

HERBICIDAL EFFECTIVENESS OF WILD POISONOUS PLANT *RHAZYA STRICTA* USING DIFFERENT MEDIA BY SANDWICH METHOD

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Abstract

Heavy doses of synthetic weed control chemicals have facilitated herbicide resistance in weeds as well as predicted to possess toxicities. Natural compounds can be screened as potential herbicides which are more cost effective, efficacious, selective and environmentally safe. Experiments were conducted on selected weeds (*Rumex dentatus*, *Euphorbia helioscopia*, *Chenopodium album*, *Avena fatua*, *Phalaris minor*) to study allelopathic potential of *Rhazya stricta*. Experiments were performed using a medium of 0.75 % (w / v) agar, filter paper and soil. Parameters studied for assessing allelopathic effects were germination inhibition (%) along reduction in radicle and plumule length (cm). Results showed that seed germination of *R. dentatus*, *P. minor* and *C. album* is inhibited by *R. stricta* allelochemicals. Minimum germination for *C. album* was noted whereas non significant effect on the germination of *E. helioscopia*, *T. aestivum* and *A. fatua* was observed. Leaf extract of *R. stricta* on the agar, filter paper, and the soil inhibited the radicle and plumule length (cm) of all the test species. Results have indicated that even though radicle length and germination of *T. aestivum* is not affected by leaf extract of *R. stricta*, the plumule length was substantially decreased. The retarding effect of growth on wheat seedlings indicates that *R. stricta* might not be an acceptable candidate for weed control under field conditions.

Key words: Weeds of wheat crop; Phytotoxicity; Plant extract; Bioassay.

Introduction

Maximizing the agricultural efficiency of the world depends on controlling pests and diseases. Weeds affect crop productivity the most negatively among pests (Dayan & Duke, 2014). The herbicides cause adverse effects on quality of product, the environment and human health (Hassaan & El Nemr, 2020; Kalyabina *et al.*, 2021). 'Natural compounds' constitutes a wide area for the environment friendly herbicides which are synthesized by compounds produced by living organisms (Soltys *et al.*, 2013). Allelochemicals and allelopathic weeds have wide prospects of application in growth of growing crops, plant's defense, and the biological control (Yan *et al.*, 2000). Crop varieties with high allelopathic ability suppress the weeds in their neighborhood by natural bioactive allelochemicals, thereby reducing reliance on synthetic herbicides (Cheng & Cheng, 2015).

Allelopathy is based on the fact that chemicals are produced by some plants and released through leaching, decomposition, exudation, etc. Such chemicals could be obtained from flowers, stem, leaves and roots etc. These chemicals are commonly called as phytotoxins or allelochemicals (Iqbal *et al.*, 2019). With many other naturally found compounds, these chemicals produces a wide range of biological effects with their capacity and can be moderately beneficial for weed management in farming systems. Bioprospecting of compounds of phytotoxic have led to marketable herbicides (e.g., pelargonic acid, triketone, glufosinate and bialaphos herbicides). From aromatic plants, (plants such as *Xanthoxylum rhetsa*, *Eucalyptus* spp., *Salvia* spp., *Laurus nobilis*, *Artemisia* spp.) essential oils, the cineoles & other monoterpenes have extracted which are phytotoxic. 'Cinmethylin' is a cineole herbicide incorporating monoterpene backbone of 1,4-cineole, with addition of benzyl ether moiety to

lower volatility of product (El-Deek & Hess, 1986). They can be perfect agrochemicals. Allelochemical characteristics which make them potentially 'natural herbicides' are; total / partial water solubility for easy application without surfactants, similar mode of action to synthetic herbicides, non-halogenated molecules that decrease the environmental half-life while preventing soil accumulation and eco environmentally chemical structures with higher nitrogen and oxygen content (Qureshi *et al.*, 2021). Allelochemicals were found in plant hormone disintegration, for example the action of ferulic acid plays role in activating the synthesis of abscisic acid (ABA) (Reigosa *et al.*, 2006). Natural herbicides that are based on allelopathic plants may help in reducing the use of synthetic herbicides. As a result, these can result to alleviate human health concerns, better agricultural products and less pollution. Benzoic acid, Cinnamic acid, glucosionates, phenoloids, flavonoids, terpenes and alkaloids are most commonly available allelochemicals (Khan *et al.*, 2014). The first step to analyze behavior of the field crops in allelopathy is laboratory bioassay. There are various bioassays available to assess crop allelopathic potential; method depends on the research target, bioassay type, the availability of analytical tools etc. However, the most critical element in research into allelopathy is the suitability of a screening process. Bioassay screening must be low cost, fast and easy to operate, reproducible and statistically effective.

Rhazya stricta Decne. belongs to family Apocynaceae, is a poisonous, evergreen shrub. *R. stricta* competes with crops including *Triticum aestivum* in fields for light and nutrient resources and hinders growth and development, eventually deteriorating yield both quantitatively and qualitatively. Alkaloids, triterpenes, volatiles, glycosides and tannins of the species *Rhazya* were reported (Baeshen *et al.*, 2016). Weeds of wheat crop are selected for Sandwich method viz. *Rumex dentatus*, *Euphorbia helioscopia*, *Chenopodium album*, *Avena fatua*, *Phalaris minor* to investigate allelopathic potential of *R. stricta*.

Methods and Materials

Collection and Mechanical processing of *R. stricta*: From Bahawalpur district (29°59'55" N latitude and 73° 15'12" E longitude, 159 m above sea level) along the roadside, the *R. stricta* leaves were collected and dried at 25°C for 4 weeks in shade after washing under tap water. Heavy duty blender was used to make fine powder of dried leaves.

Procurement of seeds of test species and surface sterilization: Seeds of *E. helioscopia*, *A. fatua*, *P. minor*, *R. dentatus*, *C. album* were collected from Barani Agricultural Research Institute (BARI), Chakwal. Sodium hypochlorite (2% w/v NaOCl) was used for surface sterilization of seeds (Ali *et al.*, 2022a,b).

Inhibition effects of *R. stricta* leaf allelochemicals on germination (%), radicle and plumule length (cm) of weeds on agar: Agar solution (0.75% w/v) (gelling

temperature 30-31°C, Nalge Nunc Intl Roskilde, Denmark) was autoclaved at 121°C for fifteen minutes. The plant material (*R. stricta* leaf powder) was weighed carefully (50, 30 mg) and rolled gradually into wells of a 6 well multi-well plate. The 1st layer of agar (5 ml) was applied by using pipette, powder rose up which was then allowed to gelatinize and a second layer of agar was applied on top of it. In each bowl, ten seeds of each species were put above agar. Aluminum foil was used to protect them from light, and kept at 25°C in the growth chamber (NTS Model MI-25S). After fifteen days, length of hypocotyl and radicle was noted for each test species. Treatment was replicated 5 times each, and results were shown as $5n \pm SD$. The percentages of germination, plumule lengths and radicle of each test species were calculated by comparison with the control.

Inhibition effects of *R. stricta* leaf allelochemicals on germination (%), radicle and plumule length (cm) of weeds on filter paper: 10 mg and 50 mg of *R. stricta* leaf powder were separately soaked in distilled water (100 ml) in a flask and agitated at 25°C for 24 h on an orbital shaker (160 rpm). With muslin cloth, the extract was strained and then filtered by Whatman filter paper No 1. To get aqueous extract, the mixture filtrate and the final volume was adjusted to 100 ml, resulting in water extract of 10 and 50%. On filter paper together with 5ml distilled water per petri dish, 5ml dried leaf powder extract *R. stricta* was added. On every sterilized petri dish, ten test species surface sterilized seeds were placed. Covered with aluminum foil and wrapped with squash tape, the petri dishes were put in the growth chamber (NTS Model MI-25S) at 25°C for 15 days. Each treatment was replicated by 5 times. By comparing with control, the percentages of germination, plumule and radicle lengths of each weed were calculated.

Inhibition effects of *R. stricta* leaf allelochemicals on germination (%), plumule and radicle length (cm) of weeds on soil: Seeds of selected test species (10 seeds for each species) were sown in petri dishes with 25 g soil. Then 15 ml of water was liquidated on the soil in control and extract. Five ml distilled water / extract was used in petri dishes as a control. Each treatment was replicated by 5 times. Glass petri plates were covered in aluminum foil, sealed with tape and incubated at room temperature for 15 days in growth chamber (NTS Model MI-25S). For each test species based on comparison with regulation, germination percentages, plumule and radicle lengths were determined.

Statistical analysis

A completely randomised design (CRD) was utilized for the experiments. Using STATISTIX 9, the statistical investigation was done and by applying the protected LSD test from Fisher, means were separated.

Results

Inhibition effects of *R. stricta* leaf allelochemicals on germination (%), plumule and radicle length (cm) of weeds on agar:

Statistical review of data relating to effect of leaf powder of *R. stricta* on plumule, radicle length and germination percentage of weed species on agar as shown in (Tables 1-3). The data revealed that 37%, 30%, and 31% germination percentage inhibition were shown by *C. album*, *P. minor*, and *R. dentatus*. *R. stricta* leaf powder (50 mg), whereas the germination percentage of *E. helioscopia*, *A. fatua* and *T. aestivum*, has no or very little

noteworthy effect. Maximum germination percentage was observed for wheat crop (97.78% germination, 2.22% inhibition), *A. fatua* (97.68% germination; 2.32% inhibition), and *E. helioscopia* (97.65% germination; 2.35% inhibition). Max germination percentage for weed species was observed for *P. minor* i.e., 68.24%. Compared to their respective control results revealed that *R. stricta* leaf powder on agar induces substantial radicle length (cm) reduction in *R. stricta* and *A. fatua* measuring 46.87% and 45.67% respectively at 50 mg, whereas in *A. fatua* and *R. dentatus* measuring 39.82% and 39.02% respectively at 10 mg radicle length (cm) reduction.

Table 1. Inhibition effects of *R. stricta* leaf allelochemicals on germination (%) of weeds.

Treatments (P _T)		Test species					
		<i>T. aestivum</i>	<i>A. fatua</i>	<i>R. dentatus</i>	<i>P. minor</i>	<i>E. helioscopia</i>	<i>C. album</i>
Media							
Filter paper	10 mg	87a (-3.33)	83a (-2.35)	61b (-31.46)	62b (-25.30)	84a (-2.32)	58b (-28.39)
	Control (P _C)	90a	85a	89a	83a	86a	81a
Soil	50 mg	88a (-3.29)	84a (-2.32)	53b (-41.11)	56b (-33.33)	85a (-2.29)	51b (-37.80)
	Control (P _C)	90a	86a	90a	84a	87a	82a
Agar	10 mg	89a (-1.11)	85a (-1.1)	58b (-34.83)	59b (-30.58)	84a (-1.17)	57b (-28.75)
	50 mg	88a (-2.22)	84a (-2.32)	56b (-37.07)	58b (-31.76)	83a (-2.35)	56b (-30.00)
	Control (P _C)	90a	86a	89a	85a	85a	80a
¹ LSD		12.722	18.46	15.97	14.11	18.45	18.698
² F-value		28.64*	25.38*	11.79*	12.68*	21.73*	10.39**

¹Germination (%) Inhibition = 100 (P_T-P_C) / P_C Where P_T and P_C represent germination in treatment and control respectively

²Means followed by different letters within column differ significantly (*p*<1%)

Table 2. Inhibition effects of *R. stricta* leaf allelochemicals on radicle length (cm) of weeds.

Treatments (P _T)		Test species					
		<i>T. aestivum</i>	<i>A. fatua</i>	<i>R. dentatus</i>	<i>P. minor</i>	<i>E. helioscopia</i>	<i>C. album</i>
Media							
Filter paper	10 mg	9.25a (-2.23)	5.59b (-38.70)	5.46b (-40.58)	8.14a (-1.80)	7.52a (-5.17)	9.16a (-1.39)
	Control (P _C)	9.46a	9.12a	9.19a	8.29a	7.93a	9.29a
Soil	50 mg	9.18a (-2.85)	5.04b (-45.86)	4.69b (-49.07)	8.22a (-2.02)	7.26a (-0.95)	9.13a (-2.03)
	Control (P _C)	9.45a	9.31a	9.21a	8.39a	7.33a	9.32a
Agar	10 mg	9.24a (-0.75)	5.56b (-39.82)	5.75b (-39.02)	8.42a (-1.40)	7.65a (-3.28)	9.84a (-0.90)
	50 mg	9.18a (-1.39)	5.02b (-45.67)	5.01b (-46.87)	8.31a (-2.69)	7.32a (-7.45)	9.64a (-2.92)
	Control (P _C)	9.31a	9.24a	9.43a	8.54a	7.91a	9.93a
¹ LSD		1.9087	1.2958	0.6098	0.6841	0.8697	0.8164
² F-value		47.98*	97.75*	214.14*	166.39*	136.19*	280.93*

¹Growth inhibition (%) = 100 (P_T-P_C) / P_C Where P_T and P_C represent radicle length of the treatment and control respectively

²Means followed by different letters within column differ significantly (*p*<5%)

Table 3. Inhibition effects of *R. stricta* leaf allelochemicals on plumule length (cm) of weeds.

Treatments (P _T)		Test species					
		<i>T. aestivum</i>	<i>A. fatua</i>	<i>R. dentatus</i>	<i>P. minor</i>	<i>E. helioscopia</i>	<i>C. album</i>
Media							
Filter paper	10 mg	4.43b (-53.51)	5.76b (-31.83)	6.25b (-31.46)	8.09a (-0.49)	9.02a (-1.09)	7.19a (-3.48)
	Control (P _C)	9.53a	8.45a	9.12a	8.13a	9.12a	7.45a
Soil	50 mg	4.05b (-55.59)	5.41b (-32.96)	5.99b (-34.17)	8.89a (-0.67)	9.20a (-1.28)	7.64a (-3.53)
	Control (P _C)	9.12a	8.07a	9.10a	8.95a	9.32a	7.92a
Agar	10 mg	4.67b (-49.73)	5.94b (-30.03)	6.46b (-28.46)	8.78a (-2.00)	9.22a (-1.17)	7.69a (-2.03)
	50 mg	4.18b (-55.00)	5.85b (-31.09)	6.13b (-32.11)	8.68a (-3.12)	9.11a (-2.35)	7.51a (-4.33)
	Control (P _C)	9.29a	8.49a	9.03a	8.96a	9.33a	7.85a
¹ LSD		2.3602	0.8448	0.7640	1.9434	1.0519	0.9319
² F-value		9.74**	70.85*	101.94*	70.9*	84.21*	205.87*

¹% Growth inhibition = 100 (P_T-P_C) / P_C Where P_T and P_C represent plumule length of the treatment and control respectively

²Means followed by different letters within column differ significantly (*p*<5%)

On the radicle length of *P. minor*, *E. helioscopia*, *C. album* and *T. aestivum*, no major effect was observed. For *R. dentatus*, it is also evident from the result that the lowest radicle length (cm) i.e., 53.13% was observed at 50 mg while for *A. fatua*, the lowest radicle length (cm) was observed at 10 mg i.e., 60.18%. The data also indicated that *T. aestivum* (98.61% growth, 1.39% inhibition); *P. minor* (97.31% growth, 2.67% inhibition), *E. helioscopia* (92.55% growth, 7.45% inhibition) and *C. album* (97.08% growth, 2.92% inhibition) were noted for maximum development for radicle length (cm). Further, the results revealed that concentration based on *R. dentatus* and *A. fatua* was an allelopathic inhibitory effect. The results also indicated that the lowest length of the plumule was 44% for *R. dentatus*. *A. fatua* and *T. aestivum* and showed inhibition of the length of the plumule at 49.73%, 30.03% and 28.46% respectively at 10 mg, while at 50 mg *T. aestivum*, *R. dentatus* and *A. fatua* showed inhibition of the length of the plumule at 55%, 32.11% and 31.09% compared to their control, though very little to no significant effect on the plumule was observed.

Inhibition effects of *R. stricta* leaf allelochemicals on plumule and radicle length (cm), germination (%) of weeds on soil and filter paper: Tables 1-3 presents data on the allelopathic effect of *R. stricta* aqueous extract on percentage radicle and plumule length, weed germination. It was observed that in *R. stricta* aqueous extract on filter paper, *P. minor*, *C. album* and *R. dentatus* exhibited 74.7, 68.54 and 71.61% germination percentage inhibition, respectively. Similarly, in soil *R. stricta* aqueous extract significantly repressed *C. album*, *R. dentatus* and *P. minor* seed germination percentage by 41.11%, 37.80% and 33.33%, respectively, compared to their respective controls. The experimental results showed that germination percentage of *E. helioscopia*, *A. fatua* and *T. aestivum* had no significant effect. Tests revealed maximum percentage germination for was observed *A. fatua* (97.65% germination, 2.35% inhibition), *T. aestivum* (96.67% germination, 3.33% inhibition) and *E. helioscopia* (97.68% germination, 2.32% inhibition), respectively. It was shown that minimum germination was found on soil and filter paper for *C. album* i.e., 71.61% & 62.20% and *R. dentatus* i.e., 68.54% & 58.89% respectively, in the present analysis. It was concluded that germination percentage reduction of *P. minor*, *R. dentatus* and *C. album* was dependent on concentration, with concentration increase, the percentage inhibitory effect increased gradually. The data further suggested that germination of *E. helioscopia*, *A. fatua* and *T. aestivum*, remained natural at any concentration *R. stricta* aqueous extract on soil. The experimental outcomes indicated that *A. fatua* and *R. dentatus* showed 40.58% and 38.70% radicle length (cm) inhibition respectively in *R. stricta* water extract on filter paper whereas no significant effect on germination of *T. aestivum*, *C. album* and *P. minor* showed opposition to the allelopathic extract. Likewise, *A. fatua* and *R. dentatus* were most susceptible to *R. stricta* aqueous extract on soil, showing substantial suppression

in radicle length (cm) measuring 49.07% and 45.86% compared to their respective regulation. The data indicates calculation of the maximum radicle length (cm) for *T. aestivum*, that is, 97.15%. The results showed that for *A. fatua* i.e., 61.30% & 54.14% and *R. dentatus* i.e., 59.42% & 50.93% for filter and soil respectively the minimum radicle length (cm) was noted. The data concluded that the *A. fatua* and *R. dentatus* decrease in length of radicle was dependent on concentration, with concentration increase, the inhibitory effect was increased. The findings of the current study showed that *A. fatua*, *T. aestivum*, and *R. dentatus* displayed an inhibition of 53.51 per cent, 31.83% and 31.46% plumule length (cm), respectively, in *R. stricta* aqueous extract on filter paper, while *C. album*, *E. helioscopia*, and *P. minor* displayed very little to no effect on length (cm) of plumule. *R. stricta* aqueous extract on soil substantially inhibited *T. aestivum* plumule length (cm), *R. dentatus* and *A. fatua* measuring 55.59%, 32.96% and 34.17% respectively. For *P. minor* (99.33% development, 0.67% inhibition), *E. helioscopia* (98.72% rise, 1.28% inhibition), and *C. album* (96.47% growth, 3.53% inhibition), the statistical analysis suggested that maximum plumule length (cm) be noted. Statistical data concluded that *T. aestivum* had a minimum plumule length (cm) of 46.49 per cent and 44.41 per cent respectively on soil and filter paper. The statistics endorsed that the suppression potential for, *A. fatua*, *T. aestivum* and *R. dentatus* was gradually diminished with the decrease in concentration. The statistical findings suggested that *C. album*, *E. helioscopia* and *P. minor* be completely resistant on filter paper to *R. stricta* leaf aqueous extract.

Discussion

Because of the negative effect of synthetic chemicals, with novel sites of action, the new classes of chemicals may be highly in demand. Biodegradable products such as plant-derived ones show structural variety. These are very rarely formed by halogenated atoms. And have potential to develop as herbicides. In the current study, maximum germination (%) for *E. helioscopia*, *A. fatua* and *T. aestivum*, noted using agar as a medium. While for *C. album* the minimum percentage of germination was noticed. For the *E. helioscopia*, *T. aestivum* *C. album* and *P. minor* the highest radicle length (cm) was noted. While for *R. dentatus* minimal radicle length (cm) was noted. Data revealed that for *C. album*, *P. minor* and *E. helioscopia* the maximum length (cm) of plumule was observed. The results further show that *T. aestivum* was illustrious for the lowest plumule length (cm). Inderjit & Duke (2003) also investigated the inhibitory effects of allelochemicals from the *R. stricta* leaf powder on the weed species. Similar allelopathic effects from other plants were noted by the findings from the current study (Hussain *et al.*, 2011). *R. stricta* phytotoxicity may be due to allelochemicals percolated in the natural surroundings from their leaves (Batish *et al.*, 2002). The data showed further that maximum

germination percentage for *T. aestivum*, *E. helioscopia* and *A. fatua* were observed for filter paper and soil as the medium. The experimental results showed that minimum germination had been observed on soil and filter paper respectively for *R. dentatus* and *C. album*. The statistical data specified that *C. album*, *E. helioscopia*, *P. minor* and *T. aestivum* displayed the highest radicle length (cm). The results also declared that *R. dentatus* and *A. fatua* measuring on soil and filter paper respectively had a significant lowest radicle length (cm). The statistical study recommended that *E. helioscopia*, *C. album* and *P. minor* should have a maximum plumule duration (cm). Statistical evidence suggested that *T. aestivum* had a total plumule length (cm). The results are in harmony with Chon *et al.* who observed that growth with *R. stricta* leaf aqueous extracts was reduced due to toxic extract factors (Chon *et al.*, 2005). Statistical analysis revealed significant variances between test treatments with various concentrations with *R. stricta* extract and power. Hegazy and Fadl-Allah and El-Khatib had noted that the vulnerability to allelochemicals enhanced and some morphological abnormalities happened in the radicle and plumule. They also stated that, contrary to filter paper, growth is more affected by allelopathic interaction on soil (El-Khatib, 2000; Hegazy *et al.*, 1995). Such observations coincided with those found by Migahid and Elkhazan noted the allelopathic effect on sesame and corn of *Zygophyllum* aqueous extracts (Migahid and El-Khazan, 2002). Shehata and El-khawas described the inhibitory effect of changing the activity of enzymes, during growth affect the transport of seed storage substances (El-Khawas & Shehata, 2005). Wardle *et al.*, (1992) note that for all weeds studied, the percentage of radicle and plumule growth and seed germination was significantly verified in *R. stricta* extracts, and this capacity for retardation depended on concentration. Additionally, *R. stricta* extracts influence osmotic factors of the weeds tested (Wardle *et al.*, 1992).

Conclusion

Biodegradable products such as plant-derived products show structural variety and have screening potential for natural weed control. Such natural compounds are friendly to the environment, and rarely consist of halogenated atoms. Therefore, these products present one such class of chemicals and are likely to be developed as herbicides. Plant-harming growth is commonly known as those that have biologically active natural products. However, the retarding effect of growth on wheat seedlings in current experiments indicates that *R. stricta* might not be an acceptable candidate for weed control under field conditions.

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