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Full Length Research Paper

Comparative tolerance of different rice varieties to sunflower phytotoxicity

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The present study was conducted to investigate the phytotixic effects of aqueous extracts of dry and fresh leaf, stem and inflorescence of sunflower (*Helianthus annuus* L.) var. Hysun 33 against germination and early seedling growth of four varieties of rice, namely Basmati Pak, Basmati Supper, Basmati 385 and IRRI-fine. Extracts of all the three parts of sunflower showed toxicity against germination and seedling growth of different rice varieties. Leaf extract exhibited the highest toxicity against germination followed by root and stem extracts, respectively. With respect to seed germination, rice varieties Basmati 385 and IRRI Fine were more resistant against various types of sunflower extracts while Basmati Pak was found to be the most susceptible one. None of the extract exhibited any significant effect on shoot length of Basmati supper and IRRI Fine. Root growth in Basmati 385 showed the most susceptible response to sunflower extracts toxicity followed by Basmati supper and Basmati Pak, respectively. Results of the present study suggest that rice variety IRRI Fine is the most tolerant to sunflower phytotoxicity followed by Basmati supper and thus may be suitable for cultivation under sunflower allelopathic stress. Basmati 385 showed highly tolerant germination behaviour and can be sown for raising of rice nursery under allelopathic stress of sunflower.

Key words: *Helianthus annuus,* phytotoxicity, rice varieties, sunflower.

INTRODUCTION

Sunflower **(***Helianthus annuus* L.), family Asteraceae, is an important oil seed crop and excellent source of proteins (kaya et al., 2005). It contains 39 to 49% oil in the seed. Sunflower oil is generally considered premium oil because of its light color, high level of unsaturated fatty acids and lack of linolenic acid, bland flavor and high smoke point. The primary fatty acids in oil are oleic and linoleic (typically 90% unsaturated fatty acids), with the remainder consisting of palmitic and stearic, the saturated fatty acids (Hassan et al., 2011). Sunflower seems to be a crop which can bridge the ever-increasing gap between our domestic edible oil production and consumption. Because it is a short duration not strictly season bounded crop, farmers have started growing it twice a year (Kamal and Bano, 2005). Many studies show that sunflower is strongly allelopathic in nature (Mehboob, 1999; Ghaffar, 1999) and its effect on subsequent crops and weeds has been reported (Azania, 2003; Macias et al., 2005). It contains numerous allelochemicals viz. phenolic compounds and terpenoids, particularly sesquiterpene lactones, heliaspirones, helibisabonoles and heliannuals with a wide spectrum of biological activities including allelopathy (Vyvyan, 2002; Macias et al., 2000b, 2003).

Rice is the second most important food crop after wheat in Pakistan. It accounts for 5.9% of the total value added in agriculture and 1.3% to GDP and is one of the main export items of the country (Anonymous, 2009). Rice is cultivated after harvesting of spring sown crop of sunflower. Sunflower residue is generally incorporated in the soil with the idea that it will add to the organic matter and fertility of the soil.

The allelochemicals released from sunflower residues in the soil are likely to cause adverse effects on the proceeding crops (Nanjappa et al., 1999). The present study was, therefore, designed to investigate the effect of aqueous extracts of different parts of sunflower on germination and seedling growth of four commonly

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cultivated rice varieties namely Basmati Pak, Basmati Supper, Basmati 385 and IRRI-fine.

MATERIALS AND METHODS

Procurement of sunflower materials

Certified seeds of commonly cultivated sunflower variety in Pakistan namely Hysun 33, were obtained from Monsanto Pakistan (Pvt) Ltd. The seeds of selected sunflower variety were sown on ridges in 2 × 2 m plots at a depth of 1 cm. Seeds were planted with inter-row and inter plant spacing of 75 and 30 cm, respectively. A basal dose of 120 kg h^{-1} N as urea, 90 kg h^{-1} P_2O_5 as triple super phosphate and 60 kg h^{-1} K_2O as potassium sulphate was applied in each plot. The plots were irrigated as recommended for sunflower. After 90 days of sowing, the mature sunflower plants were uprooted, washed thoroughly under tap water, dried with blotting paper, and root, stem and leaves were separated.

Laboratory bioassays

Fresh roots, stems and leaves of sunflower were separately crushed thoroughly in sterilized pestle and mortar and soaked in sterilized water at 20 g 100 ml^{-1} for 24 h at room temperature (25 ± 2°C). The same amounts of these plant materials were air died and similarly soaked in distilled water. After 24 h, materials were filtered through double layered thin muslin cloth and finally through Whattman filter paper No. 1. The filtrate (20% solution of each part) was designated as stock solution, from which lower concentrations of 5, 10 and 15% were made by diluting with appropriate amount of distilled water. The extracts were stored at 4°C and generally used within a weak.

Double layered sterilized Whattman filter papers No. 1 was placed in pre-sterilized Petri plates. The filter paper was moistened with 3 mL extracts of various concentrations (5, 10 and 15%) each. For control treatment, 3 ml of distilled water was used. Ten surface sterilized seeds of each of the four tested rice varieties namely Basmati Pak, Basmati Supper, Basmati 385 and IRRI-fine, were placed in these Petri plates. Each treatment was replicated thrice. Percent seed germination and early growth in terms of root/shoot length and dry weight was recorded after ten days.

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to separate the treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Analysis of variance revealed that the effect of rice varieties (V) and the extract concentration (C) was significant for all the studied parameters, viz. germination, root and shoot length and dry biomass. Similarly, the effect of extracts of different parts of sunflower (P) was significant for all the studied parameters except shoot length. However, the effect of type of sunflower material, namely; dry or fresh (M) was significant only for length and dry weight of shoot. The interactive effect of V × C was significant for all parameters while that of P × V was significant for all except parameters except root dry weight (Table 1). Leaf extract exhibited the highest toxicity against germination followed by root and stem extracts, respectively. All the three concentrations of fresh leaf extracts significantly reduced germination of Basmati Pak by 44 to 97%. Similarly, 10 and 15% fresh leaf extract significantly suppressed seed germination of Basmati Supper by 28 and 40%, respectively. However, only 15% leaf extract significantly declined germination of IRRI Fine by 35%. Dry leaf extract was comparatively less toxic against germination than fresh leaf extract. Only 10 and 15% dry leaf extract significantly reduced germination of Basmati Pak by 37 and 33%, respectively, and that of 15% extract exhibited a similar adverse effect on germination of Basmati Super and IRRI Fine resulting in the 43 and 31% reduction in the studied parameters, respectively (Table 2). Higher concentration of 10 and 15% of both fresh and dry root extracts significantly suppressed germination of Basmati Pak. Similar adverse effect of dry root extract was also observed on germination of Basmati 385 seeds. Fresh stem extract of 10 and 15% concentration significantly reduced germination of Basmati Pak by 41 and 44%, respectively.

In general, with respect to seed germination, rice varieties Basmati 385 and IRRI Fine were more resistant against various types of sunflower extracts while Basmati Pak was found to be the most susceptible one (Table 2). Recently, Sedigheh et al. (2010) reported that sunflower extracts significantly inhibited the germination of *solanum nigrum*. More than 200 natural phytotoxic compounds including phenolic compounds and terpenoids, particularly sesquiterpene lactones, heliaspirones, annuionones, helibisabonols and heliannuols, have been isolated from different cultivars of sunflower (Macıas et al., 2003; Mehmood et al., 2010), which may be responsible for germination inhibition (Kausar, 1999; Kamal and Bano, 2009).

Fresh leaf extracts of 15% significantly reduced shoot length of seedlings of Basmati Pak and Basmati 385. In contrast, 5% fresh leaf extract significantly enhanced the studied parameter. None of the extract exhibited any significant effect on shoot length of Basmati supper and IRRI Fine (Table 2). Shoot dry weight in Basmati 385 was highly susceptible to sunflower extracts toxicity where higher concentration of 15% of all the extracts significantly reduced the studied parameter. IRRI Fine was highly resistant to extracts phytotoxicity where only 15% dry leaf extract exhibited significant adverse effects on shoot dry weight. In case of Basmati supper, only extracts of fresh and dry stem of sunflower showed significant adverse effects on shoot dry weight. Similarly, 15% extract of fresh leaves significantly reduced shoot dry weight (Table 2). The results of the present study are in accordance with the findings of Javaid et al. (2007). They reported that rice varieties IRRI-8 and IRRI-Fine were more tolerant to phytotoxicity of *Cyperus rotundus* L.

Table 1. Analysis of variance for germination and early seedling growth of different rice varieties as affected by different concentrations of aqueous fresh and dry root, stem and leaf extracts of sunflower.

Source of variation	df	Mean squares				
		Germination	Shoot length	Shoot dry weight	Root length	Root dry weight
Material (M)	1	112 ^{ns}	12 ***	236 **	0.04 ^{ns}	2 ^{ns}
Plant Part (P)	2	1563 **	0.51 ^{ns}	182 **	45 ***	301 ***
Variety (V)	3	11319 **	118 ***	597 ***	134 ***	595 ***
Concentrations (C)	3	4887 **	15 ***	600 ***	141 ***	612 ***
M × P	2	591 **	1.80 ^{ns}	27 ^{ns}	13 ***	2 ^{ns}
M × V	3	500.5 **	0.72 ^{ns}	56 ^{ns}	1.21 ^{ns}	77 *
M × C	3	52 ^{ns}	1.879 ^{ns}	96 *	2.14 ^{ns}	10 ^{ns}
P × V	6	357 **	1.906 *	89 **	11 ***	59 ^{ns}
P × C	6	997 **	0.81 ^{ns}	26 ^{ns}	15 ***	60 ^{ns}
V × C	9	867 **	2.61 *	121 ***	20 ***	211 ***
M × P × V	6	399 **	1.12 ^{ns}	67 ^{ns}	1.99 ^{ns}	41 ^{ns}
M × P × C	6	230 *	6.23 ***	35 ^{ns}	4.96 **	21 ^{ns}
M × V x C	9	181 ^{ns}	1.14 ^{ns}	101 **	1.74 ^{ns}	18 ^{ns}
P × V × C	18	225 **	1.71 **	49 ^{ns}	3.16 **	49 *
M × P × V × C	18	400 **	2.82 ***	67 **	2.39 ^{ns}	46 ^{ns}
Error	192	104	0.86	33	1.61	29

ns = Non-significant. *, **, ***, Significant at P ≤ 0.05, 0.01 and 0.001, respectively.

Table 2. Effect of different concentrations of aqueous extracts of fresh and dry leaf, stem and root of sunflower on germination and shoot growth of four rice varieties.

	Sun-flower parts used	Conc. (%)	Basmati supper			Basmati Pak			Basmati-385			IRRI-Fine		
			Germination (%)	Shoot length (cm)	Shoot dry wt. (mg)	Germination (%)	Shoot length (cm)	Shoot dry wt. (mg)	Germination (%)	Shoot length (cm)	Shoot dry wt. (mg)	Germination (%)	Shoot length (cm)	Shoot dry wt. (mg)
Fresh	Leaf	0	83^{ab}	6.3^{ab}	27^{a-c}	90^{a}	3.4^{b-d}	30^{a-c}	100^{a}	5.2^{ab}	27^{a}	97^{a}	6.0^{a-d}	21^{a-c}
		5	83^{ab}	5.4^{b}	18^{cd}	50de	6.3^{a}	31^{a-c}	100^{a}	5.0^{a-c}	20^{a-d}	93^{ab}	5.6^{b-d}	18^{bc}
		10	60^{c-e}	8.1^{a}	30^{a}	50de	4.6^{b}	22^{a-d}	100^{a}	5.3^{a}	20^{a-d}	87^{ab}	6.4^{ab}	19^{bc}
		15	50de	5.8^{ab}	20^{a-d}	3^{f}	0.5^{e}	36^{e}	100^{a}	2.6^{d}	11de	63^{c}	7.5^{a}	24^{ab}
	Stem	5	83^{ab}	5.7^{ab}	19^{b-d}	73^{a-d}	3.8^{b-d}	28^{a-c}	100^{a}	5.2^{ab}	20^{a-d}	90^{ab}	6.3^{a-c}	21^{a-c}
		10	70^{b-d}	6.9^{ab}	24^{a-d}	53^{c-e}	3.7^{b-d}	36^{a}	100^{a}	5.0^{a-c}	24^{a-c}	97^{a}	5.5^{b-d}	21^{a-c}
		15	87^{ab}	4.7^{b}	17^{d}	50^{d-e}	3.3^{b-d}	35^{a}	100^{a}	4.9^{a-c}	15^{c-e}	93^{ab}	4.4^{d}	16^{c}

Table 2 contd.

Dry	Root	5	90^{ab}	5.4^{b}	21^{a-d}	83^{ab}	3.0^{b-d}	25^{a-d}	97^{a}	5.5^{a}	25^{ab}	97^{a}	6.2^{a-c}	22^{a-c}
		10	80^{a-c}	6.8^{ab}	18^{cd}	60^{b-e}	4.2^{bc}	36^{a}	97^{a}	4.8^{ac}	20^{a-d}	90^{ab}	6.2^{a-c}	22^{a-c}
		15	73^{bc}	5.8^{ab}	21^{a-d}	73^{a-d}	3.5^{b-d}	26^{a-d}	100^{a}	5.4^{a}	18^{a-e}	93^{ab}	5.9^{a-d}	25^{ab}
	Leaf	5	100^{a}	4.8^{b}	19^{b-d}	80^{a-c}	3.0^{b-d}	11de	100^{a}	4.2^{a-c}	18^{a-e}	100^{a}	5.9^{a-d}	18^{bc}
		10	70^{b-d}	7.1^{ab}	23^{a-d}	57^{b-e}	2.8^{b-d}	23^{a-d}	93^{ab}	3.7^{cd}	9^{e}	90^{ab}	5.9^{a-d}	18^{bc}
		15	47^{e}	7.1^{ab}	28^{ab}	60^{b-e}	2.7^{cd}	27^{a-d}	93^{ab}	4.4^{a-c}	12de	77^{bc}	4.7^{cd}	9^{d}
	Stem	5	100^{a}	4.9^{b}	21^{a-d}	77^{a-d}	2.9^{b-d}	15^{c-e}	97^{a}	5.2^{ab}	23^{a-c}	97^{a}	5.8^{a-d}	19^{bc}
		10	87^{ab}	5.2^{b}	20^{a-d}	73^{a-d}	4.4^{bc}	22^{a-d}	87^{bc}	4.5^{a-c}	12de	9^{7a}	5.3^{b-d}	19^{bc}
		15	70^{b-d}	6.1^{ab}	16^{d}	70^{a-d}	2.0^{de}	21^{a-e}	97^{a}	4.7^{a-c}	15^{c-e}	97^{a}	6.1^{a-c}	22^{a-c}
	Root	5	86^{ab}	6.8^{ab}	26^{a-d}	60^{b-e}	3.3^{b-d}	18^{b-e}	93^{ab}	3.9^{b-c}	17^{b-e}	100^{a}	6.4^{ab}	23^{ab}
		10	90^{ab}	5.3^{b}	18^{cd}	60^{b-e}	3.2^{b-d}	31^{a-c}	87^{bc}	5.0^{a-c}	17^{b-d}	87^{ab}	6.2^{a-c}	27^{a}
		15	97^{a}	4.5^{b}	19^{b-d}	40^{e}	0.7^{e}	33^{ab}	80^{c}	1.4^{e}	18^{a-e}	87^{ab}	4.7^{cd}	19^{bc}

In a column, values with different letters show significant difference as determined by Duncan's Multiple Range Test at P≤0.05. **Note:** The concentrations of both fresh and dry sunflower materials are based on fresh weight bases.

than various Basmati varieties, namely; Pak Basmati, Super Basmati and Basmati 385. This unequal susceptibility of various rice varieties to the sunflower extracts could be due to inherent differences in physiological and morphological characteristics of various genotypes involved. Toxicity is assumed to be associated with the presence of strong electrophilic or nucleophic systems. Action by such systems on specific positions of proteins or enzymes would alter their configurations and affect their activity (Macias et al., 1992).

Root growth was highly susceptible to various types of sunflower extracts. Root length in Basmati 385 showed the most susceptible response to sunflower extracts toxicity followed by Basmati Supper and Basmati Pak, respectively. All the concentrations of different types of sunflower extracts significantly reduced the root length of Basmati 385. Similarly, all the concentrations of fresh as well as dry leaf and stem extracts significantly reduced the root length in Basmati supper. The adverse effect of sunflower root extract on root length of Basmati supper was significant only when the higher extract concentrations were used. In case of Basmati Pak, generally higher concentrations of 10 and 15% extracts exhibited the pronounced effect resulting in significant reduction of the studied parameter. Root length in IRRI Fine generally exhibited tolerance to various types of sunflower extracts. Only 10 and 15% fresh leaf and stem extracts, and 15% dry leaf extract significantly reduced the root length in this rice variety. The response of root dry biomass to various types of sunflower extracts was generally similar to that of the response of root length (Table 3). Since roots were the first to absorb phytotoxins from the surrounding environment and thus showed their abnormal growth in response to phytotoxins resulting in more severely arrested growth as compared to shoot (Javaid and Shah, 2007; Javaid et al., 2007).

The present study concludes that IRRI Fine is more tolerant than the Basmati varieties of rice. This variety may be cultivated where soil is suffering from sunflower allelopathic stress. Among the various Basmati varieties, Basmati 385 showed that highly tolerant germination behaviour under sunflower extracts toxicity. This variety may be used for raising of rice nursery

Table 3. Effect of different concentrations of aqueous extracts of fresh and dry leaf, stem and root of sunflower on root growth of four rice varieties.

	Sunflower parts used	Conc. (%)	Basmati supper		Basmati Pak		Basmati-385		IRRI-Fine	
			Root length (cm)	Root dry wt. (mg)	Root length (cm)	Root dry wt. (mg)	Root length (cm)	Root dry wt. (mg)	Root length (cm)	Root dry wt. (mg)
		0	7.77^{a}	28^{a}	2.69^{a-c}	14^{c-d}	6.42^{a}	23^{a}	4.48^{de}	20^{a-c}
		5	4.82^{cd}	15^{c-g}	3.39^{a}	21^{ab}	3.07^{d-h}	17^{a-d}	4.97^{c-d}	16^{a-c}
	Leaf	10	3.64^{de}	13^{c-g}	1.65^{c-e}	10^{d-f}	2.03^{hi}	8^{f}	2.29^{e-g}	12^{c}
		15	0.52^{f}	17^{b-f}	0.53^{fg}	0^{h}	1.9^{h-j}	12^{d-f}	0.85^{g}	13^{bc}
		5	3.72^{de}	13^{c-g}	1.85^{c-e}	4^{gh}	3.38^{c-f}	17^{a-d}	6.06^{b-d}	22^{a-c}
Fresh sunflower material	Stem	10	1.14^{f}	14^{c-g}	0.80^{e-g}	9^{e-g}	2.63^{e-i}	14^{c-f}	2.24^{e-g}	13^{bc}
		15	0.76^{f}	7^{fg}	0.50^{fg}	18^{bc}	2.84^{e-i}	17^{a-d}	1.33^{fg}	14^{bc}
		5	7.51^{ab}	26^{ab}	1.88^{e-e}	24^{a}	3.31^{c-g}	20^{a-c}	7.65^{ab}	20^{a-c}
	Root	10	6.34^{a-c}	22^{a-d}	2.18^{b-d}	10^{d-f}	3.55^{c-e}	16^{b-e}	8.96^{a}	20^{a-c}
		15	4.87^{cd}	17^{b-f}	1.48^{d-g}	10^{d-f}	2.18^{g-i}	16^{b-e}	5.04^{b-d}	25^{ab}
		5	5.26^{b-d}	12^{c-g}	1.45^{d-g}	5^{f-h}	1.65^{ij}	16^{b-e}	5.76^{b-d}	23^{a-c}
	Leaf	10	4.40^{cd}	11^{e-g}	0.82^{e-g}	14^{c-e}	1.98^{h-i}	10^{ef}	3.52^{d-f}	14^{bc}
		15	1.05^{f}	11^{e-g}	0.39^{g}	11^{c-f}	2.38^{f-i}	12^{d-f}	1.51^{fg}	20^{a-c}
		5	4.73^{cd}	17^{b-f}	2.36^{a-d}	12^{c-e}	5.13^{b}	23^{a}	4.42^{de}	19^{a-c}
Dry sunflower material	Stem	10	4.79^{cd}	13 c-g	0.98^{e-g}	13^{c-e}	2.34^{f-i}	13^{c-f}	2.29^{e-g}	16^{a-c}
		15	1.48^{ef}	12 d-g	0.76^{e-g}	12^{c-e}	4.15^{b-d}	18^{a-d}	5.45^{b-d}	22^{a-c}
		5	7.34^{ab}	23 a-c	1.53^{d-f}	9^{e-g}	3.56^{c-e}	22^{ab}	7.41^{a-c}	27^{a}
	Root	10	5.22^{b-d}	17 b-f	2.22^{b-d}	17^{b-d}	4.37^{bc}	24^{a}	4.57^{de}	19^{a-c}
		15	1.31^{f}	6 g	0.38^{g}	11^{c-f}	0.86^{j}	15^{c-f}	5.77^{b-d}	24^{a-c}

In a column, values with different letters show significant difference as determined by Duncan's Multiple Range Test at P≤0.05. Note**:** The concentrations of both fresh and dry sunflower materials are based on fresh weight bases.

under sunflower allelopathic stress.

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