

Article

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SOIL MEDIATED ALLELOPATHIC EFFECT OF *Echinochloa colona* ON GERMINATION AND SEEDLING GROWTH OF *Zea mays*

*Efeito Alelopático Mediado Pelo Solo de Echinochloa colona sobre a
Germinação e o Crescimento de Plântulas de Zea mays*

ABSTRACT - The soil mediated allelopathic effect of *Echinochloa colona* (L.) Link (jungle rice) was investigated on seed germination and seedling growth of *Zea mays* L. (maize). The experiment was conducted in the Agronomy farm of the University of Agriculture, Faisalabad, Pakistan. This study investigated the effect of different concentrations (1, 2, 3, 4 and 5% on w/w basis) of soil incorporated *E. colona* plant residues on emergence and seedling growth of maize and compared them with residue free soil (control). The results revealed the significant increase with soil incorporated *E. colona* residues in maize seedling growth till 2% *E. colona* plant residues, and an inhibitory effect was noticed over 2% *E. colona* plant residues in comparison to the control treatment. There was maximum reduction in emergence percentage, root and shoot lengths, root and shoot dry weights and seedling vigor index of maize seedlings at 5% *E. colona* plant residues. Quercetin, ferulic acid, sinapic acid, syringic acid, cinnamic acid, m-coumaric acid were identified as water soluble phenolic in water extract of *E. colona*.

Keywords: allelopathy, emergence, *E. colona*, maize, weed residues, seedling growth.

RESUMO - Foi realizado um estudo sobre o efeito alelopático mediado pelo solo de *Echinochloa colona* (L.) Link (capim-arroz) na germinação de sementes e no crescimento de plântulas de *Zea mays* L. (milho). A experiência foi conduzida em uma fazenda do Departamento de Agronomia da Universidade de Agricultura, Faisalabad, Paquistão. Investigou-se o efeito de diferentes concentrações (1, 2, 3, 4 e 5% na razão p/p) de resíduos de plantas de *E. colona* incorporados ao solo no surgimento e crescimento de plântulas de milho, seguido de comparação com solo livre de resíduos (controle). Os resultados revelaram o efeito estimulador dos resíduos de *E. colona* incorporados ao solo em plântulas de milho de até 2% de resíduo de plantas de *E. colona*; observou-se efeito inibitório nas concentrações acima de 2% de resíduos de plantas de *E. colona* em comparação com o tratamento controle. Foi observada redução máxima em porcentagem de emergência, comprimento de raiz e parte aérea, peso seco da raiz e da parte aérea e índice de vigor de plântulas de milho na concentração de 5% de resíduos de plantas de *E. colona*. Quercetina, ácido ferúlico, ácido sinápico, ácido siríngico, ácido cinâmico e ácido m-cumárico foram identificados como fenólicos hidrossolúveis no extrato de água de *E. colona*.

Palavras-chave: alelopatia, emergência, *E. colona*, milho, resíduos de plantas daninhas, crescimento de plântulas.

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INTRODUCTION

Allelopathy is any effect that is caused by plants and microorganisms on another plant as a result of the release of chemical compounds which are named allelochemicals; further allelopathic effects on plants have no limitations because they may be direct or indirect, harmful or beneficial (Rice, 1984). Allelochemicals are produced by plants as a result of secondary metabolism products.

Weeds affect crop growth by consuming nutrients from the soil nutrient pool and utilizing environmental resources, but the major effect caused by weeds is the release of allelochemicals. Because of continuous utilization of nutrients and competition with crop plants, weeds become more harmful and are a major plant growth reduction factor. In some previous studies, the effect of weeds on crop yield reduction was well-documented (Akobundu, 1987; Swanton et al., 1993). Reduction in seed germination and seedling growth is a common problem caused by allelopathy. As with synthetic herbicides, control of the effect of allelochemicals is not easy because their mode of action is not common and there is not enough knowledge about their physiological target sites. Many experimental reports are available on the harmful effect of weeds on seed germination, growth and yield reduction of subsequent crops as a result of decaying and decomposition of weeds, which release allelochemicals in the field (Khaliq et al., 2011). Weeds influence crop germination and growth by releasing allelochemicals (Narwal, 2004).

Among cereals, maize is one of the major cereal crops consumed as a staple food by a large part of the world population. Maize crop is a major source of income for farmers in developed countries (Tagne et al., 2008). In Pakistan, maize crop ranks third in production after wheat and rice and 94% of the crop is grown in Punjab and KP provinces. In the existing cropping system of Pakistan, maize crop attained an important position because of its good economical input and its use in diversified food products such as corn oil, poultry feed, starch and glucose-D etc. (Khalil and Jan, 2002). Maize crop contributes about 2.1 percent to the value added in agriculture and up to 0.4 percent to the GDP (Pakistan, 2014).

Jungle rice has become an important grass weed, but little attention is given to its phytotoxic potential. It is native to India but now it has been reportedly found in the tropical and subtropical regions of the world. In the same way, it is also found in Africa, Australia and various parts of Asia. It is reported that it is widely distributed at sea levels up to 2000 m (Holm et al., 1977; Lazarides, 1980). Worldwide, maize is considered as one of the most important grass weeds (Holm et al., 1991; Chauhan and Johnson, 2009). Jungle rice is grown as a major weed in many crops, including rice, cotton, peanut, corn, sorghum, sugarcane and cassava (Holm et al., 1991). By this fact, yield loss up to 12-14% has been reported (Chander et al., 2008). Growth of jungle rice is best suited to sunny and light shaded moist places. However, it avoids long inundated soils. It persists in moist soils when the soil desiccates. In India and Pakistan, *E. crus-galli* (barnyard grass) and jungle rice are the most important annual weeds in summer crops (Shad and Siddiqui, 1996). Being a C4 plant, jungle rice is highly polymorphic. At the base, its branches are smaller and spread with an open type of growth as compared to barnyard grass (Williams, 1956). Jungle rice causes substantial yield reductions owing to severe infestations and rapid growth (Pons, 1985; Pons and Kruijf, 1985). Keeping in mind the importance of jungle rice and maize, the present study has been planned to find out the allelopathic effect of jungle rice on germination and seedling growth of maize.

MATERIALS AND METHODS

Field grown jungle rice plants were uprooted at maturity by using the random sampling technique and dried at room temperature ($30\text{ }^{\circ}\text{C} \pm 4$) for seven days. The dried plants were cut into 5 cm pieces with scissors. Pots whose size was 5 cm x 12.5 cm were filled with 300 g soil in different concentrations of jungle rice plant residues (0, 1, 2, 3, 4 and 5% on a weight by weight basis). Ten seeds of maize cultivar DK 6789 were sown in each pot. A uniform quantity of water was applied to the pots as per requirement. A completely randomized design was used to conduct the experiment with four replications.

Leachates from different treatments were obtained by filtering the mixture (soil and jungle rice residues) through Sartorius Minisart syringe filters of 0.45 μm pore size. These samples

were subjected to analyses for their allelochemicals characteristics. The method of Randhir and Shetty (2005) was used to determined total soluble phenolics, and final concentration was recorded by drawing a calibration curve of gallic acid and expressed as gallic acid equivalent. For identification of suspected phytotoxins and their quantification in aqueous extracts, a Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan) was used for chemical analysis. A UV detector was used for peak detection. Further, for identification and quantification of phytotoxin, standards of suspected phytotoxins (Aldrich, St Louis, USA) were run. The concentrations of isolated compounds were determined by the following equation:

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

Data collection and statistical analysis

Emergence percentage (EP)

Emergence was determined by counting germinated seeds of maize every day over a 7 days' period. Seeds were considered to be emerged when their protruding plumule reached 2 mm in length. Emergence was observed daily according to the methods of the Association of Official Seed Analysts (AOSA, 1990) and converted into emergence percentage by the following formula:

$$EP = \frac{\text{Emerged seed}}{\text{Total seed}} \times 100 \quad (\text{eq. 1})$$

Mean emergence time (MET)

The equation of Ellis and Roberts (1981) was used for calculation of mean emergence time.

$$MET = \frac{\sum Dn}{\sum n} \quad (\text{eq. 2})$$

where D represents the number of days as measured from the start of seed emergence while n represents number of seeds that had emerged on day " D ".

Time to 50% emergence (T_{50})

To calculate 50% emergence time (T_{50}), the formula of Coolbear et al. (1984), further modified by Farooq et al. (2005), was used.

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)} \quad (\text{eq. 3})$$

where N represent the final number of emerged seeds and n_j and n_i are the cumulative number of seeds emerged by adjacent counts at times t_j and t_i , respectively, where $n_i < \frac{N}{2} < n_j$.

Shoot length per plant (mg)

All seedlings from each treatment were taken and their shoot lengths were recorded in cm, from the point where root and shoot join and then to the top of seedlings. Then, an average shoot length was measured.

Root length per plant (cm)

All seedlings from each treatment were taken and their root lengths were recorded in cm, from the point where root and shoot join and then to the top of seedlings. Then, an average root length was measured.

Shoot dry weight per plant (mg)

Separated seedlings shoots were oven-dried at 70 °C for 48h and then weighed. This weight was used to calculate average shoot dry weight per plant in mg.

Root dry weight per plant (mg)

Roots of all seedlings were separated, oven-dried at 70 °C for 48h and then weighed. Average root dry weight per plant in mg was calculated.

Seedling vigor index (SVI)

Seedling vigour index (SVI) was calculated from emergence percentage and seedling length, by the formula described by Orchard (1977);

$$SVI = \text{seedling length (cm)} \times \frac{\text{germination}}{\text{emergence percentage}} \quad (\text{eq. 4})$$

All the data in the laboratory and field experiments were analyzed statistically by using Fisher's Analysis of Variance technique and calculation of significant difference of treatment means was performed by using Tukey's honest significant difference (HSD) test at $P \leq 0.5$ (Steel et al., 1997), following PROC GLIMIX procedure in Statistical Analysis Systems (SAS) version 9.4 (SAS, 2012).

The four-parameter Weibull model (Equation 5) was used to analyze the data through the RStudio software on the drc (dose response curve) package. The data of quadratic polynomial regression model (Equation 6) and their coefficient of determination (R^2) were investigated in the Microsoft Excel software version 2016. Standard errors of all the parameter estimates of the Weibull model are presented in parentheses in the respective figure; the standard error of a parameter estimate is a measure of confidence, indicating that if it is large, the parameter is poorly estimated.

Emergence percentage, mean emergence time and 50% emergence time were regressed over different concentrations of *E. colona* plant residues with the four-parameter nonlinear Weibull model.

$$Y = C + (D - C) \exp \{-\exp [K(\log X - L)]\} \quad (\text{eq. 5})$$

where X represents the concentration of *E. colona* plant residues (w/w), Y is the actual response, C is the lower asymptote or minimum limit, D is the upper asymptote or maximum limit, L is the lag phase, and the parameter K is the slope of the line (rate of change) at the lag phase.

Length of shoots and roots, their dry weights and seeding vigour index were regressed over different concentrations of *E. colona* plant residues with a quadratic polynomial regression model. This model best describes the relationship between independent variable (x-axis) and the dependent variable (y-axis).

$$y = ax^2 + bx + c \quad (\text{eq. 6})$$

In this equation, the dependent variable was represented by y and the independent variable by x , which shows jungle rice plant residues (%); c is the y intercept value of the regression line (a constant), b is the slope of linear regression line over the independent variable (x) or the

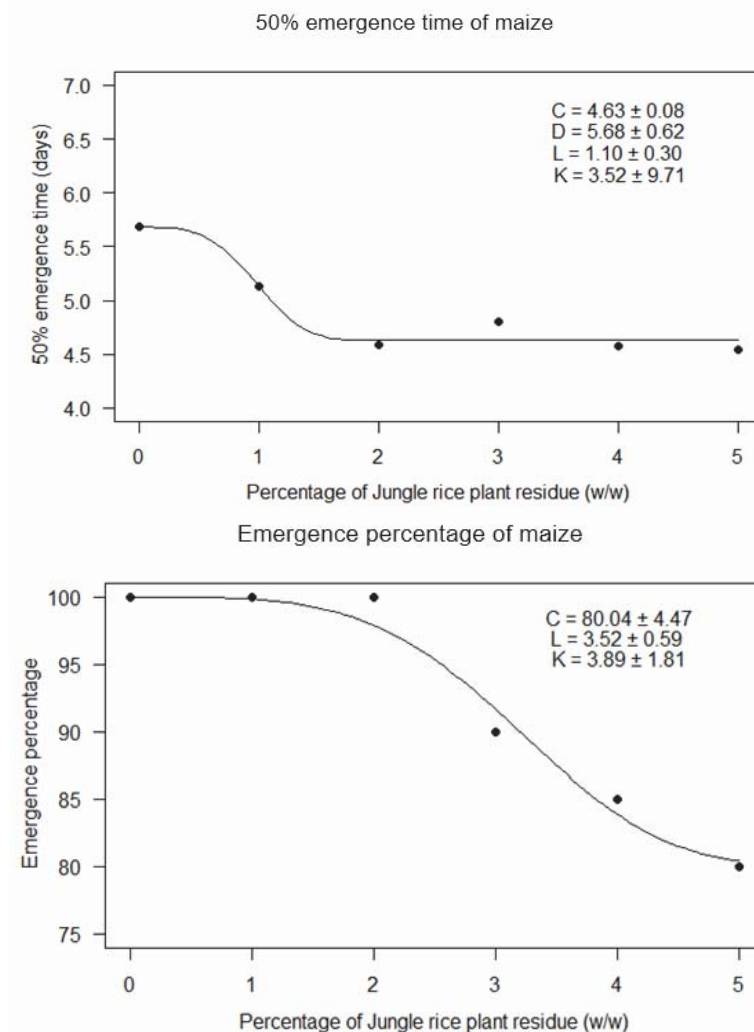
linear coefficient (increasing response of jungle rice plant residues) and a is the slope of quadratic regression line over the independent variable (x) or the quadratic coefficient (decreasing response of jungle rice plant residues).

RESULTS AND DISCUSSION

Maize emergence percentage

The effect of different concentrations of *E. colona* plant residues on emergence percentage of maize is described by the four-parameter non-linear Weibull model (Figure 1). This model depicts a decrease in maize emergence with increasing concentration of jungle rice plant residues. The minimum maize emergence (79.95%) was predicted at 5% jungle rice plant residues. Contrastingly, the maximum maize emergence (100%) was noticed in the treatment with no jungle rice plant residues. The figure clearly shows that means data points of maize percentage were similar in the 0%, 1% and 2% jungle rice plant residue treatments. Lag time, prior to a decrease in maize emergence, indicated that maize emergence tended to decrease significantly after 3.49% jungle rice plant residues. The predicted decrease rate in maize emergence at lag time was 3.69%.

This reduction in seed emergence traits was due to increased concentration of jungle rice residues, which cause less seed emergence to take place. The increased concentration of jungle



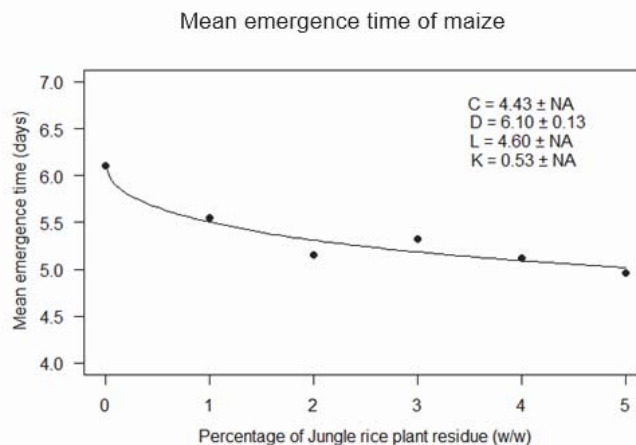
The four-parameter non-linear Weibull model fit to the data. The parameter estimates \pm standard error of this model is given in the Figure.

Figure 1 - Effect of different concentrations of *E. colona* plant residues on emergence of maize.

rice residues promoted its inhibitory effects by releasing more allelochemicals in the soil. The findings are well supported by the results of Zhang et al. (2009) and Dhole et al. (2011). They reported that *Alternanthera philoxeroides*, *Alternanthera sessilis* and *Echinochloa crus-galli* suppressed the growth of sorghum (*Sorghum bicolor*) and ryegrass (*Lolium perenne*) by both competition and release of secondary metabolites in the ecosystem. These inhibitory results are also well supported by Zuo et al. (2012), who reported that *Alternanthera* species have a strong allelopathic potential.

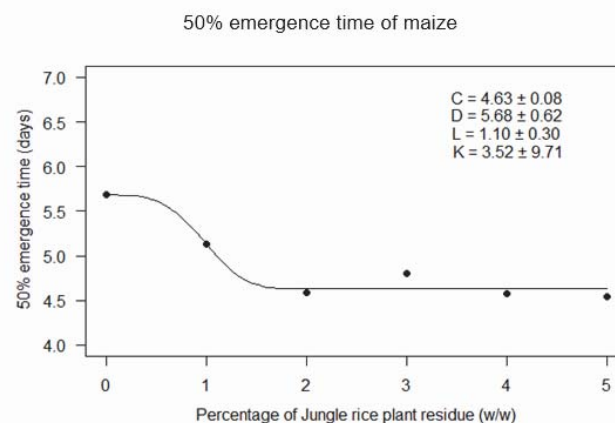
Maize mean emergence time and 50% emergence time

Lower values of mean emergence time and 50% emergence time represent enhanced germination rate. The consequence of different concentrations of jungle rice plant residues on mean emergence time and 50% emergence time are defined by the four-parameter non-linear Weibull model (Figure 2 and 3). According to this model, increasing concentration of jungle rice plant residues resulted in a slight decrease in mean emergence time and time to 50% emergence. Minimum mean emergence time and 50% emergence time were calculated at 5% jungle rice plant residues, which was statistically similar to the treatments among 2% to 5% jungle rice plant residues. Maximum mean emergence time and 50% emergence time were noticed in the treatment with no jungle rice plant residues, which was statistically similar to 1% jungle rice plant residues.



The four-parameter non-linear Weibull model fit to the data. The parameter estimates \pm standard error of this model is given in the Figure.

Figure 2 - Effect of different concentrations of *E. colona* plant residues on mean emergence time of maize.

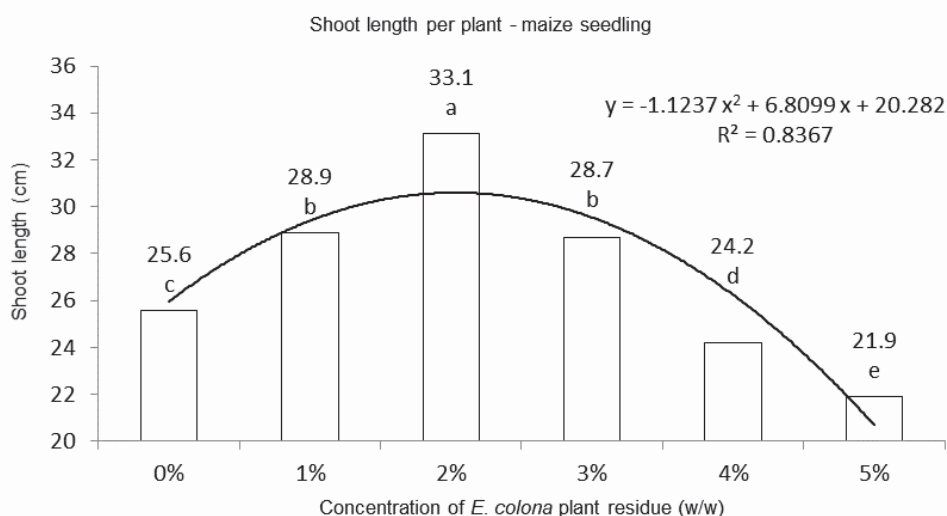


The four-parameter non-linear Weibull model fit to the data. The parameter estimates \pm standard error of this model is given in the Figure.

Figure 3 - Effect of different concentrations of *E. colona* plant residues on 50% emergence time of maize.

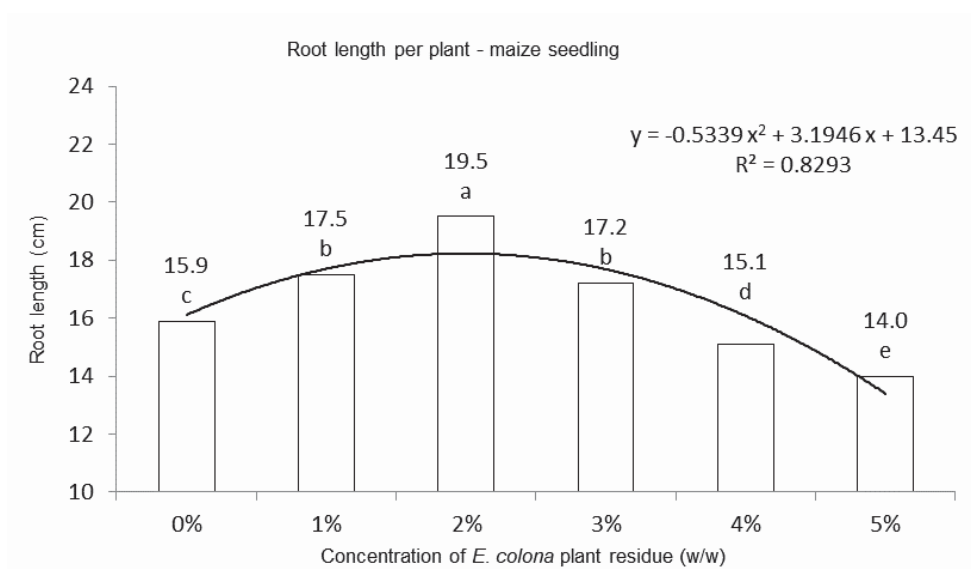
Shoot length and root length of maize seedlings

Seedling growth parameters such as shoot and root length are important in research on the effects of allelochemicals and various environmental factors on early growth and vigour of a plant. The shoot is the production centre for a plant, and it is functionally responsible for food production (photosynthesis). Plants absorb water and nutrients from the soil via roots, hence a good root system is a prerequisite for plant health. Beneficial or harmful effects of allelochemicals can be best explained through such types of seedling growth bioassays. Seedling development largely depends upon the stimulating or inhibiting effect of allelochemicals. This is due to fact that any type of allelochemicals influences the embryo of the seed at the time when it starts to emerge out in the form of seedling in order to grow into an adult plant. Data on the effect of various concentrations of jungle rice plant residues on root length and shoot length of maize seedling per plant were modelled by quadratic polynomial regression model and are shown in Figures 4 and 5.



The quadratic polynomial regression model fit to the data.

Figure 4 - Effect of different concentrations of *E. colona* plant residues on shoot length of maize seedlings.



The quadratic polynomial regression model fit to the data.

Figure 5 - Effect of different concentrations of *E. colona* plant residues on root length of maize seedlings.

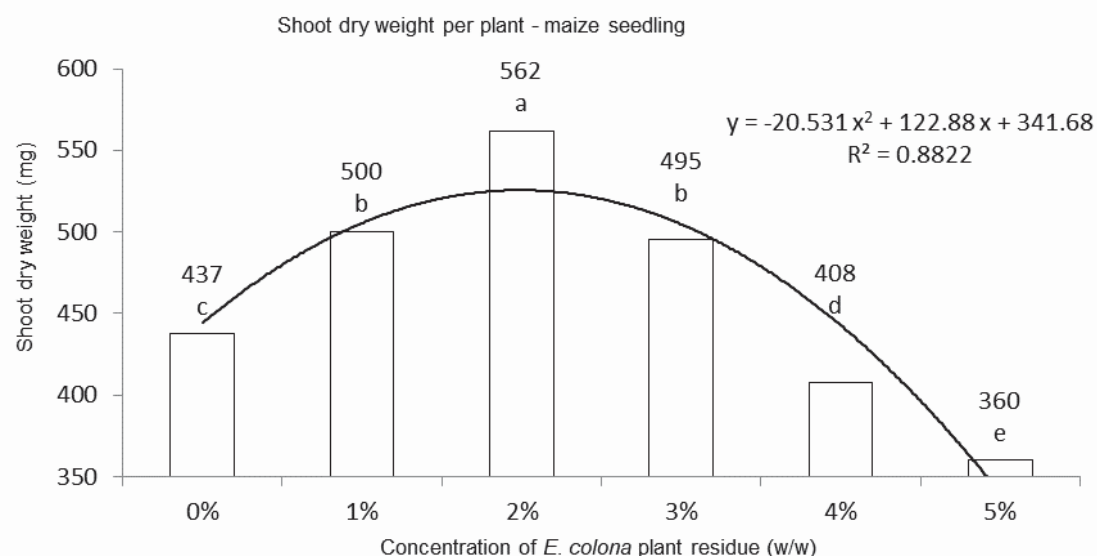
This function predicts that different concentrations of jungle rice plant residues has a different impact on shoot and root length of maize seedlings. The parameter b is the slope of the linear regression line over different concentrations of jungle rice plant residues, which suggests a linear increase of maize seedling parts (shoot and root length) at the rate of 6.81 cm and 3.20 cm per unit increase in jungle rice plant residues. This linear increase of maize seedling parts (shoot and root length) was noticed till 2% jungle rice plant residues, which was the maximum shoot and root length of maize seedling. By comparison, the parameter a is the slope of quadratic regression line on various jungle rice plant residue concentrations, predicting a decrease in shoot and root length of maize seedlings at the rate of 1.12 cm and 0.53 cm as jungle rice plant residue concentration increased onward to 2%. The coefficient of determination (R^2) indicates that this model accounts for 83.7% and 82.9% variability in the data on shoot and root length of maize seedlings around their mean. Instead of 1% and 3% jungle rice plant residues, all other jungle rice concentrations were statistically different from one another in terms of shoot and root length of maize seedlings.

These results showed that jungle rice residues release growth-promoting substances at low concentration and growth suppressing substances at higher concentration into the soil environment, which accumulated such bioactive concentrations, thus influencing the growth of maize plants. Swain et al. (2008) determined allelopathic potentials of different extracts and bioassays of jungle rice on rice and concluded that the extract at 10% decomposed plant material concentration, reducing the root and shoot growth by up to 57% and 84%, respectively. Similarly, Swain et al. (2012) found out the interactive effect of jungle rice decomposing leachates on germination and seedling growth of rice. They found that leachates decreased germination rate (90%), shoot length (43%) and root length (100%) by 10%.

Shoot dry weight and root dry weight of maize seedlings

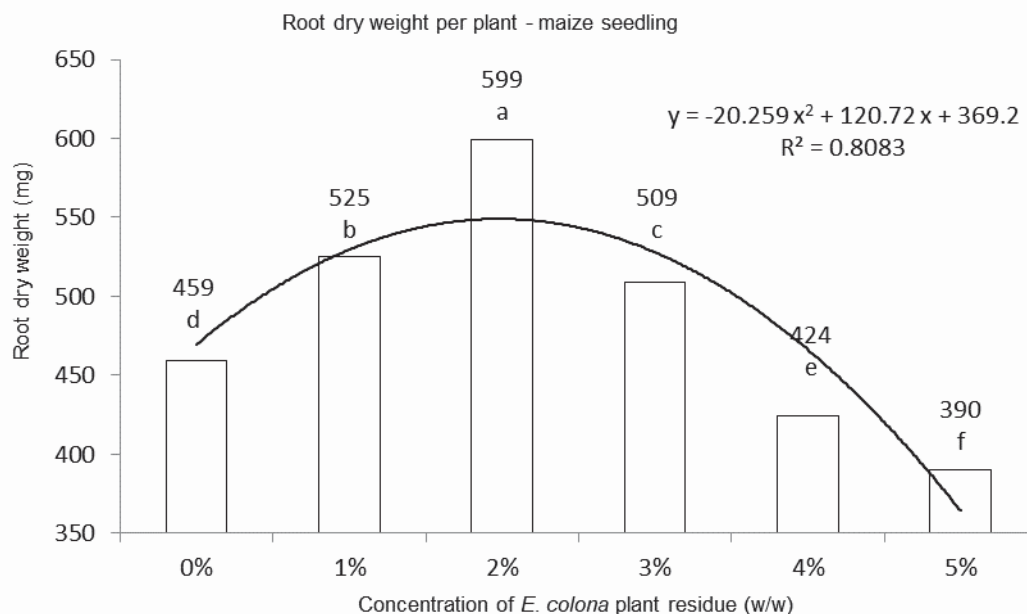
Dry matter or dry weight is a measurement of the mass of a plant when it is completely dried. Dry weight is important when studying the effect of allelochemicals on early growth and vigour of a seedling. Data on the effect of several concentrations of jungle rice plant residues on root and shoot dry weight of maize seedlings per plant were regressed by the quadratic polynomial regression model and presented in Figures 6 and 7.

This equation forecasts that different concentrations of jungle rice plant residues had a different impact on shoot and root dry weight of maize seedlings. The parameter b is the slope of



The quadratic polynomial regression model fit to the data.

Figure 6 - Effect of different concentrations of *E. colona* plant residues on shoot dry weight of maize seedlings.



The quadratic polynomial regression model fit to the data.

Figure 7 - Effect of different concentrations of *E. colona* plant residues on root dry weight of maize seedlings.

linear regression line over different concentrations of jungle rice plant residues, suggesting a linear increase in shoot and root dry weight of maize seedlings at the rate of 122.9 mg and 120.7 mg per unit increase in jungle rice plant residues. This linear increase in shoot and root dry weight of maize seedlings was noticed till 2% jungle rice plant residues, which was the maximum dry weight of shoots and roots of maize seedlings. By contrast, the parameter a is the slope of quadratic regression line on various jungle rice plant residue concentrations, predicting a decrease in shoot and root dry weight of maize seedlings at the rate of 20.5 mg and 20.3 mg as jungle rice plant residue concentration increased ahead to 2%. R^2 value predicts that the quadratic polynomial regression model describes 88.2% and 80.8% variability in shoot and root dry weight of maize seedling values around their mean. For shoot dry weight of maize seedlings, all treatments were statistically different from one another except for 1% and 3% jungle rice plant residues, whereas in all treatments, root dry weight values of maize seedlings were statistically dissimilar.

Furthermore, the presence of water soluble phenolic compounds (Table 1) such as quercetin, sinapic acid, m-coumaric acid, syringic acid, cinnamic acid, ferulic acid in jungle rice played a role in promotion and inhibition of seed germination and seedling growth of maize. The methanolic extract of jungle rice was investigated for its herbicidal potential, which slightly reduced the germination and root length of different weeds species including *E. colona* (Gomaa and AbdElgawad, 2012). The chloromethyl (CH_2Cl) fraction of the methanolic extract at 500 mg L^{-1} slightly reduced the germination of *Brachiaria reptans* (26%) and *Convolvulus arvensis* (27%) and

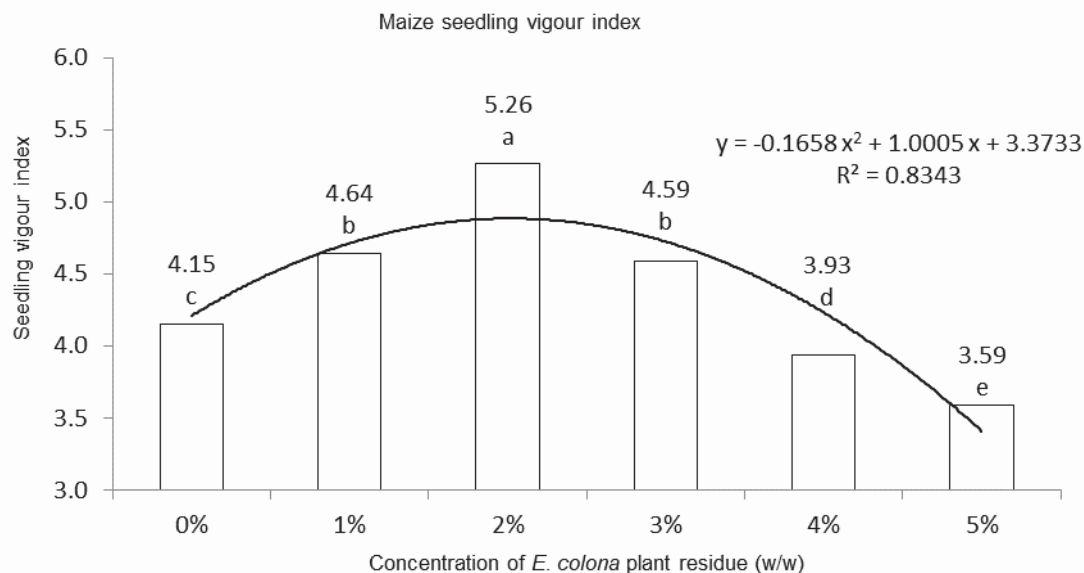
Table 1 - Phytotoxic composition of soil amended with *E. colona* plant residues at 15 days after treatment (mg L^{-1})

<i>E. colona</i> plant residues (w/w)	Quercetin	Syringic acid	Cinnamic acid	Ferulic acid	Sinapic acid	M-Coumaric acid	Total phenolics
1%	2.6	16.0	24.7	22.0	26.0	18.3	124.6
2%	2.6	16.2	25.0	22.5	26.1	18.4	125.8
3%	2.7	16.4	25.0	22.5	26.3	20.0	127.9
4%	2.7	16.7	25.3	22.6	26.3	20.6	129.2
5%	3.3	16.8	25.7	22.6	26.3	20.6	130.3

showed significant effects on root length of *Amaranthus graecizans*, *Amaranthus hybridus*, *Hibiscus trionum*, *Portulaca oleracea*, *Setaria pumila*, *B. reptans*, and *E. colona* as well. In all of the tested species, there was a high degree of inhabitation in germination, root and shoot length with the increased concentration of 1,000 mg L⁻¹, in all tested weed species. Phytotoxicity of the bio-compound from *E. colona* against *Avena fatua* was evaluated by Hegab et al. (2013) at higher concentrations of 25, 50, and 100 mM; they found that decreased starch mobilization significantly attenuated amylase activity, hampered the activity of antioxidant enzymes and resulted in suppressed germination and growth of *A. fatua*.

Maize seedling vigor index

The data on the effect of various concentrations of jungle rice on maize seedling vigour index, which is an indicator of overall germination and seedling growth performance, was described by the quadratic polynomial regression model and given in Figure 8. This model predicts that different concentrations of jungle rice plant residues had an unrelated impact on the seedling vigour index of maize. The parameter *b* is the slope of linear regression line over different concentrations of jungle rice plant residues, suggesting a linear increase in the seedling vigour index of maize at the rate of 1.0. This linear increase in the seedling vigour index of maize was noticed till 2% jungle rice plant residues, which was the maximum seedling vigour index of maize, while the parameter *a* is the slope of quadratic regression line on various jungle rice plant residue concentrations, thus predicting a decrease in seedling vigour index of maize at the rate of 0.17 as jungle rice plant residue concentration increased onward to 2%. The coefficient of determination indicates that this model accounts for 83.4% of seedling vigour index of maize data around its mean. Except for 1% and 3% jungle rice plant residues, all other treatments were statistically different from one another in terms of seedling vigour index of maize.



The quadratic polynomial regression model fit to the data.

Figure 8 - Effect of different concentrations of *E. colona* plant residues on maize seedling vigour index.

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