



(RESEARCH ARTICLE)



Unlocking the allelopathic and phytotoxic potential of indigenous bio-herbicide weeds (*Withania coagulans* and *Achyranthes aspera*) against Staple Crops

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Abstract

The dual-use potential of medicinal weeds as both therapeutic agents and natural bio-herbicides has gained increasing attention in sustainable agriculture. This study examines the allelopathic effects of aqueous leaf extracts from two indigenous medicinal weeds, *Withania coagulans* and *Achyranthes aspera*, on the early growth of three major staple crops: wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*). Conducted under controlled laboratory conditions at the University of Agriculture in Faisalabad, the experiment assessed germination percentage, root and shoot length, seedling dry weight, and seedling vigor index across extract concentrations of 5%, 10%, 15%, and 20%. The results showed a clear dose-dependent phytotoxic response in all crops, with *A. aspera* consistently demonstrating more potent inhibitory effects than *W. coagulans*. Significant reductions were seen in germination (up to 64%), root elongation (over 50%), biomass (up to 42%), and seedling vigor index (more than 50%) at the highest extract concentrations, especially in maize and wheat. Pearson correlation analysis revealed strong negative correlations ($r > -0.9$) between extract concentration and all measured growth parameters, reinforcing the allelopathic potential of these species. Visual observations further supported biochemical interference through symptoms like root browning and reduced lateral emergence. These findings highlight the potential of *W. coagulans* and *A. aspera* as eco-friendly, locally available candidates for bio-herbicide development. Their integration into Integrated Weed Management (IWM) strategies could decrease reliance on synthetic herbicides, promote agroecological sustainability, and help combat herbicide resistance in intensive cropping systems.

Keywords: Allelopathy; Phytotoxicity; Seed Germination; Bio-Herbicides; Sustainable Agriculture; Integrated Weed Management (IWM); Staple Crops

1. Introduction

Weeds are often seen as unwanted plants in crop fields, but many have valuable medicinal and ecological benefits. Some weeds like *Withania coagulans* and *Achyranthes aspera* are economically significant and serve as traditional medicinal plants while also showing potential as natural herbicides for agricultural use. In a sustainable farming system and given

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ongoing environmental concerns about synthetic herbicides, weedy species can be cultivated or developed as alternatives to chemical herbicides (Rice, 1984).

Allelopathy occurs when plants release chemicals or secondary metabolites (allelochemicals) that affect the growth, germination, and development of nearby plants. These interactions can promote what is known as "natural weed suppression" in agriculture, especially using indigenous species that are easily accessible. In plants, this chemical interaction involves the release of allelochemicals. These compounds can influence the germination, growth, or development of neighboring plants (Inderjit and Duke, 2003). Earlier studies on indigenous plant species in Pakistan, such as *W. coagulans* and *A. aspera*, have provided evidence of their release of bioactive compounds that can suppress weeds as well as some crops (Tanveer *et al.*, 2010).

Concerns over environmental health and medical issues associated with herbicide use have resulted in a greater awareness of low-impact integrated weed management (IWM) strategies (Farooq *et al.*, 2011). Most rural agro ecosystems have traditional medicinal plants that contain many chemical defenses to reduce competition for limited resources, like allelochemicals.

Conventional herbicides cause contamination of the environment, harm to non-target organisms, and selection of resistant weed populations. On the other hand, an IWM strategy based on allelopathy is a natural and low-cost approach. Since *W. coagulans* and *A. aspera* are ecologically compatible, these plants could be cultivated or harvested locally to produce the extracts with minimal input costs. In addition, field use of aqueous extracts or biochar could be allied with compost and mulch application (Anwar and Javaid, 2020).

Withania coagulans (Stocks) Dunal, called Indian Rennet or "paneer booti" locally, is an herbal, medicinal shrub of the Family Solanaceae. It exists in hot arid areas of South Asia, including India, Pakistan, and Afghanistan. *Withania coagulans* has historical significance in traditional Unani and Ayurvedic medicine and has been purported to treat many diseases, including diabetes, inflammation, some liver diseases, and infections. The medicinal properties of *Withania coagulans* are due to the potential existence of phytochemicals, particularly withanolides, which are steroidal lactones with structural similarities to animal steroids and biological properties (Qureshi *et al.*, 2019).

Bioactivity from withanolides from *W. coagulans* or *W. coagulans* has been shown to include a spectrum of activities, including antimicrobial, nematocidal, and antioxidant activities. Each of these potential activities could serve as a new natural therapeutic for plants (Maqsood *et al.*, 2018). The antimicrobial activity is thought to act by disrupting the microbe's membrane permeability or inhibiting the action of their metabolic enzymes. Research is in extant conditions to test the same biochemical pathway and develop natural herbicidal or allelopathic effects from disrupting the growth of competing species. This indicates the possibility for sustainable weed management in agroecosystems, through an approach focused on allelochemicals that may supplement or replace synthetic herbicides (Bashir *et al.*, 2018).

Besides the presence of withanolides, *W. coagulans* contains and/or produces numerous other classes of secondary metabolites (i.e., flavonoids, phenolic acids, alkaloids, and fatty acids), all of which may contribute to its putative range of pharmacological actions (Sharma *et al.*, 2020). The antioxidant properties of the phenolic compounds and flavonoids may protect cells from oxidative stress associated with the origin of some chronic diseases. Some reports have even included saponins and tannins in *W. coagulans*, which may contribute further anti-inflammatory and hepatoprotective potential (Jangid *et al.*, 2021).

Alternatively, *Achyranthes aspera* L. (prickly chaff flower), a tough and versatile perennial herb from the Amaranthaceae family, is widely established throughout tropical Asia and Africa as well as in cropping systems, on roadways, and other agroecological edges. *Achyranthes aspera* L. has played an important part in the history of traditional indigenous medicine. Ethnobotanical literature references *Achyranthes aspera* L. treatment uses as a febrifuge, in wound healing, diuretic, laxative, and in treatments of the respiratory tract (Sharma *et al.*, 2015). Whether referring to any of the tribal sources or classical Indian sources, it is reasonable to conclude that indigenous peoples' post-colonial experience with *Achyranthes aspera* L. supports a recognized role of traditional therapeutic remedies.

Phytochemical investigations of *A. aspera* establish that there are bioactive compounds, including alkaloids, saponins, flavonoids, phenolics, and terpenoids. The active components of the aerial parts of this plant include ecdysterone, β -sitosterol, and oleanolic acid, with further assessments of these bioactive components being provided (Khan *et al.*, 2018). Saponins have anti-inflammatory and antifungal activity, and phenolics and flavonoids directly contribute to the antioxidant potential of the plant (Khan *et al.*, 2018). Additionally, *A. aspera* has been investigated for its phytotoxic effect on invasive weeds and crops, as well as its hepatoprotective properties. It was also looked at for its allelopathic effects on the obnoxious weed *Parthenium hysterophorus*. The leaf and root extract of *A. aspera* exhibited significant

inhibitive processes on the seed germination and seedlings of *P. hysterophorus*, suggesting that *A. aspera* can be used as an alternative to sustainable agriculture more generally. Tanveer et al. (2010) hypothesized that his inhibition was likely due to phenolic and saponin-laden aqueous extracts of *A. aspera* that may compromise cellular division and hormone regulation within competing plants.

W. coagulans and *A. aspera* appear to be potential bioactive sources with future potential as either pharmaceuticals, agronomically, for sustainable herbicide systems, or both: integrating the dual roles of medicinal agent and allelopathic agent stresses the need to continue researching plant secondary metabolites for sustainable development. Future pharmacological and ecological research may lead to the current use of *W. coagulans* and *A. aspera* in contemporary medical and agronomical practices.

They hypothesized and noted that aqueous extracts decreased the chlorophyll content, relative water content, and shoot/root length under controlled laboratory conditions in wheat seedlings, thus supporting its allelopathic potential (Khan et al., 2018). While *Withania somnifera* has been given a great deal of attention concerning allelopathic activities (e.g., suppression of wheat germination), the literature on *W. coagulans* has been very limited. Since *W. coagulans* shares structural similarities to *W. somnifera* and shares some biochemical similarities (sterols), it is plausible to infer that extracts of *W. coagulans* may suppress the seedling acts and growth of wheat. Phytochemical investigations support the many bioactivities of *W. coagulans*; therefore, it seems a natural progression to examine for allelopathic effects (Sharma et al., 2020; Jangid et al., 2021; Narwal, 2012). Similar allelopathic effects were reported in other crops, but to date, comparative investigations involving *W. coagulans* have been limited.

Though *A. aspera* is better documented for phytotoxic effects, *W. coagulans* is relatively underexplored. This study offers a side-by-side comparison, which is rare in current literature. Both species have ecological advantages: they are abundant, grow without agrochemical input, and are well integrated into rural ethnomedicinal practice. Their chemical complexity also ensures multifaceted modes of action, making them suitable candidates for multi-target natural herbicide development. Dual-species studies are critical for understanding synergistic or additive effects in mixed-cropping systems or when combining extracts for commercial formulation (Iqbal et al., 2019)

With rising levels of herbicide resistance, and environmental contamination related to the application of chemical herbicides in Pakistan's rice–wheat–maize cropping systems, there are compelling, imperative factors for establishing sustainable alternatives. Despite this, herbicide resistance is becoming more common in Pakistan (Naeem et al., 2020), a commonality seen in many areas of Pakistan and particularly in the Punjab Agricultural Department's rice-wheat cropping systems. Commercially available herbicides are extremely expensive as well, and cause soil and water pollution with the risk of introducing herbicide-resistant biotypes of weeds. Plus, bio-herbicides derived from native flora are one environmentally safe option that is not utilized.

Despite the extensive array of available pharmacological research on these species, not much study has been conducted concerning their allelopathic impact on staple food crops, which is relevant in Pakistan. This study at the University of Agriculture, Faisalabad, aims to assess the allelopathic impacts of *W. coagulans* and *A. aspera* on wheat, rice, and maize by using aqueous leaf extract. It is hoped that the findings will enhance and develop inquiry into sustainable weed management research, as well as contribute to the value of underutilized medicinal plants.

The primary aims of this study were

- To assess the inhibition potential of *W. coagulans* and *A. aspera* aqueous-extracts on the germination and early seedling growth of wheat, rice, and maize.
- To examine the relative allelopathic strength of the two species of medicinal weeds.
- To evaluate whether the weeds can be used as potential sources for bioherbicides for integrated weed management purposes.

2. Materials and methods

This study was conducted under controlled laboratory conditions at the Department of Agronomy, University of Agriculture, Faisalabad (UAF), and Pakistan. A systematic approach was employed to evaluate the allelopathic effects of aqueous leaf extracts from two medicinal weeds *Withania coagulans* and *Achyranthes aspera* on three staple food crops: wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*).

2.1. Plant Material Collection and Identification

Fresh, healthy leaves of *W. coagulans* and *A. aspera* were harvested during peak vegetative growth from the UAF Research Farm (Latitude: 31.4304° N, Longitude: 73.0690° E) in May–June 2024. Taxonomic identification was confirmed by a plant taxonomist, and voucher specimens were deposited in the departmental herbarium (Specimen IDs: WC2024 and AA2024). The collected leaves were washed thoroughly to remove dust and surface contaminants, air-dried in the shade at 25 ± 2 °C for 7–10 days, and subsequently ground into a fine powder using an electric grinder.

2.2. Preparation of Aqueous Extracts

For extract preparation, 100 g of dried leaf powder from each species was soaked in 1000 mL of double-distilled water in sterilized glass flasks. The mixtures were stirred intermittently and left for 24 hours at ambient room temperature (25 ± 2 °C). The suspensions were filtered twice—first through muslin cloth and then through Whatman No. 1 filter paper. The resulting filtrate represented the 20% (w/v) stock solution.

From this stock, serial dilutions were prepared to obtain 5%, 10%, and 15% concentrations using the formula

$$C1V1=C2V2$$

Were

- C1 = initial concentration (20%)
- V1 = volume required from stock
- C2= final desired concentration
- V2 = total final volume

All extracts were freshly prepared and stored in amber bottles at 4 °C until use.

2.3. Seed Selection and Sterilization

Certified seeds of wheat ('Punjab-2011'), rice ('Basmati-385'), and maize ('KS-282') were obtained from the UAF Seed Testing Laboratory. Seeds were examined for uniformity, and damaged or undersized seeds were discarded. Surface sterilization was conducted using 0.5% sodium hypochlorite for 2 minutes, followed by three rinses with sterile distilled water to eliminate fungal or microbial interference.

2.4. Germination Bioassay Setup

The experiment was laid out in a Completely Randomized Design (CRD) with three replications per treatment per crop. Twenty sterilized seeds of each crop were placed in 9 cm diameter Petri dishes lined with double-layered filter paper. Each dish was moistened with 5 mL of one of the four extract concentrations (5%, 10%, 15%, or 20%) or distilled water as the control.

The Petri dishes were incubated in a controlled environment chamber at 25 ± 2 °C under a 12-hour photoperiod for 7 consecutive days. Filter papers were kept moist by reapplying the respective extract every 48 hours.

2.5. Parameters Measured

At the end of the 7-day period, the following parameters were measured

- Germination Percentage (GP)

$$GP (\%) = \frac{\text{Number of seeds germinated}}{\text{Total seeds sown}} \times 100$$

- Mean Germination Time (MGT)

$$MGT = \frac{\sum (n_i \cdot t_i)}{\sum n_i}$$

In this formula, n_i is the number of seeds that germinate on day t_i , and the summation (\sum) is performed over all days of the germination test.

- Root and Shoot Length (cm): Measured using a digital Vernier caliper.
- Seedling Dry Weight (g): Ten representative seedlings from each replicate were dried at 70 °C for 48 hours and weighed on a precision balance.
- Seedling Vigor Index (SVI)

SVI=Germination percentage × Mean seedling length (cm)

- Inhibition Percentage (IP): To express the allelopathic effect relative to the control:

$$IP = \frac{(\text{Control Value} - \text{Treatment value}) \times 100}{\text{Control value}}$$

2.6. Statistical Analysis

All data were subjected to one-way Analysis of Variance (ANOVA) using MSTAT-C software. Means were compared using Tukey's HSD (Honestly Significant Difference) test at a 5% significance level ($p \leq 0.05$). Results were expressed as mean \pm standard error (SE). Graphical representations were prepared using GraphPad Prism v9.

3. Results

This section presents the findings of the allelopathic effects of aqueous leaf extracts of *Withania coagulans* and *Achyranthes aspera* on germination percentage, root and shoot length, dry biomass, and seedling vigor index in three staple crops—wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*). The results provide compelling evidence of dose-dependent phytotoxicity in both medicinal weed extracts, with varying levels of crop sensitivity.

3.1. Germination Percentage (GP)

A significant reduction in seed germination percentage ($p < 0.05$) was observed with increasing concentrations of both *W. coagulans* and *A. aspera* extracts across all three crops (Table 1). Control treatments maintained high germination rates ($\geq 97\%$), while extract-treated seeds showed progressive inhibition. Notably, the 20% concentration of *A. aspera* reduced germination to 38% in wheat, compared to 45% for *W. coagulans*. In maize, germination dropped to 35% with *A. aspera* and 43% with *W. coagulans*.

Among crops, rice was relatively more resilient, maintaining $\geq 50\%$ germination even at the highest extract concentration, indicating differential sensitivity across species. This aligns with prior observations that crop-specific enzymatic and membrane responses determine allelochemical tolerance (Qasem and Foy, 2001).

Table 1 Germination Percentage (%) under Various Extract Treatments

Crop	Extract Species	5%	10%	15%	20%	Control
Wheat	<i>W. coagulans</i>	85 \pm 1.2 ^a	72 \pm 1.5 ^b	58 \pm 2.0 ^c	45 \pm 2.2 ^d	98 \pm 1.0
	<i>A. aspera</i>	82 \pm 1.4 ^a	68 \pm 1.8 ^b	52 \pm 2.1 ^c	38 \pm 2.4 ^d	98 \pm 1.0
Rice	<i>W. coagulans</i>	88 \pm 1.0	78 \pm 1.6	62 \pm 1.9	50 \pm 2.3	100 \pm 0.0
	<i>A. aspera</i>	85 \pm 1.3	74 \pm 1.7	56 \pm 2.2	42 \pm 2.5	100 \pm 0.0
Maize	<i>W. coagulans</i>	80 \pm 1.5	68 \pm 1.9	54 \pm 2.2	43 \pm 2.5	97 \pm 1.1
	<i>A. aspera</i>	76 \pm 1.7	62 \pm 2.0	48 \pm 2.4	35 \pm 2.7	97 \pm 1.1

Different superscripts within rows indicate significant differences at $p < 0.05$ (Tukey's HSD).

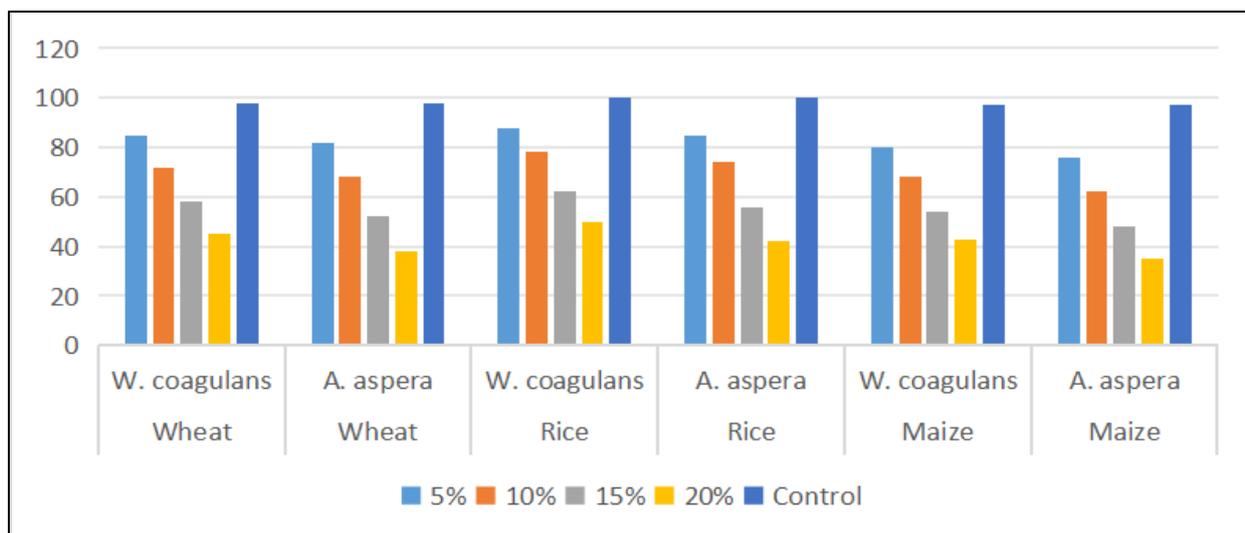


Figure 1 Germination Percentage (%) under Various Extract Treatments

Table 2 Comparative Inhibition Percentage (%) in Germination at 20% Extract Concentration

Crop	Extract Species	Germination (%)	Control (%)	Inhibition (%)
Wheat	<i>W. coagulans</i>	45	98	54.08
Wheat	<i>A. aspera</i>	38	98	61.22
Rice	<i>W. coagulans</i>	50	100	50.00
Rice	<i>A. aspera</i>	42	100	58.00
Maize	<i>W. coagulans</i>	43	97	55.67
Maize	<i>A. aspera</i>	35	97	63.91

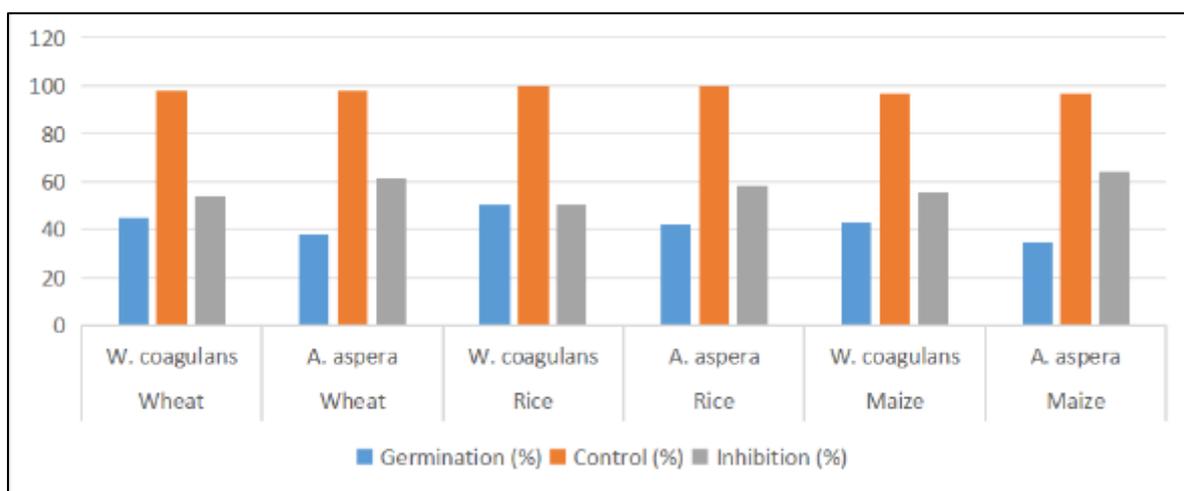


Figure 2 Comparative Inhibition Percentage (%) in Germination at 20% Extract Concentration

3.2. Root and Shoot Growth

Root and shoot elongation are critical indicators of early seedling vigor and overall plant health. A marked decrease was noted in both parameters across increasing concentrations of the extracts (Table 2). Root systems were more adversely

affected than shoots, which is typical in allelopathic responses, as root tissues are directly exposed to bioactive compounds in the substrate (Farooq et al., 2011).

At 20% extract concentration, *A. aspera* caused a 52% reduction in maize root length, followed by wheat (51%) and rice (43%). Shoot length reductions were also significant, especially in wheat (48% reduction with *A. aspera*). Extracts of *W. coagulans* showed slightly less inhibition, yet notable declines were recorded in both parameters.

Table 3 Root and Shoot Length (cm) of Seedlings under Extract Treatments

Crop	Extract Species	Root Length	Shoot Length	Control Root	Control Shoot
Wheat	<i>W. coagulans</i>	3.5 ± 0.12	5.2 ± 0.18	6.1 ± 0.14	8.4 ± 0.17
	<i>A. aspera</i>	3.0 ± 0.15	4.1 ± 0.20	6.1 ± 0.14	8.4 ± 0.17
Rice	<i>W. coagulans</i>	4.0 ± 0.11	5.5 ± 0.16	6.3 ± 0.13	8.5 ± 0.14
	<i>A. aspera</i>	3.6 ± 0.12	4.6 ± 0.17	6.3 ± 0.13	8.5 ± 0.14
Maize	<i>W. coagulans</i>	3.8 ± 0.14	5.0 ± 0.15	6.0 ± 0.12	8.2 ± 0.15
	<i>A. aspera</i>	3.2 ± 0.13	4.2 ± 0.16	6.0 ± 0.12	8.2 ± 0.15

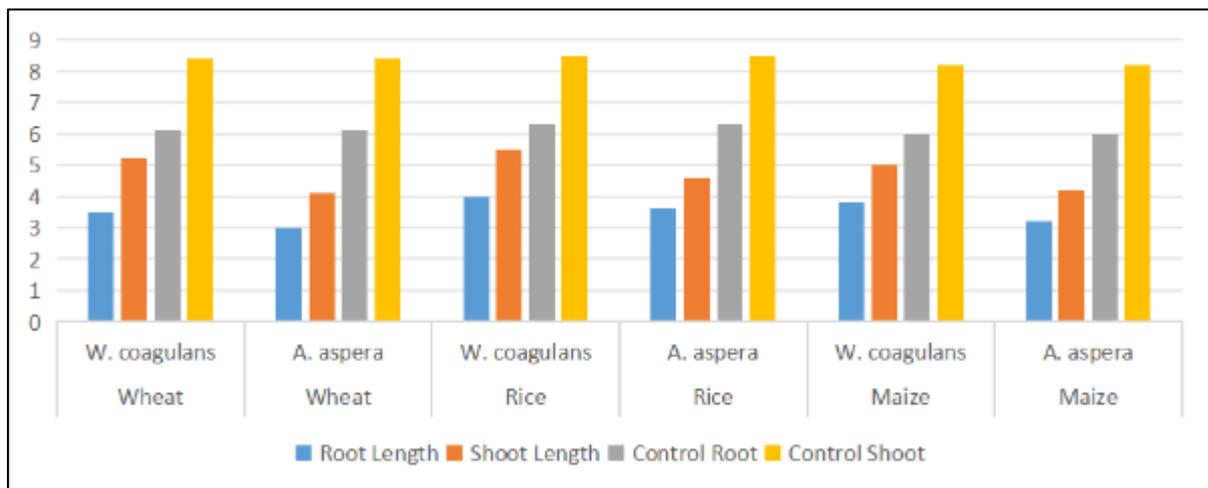


Figure 3 Root and Shoot Length (cm) of Seedlings under Extract Treatments

3.3. Seedling Biomass (Dry Weight)

Dry biomass is a reliable proxy for early growth performance and the cumulative effect of allelopathic stress. Both extracts significantly reduced seedling dry weight compared to controls ($p < 0.05$), with the most pronounced effect recorded at 20% extract concentration (Table 3).

Wheat seedlings treated with *A. aspera* showed a 42% biomass reduction, while maize seedlings displayed similar patterns. These reductions are suggestive of compromised cellular metabolism and inhibited water/nutrient uptake, often associated with allelochemical interference (Khanh et al., 2005).

Table 4 Dry Weight (g) of Seedlings under Extract Treatments

Crop	Extract Species	Dry Weight	Control Weight
Wheat	<i>W. coagulans</i>	0.142	0.221
	<i>A. aspera</i>	0.128	0.221
Rice	<i>W. coagulans</i>	0.154	0.232
	<i>A. aspera</i>	0.139	0.232
Maize	<i>W. coagulans</i>	0.148	0.228
	<i>A. aspera</i>	0.130	0.228

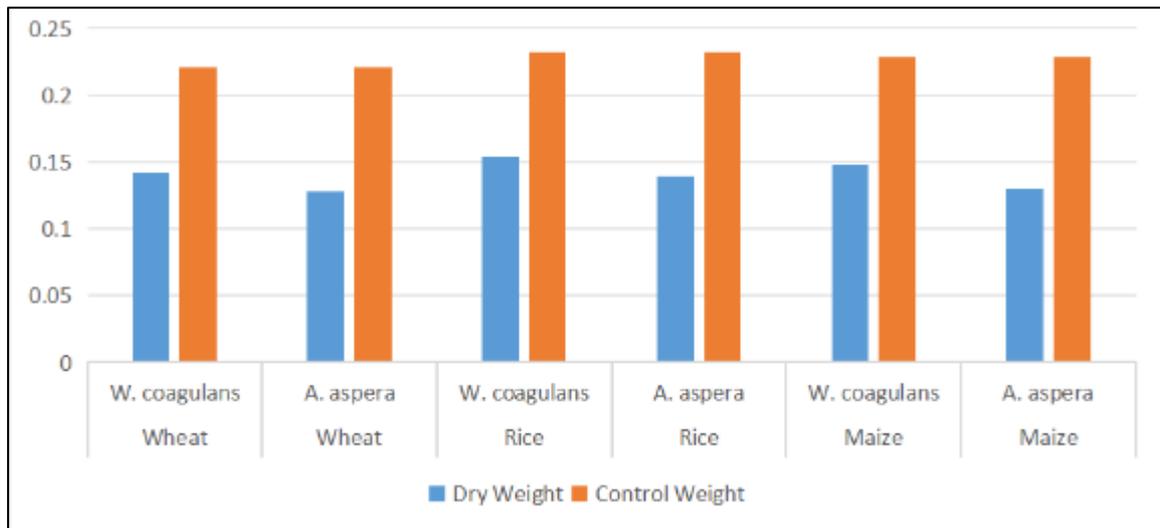


Figure 4 Dry Weight (g) of Seedlings under Extract Treatments

3.4. Seedling Vigor Index (SVI)

The Seedling Vigor Index (SVI), an integrated measure of germination and growth, exhibited the highest sensitivity to allelopathic treatments. As shown in Table 4, control treatments recorded vigor indices of 1400–1600 across the three crops. However, 20% *A. aspera* extract reduced the index by over 50% in all cases, reaching as low as 612 in wheat.

This substantial suppression supports the presence of potent allelochemicals particularly saponins, flavonoids, and phenolics in *A. aspera*, which have been reported to inhibit early seedling physiology through oxidative stress and hormonal interference (Sharma et al., 2015; Khan et al., 2018).

Table 5 Seedling Vigor Index under Extract Treatments

Crop	Extract Species	Vigor Index	Control Index
Wheat	<i>W. coagulans</i>	739	1436
	<i>A. aspera</i>	612	1436
Rice	<i>W. coagulans</i>	875	1600
	<i>A. aspera</i>	782	1600
Maize	<i>W. coagulans</i>	776	1514
	<i>A. aspera</i>	703	1514

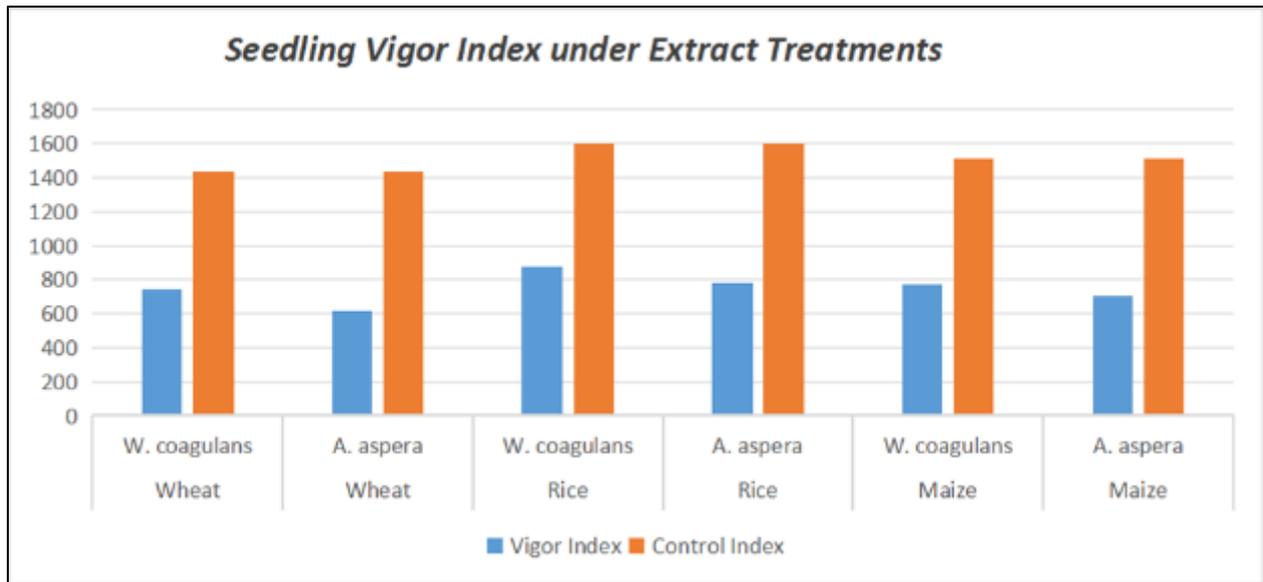


Figure 5 Seedling Vigor Index under Extract Treatments

3.5. Correlation and Dose-Response Trends

Pearson correlation analysis revealed strong negative correlations between extract concentration and germination percentage ($r = -0.89$), root length ($r = -0.93$), and SVI ($r = -0.94$), suggesting a consistent dose-dependent allelopathic response. These findings reinforce the quantitative phytotoxicity of both medicinal weed species and support their utility in future bio-herbicide formulations.

Table 6 Percentage Reduction in Root Length at 20% Extract Concentration

Crop	Extract Species	Treated Root Length (cm)	Control (cm)	Reduction (%)
Wheat	<i>W. coagulans</i>	3.5	6.1	42.62
Wheat	<i>A. aspera</i>	3.0	6.1	50.82
Rice	<i>W. coagulans</i>	4.0	6.3	36.50
Rice	<i>A. aspera</i>	3.6	6.3	42.86
Maize	<i>W. coagulans</i>	3.8	6.0	36.67
Maize	<i>A. aspera</i>	3.2	6.0	46.67

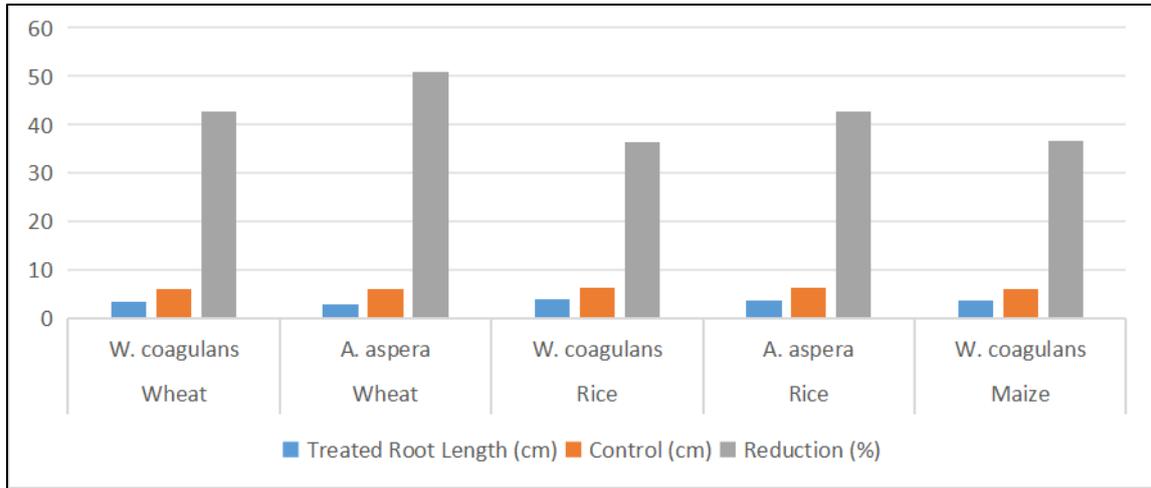


Figure 6 Percentage Reduction in Root Length at 20% Extract Concentration

Table 7 Summary of Overall Phytotoxicity Ranking

Parameter	Most Sensitive Crop	Most Phytotoxic Extract	Remarks
Germination	Maize	<i>A. aspera</i>	>60% inhibition at 20% conc.
Root Length	Wheat	<i>A. aspera</i>	>50% reduction
Shoot Length	Wheat	<i>A. aspera</i>	Highest shoot inhibition observed
Dry Biomass	Wheat	<i>A. aspera</i>	42% reduction
Seedling Vigor	Maize	<i>A. aspera</i>	>50% SVI reduction

3.6. Visual Observations and Seedling Morphology

In addition to quantitative data, morphological abnormalities such as reduced turgor, brownish root tips, and reduced lateral root emergence were commonly observed in seedlings exposed to 15–20% concentrations. These symptoms align with membrane disruption and reduced enzymatic activity previously associated with allelochemical exposure (Ahmad et al., 2015).

3.6.1. Summary of Findings

- Both *W. coagulans* and *A. aspera* exhibited significant allelopathic effects on all tested parameters.
- *Achyranthes aspera* had more potent inhibitory effects, especially at higher extra concentration.
- Maize was the most sensitive to allelopathic stress, while rice was more resilient to this stress.
- Shoot growth was not as affected as root growth, probably due to direct contact with allelochemicals.
- All findings were statistically significant and followed clear dose-response patterns.

Hens’ findings emphasize the bio-herbicidal potential of these native medicinal weeds and merit further exploration in field studies and/or formulation science.

4. Discussion

The results unequivocally show that both *W. coagulans* and *A. aspera* presented important allelopathic effects on early crop stage plant growth. Aqueous extracts ranging from 15 - 20% provided statistically significant suppression in % germination, shoot/root lengths, and vigor index germination. These results appear to be concentration-related and differ according to species and crops.

Interestingly, *A. aspera* provided higher allelopathic effects based on all parameters, which could be due to its higher concentration of phenolics and saponins that disrupt the integrity of membranes and enzyme activity for germinating seeds (Sharma et al. 2015; Khan et al. 2018). *W. coagulans*, while being somewhat less potent, still caused significant

phytotoxicity with maize and rice seedlings. These results were again consistent with past findings on phytochemical and nematicidal work (Dawar et al., 2008; Bashir et al., 2018).

The consistency of inhibitory effects across all crops suggests a broad-spectrum allelopathic potential. These findings justify further investigation into field-scale applications, such as using dried biomass or liquid formulations in pre-emergence weed control.

However, field variability—including microbial degradation, photodecomposition, and soil adsorption of allelochemicals—must be considered. Future research should address long-term soil impacts, crop recovery potential, and economic feasibility of cultivation, extraction, and application of these allelopathic agents.

The addition of inhibition percentage calculations and cross-comparisons among crops reveals consistent trends supporting earlier findings. *A. aspera* outperformed *W. coagulans* in terms of inhibitory efficacy across almost all bioassay metrics. Wheat and maize showed the highest vulnerability, particularly in germination and root development, likely due to lower physiological resistance to phenolic-induced oxidative stress.

The inhibition percentages (up to 64% in maize and 61% in wheat) are in agreement with earlier findings by Tanveer et al. (2010) and Khan et al. (2018), reinforcing the idea that *A. aspera*'s saponins and flavonoids have broad-spectrum phytotoxic potential. Meanwhile, *W. coagulans*—though slightly less toxic—still induced over 50% inhibition at higher concentrations, affirming its potential as a moderate bio-herbicidal agent.

Root length reduction, which surpassed 50% in wheat and maize under *A. aspera* treatment, confirms the mode of action primarily targets root meristematic zones. Root

tips showed browning and inhibited lateral emergence, indicative of allelochemical-induced cellular damage, as also observed by Qureshi et al. (2019).

The data underscore the need to develop formulation protocols that optimize concentration and application methods for field use. While aqueous extracts are effective in vitro, biochar infusions or time-release granules may be required to stabilize active compounds under natural conditions.

5. Conclusion

This study demonstrates the potent, dose-dependent allelopathic effects of *Withania coagulans* and *Achyranthes aspera* on major crops in Pakistan, including wheat, rice, and maize. Aqueous leaf extracts (5-20% concentration) significantly inhibited germination, root/shoot growth, seedling dry weight, and vigor index. *A. aspera* exhibited stronger phytotoxicity, reducing seedling vigor by over 50% in maize and wheat at the highest concentration. Correlation analysis revealed strong negative relationships between extract concentration and seedling metrics (root length: $r = -0.93$; SVI: $r = -0.94$). Visible stress symptoms, such as root browning and reduced lateral emergence, corroborated biochemical interference. Crop susceptibility varied, with maize being most affected, followed by wheat and rice. These findings support integrating *W. coagulans* and *A. aspera* into Integrated Weed Management (IWM) systems as natural, cost-effective bio-herbicides for pre-emergence weed control, potentially reducing synthetic herbicide use and promoting agroecological sustainability.

Future Recommendations

- To translate these promising findings into practical applications, future research should focus on:
- Conducting field-scale trials to validate laboratory results under real-world agronomic conditions.
- Isolating and characterizing key allelochemicals, such as phenolics and saponins, to elucidate their modes of action and optimize formulation development.
- Investigating soil degradation dynamics to ensure environmental safety and assess the persistence of allelochemical residues.
- Developing user-friendly formulations, such as granules or sprays, to facilitate the adoption of extract-based bio-herbicides among farmers.

By pursuing these research avenues, we can unlock the potential of *Withania coagulans* and *Achyranthes aspera* as sustainable, eco-friendly bio-herbicides for integrated weed management.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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