Effect of Crop Residues Applied Isolated or in Combination on the Germination and Seedling Growth of Horse Purslane (*Trianthema portulacastrum*)[1]

*Efeito de Resíduos Vegetais Aplicados Isolados ou em Associação na Germinacão e Crescimento de Plântulas de (**Trianthema portulacastrum**)*

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ABSTRACT - Soil incorporation of crop residues can lead to weed suppression by posing allelopathic and physical effects. Allelopathic potential of the crops sorghum, sunflower, brassica applied as sole or in combination for horse purslane (*Trianthema portulacastrum*) suppression was evaluated in a pot investigation. Chopped crop residues alone and in combination were incorporated at $6\,g\,kg^{-1}$ soil ($12\,t\,ha^{-1}$), and a weedy check was maintained. Germination traits time to start germination; time to 50% emergence, mean emergence time, emergence index and final germination percentage were negatively influenced by residue incorporation. Crop residues also exerted a pronounced negative influence on the shoot and root length of horse purslane. Significant suppression in leaf and root score and leaf area per plant was also observed. A combination of sorghum and sunflower residues accounted for maximum (71%) seedling mortality. Soil incorporation of allelopathic crop residues can be employed for horse purslane management.

Keywords: residues, allelopathic, sorghum, sunflower, brassica, *Trianthema portulacastrum*, germination/seedling growth, suppression.

*RESUMO - A incorporação de resíduos vegetais pode levar à supressão de plantas daninhas devido a efeitos físicos, químicos e alelopáticos. O efeito alelopático potencial de resíduos de sorgo, girassol e canola, aplicados isolados ou em combinação, foi avaliado em relação à germinação e crescimento inicial de beldroegas-cavalo (**Trianthema portulacastrum**) em experimento em vasos. Os resíduos vegetais foram picados e incorporados no solo na quantidade de 6 g kg⁻¹ de solo, que é equivalente a 12 t ha⁻¹; manteve-se também um tratamento sem resíduos. O tempo para iniciar a germinação, tempo de emergência de 50% (E50), tempo médio de emergência, índice de emergência e porcentagem final de germinação foram influenciados negativamente pela incorporação dos resíduos. A palhada também exerceu forte influência negativa sobre o crescimento da parte aérea e comprimento radicular de beldroegas-cavalo. Também foi observada supressão significativa na contagem de folhas e raízes e área foliar. A combinação de resíduos de sorgo e girassol causou a máxima mortalidade de plântulas, que foi de 71%. A incorporação de resíduos vegetais de culturas pode ser utilizada para o manejo de beldroegas-cavalo.*

Palavras-chave: alelopatia, canola, girassol, resíduos vegetais, sorgo, *Trianthema portulacastrum*.

INTRODUCTION

Horse purslane (*Trianthema portulacastrum*) is an annual indigenous plant of South Africa that is widely distributed in South East and West Asia, Africa and Tropical America. It is one of the problematic terrestrial weed by virtue of its competitiveness as a C_4 species.

In Pakistan and India, it is among the most common weeds during summer season in the major field crops such as pulses, cotton, sugarcane, direct seeded rice and maize. Its infestation in cotton, maize and direct seeded rice especially in rainy season is a matter of great concern and could reduce crop yields by 32-60% (Baylan & Malik, 1989). Horse purslane is also a major biological constraint to the large scale adoption of dry-direct-seeded rice in Pakistan and has endangered its sustainability. Significant losses in maize and peanut yield are also attributed to this weed (Gricher, 2008). Horse purslane has indeterminate growth habit which makes it highly competitive. Allelopathic growth inhibition of crop plants owing to horse purslane has also been reported (Sethi & Mohnot, 1988). Enormous seeding capacity or very little dormancy allows the mature seed to germinate immediately thus producing multiple generations in the same season.

Horse purslane is easily killed by herbicides but once more it germinates with subsequent irrigations due to large seed bank. Although chemical weed control is effective and offers prompt solution to many of the weed related problems yet it has resulted in many herbicide resistance as well as environmental issues. These problems require the development and utilization of eco-friendly methods for horse purslane control. Allelopathy promises to be one such strategy which can be applied to manage many of the weed problems in agricultural systems.

Soil incorporation or surface application as mulch of allelopathic crop residues affects weed dynamics by reducing or delaying seed germination and establishment, suppressing individual plant growth resultantly contributed to overall decline in the density and vigor of the weed community (Gallandt et al., 1999). Allelopathic crop residues on decomposition produce a variety of phytotoxins, particularly the phenolics, in the soil causing side effects on the other plants (Nelson, 1996) and have potential to pose a chemical as well as a physical effect on the growth and development of subsequent crops and weeds (Purvis et al., 1985; Thorne et al., 1990), and can be managed for weed suppression (Weston, 1996). Phyototoxicity of dried sunflower residues and

leaf powder has been reported (Batish et al., 2002). Incorporation of (*in situ*) whole sorghum plant or its various parts alone or mixed with each other was found to suppress weed growth in wheat (Cheema & Khaliq, 2000). *Brassica compestris* also exhibited inhibitory effect on weed density in the following year due to decomposition of its residues. Tarahumara Indians in North Mexico use this crop for weed suppression (Chacon & Gliessman, 1982; Norsworthy et al., 2005).

Although the exploitation of allelopathy as a natural weed management tool is eye-catching as an eco-friendly weed management approach yet contrary to bioassays, the suppression achieved under field conditions is often negligible (Bhowmik, 1992; Duke et al., 2001). Inderjit (2001) discussed the significance of soils in the expression of allelopathy that may be explained under field or pot experiments, and in terrestrial systems any bioassay without involving soil has no ecological relevance (Foy, 1999). In the absence of soil, any growth bioassay may have little or no significance. It is almost impossible to simulate exact field conditions in laboratory experiments, however an effort is made in present studies to simulate field conditions to the maximum extent mainly by using soil as a growing medium with the objectives of maintaining the physical, chemical, and biological soil factors of the natural setting (Schmidt & Ley, 1999). Moreover, the pots were placed in a screen house under open environments.

Different pant species contain allelo-chemicals that vary in type and concentration. Release of allelochemicals by mixed residues can have a synergistic and/or additive effect on the target species. The present studies were carried out to investigate the suppressive effect of allelopathic sorghum, sunflower and brassica residues on germination dynamics and seedling growth of horse purslane.

MATERIALS AND METHODS

Plant residues

Field grown mature plants of sorghum (cv. JS-263), sunflower (Hysun-33) and brassica (Rainbow) were collected. These plants were

chopped into 3-5 cm pieces with a fodder cutter and dried in an oven at 70 °C for 48 h. Whole plant residues were mixed into the soil *in situ.*

Bioassay

Seed of horse purslane was cleaned manually to ensure physical purity. This seed was surface sterilized with water: bleach solution (10:1) for 15 min and rinsed four times with distilled water.

Plastic pots of 29 x 18 cm and 10 kg capacity were filled with air dried, sieved, well mixed soil taken from the Agronomic Research Area. Soil belongs to Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification and Haplic Yermosols in FAO classification). The pH of saturated soil paste was 7.6 and total soluble salts were 1.2 dS m^{-1}. Organic matter, total nitrogen, available phosphorus and potassium were 0.71%, 0.062%, 13.1 ppm, and 179 ppm, respectively. Crop residues (sorghum, sunflower and brassica) alone and their combination in equal ratio were incorporated to have a total of 6 g kg^{-1} of soil (12 t ha^{-1}). Pots without residue were included as control. Five days after incorporation of residues, thirty seeds of horse purslane were sown in each pot. The pots were placed in a screen house under natural solar radiations with an average temperature of 35 ± 5 °C, and were irrigated as necessary to keep the soil moist and avoid water stress. Experiment was repeated four times. The experiment was visited daily to record emergence count (AOSA, 1990) until a constant count was achieved. Time taken to 50% emergence (E_{50}) was computed by following modified formula of Farooq et al. (2005):

$$E_{50} = t_i + \frac{\left(\dfrac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

where N is the final number of emerged seeds, and n_i and n_j are the cumulative number of seeds emerged by adjacent counts at times t_i and t_j where $n_i < N/2 < n_j$.

Mean emergence time (MET) was calculated according to Ellis & Robert (1981) as:

$$MET = \frac{\sum Dn}{\sum n}$$, where n is the number of seeds, which were emerged on day D and D is the number of days counted from the beginning of sprouting. Emergence index was calculated as described by AOSA (1983):

$$EI = \frac{No.\,of\,emerged\,seeds}{Days\,of\,first\,count} + - - - - - - + \frac{No.\,of\,emerged\,seeds}{Days\,of\,final\,count}$$

Root and shoot lengths were measured after 28 d. Plants were uprooted after wetting in water and washing under tap and separated into roots and shoots from each pot. Number of leaves and secondary roots were counted manually and averaged. A sub-sample of 2 g leaves was used to measure the leaf area using a leaf area meter (Licor, Model LI-3000). Harvested plant material was oven-dried at 70 °C for 48 h and dry biomass of root and shoot was recorded. Total seedling biomass was taken as sum of root and shoot biomass. Seedling mortality was calculated as under:

$$Seedling\,mortality\,(\%) = \frac{No.\,of\,seedlings\,emerged - No.\,of\,seedling\,survived}{No.\,of\,seedlings\,emerged} \times 100$$

Total phenolics were determined as described by Randhir & Shetty (2005) and are expressed as Gallic acid equivalents. Photosynthetic pigments (Chl.a and Chl.b) were extracted in in 80% ice cold acetone and read out at 663 and 645 nm wavelength in a UV-spectrophotometer (UV-4000, ORI, Germany). These are expressed as mg g^{-1} fresh leaf weight (Lichtenthaler & Wellburn, 1987).

Experimental design and statistical analysis

Experimental pots were arranged in completely randomized design with three replicates. Data were analyzed following an analysis of variance and treatment means were separated using least significant differences (LSD) at P≤0.05.

RESULTS AND DISCUSSION

Incorporation of allelopathic crop residues significantly suppressed the germination traits of horse purslane as compared with control (Table 1). Significant (P≤0.05) increase

KHALIQ, A. et al.

Table 1 - Influence of crop residues incorporated alone or in combination on germination dynamics of *Trianthema portulacastrum*

	Treatment	Time to start germination (days)	Final germination (%)	Time to 50 % emergence (days)	Mean emergence time (days)	Emergence index
T_1	Control	3.00 c[1/]	87.78 a	3.80 e	5.97 d	20.51 a
T_2	Sorghum residues incorporated at 6 g kg^{-1} soil	3.33 bc (11)[2/]	58.89 d (-33)	4.60 de (21)	6.69 bcd (12)	10.80 bc (-47)
T_3	Sunflower residues incorporated at 6g kg^{-1} soil	4.00 ab (33)	63.33 d (-28)	5.08 cd (34)	6.58 cd (10)	10.18 bc (-50)
T_4	Brassica residues incorporated at 6 g kg^{-1} soil	3.33 bc (11)	71.11 bcd (-19)	5.14 bcd (35)	7.11 abc (19)	13.61 abc (-34)
T_5	Sorghum + sunflower residues each incorporated at 3 g kg^{-1} soil	3.33 bc (11)	58.89 d (33)	5.19 bcd (37)	6.73 abcd (13)	15.12 ab (-26)
T_6	Sorghum + brassica residues each incorporated at 3 g kg^{-1} soil	4.00 ab (33)	63.33 d (-28)	5.75 abc (51)	7.05 abc (18)	11.70 bc (-43)
T_7	Sunflower + brassica residues each incorporated at 3 g kg^{-1} soil	4.33 a (44)	71.11 bcd (-19)	6.05 ab (59)	7.53 a (26)	10.48 bc (-49)
T_8	Sorghum + sunflower + brassica residues each incorporated at 2 g kg^{-1} soil [1]	4.00 ab (33)	65.56 cd (-25)	6.31 a (66)	7.43 ab (24)	7.98 c (-61)
	LSD	0.71	13.73	0.95	0.85	6.99

[1/] Means with different letters differ significantly at 5% level of probability. [2/] Figures given in parenthesis show percent change over control.

in time to start germination and E_{50} was caused by incorporation of all residues which exerted more pronounced negative influence on E_{50} (ranging from 37 to 66%) when used in combination than their sole application (21-35%) over control. Incorporation of brassica residue either alone or in combination with rest of the residues, significantly prolonged mean emergence time (MET) over control. Final germination was suppressed by incorporation of all residues over control and suppression was similar where the sunflower and sorghum residues were applied at 6 g kg^{-1} soil. Interestingly, these two residues when applied in combination each at 3 g kg^{-1} soil or at 2 g kg^{-1} soil with brassica at 2 g kg^{-1} soil had similar level of germination inhibition of horse purslane. Emergence indices of horse purslane were significantly (P≤0.05) reduced with the incorporation of residues, and combination of residues was more inhibitory than their sole application. Germination dynamics were reflective of the suppressive effects of sorghum, sunflower and brassica residues. Sorghum (Cheema & Khaliq, 2000), sunflower (Batish et al., 2002) and brassica (Chacon & Gliessman, 1982) residues released phytochemicals during decomposition process (Thorne et al., 1990; Nelson, 1996) that were reported to be inhibitory to germination

of a number of species (Purvis et al., 1985; Thorne et al., 1990; Cheema & Khaliq, 2000; Batish, 2002; Norsworthy et al., 2005).

Seedling growth of horse purslane was also significantly (P≤0.05) suppressed by the crop residues applied either alone or in combination (Table 2). The combination of sorghum, sunflower, brassica residues each at 2 g kg^{-1} soil, resulted in the highest inhibition of shoot (88%) and root (59%) lengths. Dry matter accumulation in shoot and root was also reduced. Sole application of brassica scored 86 and 91% reductions in shoot and root dry weight per seedling, respectively. It was statistical similar (P≤0.05) with other combinations of crop residues. Sorghum, sunflower, brassica residues exerted drastic influence on leaf score and suppressed it by 94% (Table 2). Incorporation of crop residues also restricted lateral spread and proliferation of roots so that combination of sorghum and sunflower recorded 91% reduction in number of lateral roots per plant over control. Besides leaf score, leaf expansion in horse purslane was also reduced. Leaf area was reduced to higher extent with increasing number of crop residues. Sorghum and sunflower residue each at 3 g kg^{-1} soil caused 71% seedling mortality, which was at par with that (64%) recorded for brassica residue alone at 6 g kg^{-1}

Effect of crop residues applied isolated or in combination on ...

125

soil (Table 2). The inhibition of horse purslane germination and growth may be attributed to the presence of several phytotoxins in sorghum such as Gallic acid, protocatechuic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, p-coumaric acid, benzoic acid, ferulic acid, m-coumaric acid, caffeic acids, p-hydroxybenzaldehyde and sorgoleone (Netzly & Butler, 1986; Cheema et al., 2009). Several studies have identified allelochemicals from sorghum, their secretion mechanisms and genes regulating them and also confirmed sorghum allelopathic potential under natural and controlled conditions (Weston & Duke, 2003; Weston, 2005). Sunflower contains allelochemicals viz. chlorogenic acid, isochlorogenic acid, á-naphthol, scopolin, and annuionones (Macias et al., 2002; Anjum & Bajwa, 2005). The members of Brassicaceae family contain glucosinolates that upon

hydrolysis yield isothicynates (Jiménez-Osornio & Gliessman, 1987), and isoprenoids and benzoids (Tollsten & Bergstrom, 1988) which exert allelopathic effects on germination and growth of other species (Norsworthy et al., 2005). Residues species varied in their severity against horse purslane eliciting brassica as the more toxic one. This shows the susceptibility of horse purslane to isothiocyanates. The variable influence of sorghum, sunflower and brassica residues on the horse purslane germination and seedling growth may be due to the type and concentration of allelochemicals present in these species (Xuan et al., 2005).

Horse purslane seedling grown in soil amended with allelopathic crop residues had reduced chlorophyll a and b in their leaves (Table 3). Total chlorophyll was reduced to

Table 2 - Influence of crop residues incorporated alone and in combination on seedling growth of *T. portulacastrum*

Treat.	Shoot length	Root length	Shoot dry weight	Root dry weight	Total dry weight	Leaf score per plant	Lateral roots per plant	Leaf area	Seedling mortality
	(cm)		(g seedling^{-1})					(cm^2)	(%)
T_1	26.00 a[1]	9.62 a	2.27 a	0.16 a	2.42 a	182.21 a	13.19 a	58.74 a	-
T_2	8.21 d (-68)[2]	5.22 d (-46)	0.97 b (-57)	0.03 c (-80)	1.00 b (-59)	52.57 c (-71)	4.05 bc (-69)	37.50 bc (-36)	43.86 bc
T_3	9.21 cd (-65)	7.07 b (-27)	1.05 b (-54)	0.09 b (-41)	1.15 b (-53)	54.15 c (-70)	3.06 cd (-77)	36.25 c (-38)	56.70 abc
T_4	9.94 bc (-62)	5.99 cd (-38)	0.31 c (-86)	0.02 c (-91)	0.33 c (-86)	48.42 c (-73)	3.85 bc (-71)	37.73 bc (-36)	64.18 ab
T_5	5.47 e (-79)	6.08 cd (-37)	0.36 c (-84)	0.02 c (-92)	0.38 c (-84)	22.73 d (-88)	1.15 e (-91)	36.07 c (-39)	70.62 a
T_6	11.31 b (-57)	6.61 bc (-31)	0.79 b (-65)	0.02 c (-84)	0.82 b (-66)	66.19 b (-64)	5.28 b (-60)	38.32 bc (-35)	35.58 c
T_7	5.95 e (-77)	5.75 cd (-40)	0.21 c (-90)	0.01 c (-92)	0.23 c (-91)	29.04 bd (-84)	1.81 de (-86)	39.81 b (-32)	32.36 c
T_8	3.09 f (-88)	3.93 e (-59)	0.13 c (-95)	0.01 c (-94)	0.14 c (-94)	11.49 e (-94)	1.69 de (-87)	29.87 d (-49)	42.46 bc
LSD	1.70	0.92	0.29	0.05	0.33	10.15	1.68	2.92	24.82

[1] Means with different letters differ significantly at 5% level of probability. [2] Figures given in parenthesis show percent change over control.

Table 3 - Influence of crop residues incorporated alone and in combination on photosynthetic pigments and total soluble phenolics in *T. portulacastrum*

Treatment	Pigment (mg g^{-1} fresh weight)				Total soluble phenolics (mg g^{-1} fresh weight)
	Chlorophyll a	Chlorophyll b	Chlor. a/Chlor. b	Total Chlorophyll	
T_1	3.16 a[1]	1.41 a	2.24	4.57 a	1.41 c
T_2	2.55 ab	1.12 abc	2.28	3.67 ab	2.59 b
T_3	2.47 ab	1.26 ab	1.96	3.73 ab	2.54 b
T_4	1.38 c	0.63 d	2.19	2.01 c	2.74 b
T_5	1.73 bc	0.84 cd	2.06	2.57 bc	2.79 b
T_6	1.67 bc	0.92 bcd	1.82	2.59 bc	2.70 b
T_7	2.03 bc	0.88 bcd	2.31	2.91 bc	2.60 b
T_8	1.36 c	0.63 d	2.16	1.99 c	3.40 a
LSD	1.07	0.38	-	1.26	0.31

[1] Means with different letters differ significantly at 5% level of probability.

the highest level (63%) with incorporation of sorghum, sunflower and brassica residues each at 2 g kg⁻¹ soil. However, sole incorporation of brassica residue at 6 g kg⁻¹ soil had similar suppression (56%) in total chlorophyll. There was a mixed response regarding ratio of chlorophyll a/b, and this seemed to be influenced by the amount of individual residues. Significantly higher phenolic contents were observed in horse purslane seedlings that were grown in soil amended with crop residues. A combination of all three crop residues produced highest water soluble phenolics (Table 3). Chlorophyll content in leaves of horse purslane decreased in soil amended with allelopathic crop residues (Table 3). Reduced chlorophyll content in allelochemical-treated plants has been reported elsewhere (Singh et al., 2010). Soybean plants treated with phenolic acids such as ferulic, p-coumaric, and vanillic acids exhibited reduced biomass due to lower chlorophyll content in leaves (Patterson, 1981). Allelochemicals released by decomposing crop residues might have inhibited chlorophyll by interfering with biosynthesis of photosynthetic pigments or enhancing their degradation or through the integration of both. Yang et al. (2002) investigated the effects of three phenolic acids on the concentration of chlorophyll and its three biosynthetic porphyrin precursor's proto, Mg-Proto and Pchlide. They argued that tested phenolic acids might have Mg-chelatase, an enzyme responsible for the conversion of Proto into Mg-Proto as their major target. In contrast, allelochemical mediated suppression in the chlorophyll content from the trichomes of *Parthenium hysterophorus* was attributed to enhanced chlorophyll degradation (Kanchan & Jayachandra, 1980). Reduction in chlorophyll was associated with higher total phenolics in horse purslane seedling (Figure 1). Allelochemical-mediated reduction in seedling photosynthetic pigments primarily due to phenolic acids has been reported (Inderjit & Dakshini, 1992).

Mixed application of crop residues showed higher inhibition of horse purslane germination and seedling growth than their isolated treatments (Tables 1 and 2). It seems that apparently a combination of residues with variety of allelochemicals each
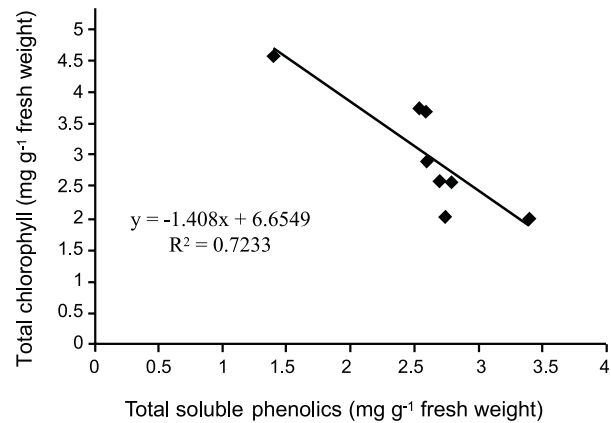

Figure 1 - Relationship between total soluble phenolics and total chlorophyll content in *Trianthema portulacastrum*.

having a different mode and site of action was more effective than application of individual crop residues (Jamil et al., 2009). This might had increased not only the number of susceptible sites but also the concentration and uptake of allelochemicals. A number of experiments have established that combinations of allelochemicals act additively or synergistically to inhibit plant growth (Duke & Lydon, 1993; Einhelling, 1996).

Present work concluded that combined application of sorghum, sunflower and brassica residues have potential to suppress germination and seedling growth of horse purslane. Khanh et al. (2005) asserted the use of a combination of different crop plants may help control more weed species than a single crop material. Blum (1996) reported that mixtures of phenolic allelochemicals and other organics present in the substratum could cause inhibitory effects even though concentration of the individual allelochemicals is below the required level to cause an effect. These residues can be used as eco-friendly approach for management of this weed, provided phytotoxins entering into the soil encounter the early growth and development of horse purslane. Investigations may further be conducted to evaluate suppressive efficacy of such residues applied alone and in combination with each other under field conditions. Sorghum, sunflower and brassica crop allelopathy may also be evaluated in different crop rotations for managing the spread of horse purslane.

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