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Phytotoxic effects of selected medicinal plants collected from Margalla Hills, Islamabad Pakistan

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The present studies cover the phytotoxic effects of the crude methanolic extracts of different parts of 13 medicinal plants viz. *Woodfordia fruiticosa*, *Adhatoda vasica*, *Chenopodium ambrosoides*, *Viburnum cotinifolium*, *Euphorbia hirta*, *Vitex negundo*, *Peganum harmala*, *Broussonetia papyrifera*, *Taraxacum officinale*, *Urtica dioica*, *Verbascum thapsus*, *Caryopteris grata* and *Mimosa rubicaulis* collected from different localities of Margalla Hills on the germination of radish seeds to study the germination %, growth inhibition %, root shoot length, velocity of germination, biomass fresh weight, dry weight and moisture content (%) at two concentration levels. Germination velocity was decreased by the application of the extracts, however more pronounced effect was seen at 10 mg/ml concentration. Maximum decrease in germination velocity of radish seed was exhibited by methanolic extract of *W. fruticosa* (25.23) and minimum by *V. negundo* (39.06). Maximum inhibition of radish seed germination was caused by *B. papyrifera* (53.33%), *W. fruticosa* (52%), *V.thapsus* (48.89%) and minimum by *M. rubicaulis* (13.33%). At higher concentration that is, 10 mg/ml, the methanolic extract of *W. fruticosa* was most effective in decreasing the shoot fresh weight (0.15 gm), followed by *B. papyrifera* (0.29 gm), *Caryopteris grata* (0.39 gm), *U. dioica* (0.49 gm), *V.thapsus* (0.50 gm) and *P. harmala* (1.44 gm). The extract of *W. fruticosa* was more pronounced in decreasing the shoot dry weight (0.07 gm) followed by *B. papyrifera* (0.08 gm), *V. thapsus* (0.1 gm) and the least effective was the *M. rubicaulis* (0.21 gm). However at concentration 1 mg/ml, *T. officinale* exhibited maximum decrease in germination velocity (35.65) and maximum inhibition of seed germination was caused by methanolic extract of *U. dioica* (42.22%), followed by *V. thapsus* (40%). Lower concentrations of *V. negundo* and *T. officinale* exhibited similar effects on germination velocity. The intensity of decrease in moisture content at concentration 1mg/ml was lower than that at 10 mg/ml. Maximum reduction in seedling moisture content was also recorded at concentration 1 mg/ ml for *W. fruticosa* (83.1%), followed by *V. cotinifolium* (84.51%) and *B. papyrifera* (86.42%) and minimum for that of *V. thapsus* (90%). The *P. harmala* at low concentration (1 mg/ ml) promoted the growth rather showing the allelopathic effects.The phytotoxic activity of the selected medicinal plants on radish seed germination was dose dependent.

Key words: Medicinal plants, Margalla hills, phytotoxicity.

INTRODUCTION

Weed species are frequently considered to be competitive because they grow vigorously in crops and affect the crop yields. The possession of certain biological characteristics has the potential to predispose a species to exhibiting weediness. An ideal weed has the ability to show stronger and also interspecific competition via special mechanisms such as allelopathic processes (Mortimer, 1990). Allelopathic chemicals play an important role in determination of the persistence and abundance of the weed species in mixtures of the plants. The allelopathic effects of weeds on crops have been extensively studied since 1970 (Rice, 1979). According to Rice, the modification of seed germination and plant growth is one of the obvious manifestations of allelopathy, and germination is one of the important tools for the study of allelopathy. Allelopathy is a mechanism in

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Table 1. Preparation of stock solution for phytotoxicity against radish seed germination.

S. No	Concentration (mg/ml)	Stock solution (ml)	Methanol (ml)	Final volume (ml)
1	10	30	0	30
2	1	3	27	30

which chemicals produced by weed plants may increase or decrease the associated plant growth. Molish (1937) coined the term "allelopathy" as an interaction among the plants and the microorganisms. Rice (1984) defined allelopathy as the effects of one plant (including microorganisms) on another plant via the release of chemicals into the environments. Allelopathy is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth (Harper, 1977; May and Ash, 1990). Allelopathy may also play an eminent role in the intraspecific and interspecific competition and may determine the type of interspecific association.

The plant may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in the immediate vicinity. It offers potential for selective biological weed management through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots of living or dead decomposing plant materials (Weston, 1996). The term allelopathy refers to biochemical interactions among the plants, including those mediated by microorganisms. This broad definition of allelopathy is appropriate as considerable research has indicated the involvement of microorganisms and lower plants in production of phytotoxins (Garlado and Chilton, 1992).

Exotic and native species in competition produce a large amount of toxins (Allelochemical substances) which effectively repel other species and thus their ability to invade the whole plant community is increased (Indergit and Dakshini, 1998). Many secondary metabolites acting as allelochemicals include alkaloids, phenols and terpenoids. Phenols are the most abundant substances under the field conditions that affect the seed germination, seedling growth, cell division and fungal activity (Lodhi, 1976). Many laboratory techniques have been developed for the measurement and quantification of allelopathy without interfering the resource competition (Leather and Einhelling, 1986; Navarezand-Olofsdotter, 1996; Kawaguchi et al., 1997). Large screenings of germ plasm collection require reliable test species. It is a common tradition that easily grown but sensitive reliable species like *Lemna minor*, Lettuce (*Lactuca sativa*) and radish (*Raphanus sativa*) seeds have been used as test plants in allelopathic studies (Putnam et al., 1983; Einhelling et al., 1985; Leather and Einhelling, 1985). This assay has a wide range of application in research towards the discovery of active principles in plants (Arzu et al., 2002).

MATERIALS AND METHODS

The method was followed as reported by Atta-ur-Rehman (1991) and Arzu (2000). Radish (*R. sativus* L.) seed germination and root length parameters were assayed and two different concentrations were used for this purpose. The assay was carried out in laminar flow to avoid contamination.

Preparation of stock solutions

500 mg methanolic plant extract was dissolved in 50 ml of methanol to get a stock solution 10 mg/ml that is, 10,000 mg/L or 10,000 ppm concentration. Stock solution was further diluted to 1000 ppm with methanol (Table 1) Autoclaved distilled water and pure methanol were used as positive and negative control, respectively.

Surface sterilization of radish seeds

0.1% $HgCl_2$ (mercuric chloride) solution was prepared in a beaker. Radish seeds were put in it for 2 to 3 min rinsed with autoclaved distilled water and finally dried them with sterilized blotting paper.

Bioassays for inhibition of root length

0.5 ml of each concentration was put in sterilized (autoclaved) 10 cm petriplate containing a sterilized filter paper (Whatman # 1). Methanol was vacuum evaporated and then 5 ml autoclaved distilled water was added to each petriplate. Three replicates were prepared for each concentration. For negative control, 5 ml methanol was added to the plate, it was vacuum evaporated and then 5 ml autoclaved distilled water was added to it. For positive control only 5 ml autoclaved distilled water was added to each plate. Three replicates were prepared for each control. Sterilized 15 Radish seeds were placed at sufficient distances with sterilized forecep in each plate. Petri plates were incubated in dim light at 25°C. Root length was measured with the help of scale after 3 and 5 days and percentage age inhibition of the root length was calculated as:

$$\% \text{ inhibition of the root length} = \frac{\text{Root length in test sample}}{\text{Root length in control}} \times 100$$

The number of seeds germination was counted daily till completion of germination. % germination and speed of germination "S" was calculated following Khandakar and Bradbear (1983) as:

$$S = [N_1/1 + N_2/2 + N_3/3 \text{----------} N_n/n] \times 100/1$$

Where N_1, N_2, N_3-------- N_n = proportion of seeds which germinated on day 1, 2, 3-----n following set up of the experiment. 'S' varies from 100 (if all the seeds germinated on the first day following set up) to 0 (if no seeds are germinated by the end of the experiment).

Table 2. Phytotoxic effects of selected medicinal plants collected from Margalla Hills on seed germination (%), germination velocity, shoot fresh and dry weight (g) of radish at 10 mg/ml concentration of methanolic extract. The data represents mean of three replicates.

Plant species	Phytotoxicity against radish seeds (10 mg/ml)					
	Germination velocity	Germination (%)	Inhibition (%)	Fresh weight	Dry weight	Moisture (%)
Woodfordia fruticosa	25.23±0.14	48.88±4.44	52±0.00	0.15±0.01	0.07±0.00	55.33±1.7512
Adhatoda vasica	37.33±1.36	64.44±5.87	35.56±5.87	0.97±0.08	0.18±0.02	81.58±0.83
Chenopodium ambrosoides	32.77±1.32	55.55±4.44	44.44±4.44	0.53±0.03	0.12±0.00	76.82±2.09
Viburnum cotinifolium	36.56±0.35	62.21±4.443	37.78±4.44	1.07±0.10	0.17±0.029059	82.74±4.476101
Euphorbia hirta	32.58±1.53	73.33±16.77	26.67±16.77	0.89±0.11	0.15±0.05	83.95±3.93
Vitex negundo	39.06±0.80	73.33±3.85	26.67±3.85	1.06±0.11	0.17±0.01	83.57±0.34
Peganum harmala	35.51±0.46	66.66±3.84	33.33±3.84	1.44±0.01	0.15±0.01	89.78±0.88
Broussonetia papyrifera	34.16±4.31	46.66±3.84	53.33±3.84	0.29±0.04	0.08±0.01	68.05±8.88
Taraxacum officinale	37.75±0.34	71.11±4.44	28.89±4.44	1.34±0.08	0.14±0.02	89.41±0.73
Urtica dioica	37.71±0.40	64.44±2.22	35.56±2.22	0.49±0.03	0.16±0.01	67.26±0.75
Verbascum thapsus	29.25±4.05	51.11±18.98	48.89±18.98	0.50±0.16	0.1±0.01	74.85±8.48
Caryopteris grata	34.63±0.72	62.22±2.22	37.78±2.22	0.39±0.04	0.11±0.01	72.85±0.76
Mimosa rubicaulis	35.09±10.78	86.66±24.37	13.33±8.00	1.43±0.05	0.21±0.01	85.27±0.30
Control	38.63±0.31	100±0.00	0±0.00	1.99±0.00	0.55±0.17	79.39±1.86

This has advantage over percentage germination, because it is usually more sensitive ‘’S’’ indicator of allelopathic (Wardle et al., 1991).

RESULTS AND DISCUSSION

Phytotoxicity is an important attribute in determination of allelopathic potential of a plant species. Determination of phytotoxicity of a plant species help in the formulation of natural plant growth regulators or biological herbicides. Losses caused by weeds are well documented in many studies (King, 1966). During the present investigation, the phytotoxicity of selected 13 medicinal plant species was checked on radish. It was found that the germination velocity of radish seeds was decreased by methanolic extracts of selected plant species. However, their effect was more pronounced at 10 mg/ml concentration. Maximum decrease in germination velocity was exhibited by methanolic extract of *Woodfordia fruticosa* as compared to respective control and other plant species at 10 mg/ml. The ranking of selected plant species for their phytotoxic effects on germination velocity of radish seed at 10 mg/ml was as follows: *W. fruticosa*> *Verbascum thapsus* > *Euphorbia hirta*> *Chenopodium ambrosoides*> *Broussonetia papyrifera* > *Caryopteris grata* > *Mimosa rubicaulis* > *Peganum harmala* > *Viburnum cotinifolium* > *Adhatoda vasica* > *Urtica dioica* > *Taraxacum officinale* > *Vitex negundo* (Table 2). The results showed that maximum inhibition of radish seed germination was caused by *W. fruticosa* as compared to control and other plant species.

On the basis of inhibitory effect of methanolic extract at 10 mg/ml concentration on seed germination of radish, the selected plant species were ranked as follows: *B. papyrifera* > *W. fruticosa V. thapsus* > *C. ambrosoides* > *V. cotinifolium* = *C. grata* > *A. vasica* = *Urtica dioica* > *P. harmala* > *T. officinale* > *E. hirta* = *V. negundo* > *M. rubicaulis* (Table 2). The results presented in Table 2 and 3 showed that at higher concentration, shoot fresh weight was decreased by application of methanolic plant extracts as compared to control. It was found that methanolic extract of *W. fruticosa* were highly effective in decreasing the shoot fresh weight of redish as compared to other plant species. According to their phytotoxic effects on shoot fresh weight, the selected medicinal plant species were ranked as: *W. fruticosa* > *B. papyrifera* > *C. grata*> *U. dioica*> *V. thapsus* > *C.ambrosoides* > *E. hirta* > *A. vasica* > *V. negundo* > *V. cotinifolium* > *T. officinale* > *M. rubicaulis* > *P. harmala*. Like shoot fresh weight, the dry weight of radish was also decreased by the application of methanolic plants extract. Maximum phytotoxic effects on shoot dry weight of radish were exhibited by methanolic extract of *W. fruticosa* as compared to control and other plant species which also exhibited greater reduction in shoot fresh weight.

The ranking of selected medicinal plants for their phytotoxic effects on radish seedling dry weight was as follows: *W. fruticosa* > *B. papyrifera* > *V. thapsus* > *C. grata* > *C. ambrosoides* > *T. officinale* > *E. hirta* = *P. harmala* > *U. dioica* > *V. negundo*= *V. cotinifolium* > *A. vasica* > *M. rubicaulis* (Table 2). The moisture content of radish seedling with respect to their fresh and dry weight was highly affected (less than 70%) by methanolic extract of *W. fruticosa* followed by *U. dioica, B. papyrifera.* The methanolic extracts of *C. grata, V. thapsus, C. ambrosoides, A. vasica, V. cotinifolium* exhibited moderate (more than 70%) effects on moisture content (Table 2). The results showed that phytotoxic activity of selected medicinal plants on radish was dose dependant. It was found that at 1 mg/ml of extract concentration,

Table 3. Phytotoxic effects of selected medicinal plants collected from Margalla Hills on seed germination (%), germination velocity, shoot fresh and dry weight (g) of radish at 1 mg/ml concentration of methanolic extract. The data represents mean of three replicates.

Plant species	Phytotoxicity against radish seeds (1 mg/ml)					
	Germination velocity	Germination (%)	Inhibition (%)	Fresh weight	Dry weight	Moisture (%)
Woodfordia fruticosa	38.85±1.19	82.21±4.44	17.79±0.00	1.12±0.15	0.18±0.02	83.11±1.16
Adhatoda vasica	36.71±1.44	77.77±4.44	22.23±4.44	1.91±0.10	0.24±0.03	87.50±1.07
Chenopodium ambrosoides	37.35±0.42	73.33±10.18	26.67±10.18	1.61±0.0	0.16±0.01	90.07±0.626906
Viburnum cotinifolium	36.54±1.14	77.77±4.44	22.22±4.44	1.53±0.06	0.19±0.01	84.51±2.24
Euphorbia hirta	36.31±0.89	62.21±4.44	37.78±4.44	1.26±0.01	0.13±0.00	89.39±0.647311
Vitex negundo	37.13±0.42	93.33±0.00	6.67±0.00	1.74±0.06	0.19±001	88.69±0.38
Peganum harmala	39.77±0.32	95.55±2.22	4.44±2.22	1.99±0.13	0.20±0.00	89.77±0.22
Broussonetia papyrifera	38.10±0.26	84.44±2.22	15.56±2.22	1.45±0.07	0.19±0.01	86.42±0.18
Taraxacum officinale	35.65±0.82	93.33±6.66	6.67±6.66	1.78±0.06	0.21±0.01	88.17±0.54
Urtica dioica	36.93±0.67	57.78±2.22	42.22±2.22	1.34±0.00	0.14±0.00	89.3±0.25
Verbascum thapsus	36.09±0.52	60±0.00	40±0.00	1.28±0.09	0.13±0.01	90.1±0.16
Caryopteris grata	37.88±0.86	71.11±2.22	28.89±2.22	1.19±0.01	0.15±0.00	87.18±0.15
Mimosa rubicaulis	39.49±0.08	82.21±4.44	17.78±4.44	1.65±0.16	0.2±0.02	87.89±0.53
Control	38.63±0.31	100±0.00	0±0.00	1.99±0.01	0.55±0.17	79.39±1.86

± represent the value of standard error.

the methanolic extract of *T. officinale* exhibited maximum decrease in germination velocity of radish. The sequence of different medicinal plants for their phytotoxic effects on germination velocity of radish at 1 mg/ml concentration was as follows: *T. officinale* > *V .thapsus* > *E. hirta* > *V. cotinifolium* > *A. vasica* > *U. dioica* > *V. negundo* > *C. ambrosoides* > *C. grata* > *B. papyrifera* > *W. fruticosa* > *M. rubicaulis* > *P. harmala* (Table 3). The results established that maximum inhibition of seed germination in radish was caused by methanolic extract of *U. dioica* followed by *V. thapsus*, *E. hirta* and *C. grata,* respectively.

The results further revealed that lower concentrations of *V. negundo* and *T. officinale* exhibited similar effects on germination velocity of radish. It was found that methanolic extract of *W. fruticosa* showed greater decrease in shoot fresh weight of radish seedling as compared to control and other plant species. On the basis of reduction in shoot fresh weight of radish seedling, the selected plant species were ranked accordingly (Table 3) *W. fruticosa* > *C. grata* > *E. hirta* > *V. thapsus* > *U. dioica* > *B. papyrifera* > *V. cotinifolium* > *C. ambrosoides* > *M. rubicaulis* > *V. negundo* > *T. officinale* > *A. vasica* > *P. harmala*. Like shoot fresh weight, the shoot dry weight was also decreased in radish by application of methanolic extracts of selected medicinal plant species as compared to control. Maximum reduction in shoot dry weight was exhibited by methanolic extract of *E. hirta* and *V. thapsus* as compared to other plant species. The ranking of selected plant species for their effect on shoot dry weight of radish was as follow: *V. thapsus* = *E. hirta* > *U. dioica* > *C. grata* > *C. ambrosoides* > *W. fruticosa* > *V. cotinifolium* = *V. negundo* = *B. papyrifera* > *P. harmala* = *M. rubicaulis* > *T. officinale* > *A. vasica* (Table 3). The moisture content of radish seedling with respect to their fresh and dry weight at 1 mg/ml was lower in radish seedling treated with methanolic extracts of selected medicinal plants as compared to control. However, the intensity of decrease was lower than that at 10 mg/ml concentration. Maximum reduction in seedling moisture content was recorded in seedlings treated with *W. fruticosa* followed by *V. cotinifolium* > *B. papyrifera* > *C. grata* > *A. vasica* > *M. rubicaulis* > *T. officinale* > *V. negundo* > *U. dioica* > *E. hirta* > *P. harmala* > *C. ambrosoides* > *V. thapsus* (Table 3). Pardo et al. (1997) isolated iridoid glucosides, lateroside, harpagoside, ajugol and aucubin from the ethanolic etracts of roots of *V. thapsus* which exhibited anti germination activity on the barley (*Hordeum vulgare*) seeds. Cameron et al. (1984) tested isolated irridoides at 1 - 5 mM concentration for anti germination tests and effects on embryo growth activity. During present investigation, it was also found that methanolic extracts of *V. thapsus* were highly effective in decreasing the germination velocity and germination percentage of radish seed. Similarly, the methanolic extracts of *W. fruticosa* were highly effective in decreasing the fresh and dry weight of radish seedling. From the results it can be concluded that methanolic extracts of *W. fruticosa*, *V. thapsus*, *B. papyrifera* and *C. ambrosoides* could be utilized in the formulation of biological weedicides. However, further researches in this direction are needed.

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