Article

Suppression of Maize (*Zea mays*) Seedling Growth by Invasive Alligatorweed (*Alternanthera philoxeroides*) Residues

*Supressão do Crescimento de Plântulas de Milho por Resíduos de Erva-de-Jacaré (**Alternanthera philoxeroides**)*

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ABSTRACT - To investigate the allelopathic influence of soil incorporated plant residues of alligatorweed on emergence and seedling growth of maize (*Zea mays*), an experiment was conducted under laboratory conditions in the 2014-15 period. The effect of soil amended with whole plant residues of alligatorweed at different concentrations (1, 2, 3, 4 and 5% on w/w basis) was compared with residue free soil (control). Emergence traits, such as emergence percentage, time to 50% emergence, mean emergence time and emergence index were negatively influenced by weed residue incorporation as compared with weed residue free soil. Alligatorweed residues also exerted a pronounced negative influence on root and shoot lengths, root and shoot dry weights and seedling vigor index of the maize crop. The results revealed that the inhibitory effect was directly proportional to the increasing concentrations (1, 2, 3, 4 and 5%) of the weed residues as compared to control treatment. It was observed that soil infested with 4% and 5% residues of alligatorweed caused a maximum reduction in emergence (76-87%), root and shoot lengths (58-73% and 42-61%), root and shoot dry weights (49-58% and 40-48%) and seedling vigor index (87-95%) of the maize crop. Chloro-genic acid, ferulic acid, 4-hydroxy-3-methoxybenzoic acid, trans-4-hydroxy 3-methoxy cinnamic acid, gallic acid, *p*-coumaric acid, querceitin and syringic acid were identified as water soluble phenolics in alligatorweed residue soil saturated solution. This study indicates that the allelopathic potential of alligatorweed may play an important role in suppressing maize seedling growth.

Keywords: invasive weed, allelopathy, emergence, maize, weed residues, seedling growth.

RESUMO - *A fim de investigar a influência alelopática de resíduos vegetais de erva-de-jacaré incorporados no solo na emergência e no crescimento de plântulas de milho (**Zea mays**), foi conduzido um experimento em condições de laboratório no período 2014-15. O efeito do solo corrigido com resíduos vegetais inteiros de erva-de-jacaré em diferentes concentrações (1, 2, 3, 4 e 5% em uma razão p/p) foi comparado com o do solo livre de resíduos (controle). As características de emergência, como porcentagem de emergência, tempo de emergência de 50% das plântulas, tempo médio de emergência e índice de emergência, foram influenciadas negativamente pela incorporação de resíduos de plantas daninhas, em comparação com o solo livre destas plantas. Os resíduos de erva-de-jacaré também exerceram influência negativa acentuada sobre os comprimentos da raiz e da parte aérea, os pesos secos da raiz e da parte aérea e o índice de vigor das plântulas da cultura de milho. Os resultados revelaram que o efeito inibitório foi diretamente proporcional ao aumento das concentrações (1, 2, 3, 4 e 5%) dos resíduos de plantas daninhas,*

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em comparação com o tratamento controle. Observou-se que o solo infestado com 4% e 5% de resíduos de erva-de-jacaré causou redução máxima na emergência (76-87%), no comprimento da raiz e da parte aérea (58-73% e 42-61%), no peso seco da raiz e da parte aérea (49-58% e 40-48%) e no índice de vigor de plântulas (87-95%) da cultura de milho. Ácido clorogênico, ácido ferúlico, ácido 4-hidroxi-3-metoxibenzoico, ácido-4-hidroxi-3-metoxi-trans-cinâmico, ácido gálico, ácido p-cumárico e ácido siríngico foram identificados como fenólicos solúveis em água. O presente estudo indica que o potencial alelopático da erva-de-jacaré pode desempenhar papel importante na supressão do crescimento de plântulas de milho.

Palavras-chave: plantas invasoras, alelopatia, emergência, milho, resíduos de plantas daninhas, crescimento de plântulas.

INTRODUCTION

Invasive plants species are responsible for disrupting the stability of natural ecosystems and native biodiversity and for causing major economic losses in agriculture, forestry, livestock husbandry, and many other industries worldwide (Pimentel et al., 2005; Holmes et al., 2009). These plants can grow, spread and dominate in a new range to cause detrimental effects on pre-existing native ecosystems (Mack et al., 2000). There are several characteristics which explain the success of invasive species over native species, e.g., growth rate or resource use (Maron et al., 2004), rapid evolution in the introduced range, phenotypic plasticity, high seed production and successful dispersal mechanisms, reproduction, and longer photosynthetic periods (Ehrenfeld et al., 2001; Bossdorf et al., 2005). Recently, it has been reported that allelopathy plays a key role in weed invasions, particularly when invaders release compounds that reduce germination, growth and/or reproduction of native plants (Hierro and Callaway, 2003; Inderjit et al., 2008). The mode of entry of allelopathic substances into the environment plays an important role in the expression of their phytotoxic properties (Zimdahl, 2007).

Several reports have documented the deleterious effect of decaying and decomposing weed residues on germination, growth and yield of subsequent crops in the field (Khaliq et al., 2011). The mechanisms for the allelochemicals to be released from plants into the environment have a significant role in affecting other plants in the vicinity (Safdar et al., 2014). These allelochemicals may be washed directly from the weed residues or may become more toxic after being transformed by microbial activity during decomposition (Kumar et al., 2006).

Maize (*Zea mays*) is the third most important cereal crop after wheat and rice on the basis of grain production in the world. Among various factors considered for yield reduction in maize, weeds have supreme importance as they decline maize yield up to 90% depending on degree of weed infestation (Chikoye and Ekeleme, 2003; Dalley et al., 2006). The main harmful weeds causing significant yield reduction in maize are *Amaranthus viridis* (wild amaranth), *Cyperus rotundus* (nut grass), *Cynodon dactylon* (bermuda grass), *Echinochloa colona* (jungle rice) and *Trianthema portulacastrum* (horse purslane) (BCS, 2015)**.**

In addition to these weeds, alligator weed is now emerging as an invasive weed in maize crops of Pakistan which seems to be more proliferating and problematic than conventional weeds. It is native to southern South America (Argentina, Paraguay, Uruguay and Brazil). It is a problematic weed of 30 countries in the world including USA, Puerto Rico, New Zealand, Burma, China, Thailand, Indonesia and Australia, where it has been considered as an invasive alien weed. It is listed among the 20 weeds of national significance in Australia (Gunasekera and Bonilla, 2001).

Alligator weed can invade agricultural crops, leading to crop failure. It is considered as an invasive and troublesome weed in rice, maize, cotton, soybean, vegetables and fruit trees in China (Lu et al., 2002; Ye et al., 2003). Alligator weed has been proliferating in different field crops in that country because the local ecological conditions favor its growth. The literature shows that no information is available about the allelopathic effects of alligator weed residues on emergence and seedling growth of maize. It is hypothesized that alligator weed residues may hamper the emergence and seedling growth of maize plants by releasing water-soluble phenolics.

Therefore, the present study was planned with the objective to assess the allelopathicity of alligator weed against the emergence and early seedling growth of maize.

MATERIALS AND METHODS

Collection and mechanical processing of plant material

Field grown plants of alligator weed were uprooted at maturity from the Agronomic research area, University of Agriculture, Faisalabad, Pakistan, in August and repeated in September 2014. These weed plants were washed in distilled water for removal of dust and soil particles and then dried at room temperature (30 ℃±4) for seven days. The well-dried plants were chopped into about 5 cm pieces with a pair of scissors. In order to study the effect of alligator weed residues on emergence and early seedling growth of maize, the soil was collected from a profile of 0 to 25 cm on a weed free site. The soil was air dried, sieved and stored in plastic bags at room temperature. The soil was sandy loam.

Seedling emergence and growth bioassay in soil amended with alligator weed residues

Under natural field conditions, residues of alligatorweed fall on the soil surface and get mixed with the soil with subsequent tillage practices. In order to simulate these natural conditions, 3, 6, 9, 12 and 15 g of alligator weed residues were added and mixed with 300 g of soil already collected so as to get 1, 2, 3, 4, and 5% (w/w) concentrations, respectively. The pots measured 5 cm x 12.5 cm and were filled with soil already mixed with alligator weed residues (0, 1, 2, 3, 4 and 5% on w/w basis). Then, 100 mL of distilled water was added to each pot and pots were kept at room temperature for 15 days. As a control, 300 g of unmodified soil was soaked in 100 mL of water for 15 days. Ten seeds of maize hybrid "30R50" were sown in each pot. Pots were placed on a laboratory bench. Each pot was watered with 20 mL of water daily. The experiment was laid out in a completely randomized design with four replications. A maximum temperature of 32 ℃ and a minimum temperature of 28 ℃ were recorded throughout the course of the experiment.

Fifteen-day old seedlings were uprooted and washed with water and their shoot and root lengths and dry weights were recorded after drying in an electrical oven at 70 ℃ for 48 hours.

Data collection and statistical analysis

The number of seeds emerged was counted daily up to fifteen days. Seedlings were considered emerged when the coleoptile was first visible at the soil surface. Emergence was observed daily in accordance with the methods of the Association of Official Seed Analysis (AOSA, 1990). Emergence count data were used to calculate various seed emergence and vigor parameters as detailed below:

Emergence percentage (EP) was calculated by the following formula:

$$EP = [N_T \times 100]/N$$

where N_T: the proportion of emerging seeds in each treatment for the final measurement, and N: number of seeds used in the bioassay.

Mean Emergence Time (MET) was calculated as per the equation of Ellis and Roberts (1981).

$$MET = \Sigma\, Dn / \Sigma\, n$$

where n is the number of seeds that had emerged on day D and D is the number of days counted from the beginning of emergence.

Time taken to 50% emergence (E_{50}) was calculated by using the formula given by Coolbear et al. (1984) as modified by Farooq et al. (2005).

$$T_{50} = t_i + \{(N/2) - n_i\}\,(t_j - t_i)\,/n_j - n_i$$

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where N is the final number of germinated seeds while nj and ni are the cumulative numbers of seeds germinated by adjacent counts at times tj and ti, respectively, where $ni<N/2<nj$.

Emergence index (EI) was calculated as given by following the formula described by Scott et al. (1984):

$$EI = N1 / D1+....................+NL / DL$$

where N1: number of seeds emerged on 1st count, D1: days to 1st count, NL: number of seeds emerged on the last count, and DL: days to last count.

Seedling vigor index (SVI) was calculated by the following formula as described by Orchard (1977):

$$SVI = seedling\ length\ (cm) \times emergence\ percentage$$

Emergence energy (EE) was recorded on the 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan et al., 2002).

Soil aqueous extracts were tested for their biochemical characteristics. Those were prepared by soaking 10 g soil in 100 mL distilled water for 24 hours. The soil extracts were then filtered by the use of micro-syringe filters. Total soluble phenolics were determined as described by Randhir and Shetty (2005) and were expressed as gallic acid equivalents. For identification and quantification of their suspected phytotoxins, aqueous extracts were chemically analyzed (Table 1) on a Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). The peaks were detected by a UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification and quantification.

Table 1 - Phytotoxic composition of soil amended with alligatorweed residues (mg L^{-1})

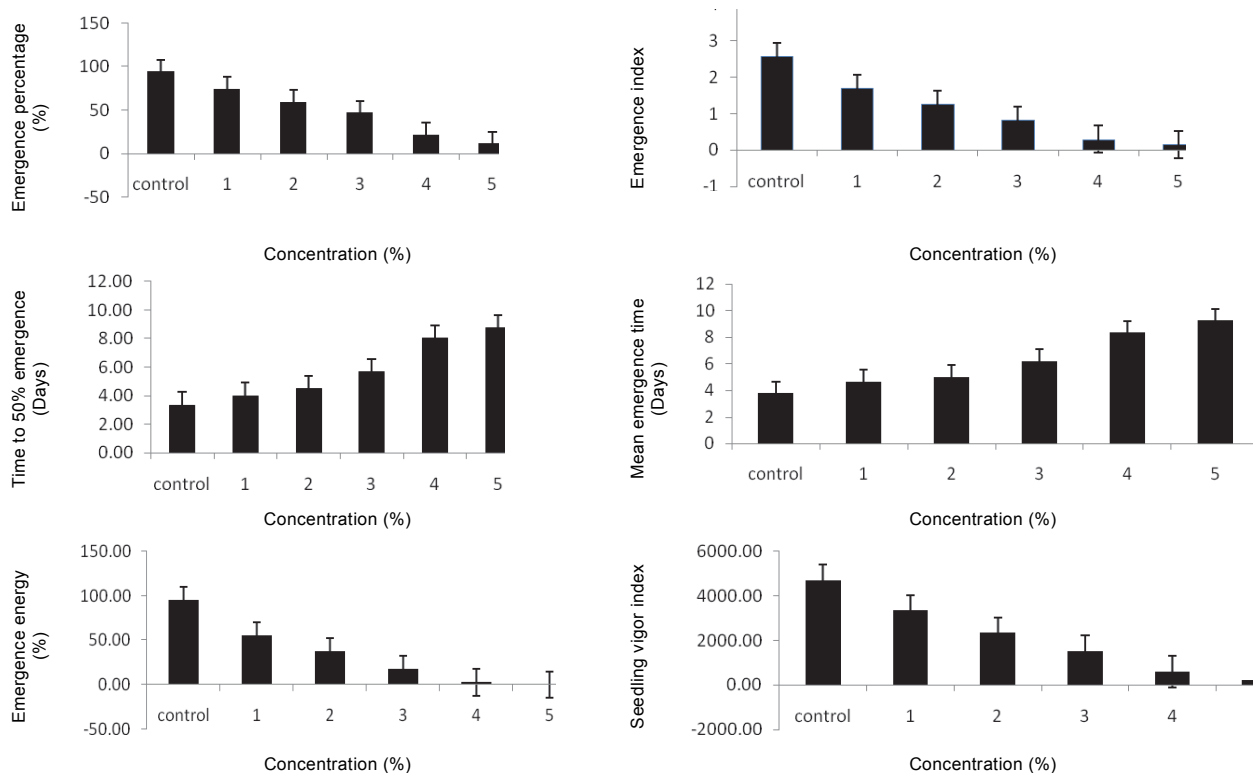| Residue conc. (%)	Gallic acid	4-hydroxy-3-methoxyben-zoic acid	*p*-coumaric acid	Chlorogenic acid	Quercetin	trans-4-hydroxy 3-methoxy cinnamic acid	Ferulic acid	Syringic acid
0	0	20.84	1.33	0	0.47	8.36	30.27	0
1	0	25.72	1.44	8.73	0.41	14.25	29.88	0
2	0	30.54	1.67	9.62	0.16	29.23	20.32	0
3	0.65	28.42	1.83	10.72	0.12	33.27	17.78	1.23
4	0.82	33.26	2.79	13.24	0.23	44.42	18.36	1.38
5	0.9	34.56	2.96	14.31	0.3	39.21	15.58	0.82

Concentration of each isolated compound was determined by the following equation:

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

The average data collected in study were analyzed statistically using Fisher's analysis of variance techniques (Steel et al., 1997) using the computer software Statistix 8.1. Tukey's Honestly Significant Difference (HSD) test was applied at 5% probability level to test the significance of treatment means. The experiment was repeated twice. The results have been presented and described for the first experiment conducted because the repeated experiment showed similar results.

RESULTS AND DISCUSSION

Data on various emergence and seedling growth parameters of maize as influenced by the residue amended soils of alligator weed are shown in Figures 1 and 2. The results revealed that

Bars indicate standard error of means.

Figure 1 - Means of emergence parameters of maize as influenced by soil amended with alligator weed residues.

alligator weed residues affected all the emergence traits of the maize crop. The residues of alligator weed at their 5% concentration caused maximum inhibition in emergence percentage, emergence energy (EE) and emergence index (EI) of maize.

In the soil amended with alligator weed residues, emergence percentage of maize was reduced to 21, 37, 50, 76 and 87% at their 1, 2, 3, 4 and 5% concentrations, respectively, when compared with the control.

Emergence index (EI) is a measure of both percentage and speed of germination. Early-grown seeds have more EI than late-grown seeds. It means maximum weightage is given to the seeds emerging on the first day. Figure 1 showed that alligator weed residues reduced EI with increasing residues concentration as compared to the unmodified soil (control). Minimum emergence index (0.14) was recorded with seeds sown in soil amended with alligator weed residue with 5% concentration.

The lowest EE (2.5 and 0) was found by the treatments containing soils with 4 and 5% concentrated alligator weed residues (Figure 1).

Data shown in Figure 1 revealed that alligator weed residues of all concentrations significantly delayed time to 50% emergence and mean emergence time as compared with the control.

Seedling vigor index (SVI) values were significantly reduced in response to alligator weed residues (Figure 1). The minimum seedling vigor index (220) was recorded where alligator weed residues were 5%.
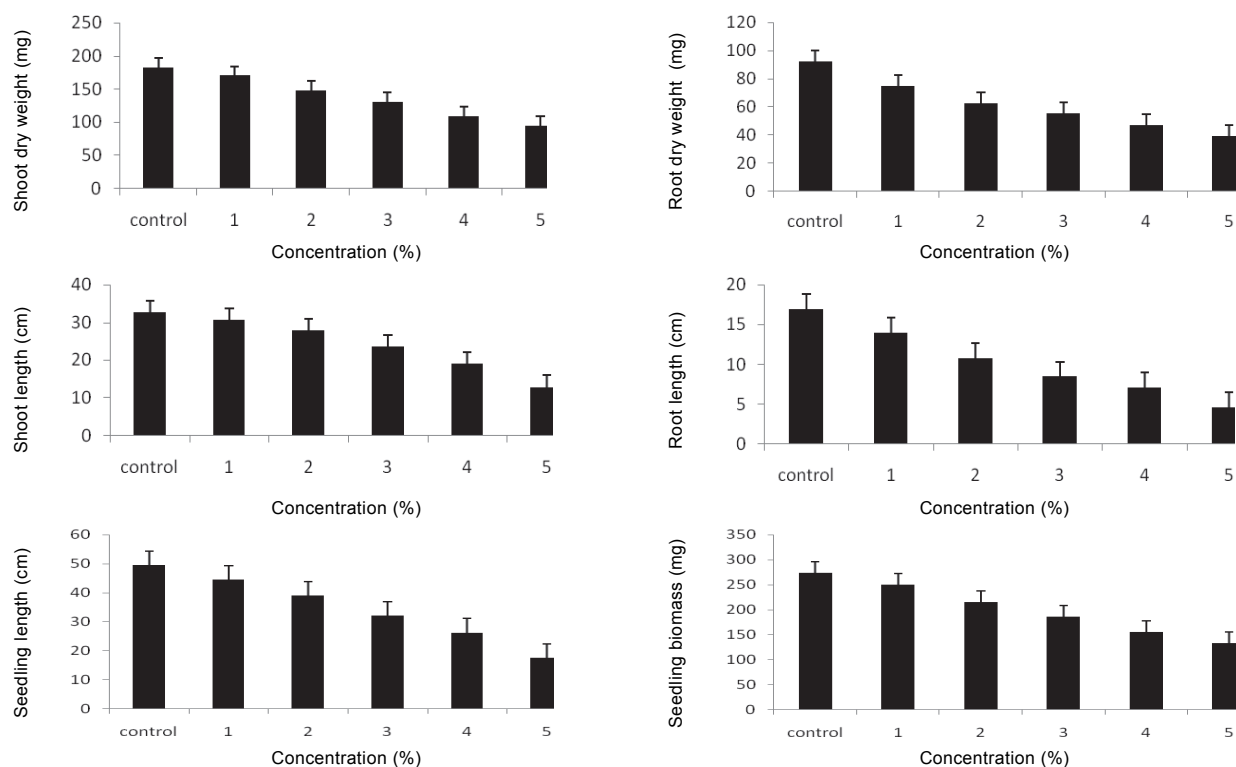
The inhibition in all the seed emergence traits of maize by increasing the concentration of alligator weed residues in soil was probably due to the phytotoxic influence of gallic acid, 4-hydroxt-3methoxybenzoic acid, *p*-coumaric acid, chlorogenic acid, trans-4-hydroxy-3-methoxy cinnamic acid and syringic acid because these allelochemicals showed a consistent increase in soil by increasing alligator weed residue concentration (Table 1). These results are supported by those of Zuo et al. (2012), who reported that the species *Alternanthera* grown under aquatic

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conditions have strong allelopathic potential and produce more allelopathic substances. Similar findings were also reported by Zhang et al. (2009) and Dhole et al. (2011). They revealed that *A. philoxeroides*, *A. sessilis* and *E. crus-galli* suppressed the growth of sorghum and ryegrass by both competition and release of secondary metabolites in the ecosystem.

Data on various maize seedling growth parameters as affected by the different concentrations of alligator weed residues are shown in Figure 2, which revealed that alligator weed residues have significant inhibitory effects on seedling root and shoot lengths, and their dry weight at all concentrations as values of these parameters were gradually reduced in response to increasing concentration of residues in soil as compared with the control soil. The lowest values for shoot length (12.88 cm) and dry weight (95.00 mg), root length (4.63 cm) and dry weight (38.75 mg), seedling length (17.50 cm) and dry weight of maize seedlings (133.75 mg) were recorded with alligator weed residues at 5% concentration.

The deleterious phyto-inhibitory influence on seedling growth of maize in response to increasing concentration of alligator weed residues seems to be due to an increase in the concentrations of gallic acid, 4-hydroxt-3-methoxybenzoic acid, *p*-coumaric acid, chlorogenic acid, trans-4-hydroxy-3-methoxy cinnamic acid and syringic acid in soil (Table 1). Alligator weed residues released growth-suppressing substances into the soil environment, which accumulate in such bioactive concentrations that they adversely influence the growth of maize plants. Similar results were recorded by Liu-Qing et al. (2007), who reported that alligator weed inhibited the root growth of lettuce (*Lactuca sativa*) and barnyard grass (*E. crus-galli*) by releasing growth-suppressing substances. Abbas et al. (2014) also published similar findings that aquatic weeds (*A. philoxeroides*, *A. sessilis*, *Conyza stricta*, *Polygonum barbatum* and *E. crus galli*) released allelochemicals which affected the growth of wheat in a rice-wheat cropping system.

Channappagoudar et al. (2005) recorded that phenolic compounds are the main allelochemicals which cause inhibition in seed emergence and early seedling growth of plants. A further analysis showed that with increasing concentration of residues, there is a higher rate of release of phenolics. The range of total phenolic concentration was 277-565 ug g^{-1} with alligator

Bars indicate standard error of means.

Figure 2 - Means of seedling growth of maize as influenced by soil amended with alligator weed residues.

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weed residues range of 0-5% on a w/w basis (Table 2). This study is in agreement with earlier studies reported by Abbas et al. (2015). They concluded that alligator weed residues have water soluble phenolics in their tissues which influence the sustainability of rice ecosystems and rice-wheat cropping systems. On the basis of these results, we can conclude that alligator weed residues have phyto-toxic substances released after decomposition into the soil which can inhibit the emergence and seedling growth of maize crops. However, more detailed research is needed so that their allelopathic based crop growth inhibition can be studied in different crops.

Table 2 - Total amount (ug g^{-1}) of water soluble phenolics in soil amended with alligator weed residues

Concentration (%)	Total phenolic concentrations
0 (control)	277
1	307
2	311
3	362
4	454
5	565

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