RESEARCH

ALLELOPATHIC ACTIVITY OF CROP RESIDUE INCORPORATION ALONE OR MIXED AGAINST RICE AND ITS ASSOCIATED GRASS WEED JUNGLE RICE (*Echinochloa colona* [L.] Link)

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Weed suppression is one of the several benefits achieved by soil incorporation of crop residues and such suppression is believed to be allelopathic in nature. The allelopathic potential of different crop residues: viz. sorghum (*Sorghum bicolor* [L.] Moench), sunflower (*Helianthus annuus* L.), brassica (*Brassica campestris* L.) was evaluated in rice (*Oryza sativa* L.) and jungle rice (*Echinochloa colona* [L.] Link). Chopped crop residues were soil-incorporated alone and mixed at 6 g kg⁻¹ soil (12 t ha⁻¹) and compared with a control without residues. Soil incorporation of residues substantially delayed germination of jungle rice. The time to start germination, time to 50% emergence, mean emergence time, emergence index, and final germination percentage were all depressed by residue incorporation. Final germination of rice and jungle rice dropped by 11 to 15% and 11 to 27% with residue application alone and by 18 to 22% and 8 to 34% with a combination of crop residues, respectively. Residues were more suppressive to germination dynamics of jungle rice than rice. Crop residues exerted a pronounced negative influence on the shoot (25 to 100% and 14 to 44%) and root lengths (22 to 100% and 10 to 43%) of rice and jungle rice, respectively. Shoot and root dry weight of both rice and jungle rice also decreased significantly. An appreciable quantity of phenolics was recorded in soil amended with sorghum+sunflower+brassica residues. Since soil incorporation of allelopathic crop residues was detrimental to both rice germination and seedling growth, it is suggested that the time of residue application for jungle rice suppression and rice seeding time need to be adjusted so as to minimize rice crop damage.

Key words: Residues, allelopathy, sorghum, sunflower, brassica, *Oryza sativa, Echinochloa colona*, weed control.

Modern agriculture is productivity-oriented and relies primarily on synthetic inputs to tackle weeds and other pest problems (Sadeghi *et al.*, 2010). Intensive herbicide use to control weeds over the last few decades is posing serious ecological and environmental threats to the planet and its inhabitants. Herbicide residues in produce, soil and ground water, shifts in weed populations, evolution of resistant weed biotypes, and associated health hazards have diverted the attention of researchers to discover and establish alternative weed management strategies. There is an increasing thrust for organically-produced commodities worldwide (Jamil *et al.*, 2009). Allelopathy, an important ecological phenomenon that explains interference among species through biochemical pathways is a tool that can be manipulated to manage weeds in agroecosystems (Khanh *et al.*, 2005). Use of allelopathic properties of native plant/crop species offers promoting opportunities for this purpose. Allelopathy can regulate plant biodiversity through its impact on plant adaptation, survival, and community organization (Chou and Lee, 1991).

Soil incorporation or surface application, such as mulch of allelopathic crop residues, affects weed dynamics by reducing/delaying seed germination and establishment, in addition to suppressing individual plant growth resulting in an overall decline in the density and vigor of the weed community (Gallandt *et al.*, 1999). Allelopathic crop residue decomposition produces a variety of phytotoxins in the soil causing adverse effects on other plants (Nelson, 1996), and have the potential to sustain a chemical as well as physical effect on the growth and development of subsequent crops and weeds (Reddy, 2001). Allelopathic crop residues can be exploited for weed suppression, and can thus be helpful in reducing reliance on herbicides (Weston, 1996).

Sorghum (*Sorghum bicolor* [L.] Moench), sunflower (*Helianthus annuus* L.), and brassica (*Brassica campestris* L.) are potent allelopathic crops grown in rice-based cropping systems in South Asia, in general, and more particularly in the Indo-Pak region. Phytotoxicity of dried sunflower residues and leaf powder has been reported (Batish *et al.*, 2002). Incorporation (*in situ*) of whole sorghum plant or its various parts, alone or mixed, was found to suppress weed growth in wheat (*Triticum aestivum* L.) (Cheema and Khaliq, 2000). *Brassica campestris* exhibited an inhibitory effect on weed density in the following year due to its residue decomposition

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Received: 24 December 2010.
Accepted: 15 April 2011.
(Norsworthy et al., 2005). Boydston and Hang (1995) found that incorporating *Brassica napus* residue reduced weed count and biomass by 73 to 85% and 50 to 96% in a potato (*Solanum tuberosum* L.) field.

Jungle rice (*Echinochloa colona* [L.] Link) is found to be a predominant weed in transplanted and direct-seeded rice (Rao et al., 2007), but is more competitive in the latter (Dubey, 2004). Besides rice, it also infests maize fields. It is the major grass weed with an intensified seed bank encountered early in the season in dry-seeded rice in Pakistan and is highly competitive (Caton et al., 2004).

Different plant species contain allelochemicals (phenolics being most abundant) that vary in type and concentration (Xuan et al., 2004). Weeds can be better controlled by incorporating plant residues that release a greater fraction of allelochemicals in the soil (Elijarrat and Barcelo, 2001). Release of allelochemicals by mixed residues can have a synergistic and/or additive effect on target species (Matloob et al., 2010). Although useful levels of herbicidal activity through residue incorporation have been achieved, there are still undesirable instances of phytotoxicity to subsequent crops (Holmes and Mayberry, 1996; Mitchell et al., 2000). As a practical approach, residue-mediated phytoxins must selectively target weed species and not crop plants (Kruidhof et al., 2010). The present studies were carried out to investigate the possible suppressive effects of allelopathic sorghum, sunflower, and brassica residues against the germination dynamics and seedling growth of rice, and jungle rice, its associated grass weed. Moreover, phenolic release by the incorporated residues was quantified over time intervals and related to their suppressive activity.

**MATERIALS AND METHODS**

**Plant residues**

Field-grown mature sorghum (‘JS-263’), sunflower (‘Hysun-33’), and brassica (‘Rainbow’) plants were collected from the Agronomic Research Area, University of Agriculture, Faisalabad. Plants were chopped into 3 to 5-cm pieces with a fodder cutter and oven-dried at 70 °C for 48 h. Whole plant residues were mixed *in situ* into the soil.

**Bioassay**

Rice and jungle rice seeds were cleaned manually to ensure physical purity. These were surface sterilized with a water:bleach solution (10:1) for 15 min and rinsed four times with distilled water. Plastic pots (29 × 18 cm, 10 kg capacity) were filled with air-dried, sieved, and well-mixed soil taken from the Agronomic Research Area. Soil belongs to the Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid) in the USDA classification and Haplic Yermosols in the FAO classification (Cheema and Khaliq, 2000). The pH of the saturated soil paste was 7.6 and total soluble salts were 1.2 dS m⁻¹. Organic matter, total N, available P and K were 0.71%, 0.062%, 13.1 mg kg⁻¹, and 179 mg kg⁻¹, respectively. Crop residues (sorghum, sunflower, and brassica) alone and combined in equal ratios were incorporated to obtain a total of 6 g kg⁻¹ of soil (12 t ha⁻¹). Pots without residue were included as a control. Two days after residue incorporation, 30 rice and jungle rice seeds were sown in each pot and these were placed in a screen house under natural solar radiation with an average temperature of 35 ± 5 °C and irrigated when required to keep the soil moist and avoid water stress. The experiment was repeated four times and visited daily to record emergence count (AOSA, 1990) until a constant count was achieved. The time lapse to 50% emergence (E₅₀) was computed by following the modified formula of Farooq et al. (2005):

\[
E_{50} = t \times \frac{\frac{N}{2} - n_l}{n_i - n_l}
\]

where \(N\) is the final number of emerged seeds, \(n_l\) and \(n_i\) are the cumulative number of seeds emerged by adjacent counts at times \(t\) and \(t\), and \(n < N/2 < n\). Mean emergence time (MET) was calculated according to Ellis and Roberts (1981) as:

\[
MET = \frac{\sum D_n \times n}{\sum n}
\]

where \(n\) is the number of seeds emerged on day \(D\), and \(D\) is the number of days counted from the beginning of sprouting. The emergence index was calculated as described by AOSA (1983):

\[
EI = \frac{\text{No of emerged seeds}}{\text{Days of first count}} + \frac{\text{No of emerged seeds}}{\text{Days of final count}}
\]

Root and shoot lengths were measured after 28 d. Plants were uprooted after wetting with water, washed under the tap, and separated into roots and shoots from each pot. The number of leaves and secondary roots were counted manually and averaged. Harvested plant material was oven-dried at 70 °C for 48 h and dry root and shoot biomass was recorded. Total seedling biomass was taken as the sum of root and shoot biomass. Seedling mortality was calculated as:

\[
\text{Seedling mortality (\%)} = \frac{\text{No of seedlings emerged} - \text{No of seedlings survived} \times 100}{\text{No of seedlings emerged}}
\]

Total water-soluble phenolic content of residue-amended soil was determined at 700 nm wavelength in a UV-spectrophotometer (UV-4000, ORI, Hamburg, Germany) according to Swain and Hillis (1959) with Folin-Ciocalteu reagent and expressed as ferulic acid equivalents.

**Experimental design and statistical analysis**

Experimental pots were arranged in a completely randomized design with three replicates. Two separate
experiments were conducted. Data were analyzed by ANOVA with MSTAT-C software (Freed and Scott, 1986). Treatment means were separated by the least significant differences (LSD) at P ≤ 0.05.

RESULTS AND DISCUSSION

Germination traits

Germination traits of rice and jungle rice were negatively influenced by crop residue incorporation (Table 1). Significant (P ≤ 0.05) delay in the time to start germination and ESI over the control was provoked by all the treatments on jungle rice. Germination was delayed by >1 d in jungle rice while it remained unaffected for rice. The time to start germination of jungle rice was delayed to the maximum by the combined application of sunflower+brassica residues and was at par with the incorporation of only sunflower residues, sorghum+brassica residues, or a combination of all three residues (sorghum+sunflower+brassica). Combinations of crop residue incorporation exerted a marked negative influence on time needed for 50% emergence (12 to 34 and 12 to 21%) than when applied alone (1 to 11 and 11 to 14%) for rice and jungle rice, respectively. Final rice germination was suppressed by 11 to 27%. Sunflower residues incorporated alone scored 15% inhibition while combined with sorghum accounted for 27% suppression. Final germination of jungle rice dropped from 8 to 34%. Interestingly, sorghum+sunflower residues were the least effective in retarding jungle rice germination (-8%); maximum inhibition was noticed with all three crop residues, which was at par with that achieved by incorporating only sunflower and brassica residues (22 and 21%). Incorporation of only sorghum, sunflower, and brassica provided statistically similar suppression of final germination in both rice and jungle rice. In various combinations, sorghum+sunflower scored 33% followed by 25% inhibition recorded when all three residues were combined. Mean germination time (MGT) also reflected the allelopathic influence of crop residues on germination events and a significant increase over the control was recorded for both test species. A combination of sorghum+sunflower scored maximum (84%) delay in MGT for rice while a similar suppression value for jungle rice was attributed to mixing all three crop residues. Significantly (P ≤ 0.05) lower emergence index (EI) values, except for the control, were also noticed with minimum EI observed when all three residues were incorporated integratively.

A critical look into the data obtained confirmed the inhibitory effects of crop residues on germination and seedling growth of the test species. Such suppressive actions are believed to originate through the release of phytotoxins (allelochemicals) from incorporated crop residues either by leaching or decomposition (Birkett et al., 2001). Allelopathic compounds in crop residues were rapidly solubilized and imbibed by the germinating seeds, retarded/delayed emergence, and adversely affected subsequent seedling growth. Germination suppression of test species influenced by allelopathic crop residues is in line with Matloob et al. (2010).

Seedling growth

Rice and jungle rice root and shoot length were also significantly (P ≤ 0.05) suppressed by crop residues and various combinations thereof (Table 2). These residues were significantly reduced in the root (22 to 100 and 10 to 43%) and shoot (25 to 100 and 14 to 44%) length over the control in rice and jungle rice, respectively. A combination of sorghum+sunflower+brassica recorded

Table 1. Influence of crop residues applied alone or combined on rice and jungle rice germination traits.

| Treatments                          | Rice Time to start germination (d) | Rice Final germination (%) | Jungle Time to 50% emergence (d) | Jungle Mean germination time (d) | Jungle Emergence index |
|------------------------------------|-----------------------------------|-----------------------------|---------------------------------|---------------------------------|------------------------|
| Control                            | 4.00ns                            | 8.00c                       | 86.67a                          | 78.89a                          | 4.96d                  | 10.55c                 | 5.45b                | 12.38e               | 13.19a               | 8.16a               |
| T1 Sorghum residues incorporated at | 6 g kg⁻¹ soil                     | (0.00)                      | (4.17)                          | (-14.11)                        | (11.41)                | (1.16)                | (14.42)             | (4.15)              | (10.58)             | (-22.95)            | (-27.37)            |
| T2 Sunflower residues incorporated at | 6 g kg⁻¹ soil                     | (0.00)                      | (12.50)                         | (-15.39)                        | (22.54)                | (9.07)                | (13.18)             | (24.01)             | (6.97)              | (-15.42)            | (-36.32)            |
| T3 Brassica residues incorporated at | 6 g kg⁻¹ soil                     | (0.00)                      | (8.33b)                         | (-11.54)                        | (-21.13)               | (10.95)               | (11.09)             | (24.55)             | (6.06)              | (-12.48)            | (-27.49)            |
| T4 Sorghum+sunflower residues each incorporated at 3 g kg⁻¹ soil | (16.67)                          | (4.17)                      | (-26.93)                        | (-8.45)                         | (28.97)                | (12.26)               | (84.36)             | (8.56)              | (13.81ab)           | (6.05bc)            |
| T5 Sunflower+brassica residues each incorporated at 3 g kg⁻¹ soil | (4.67)                            | 9.33a                       | 76.67b                          | 66.67bc                         | 5.57cd                 | 11.95b                | 7.29ab              | 13.81ab             | 11.38ab             | 6.05bc               |
| T6 Sorghum+sunflower+brassica residues each incorporated at 2 g kg⁻¹ soil | (4.67)                            | 9.67a                       | 71.11b                          | 52.22d                          | 5.81bc                 | 12.32ab               | 7.47ab              | 14.00ab             | 11.54ab             | 5.87bc               |
| T7 Sorghum+sunflower+brassica residues each incorporated at 2 g kg⁻¹ soil | (16.67)                          | (20.83)                     | (-17.95)                        | (-33.80)                        | (34.68)                | (21.23)               | (32.01)             | (13.97)             | (-36.26)            | (-45.26)            |

LSD (P ≤ 0.05) = 0.86 17.51 10.86 0.76 0.79 3.15 0.64 2.84 2.06

Means with different letters differ significantly at 5% probability level; *Figures shown in parenthesis show percent change over the control, as: non significant. LSD: least significant differences.
maximum suppression of these parameters in both test species. Shoot and root dry weight was also reduced under all treatments over the control. Among the application of residues alone, sunflower scored the maximum reduction in shoot (59 and 38%) and root (56 and 39%) dry weight of rice and jungle rice, respectively. A combination of all treatments over the control. Among the application of residues alone, sunflower scored the maximum reduction in shoot (59 and 38%) and root (56 and 39%) dry weight of rice and jungle rice, respectively. A combination of all treatments over the control.

A number of allelochemicals responsible for inhibitory allelopathic activity at specific quantities have been reported for sorghum (Cheema et al., 1999), sunflower (Macias et al., 2002; Anjum and Bajwa, 2005), and brassica spp. (Al-Khatib and Boydston, 1999). Residue species varied in their severity against test species which also reflected variable susceptibility to different crop residue species and various combinations thereof. Such a variable influence of sorghum, sunflower, and brassica residues can be due to the type and concentration of allelochemicals involved. Allelopathic interactions are characterized by species specificity of both the donor and receiver (Rice, 1984), and manifested owing to the concerted action of numerous allelochemicals in the substratum (Einheilig, 1996). Blum (1996) reported that phenolic allelochemical mixtures and other organics present in the substratum could cause inhibitory effects even though the concentration of individual allelochemicals is below the required level to cause an effect. It is assumed that the increase in the magnitude of an increasing number of crop residues in the mixture in these studies was on account of the compound effect of the variety of allelochemicals (Matloob et al., 2010).

The variable influence of crop residues is also believed to be due to the differential seed size of the test species. Petersen et al. (2001) pointed out that small-seeded species are more subject to the adverse effects of phytotoxic crop residues. Several explanatory mechanisms and hypotheses have been proposed for this differential suppression. Firstly, small-seeded species have more root length per unit of root mass (Leishman et al., 2001) that provides a more absorbing surface area for allelochemical uptake. Secondly, bold-seeded species contain large seed reserves which are positively correlated with seed size. Thus, they enjoy a competitive edge over small-seeded species because of better seedling respiration under stress-induced carbon deficit conditions (Westoby et al., 2002). Liebman and Sundberg (2006) proposed that species with large reserves are also better able to tolerate and detoxify allelopathic agents. On the contrary, rice appeared more susceptible to phytotoxins than jungle rice in our study. Although it showed prompt germination, even under residue influence, seedling decay was observed with higher mortality rates. Shoot and root elongation, as well as dry matter accumulation in these parts, was relatively less than that recorded for jungle rice under the same set of treatments. This might be attributed to morphological and physiological divergence present in the test species. Moreover, given that weeds are more versatile, aggressive, and tough, they are better able to adapt to changing or adverse external conditions than crops.

### Phenolic dynamics

Since phenolics are the major category of water-soluble allelochemicals responsible for most allelopathic activity,
their presence was quantified over time after incorporation. An appreciable quantity of phenolics was encountered in soil amended with sorghum+sunflower+brassica residues (Figure 1). Phenolic content showed a periodic increase over time achieving peaks after 96 h after which a decrease in phenolic content was observed. Thus, releasing allelochemicals into the rhizosphere is a function of time, as well as quantity and type of residue involved. The decline in phenolic content of residue-amended soil is due to the variation of physical-chemical and biological transformations when entering into the soil phase as proposed by Blum et al. (1999). This confirms the hypothesis by Kruidhof et al. (2010), who proposed that residue-mediated inhibition can occur only if the susceptibility period of the receptor plant coincides with the inhibitory allelopathic potential peak period. Our studies suggest that jungle rice was more susceptible to residue allelopathy during germination while rice sensitivity was attributable to seedling suppression. Khanh et al. (2005) affirmed that combining different crop plants can help to control more weed species due to its greater inhibitory potential, and that it must find weed initial growth in order to be effective.

**CONCLUSION**

These studies conclude that integrating sorghum, sunflower, and brassica residues has the potential to suppress jungle rice germination and seedling growth. These residues can be used as an eco-friendly approach to manage this weed, provided that maximum levels of phytotoxins entering into the soil find the early growth and development of this weed. Waiting 1 wk between residue incorporation and seeding a sensitive crop such as rice can increase crop safety and reduce the weed burden before seeding. However, field studies need to be conducted to evaluate the suppressive efficacy of such residues applied alone and combined with each other under natural conditions. Moreover, sorghum, sunflower, and brassica crop allelopathy can play an important role in different crop rotations to manage the spread of jungle rice.

**ACKNOWLEDGEMENTS**

The authors are grateful to the Higher Education Commission of Pakistan for providing financial support for these studies under the National Research Program.

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