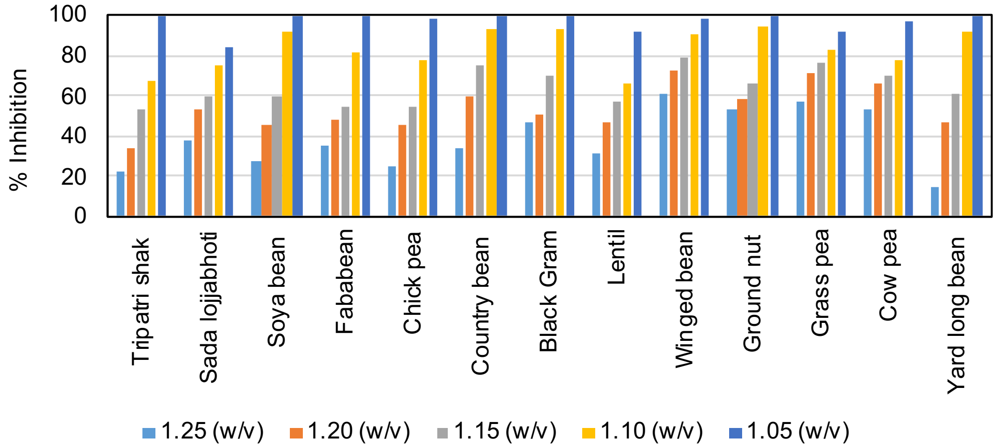

Figure 5. Effect of aqueous leaf extracts of legume herbs on the root growth of *Raphanus sativus* over control

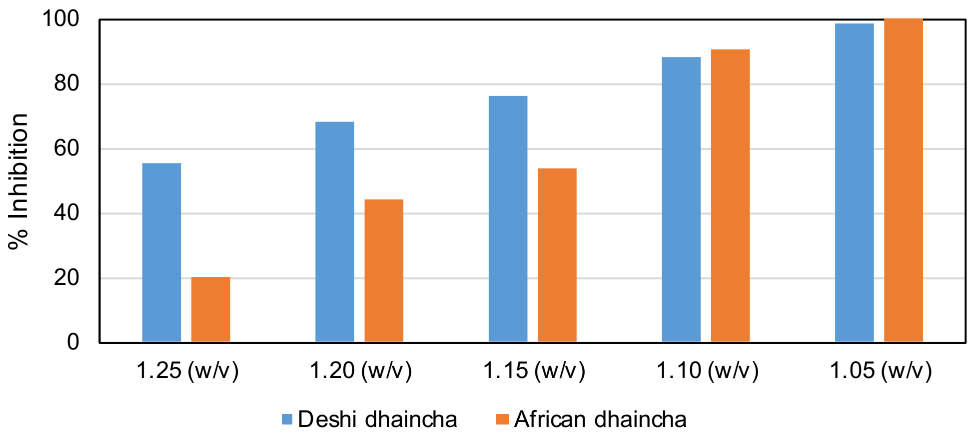

Figure 6. Effect of aqueous leaf extracts of legume shrubs on the root growth of *Raphanus sativus* over control

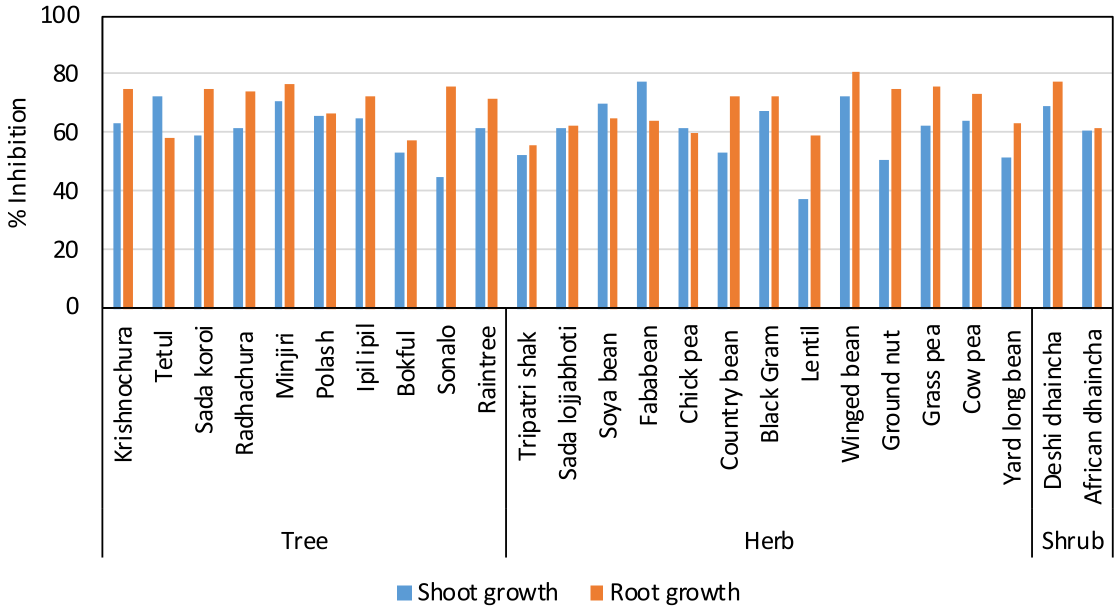

Figure 7. Average shoot and root growth inhibition of *R. sativus* by the legume plant extracts

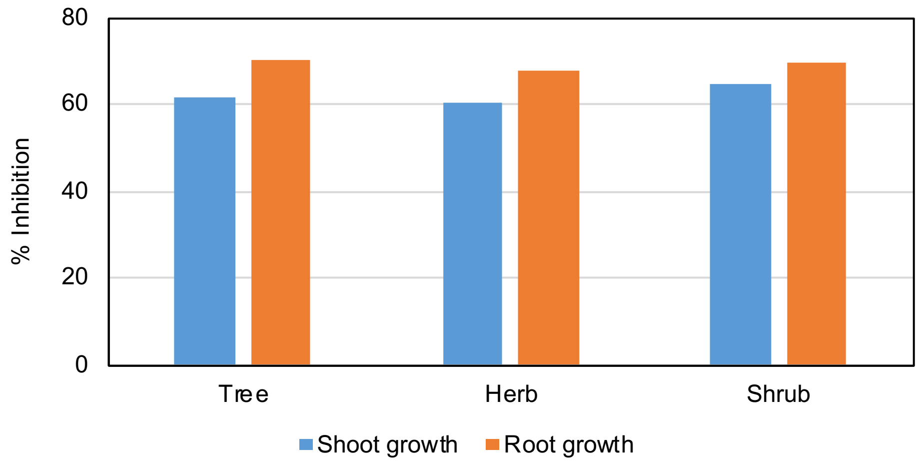

Figure 8. Average shoot and root growth inhibition of *R. sativus* by the aqueous leaf extract of different categories of legume plant species

the lowest (68%) effect on *R. sativus* root growth and tree species had the most (70%) (Fig. 8).

4 Discussion

The concentration-dependent downregulation of the aqueous leaf extracts of 25 species of legume plants on the development of *R. sativus* seedlings was noticed. Aoki et al. (1997) noted that the proportion of compounds in the extracts determines the strength of allelopathic effects, which was also witnessed in this investigation. Inhibiting action that is concentration driven was also documented by Islam and Kato-Noguchi (2014), Islam et al. (2018a), Islam et al. (2019b,a), Charoenying et al. (2022) and Kyaw et al. (2022). This form of inhibitory activity by the extracts of allelopathic species was documented by An et al. (2005), Islam et al. (2019b,a), Motmainna et al. (2021) and Satapathy et al. (2022). The numerous bio-chemicals engaged in the mechanism may have intrinsic variations that account for the unsymmetrical sensitivity of *R. sativus* to diverse legume plants extracts. According to Rice (1984), the consequences of allelopathy may affect plant water relations, photosynthesis, respiration, protein synthesis, lipid metabolism, and organic acids, along with cell division, stretching, and ultrastructure in addition to stomatal opening.

The location of where nutrients and water are actively absorbed is determined by the plant root zone (Jackson et al., 1997). The root development of *R.*

sativus was more responsive to the aqueous leaf extracts of 25 legume plants than the shoot development was. Phytotoxicity can be detected by the reduction in root length of the target plant species in reaction to allelochemicals. Higher quantities of the plant extracts resulted in a considerable drop in root length, according to prior investigations (Andrew et al., 2015).

Reduction in cell division, elongation, and expansion rates, which are growth prerequisites, could be the cause of *R. sativus* growth suppression in the presence of allelochemicals (Rice, 1984; Einhellig, 1994). Additionally, allelochemicals impair a number of physiological aspects of plants, including photosynthetic rate, stomatal conductance, transpiration, chlorophyll and carotenoid content (Motmainna et al., 2021), absorption of ions (Qasem and Hill, 1989), function of enzymes (Sato et al., 1982), production of plants endogenous hormones, proteins (John and Sarada, 2012), phytochromes alternation, germination regulation (Leather and Einhellig, 1988) and thus, leads to halted plant development. Allelochemical may produces more than one effect of the above on the cellular processes that could be responsible of the biochemical mechanism through which allelochemicals extract a toxic effect on the growth of any plant species are still not well known (Zhou et al., 2013).

The elevated use of herbicides provokes serious environmental issues, such as the decay of agricultural land caused by the eradication of soil biota (Peng et al., 2004), groundwater pollution (Aktar et al., 2009), the decline of fish stocks (Khan et al., 2011), and the emergent of herbicide-resistant biotypes of weeds (Vyvyan, 2002). Therefore, it is imperative to create innovative and eco-friendly methods to effectively eradicate weeds. In this sense, our investigation may offer some helpful tips to the scientists looking to create novel natural weed-controlling herbicides utilizing allelochemicals from legume plant species.

5 Conclusion

Allelopathic plants may play a vital role and could reduce the heavy dependence on synthetic chemical herbicides. Therefore, the purpose of this study was to evaluate the allelopathic activity of 25 Bangladeshi legume plants against the seedling development of the allelopathically susceptible *Raphanus sativus*. At a concentration of 1:05 (*w/v*), lentil (Lens culinaris) leaf extract provided the least suppression of *R. sativus* shoot elongation (70%) whereas African dhaincha induced the strongest suppression (100%) with the same concentration. In case of root, highest inhibition (100%) was obtained from Tetul, Radhachura, Minjiri, Polash, Bokul, Ipilipil, Tripatrishak, Soybean, Fababean, Country bean, Black gram, Groundnut, Yardlong bean and African dhaincha with the concentration of 1:05 (*w/v*). Sada lojjabhoti provided the lowest percentage of root inhibition (85%) at the same dose. When compared to root growth, which was inhibited to a greater extent by the aqueous leaf extracts of the legume plant (56–81%), the shoot growth was less inhibited (ranged 37–77%). According to the findings of this study, African dhaincha is the best potential plant among the examined legume plant species with strong allelopathic qualities, followed by Soybean, Faba bean, Blackgram, and Winged bean. The selective behavior of these species toward other distinct plant species, such as weeds and crops in the field, must thus be investigated. Furthermore, by further isolating and characterizing allelochemicals, these legume plant species may be exploited to create natural product-based herbicides as an alternative to synthetic herbicides.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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