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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (7): 1141-1143, 2007
ISSN 1028-8880

Allelopathic Potential of *Trifolium resupinatum* and *T. alexandrium* on Seed Germination of Four Weed Species

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Abstract: Laboratory studies were conducted to determine the seed germination of *Amaranthus retroflexus*, *Convolvulus arvensis*, *Secale cereale* and *Sinapis arvensis* as affected by water-and methanol-soluble constituents of Persian and Berseem clovers. Aboveground tissues of the clovers were collected during flowering stage and dried. Three concentrations of aqueous and methanolic extracts were used: full-strength (33.3 g L^{-1}), half-strength (16.7 g L^{-1}) and quarter-strength (8.3 g L^{-1}). The weed seeds were placed in petri dishes contained the legume extract, or distilled water (control). Percent seed germination was taken after a week. In general, seed germination of the weed species declined progressively with increasing concentration of the clover extracts. It was found that wild mustard exhibited the greatest sensitivity to both the legume extracts. Compared with aqueous extract, methanolic extract caused more decline in seed germination of the weeds. In addition, compared to Persian clover, Berseem clover was stronger inhibitor of seed germination. Therefore, the amounts of allelochemicals may be different in these clovers. Field bindweed showed the least sensitivity to both the legume extracts. Therefore, field bindweed had probably more tolerance to the allelochemicals produced by the clover species.

Key words: Allelopathy, seed germination, weed species, clover, *Trifolium* spp.

INTRODUCTION

Cover crops are fundamental sustainable tools used to manage soil fertility, soil quality, water, weeds, pests and diseases in agroecosystems (Lue *et al*., 2000). Thick cover crop stands often compete well with weeds during the cover crop growth period and can prevent most germinated weed seeds completing their life cycle and reproducing. Furthermore, even when seeds germinate, they often run out of stored energy for growth before builiding the necessary structural capacity to break through the cover crop mulch layer (Kobayashi *et al*., 2003). In addition to competition-based or physical weed suppression, certain cover crops are known to suppress weeds through allelopathy (Singh *et al*., 2003; Kamo *et al*., 2003). Some well known examples of allelopathic cover crops are *Secale cereale* (rye), *Vicia villosa* (hairy vetch), *Trifolium* spp., (clover species) *Sorghum bicolor* (sorghum-sudangrass) and species in Brassicaceae family, particularly mustards (Haramoto and Gallandt, 2004). The use of cover crops for weed control is an alternative that is less harmful to the environment than pesticides or other chemical practices. Numerous authors have considered the role of cover crops as a component of integrated weed management (Uchino *et al*., 2005; Qasem, 1995).

Allelopathy is a phenomenon of chemical regulating and controlling in natural ecological systems and is a mechanism of organisms accommodating environment (Peng *et al*., 2004). Recent years, a lot of research has been conducted in the allelopathy of plants worldwide (Mo *et al*., 2005). The objective of this study was to determine if these clovers contain water- and methanol-soluble phytotoxic constituents that affect the seed germination of *Amaranthus retroflexus*, *Convolvulus arvensis*, *Secale cereale* and *Sinapis arvensis*.

MATERIALS AND METHODS

The experiment was done during 2004 in Plant Pests and Diseases Research Institue. Aboveground plant tissue was collected at flowering stage from field-grown Persian and Berseem clovers in Karaj. Three concentrations of aqueous and methanolic extracts were

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used: full-strength (33.3 g L^{-1}), half-strength (16.7 g L^{-1}) and quarter-strength (8.3 g L^{-1}). Water and methanol extract preparation and seed germination studies were done using White *et al.* (1989) method.

The data was analyzed with analysis of variance (ANOVA) for factorial design (factor A: clover species, factor B: weed species, factor C: water extract concentration, factor D: methanolic extract concentration) with 3 replicates using ANOVA program of MSTATC. The results were subjected to a statistical analysis using Duncan Multiple Range Test.

RESULTS

In Persian clover, weed seed germination declined with increasing concentration of water extract. Mustard, rye and amaranth germination were inhibited completely by full-strength. Mustard germination was also inhibited completely by half-strength. Rye germination was suppressed completely by all water extract concentrations. Half-and quarter-strength extracts, declined germination of amaranth and mustard by 61 and 52%, respectively (Table 1).

As shown with aqueous extract, weed germination decreased progressively with increasing concentration of the metanolic extract. Full-strength extract inhibited completely rye germination. Mustard germination was inhibited completely at all extract concentrations. Germination of field bindweed and amaranth declined 60 and 53% of control at full-strength extract. In response to half-strength extract, rye germination decreased by 56% (Table 2).

As Persian clover, in Berseem clover weed germination was also decreased significantly as the concentration of the aqueous extract increased. Full-strength extract, suppressed completely germination of amaranth, rye and mustard. In response to this concentration, field bindweed germination, decreased by 74%. Half-strength extract suppressed amaranth germination by 62% and inhibited completely rye and mustard germination. The seed germination of rye and mustard were inhibited by quarter-strength extract, to an extent of 51 and 9%, respectively (Table 3).

According to Table 4, mustard germination was inhibited completely in response to all of methanolic extract concentrations. Full-and half-strength extracts, suppressed completely amaranth germination. Full-strength extract, reduced germination of field bindweed and rye, by 55 and 56%, respectively. Quarter-strength extract, inhibited field bindweed, amaranth and rye germination by 81, 15 and 40%, respectively (Table 4).

Table 1: Changes in seed germination percent of the weeds in response to different concentrations of aqueous extract of Persian clover

Aqueous extract concentration (g L^{-1})	Field bindweed	Amaranth	Rye	Mustard
0	86	85.3	18.3	86.67
8.3	70.67	77.3	0	44.67
16.7	50	52	0	0
33.3	45.3	0	0	0

Table 2: Changes in seed germination percent of the weeds in response to different concentrations of methanolic extract of Persian clover

Methanolic extract concentration (g L^{-1})	Field bindweed	Amaranth	Rye	Mustard
0	76	86.67	16.67	81.33
8.3	77.33	85.33	7.33	0
16.7	56	61.33	9.33	0
33.3	45.3	46	0	0

Table 3: Changes in seed germination percent of the weeds in response to different concentrations of aqueous extract of Berseem clover

Aqueous extract concentration (g L^{-1})	Field bindweed	Amaranth	Rye	Mustard
0	86	85.3	18.3	86.7
8.3	78.7	69.3	9.3	7.7
16.7	75.3	52.7	0	0
33.3	63.3	0	0	0

Table 4: Changes in seed germination percent of the weeds in response to different concentrations of methanolic extract of Berseem clover.

Methanolic extract concentration (g L^{-1})	Field bindweed	Amaranth	Rye	Mustard
0	72.7	86.7	16.7	80.7
8.3	58.7	13.3	6.7	0
16.7	56.7	0	9.3	0
33.3	40	0	9.3	0

DISCUSSION

Cover crops may play an important role in sustainable agriculture because of their ability to reduce soil erosion and nitrate leaching and through increasing soil water infiltration rate, soil organic matter content and nutrient availability. An additional feature of cover crops is their ability to suppress weeds. Residues and extracts of cover crops were found to cause allelopathic suppression of certain weed species. These data suggest that a properly managed cover crop may be utilized as an additional component in weed management strategies for no-till cropping systems (White *et al.*, 1989).

The present research with Persian and Berseem cloves revealed that these species imposed an allelopathic influence on weed species. Water and methanolic extracts, had negative impact on weed germination through this mechanism. According to Williams *et al.* (1998), cover crop residues affect weed suppression. In the presence of *Trifolium subterraneum* residues, weed biomass or density was reduced for *Amaranthus retroflexus*, *Lolium perenne* and *Sinapis arvensis.* Dyck and Liebman (1994) found that time to 50% emergence of *Amaranthus* was delayed 3.4 day in the presence of soil-incorporated Crimson clover residue. Crimson and subterraneum clovers have previously been shown to inhibit weed growth and germination and allelopathy was implicated as the cause (Lehman and Blum, 1997). The presence of allelochemicals like phenolic acids in the clover biomass, may be the reason for poor germination of the weeds (Challa and Ravindra, 1998). Results of the experiments done by Qasem (1995), confirmed the harmful effects that clover species imposed on weed species and the ecological significance of such effects. Toxic chemicals released through clover shoot exudates to the growing media might have an important role in the dominance of clover species. Allelopathy through the production and activity of allelochemicals play a major role in weed dynamics (Challa and Ravindra, 1998).

It seemed that the extracts of Berseem clover had relatively higher phytotoxicity. Because, Berseem clover was stronger inhibitor of weed germination. According to Challa and Ravindra (1998), quantities of allelochemicals vary with plant species, tissue, phenology and environmental conditions. Differences in allelopathic potential of different clover species expected due to variations in the nature and amount of allelopathic agents and their concentrations (Qasem, 1995). Differences in the sensitivity of the weed species to allelopathic potential, were clear and mustard was the most affected weed with the extracts of the clovers. Qasem (1995), has also observed that mustard was the most affected weed with other weeds extracts. The most tolerant weed, was field bindweed. Several workers have also reported differences between weeds in their tolerance to allelopathic effects confirming that allelopathy was mainly a selective mechanism (Williams *et al*., 1998).

As compared to water extracts, methanolic extracts caused more decline in weed germination. It seemed that more allelochemicals of the both clover species are soluble in organic solvents such as methanol.

CONCLUSIONS

The results of this study provide evidence that the extracts of Persian and Berseem clovers have allelopathic potential and that some of the phytotoxins are water-soluble and others are soluble in organic solvents such as methanol. Identification of the compound(s) for the effects observed, would further define allelopathic interaction. This study demonstrates that the effect of cover crop extracts on weed germination varies depending on weed and cover crop species, the concentration and the kind of the extracts.

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