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PHYTOTOXIC EFFECT OF WATER SOLUBLE PHENOLICS FROM FIVE LEGUMINOUS WEEDS ON GERMINATION AND SEEDLING GROWTH OF RICE

Ali Zohaib[1], Asif Tanveer[1], Abdul Khaliq[1] and Muhammad Ehsan Safdar[2]

ABSTRACT

Allelopathic effects of five leguminous weeds viz., Lathyrus aphaca L., Medicago polymorpha L., Melilotus indica L., Vicia sativa L. and Trigonella polycerata L. against the germination and seedling growth of rice was studied. The 2.5% and 5% (w/v) aqueous extracts and 2% (w/w) soil incorporated residues with varying decomposition durations (0, 15, 30 days) of these weeds were used. Inhibitory effects against rice were shown by water extracts and residues of these weeds. The 100% germination inhibition was caused by 5% aqueous extract of M. indica. However, among others, 5% aqueous extracts of T. polycerata showed significantly lower germination percentage (20), germination index (0.40), root length (1.01 cm) and seedling vigor index (19.0) whereas V. sativa and L. aphaca resulted in higher mean germination times (6.92 and 6.75 days) of rice, respectively. Medicago polymorpha aqueous extract with 5% concentration and residues decomposed for 30 days produced significantly lower shoot lengths (3.8 and 4.72 cm) and seedling dry weight (4.49 and 4.84 mg) while significantly lower root lengths (1.01 and 2.36 cm) and seedling vigor indices (19.0 and 53.43) of rice seedlings were noted in response to 5% aqueous extract of T. polycerata and residues of V. sativa decomposed for 30 days which also resulted in minimum emergence percentage (22.5), emergence index (0.26) and mean emergence time (9.08 days). Presence of phenolic compounds such as caffeic acid, gallic acid, chlorogenic acid, vanillic acid, 4-hydroxy-3-methoxybenzoic acid, ferulic acid, p-coumaric acid, m-coumaric acid and syringic acid seem to be the cause of phytotoxity of these allelopathic weeds.

Keywords: Allelopathy, germination, leguminous weeds, phenolics, residues, rice.

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[1]Dept. of Agronomy, University of Agriculture, Faisalabad, Pakistan
[2]Dept. of Agronomy, University College of Agriculture, University of Sargodha, Pakistan
*Corresponding author's email: drasiftanveeruaf@hotmail.com

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INTRODUCTION

The plants growing together interact with each other through direct or indirect allelopathic interactions and exert inhibitory or stimulatory effects on growth of each other through releasing compounds known as allelochemicals (Kumar *et al*., 2006; Batish *et al*., 2007). Stimulatory or inhibitory effect of allelochemicals is dependent on their concentration (Hill *et al*., 2006). Allelochemicals are commonly found in decomposing plant residues, living plant exudates and volatile compounds released from leaves, roots and rhizomes (Narwal *et al*., 2005). Allelochemicals released from plants affect other plants mainly at their germination and seedling growth stages (Oyerinde *et al*., 2009). Allelopathic effects of water extracts of numerous weeds have been observed on crops (Sayed *et al*., 2012; Yasin *et al*., 2012). Similarly, harmful effects of decomposing weeds residues on emergence and growth of crops have also been documented (Singh *et al*., 2005; Thapar and Singh, 2006; Samad *et al*., 2008).

Weeds of Fabaceae family including *Lathyrus aphaca* L. (Yellow vetchling), *Medica gopolymorpha* L. (bur clover), *Melilotus indica* L. (Indian sweet clover), *Vicia sativa* L. (common vetch) and *Trigonella polycerata* L. (trefoil) are found abundantly in European, American, Australia, Africa and Asian countries including India and Pakistan. These occur as associated weeds of winter crops and vegetables in Pakistan. However, these are observed mostly in wheat (Ahmad and Shaikh, 2003). These weeds grow in October/November and complete their life cycle in March, thus leaving their residues in the field. Rice is cultivated after wheat in Pakistan in rice-wheat cropping system. The decomposing residues of these weeds release allelochemicals which may adversely affect the rice crop. Information related to allelopathic action of the plant leachates and decomposition products of these weeds on succeeding rice crop is not available. Such type of knowledge may act as a precaution forrice farmers to avoid any deleterious section of these weeds present in preceding winter crop. The present study was therefore, carried out to ascertain the allelopathic effect of water extracts and decomposing residues of these weeds at different concentrations and decomposing periods on germination/ emergence and seedling growth of rice.

MATERIALS AND METHODS

Laboratory experiments were carried out in Weed Science Laboratory(Latitude 31.25°N, longitude 73.09°E and altitude 184.4 m), Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during 2012-13 to study allelopathic effect of 5 weed species viz., *Lathyrus aphaca* L., *Medicago polymorpha* L., *Melilotus indica* L., *Vicia sativa* L. and *Trigonella polycerata* L. on germination and seedling growth of rice.

Preparation of water extracts

Whole plants of *L. aphaca*, *M. polymorpha*, *M. indica*, *V. sativa* and *T. polycerata* were uprooted from field in March, 2012. The plants were dried under shade and then in oven at 70°C for 24 hours. Small pieces (1-3 cm) of each dried weed were soaked separately in distilled water in a ratio of 1:20 (w/v) for 24 hours at room temperature. Water extracts were then filtered with a sieve and then finally with Whattman no. 1 filter paper. The extracts were stored in separate bottles and used as stock solutions. Dilution was made to carry out experiment at different concentrations.

Determination of types of phenolics

For identification of suspected phytotoxins, aqueous extracts of all weeds were chemically analyzed on Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). The peaks were detected by UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification. Concentration of each isolated compound was determined by the following equation. The phenolics identified in weeds water extracts are presented in Table-1.

$$\text{Concentration (ppm)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \text{ x Conc. of the standard x Dilution factor}$$

Experiment 1

Petri dish bioassay

Water extracts of all weeds with 2.5% (1:40 w/v) and 5% (1:20 w/v) concentrations were evaluated for their allelopathic effect on rice. Ten seeds of rice were placed in a 9 cm diameter petri dish lined with filter paper. At start of experiment, 7 ml of 2.5% and 5% aqueous extract of each weed species was poured in each petri dish whereas equal volume of distilled water was used in control treatment. Petri dishes were placed on laboratory shelves and experiment was laid out in completely randomized design (CRD) with factorial arrangement and each treatment was replicated four times. Daily germination count was noted for a period of 16 days and then seedling growth parameters were recorded. Seeds were considered germinated when radical size was 2 mm. Minimum and maximum temperatures during the course of experiment were29°C and 31.8°C, respectively.

Experiment 2

Pot cultured bioassay

Residues of each weed species in the form of their dried plant parts were incorporated in soil with a residue: soil ratio of1:50 (w/w) to get 2% (w/w) mixture. Plastic pots of 11-cm diameter and 5-cm depth were filled with equal weight of residue-soil mixture and kept for 0, 15 and 30 days for decomposition. Soil without any residue was used as control. Ten seeds of rice were sown in each pot. Pots were placed on laboratory floor. Soil was kept moist during whole duration of experiment with distilled water. Experiment was laid out in completely randomized design (CRD) with factorial arrangement and each treatment was replicated four times. Emergence count was taken daily for a period of 16 days after which seedlings were uprooted and seedling growth parameters were recorded. Minimum and maximum temperatures during the course of experiment were 32.5°C and 37°C, respectively.

Procedures for data recording

Germination/emergence was calculated following the procedure of seedling evaluation in the Handbook of Association of Official Seed Analysts (AOSA, 1990) by using the formula:

$$\text{Germination \%} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds}} \times 100$$

Mean germination/emergence time was calculated using the formula of Ellis and Roberts (1981):

$$\text{Mean germination time} = \frac{\sum \text{Dn}}{\text{N}}$$

Where, *n* denotes the number of seeds germinated or seedlings emerged on day *D* and *N* is the total number of germinated/emerged seeds.

Germination/emergence index was calculated using the following formula as per Association of Official Seed Analysts (AOSA, 1983):

$$\text{Germination Index} = \frac{\text{Ni}}{\text{Di}} + \cdots + \frac{\text{Nf}}{\text{Df}}$$

Where *Ni* is number of seeds germinated/emerged on Di (days of first count) and *Nf* is the number of seeds germinated/emerged on Df (days of final count).

At the end of germination period, seedlings were uprooted and their root and shoot lengths (cm) and seedling dry biomass (mg) were measured. Seedlings were oven dried at 70°Ctill constant weight. Seedling vigour index (SVI) was calculated using the equation of Abdul-Baki(1980):

$$\text{SVI} = \text{Germination \%age} \times \text{Radicle length}$$

Statistical analysis

Data obtained was analyzed using Fisher's analysis of variance (ANOVA) procedure and comparison of treatments' means was carried out using least significant difference test (LSD) at 0.05% probability level (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Allelopathic effect of water extracts of weeds on germination percentage (GP), germination index (GI) and mean germination time (MGT) and seedling growth of rice have been shown in Tables-2 and -3 which revealed that rice germination and seedling growth were significantly suppressed by application of the water extracts compared with control. Moreover, phytotoxic inhibition increased with increasing concentration of these extracts. No germination of rice seeds could take place by application of 5% aqueous extract of *M*. *indica*. The 5% aqueous extracts of *T. polycerata* and *L. aphaca* can be ranked at second position in imposing inhibitory effect on germination traits as it produced significantly lower GP (20.0and 25.0) and GI (0.40 and 0.49). However, longest MGT (6.92 days) was recorded for5% aqueous extract of *V. sativa*, which was statistically at par with that in case of *L. aphaca* 5% aqueous extract (Table-2). Data presented in table 3 indicates that maximum allelopathic inhibitory effect in terms of root length (1.01 cm) and seedling vigor index (19.0) was shown by 5% aqueous extract of *T. polycerata.* However, significantly lower shoot length (3.80 cm) and seedling dry biomass (4.49 mg) were recorded in 2.5% and 5% aqueous extracts of *M. polymorpha,* respectively (Table-3). Kumar *et al*. (2007) observed that leaf extracts of *Eupatorium odoratum* and *Ageratum conyzoides* showed negative effect on GP, GI, MGT, radicle and plumule elongation and seedling vigour index of *Oryza sativa*, *Glycine max* and *Brassica campestris*. In another study, Suwal *et al*. (2010) also noticed phytotoxic suppressive action of water extract of *Chromolaena odorata* on germination and seedling growth ofrice and barnyard grass. The inhibition of germination and seedling growth is attributed to the allelochemicals present in the weed tissues (Heidarzade *et al*., 2012). Inhibition of germination and seedling growth by phenolic compounds is due to their interference with plant hormonal activity, photosynthesis, respiration, cell division and enlargement (Li *et al*., 2010). The inhibition of plant hormones and respiration inhibits the germination partially or fully.

Increase in phytotoxic inhibitory effects of weeds by increasing concentration of their aqueous extracts has also been reported by Hossain and Alam (2010) while studying effect of *Lantana camara*water extract at different concentrations on germination and

seedling length of *O*. *sativa*, *Triticum aestivum* L., *Vigna sinensis* L., *Cucurbita pepo* L., *Abelmoschus esculentus* (L.) Moench, *Amaranthus tricolor* L., *Acacia auriculiformis* A. Cunn. exBenth., *Paraserianth esfalcataria* (L.) Nielsen and *Albiziaprocera* (Roxb.) Benth. They found that inhibition increased with an increase in concentration while lower concentrations caused stimulatory effect.

Data revealed that residues containing soils caused significant inhibitory effects on emergence and seedling growth of rice compared with soil without any residue (Table-4 and -5). This may be due to allelochemicals released by decomposition of weed residues. *Vicia sativa* and *T. polycerata* residues after 30 days of their decomposition showed maximum suppressive action on germination of rice by producing significantly lower EP (22.5 and 25.0), EI (0.26 each)and MET (9.08 and 9.96 days), respectively (Tables 4 and 5). However, stimulation of emergence index was found by the effect of *M*. *polymorpha* residues with 15 days of decomposition. Table-5 highlighted that significantly lower shoot length (4.72 cm) and seedling dry weight (4.84 mg) were noted in rice seedlings emerged from *M*. *polymorpha* residue incorporated soil, decomposed for a period of 30 days. However shoot length was statistically similar with those observed in case of *T. polycerata* (4.85 cm) and *V. sativa* (5.54 cm). It was statistically at par with *T. polycerata* and *V*. *sativa* residues with 30 days of decomposition. Interestingly, it was noticed that shoot length of rice seedlings was increased in pots filled with soil incorporated with fresh *L*. *aphaca* residues (Table-5). Contrastingly, the lowest root length (2.25 cm) of rice seedlings was caused by *T*. *polycerata* residue with zero days of decomposition which was not different from that obtained in case of *V. sativa* (2.36 cm) residue decomposed for 30 days (Table-5). Seedling vigour index was inhibited most by *V*. *sativa* residue with 30 days of decomposition (Table-5). Hamidi *et al*. (2008) observed the inhibitory allelopathic effect of soil incorporated residues of *Hordeum spontaneum* on seedling length and dry weight of *T. aestivum*. Ismail and Siddique (2011) also found that seedling length and weight of *O*. *sativa*was suppressed by residues of *Cyperus iria* in soil. However, at some instances, stimulatory effect was also observed on shoot length and seedling dry biomass of rice by fresh residues or residues decomposed for lesser time of some weeds as compared to control (Hussain *et al*., 2007). This was due to fact that phytotoxic compounds take time to be released from plant material and at early stages of decomposition, low quantity of these allelochemicals impart growth promontory rather than inhibitory action (Mubarak *et al*., 2011).

The higher germination and seedling growth inhibitory effects of *M. indica, V. sativa, T. polycerata, L. aphaca*and *M. polymorpha*may be

due to presence of caffeic acid, gallic acid, Chlorogenic acid, vanilic acid, 4-hydroxy-3-methoxybenzoic acid, ferulic acid, *p*-coumaric acid, *m*-coumaric acid and syringic acid as detected by HPLC analysis of their aqueous extracts (Table-1). These phenolics have been reported to cause inhibition of germination and seedling growth of recipient plants (Gao *et al*., 2011). However, the action of these phenolics seems to be different in water extracts and soil incorporated residues as exhibited by differential response of rice seeds and seedlings sown in petri-dish and pot-cultured bioassays. This could be due to differential contact of these allelochemicals with seed or root in different growing media. Allelochemicals present in extracts come in direct contact with seed or plant while several factors govern the availability and uptake of allelochemicals in soil by plants (Kruse *et al*., 2000) as adsorption of allelochemicals with soil particles, microbial decomposition and transformation of allelochemicals, redox reactions, soil texture and leaching influence the availability and activity of allelochemicals in soil (Jennings and Nelson, 1998; Inderjit *et al*., 2001).

CONCLUSION

The bioassay studies conclude that phytotoxic compounds from leguminous weeds *L. aphaca*, *M. polymorpha*, *M. indica*, *V. sativa* and *T. polycerata* pose allelopathic action against germination and seedling growth of rice either in the form of leachates or decomposing remains. Therefore these weeds are hazardous to rice in rice-wheat cropping pattern and should be removed from the fields either in presence or absence of rice.

Table-1. Phenolics types identified in water extracts of leguminous weeds

Weeds	Phenolics
Lathyrus aphaca	Caffeic acid, Gallic acid, m-Coumaric acid, Syringic acid
Medicago polymorpha	4-Hydroxy-3-Methoxybenzoic acid, m-Coumaric acid, p-Coumaric acid, Vanilic acid
Melilotus indica	Caffeic acid, Chlorogenic acid, Ferulic acid
Trigonella polycerata	4-Hydroxy-3-Methoxybenzoic acid, m-Coumaric acid, Syringic acid
Vicia sativa	4-Hydroxy-3-Methoxybenzoic acid, Ferulic acid, p-Coumaric acid

Table-2. Influence of water extracts of winter leguminous weeds on germination traits of rice

Treatments	Germination %		Germination index		Mean germination	
	2.5%	5%	2.5%	5%	2.5%	5%
Control	92.50 a	92.50 a	3.81 a	3.81 a	2.70 f	2.70 f
V. sativa	65.00 bc	30.00 e	2.11 c	0.49 ef	3.52 de	6.92 a
T. polycerata	57.50 c	20.00 f	1.85 c	0.40 f	3.35 def	4.08
L. aphaca	70.00 b	25.00 ef	2.49 b	0.49 ef	3.30 ef	6.75 a
M. polymorpha	30.00 e	57.50 c	0.75 e	1.11 d	4.79 bc	5.51 b
M. indica	45.00 d	NG	1.11 d	NG	5.44 b	NG
LSD	8.279		0.296		0.746	

Means with no similar letter differ significantly at 0.05 probability level. NG = not germinated

Table-3. Influence of water extracts of winter leguminous weeds on seedling growth of rice

Treatments	Shoot length (cm)		Root length (cm)		Seedling dry weight		Seedling vigour index	
	2.5%	5%	2.5%	5%	2.5%	5%	2.5%	5%
Control	7.13 a	7.13 a	4.15 a	4.15 a	7.20 b	7.20 b	383.55 a	383.55 a
V. sativa	4.73 bc	4.58 bc	2.83 c	3.53 b	6.13 ef	6.68 cd	183.86 c	105.90 ef
T. polycerata	4.97 b	4.56 bc	2.80 c	1.01 e	6.47 de	5.72 f	161.77 cd	19.00 h
L. aphaca	4.59 bc	4.33 cd	2.57 cd	2.30 d	5.98 f	7.93 a	180.46 c	57.00 g
M. polymorpha	3.80 d	5.18 b	2.85 c	3.87 ab	5.77 f	4.49 g	86.62 fg	224.41 b
M. indica	4.88 bc	NG	2.94 c	NG	6.90 bc	NG	132.61 de	NG
LSD	0.626		0.417		0.424		36.446	

Means with no similar letter differ significantly at 0.05 probability level. NG = not germinated

Table-4. Influence of residues of winter leguminous weeds on emergence traits of rice

Treatments	Emergence percentage (%)			Emergence index			Mean emergence time (days)		
	0	15 days	30 days	0 days	15 days	30 days	0 days	15 days	30 days
Control	95.00	95.00 a	95.00 a	1.60 ab	1.60 ab	1.60 ab	6.36 gh	6.36 gh	6.36 gh
V. sativa	67.50	50.00 e	22.50 g	1.11 de	0.75 g	0.26 i	7.43 efg	8.75 bcd	9.08 abc
T. polycerata	75.00	75.00 bc	25.00 fg	1.06 e	1.28 cd	0.26 i	8.87 bc	6.64 fg	9.96 a
L. aphaca	60.00	67.50 cd	45.00 e	0.82 fg	1.04 e	0.50 h	7.52 ef	8.11 cde	9.48 ab
M. polymorpha	60.00	77.50 b	32.50 f	1.02 e	1.67 a	0.38 hi	6.74 fg	4.71 i	9.23 ab
M. indica	72.50	60.00 d	45.00 e	1.44 bc	0.97 ef	0.52 h	5.55 hi	7.69 def	9.21 ab
LSD	9.151			0.198			1.073		

Means with no similar letter differ significantly at 0.05 probability level.

Table-5. Influence of residues of winter leguminous weeds on seedling growth of rice

Treatments	Shoot length (cm)			Root length (cm)			Seedling dry weight (mg)			Seedling vigour index		
	0 days	15 days	30 days	0 days	15 days	30 days	0 days	15 days	30 days	0 days	15 days	30 days
Control	10.64 d	10.64 d	10.64 d	7.95 a	7.95 a	7.95 a	10.54 a	10.54 a	10.54 a	754.63 a	754.63 a	754.63 a
V. sativa	9.71 e	7.49 g	5.54 h	3.20 d	2.51 fg	2.36 g	6.46 gh	7.55 ef	9.76 b	216.85 d	123.98 ghi	53.43 j
T. polycerata	7.38 g	10.1 de	4.85 h	2.25 g	5.36 b	3.24 d	5.88 h	10.98 a	8.71 cd	169.93 ef	402.13 b	81.08 ij
L. aphaca	13.39 a	8.76 f	6.82 g	4.89 c	3.03 de	3.32 d	7.12 fg	8.13 de	7.98 de	291.88 c	204.60 de	149.95 fg
M. polymorpha	12.33 bc	11.68 c	4.72 h	2.48 fg	4.93 c	2.56 fg	7.59 ef	9.13 bc	4.84 i	148.70 fg	382.25 b	83.33 hij
M. indica	12.72 ab	8.74 f	7.21 g	2.29 g	3.35 d	2.81 ef	9.21 bc	7.90 e	7.49 ef	167.79 efg	202.25 de	126.40 fgh
LSD	0.822			0.388			0.734			44.558		

Means with no similar letter differ significantly at 0.05 probability level.

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