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DEGRADATION OF PHENOLICS IN *DIGERA MURICATA*: PHYTOTOXIC EFFECTS OF ROOT AND SHOOT LEACHATE PLUS N FERTILIZATION ON THE GROWTH OF MILLET

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Abstract

Digera muricata was found to be highly allelopathic in nature. Phenolic compounds (quercetin, sinapic and ferluic acid), alkaloids (cystine and berbine) and a terpenoid (limonene) was detected from root and shoots of *D. muricata*. Decaying plant material of *D. muricata* inhibit not only the germination and growth of neighbouring species but it is also self inhibitory. When plant matures and dies down, the leachates having greater concentrations of phenols enter the soil. At this time the leachate is highly concentrated but with the passage of time it undergoes degradation and level of phenolic declines. Thus allowing the germination of other plants. Therefore, the present study was designed to reveal the degradation process of phenolic compounds in the soil Moreover, adverse effects of phenolic compounds on the growth of bullrish millet, the most susceptible crop plant.was also studied. For this purpose different levels of nitrogen fertilization (0 mM, 1 mM and 5 mM) were added to the soils having different dilutions of decaying root and shoot leachate of *D. muricata* (full-strength, 1/2 strengthand 1/4 strength). Addition of nitrogen to the soil showed significant decline of phenolic compounds with time, because nitrogen might increase the microbial activites in the soil, which lowered the phenolic levels, thus ameliorating growth rates. Concentration of phenolic compounds were greater in the soil having full-strength root/shoot leachate with 5 mM N fertilization, followed by by 1 and 0 mM N fertilization. Growth of millet was significantly higher in the controls having no plant leachate followed by 1/4 and 1/2 strength root/shoot leachate. Dry weights were considerably lower in full-strength plant leachate. Whereas, 5 mM N fertilization showed maximum growth followed by 1 and 0 mM N fertilization. Relatively root leachate was found to be more toxic than shoot leachate.

Key words: Allelopathy, Degradation of phenolics, Nitrogen fertilization, Plant leachate.

Introduction

Allelopathy plays an important role in the distribution of plants in any area. According to Han *et al*. (2013), allelopathy is an environmental friendly technique which act as a tool for weed management. Common compounds involved in allelopathy are water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, ketones, simple unsaturated lactones, long-chain fatty acids, napthoquinones, anthraquinone, complex quinines, terpenowing nearoids, steroids, simple phenols, benzoic acid derivatives, cinnamic acid derivatives, coumarins, flavonoids, tannins, amino acids and polypeptides, alkaloids and cynohydrins, sulfides and mustard oil derivatives, purines and neucleosides, etc. (Whittaker & Feeny, 1971; Rice, 1984).

Some crop residues are harmful for crop plants. For instance, residues of oat, wheat, sorghum and corn were shown to be inhibitory to the growth of wheat seedlings (Guenzi & Mc Calla, 1966, a, b). According to Harper (1977), allelochemical substances from plant debris or leachate result in reduced nitrogen levels. Lyon *et al*. (1923), reported depressive influence of maize, wheat and oats on the accumulation of nitrates in the soil, because these plants liberate carbonaceous matter into the soil. Boughey *et al*. (1964), reported that two species of grass *Hyparrhenia*, in savanna, secrete a toxin that suppresses the growth of nitrifying bacteria. (Rice, 1964), also reported the inhibition of nitrogen fixing and nitrifying bacteria by some plants of Oklahoma. Rice *et al*. (1981), reported reduced nitrogen fixation by *Rhizobium* due to decomposing rice straws.

Certain members of the family Amaranthaceae, including the genus Amaranthus and *Digera* (*D. alternifolia, D. arvensis, D. muricata*) (Rice, 1984) are known to be allelopathic. Greater amounts of phenolic compounds (Quercetin, sinapic and ferluic acid), alkaloids (cystine and berbine) and a terpenoid (limonene) were detected from plant material of *D. muricata* (Aziz & Shaukat, 2013). Phenolic compounds are present in much higher amounts than alkaloids and a terpenoid. Phenolic compounds play an important role in the deterioration of the environment, and are considered as the most common air and water pollutants, showing high allelopathic effect. Microbial activity is helpful in decreasing the quantity of phenols in the soil, thus lowering it's self inhibitory effects on the germination and successful establishment of the plants. The degradation of phenol and it's derivatives have also been reported by Sharma *et al.* (2012) and Krastanov *et al*. (2013). According to Claussen (2005), proline is a compatible solute and serves as an energy or nitrogen source. Albert *et al*. (2012), provide evidence that there was less proline accumulation in lesves of *Brassica napus* in N-deficient plants. Similarly, Sanchez *et al*. (2001), reported proline accumulation as an indicator of excess N in green bean plants. The present study focuses on the degradation rate of phenolic compounds with the passage of time in soils amended with different dilutions of plant leachate plus different levels of nitrogen fertilization. Moreover, the effect of different dilutions of root/shoot leachate of *D. muricata*, along with N fertilization (0mM, 1mM and 5 mM) were also studied on millet (*Pennisetum americanum* (L.), Schumann , the most susceptible crop plant.

Materials and Method

Preparation of root and shoot leachate amended with N fertilization: To detect the phytotoxic effects of *D. muricata* on millet (*Pennisetum americanum* (L.), Schumann, roots and shoots of *D. muricata* was air dried for 96 h. From this material 15 g root and shoot material of *D. muricata* was separately soaked in 220 ml of distilled water for 72 h. This mixture was filtered and identified as full-strength (FS) root and shoot leachate. From this leachate dilutions of 1/ 2 strength and 1/4 strength were prepared. N fertilization levels were prepared by dissolving appropriate amount of NH_4NO_3 in distilled water. From this solution 1mM and 5 mM N fertilization levels were prepared.

Phytotoxic effects of root and shoot leachate of *D. muricata* plus N fertilization on the growth of millet: 300 g soil was amended separately with 50 ml FS root and shoot leachate plus 0 mM, 1 mM and 5 mM N fertilization. Similarly, 50 ml 1/ 2 strength of root/shoot leachate and 1/4 strength root/shoot leachate solutions were also mixed separately with 300 g soil plus 0 mM, 1 mM amd 5 mM N fertilization. 300 g soil having 50 ml distilled water was used as control. Each treatment was replicated five times. All the soil samples were taken in 9 cm diameter plastic pots. Ten seeds of millet were sown in each pot. Dry weights were measured after 12 days of growth.

Degradation of phenolics: 10 g soil was amended with 5 ml of full strength (FS), 1:2 strength leachate and 1:4 strength leachate of *D. muricata* (root plus shoot) along with 0 mM, 1 mM and 5mM nitrogen fertilization. After an incubation period of 0, 24, 48, 72, 96 and 120 h, each treatment was extracted with 15 ml distilled water. Water-soluble phenolics were determined spectrophotometerically using the method of Folin-Ciocalteu's reagent as modified by Swain and Hillis (1959). 0.2 ml Folin-Ciocalteu's reagent was added to 0.1 ml of extract in 5 ml distilled water. After shaking the tubes for 3 minutes, 1 ml of saturated $NaHCO_3$ solution was added. Tubes were incubated at 28°C for 30 minutes. Optical density was recorded at 660 nm against the reagent blank. For reagent blank, 0.1 ml of distilled water was taken in the separate test tube and treated in the same manner as the phenol solution. Total phenols were estimated with the help of standard curve.

Results

Phytotoxic effects of either root or shoot leahcate plus nitrogen fertilization on growth of millet plants

Root leachate: Dry weights of millet plants surviving in the soils amended with different dilutions of root leachates of *D. muricata* plus different levels of nitrogen fertilization showed significant variations in root ($F = 155.67$, $p<0.001$) and shoot dry weights ($F = 107.71$, $p<0.001$). Two-way interaction between different dilution of root leachate x N fertilization was also showed significant differences ($F = 6.54$, $p<0.001$). Relatively, greater plant dry weights of millet were recorded in 5 mM N fertilization followed by 1 mM and 0 mM N fertilization. Moreover, reduction in dry weights were highest in FS, followed by 1/2 strength root leachate, followed by 1/ 4 strength. Plants having no root leachate i.e., controls exhibited maximum dry weights (Table 1).

Shoot leachate: Root dry weights of millet plants grown in shoot leachate of D. muricata exhibited significant ($F = 24.55$, $p<0.001$) variations at different dilutions and N fertilization levels ($F = 20.23$, $p<0.001$). Whereas, two-way interaction of shoot leachate dilutions and N fertilization was non- significant ($F = 2.09$, n.s.). Whereas, shoot dry weights of millet exhibited significant variations at different dilutions of shoot leachate ($F = 10.05$, $p<0.001$) and different levels of N fertilization ($F = 13.44$, $p<0.001$). Two-way interaction was also found to be significant ($F = 5.85$, $p<0.01$). In general, plants growing in shoot leachate also exhibited similar pattern as those growing in root leachate (Table 2). However, on an overall basis, dry weight reduction was higher for root leachate and relatively lower in shoot leachate.

Table 1. The effects of soil amended with different dilutions of root leachate plus different levels of nitrogen fertilization on dry weights of millet (mean ± standard error).

N fertilization	Plant leachate	Root weight (g)	Shoot weight (g)
0 mM	Full-strength	0.08 ± 0.13	1.37 ± 0.28
	1 / 2 strength	1.03 ± 0.12	1.85 ± 0.46
	1 / 4 strength	1.40 ± 0.32	2.42 ± 0.52
	Control	1.72 ± 0.38	3.80 ± 0.71
1 mM	Full-strength	1.16 ± 0.22	1.50 ± 0.34
	1 / 2 strength	1.45 ± 0.30	2.12 ± 0.45
	1 / 4 strength	1.69 ± 0.35	2.66 ± 0.53
	Control	2.05 ± 0.44	4.25 ± 1.97
5 mM	Full-strength	1.48 ± 0.32	2.08 ± 0.46
	1 / 2 strength	1.81 ± 0.44	2.30 ± 0.50
	1 / 4 strength	2.08 ± 0.65	2.93 ± 0.62
	Control	2.45 ± 0.51	4.66 ± 1.02

Table 2. The effects of soil amended with different dilutions of shoot leachate plus different levels of nitrogen fertilization on dry weights of millet (mean ± standard error).

N fertilization	Plant leachate	Root weight (g)	Shoot weight (g)
0 mM	Full-strength	1.50 ± 0.33	1.90 ± 0.78
	1 / 2 strength	1.70 ± 0.39	2.60 ± 0.55
	1 / 4 strength	2.15 ± 0.45	3.10 ± 0.34
	Control	2.40 ± 0.53	4.50 ± 0.80
1 mM	Full-strength	1.70 ± 0.39	2.51 ± 0.55
	1 / 2 strength	2.10 ± 0.48	2.90 ± 0.64
	1 / 4 strength	2.40 ± 0.60	3.72 ± 0.75
	Control	2.70 ± 0.88	4.93 ± 1.22
5 mM	Full-strength	1.99 ± 0.46	3.20 ± 1.06
	1 / 2 strength	2.50 ± 0.52	3.80 ± 0.78
	1 / 4 strength	2.70 ± 0.63	4.00 ± 1.15
	Control	3.00 ± 0.74	5.30 ± 1.31

Table 3. Degradation of phenolics in soils amended with *D. muricata* leachate during 8 days of incubation (mean ± standard error).

N fertilization	Plant leachate	Phenolics (ug / g) Time (days)			
		2	4	6	8
0 mM	Full-strength	19.6 ± 2.3	18.0 ± 2.5	15.3 ± 2.3	13.6 ± 1.7
	1/2 strength	15.6 ± 1.6	13.3 ± 1.2	12.8 ± 0.5	09.6 ± 1.5
	1/4 strength	12.0 ± 0.9	09.6 ± 0.6	06.6 ± 0.5	07.0 ± 0.2
	Control	09.3 ± 0.3	07.5 ± 0.5	05.6 ± 0.3	03.6 ± 0.4
1 mM	Full-strength	17.3 ± 2.3	15.0 ± 1.5	13.0 ± 0.9	11.3 ± 1.0
	1/2 strength	12.6 ± 1.8	10.3 ± 1.3	07.3 ± 1.2	06.6 ± 0.4
	1/4 strength	10.0 ± 2.1	06.3 ± 0.7	04.5 ± 0.7	03.6 ± 0.8
	Control	08.3 ± 0.3	05.7 ± 0.4	03.0 ± 0.6	02.3 ± 0.7
5 mM	Full-strength	14.3 ±1.3	12.0 ± 1.6	12.3 ± 1.2	07.3 ± 0.3
	1/2 strength	10.0 ± 1.6	08.0 ± 0.5	05.6 ± 0.3	03.6 ± 0.4
	1/4 strength	07.0 ± 0.5	05.3 ± 0.9	04.0 ± 0.5	02.2 ± 1.3
	Control	04.3 ± 0.3	02.6 ± 0.4	02.8 ± 0.6	01.4 ± 0.8

Degradation of phenolics: Degredation of phenolic compounds with time at different levels of N fertilization and in various strengths of plant leachate was noted. Amount of phenolics greatly declined in the soil from 0 to 8 days (Table 3). Concentration of phenolic compounds was greater in the FS followed by 1/2 and 1/4 strength. Highest amounts of phenolic compounds were recorded in 0 mM N fertilization and lowest in 5 mM N fertilization. Two-way interaction of time x N fertilization ($F = 2.52$, $p<0.05$), time x leachate ($F = 4.56$, $p<0.001$) showed significant variations. While N fertilization x leachate exhibited non-significant ($F = 1.57$, n.s.) differences. Three-way interaction (time x N fertilization x leachate) also showed significant ($F = 3.89$, $p<0.001$) differences.

Discussion

Decline in phenolics with time was noted in *D. muricata*. This could be due to chemical and microbial degradation or due to sorption of phenolic compounds in soil with time as reported by Inderjit *et al*. (1999). According to Tanrisever *et al*. (1987), chemical degrade with time, and in allelopathy, the degradation products are often believed to be the inhibitory compounds. Such degradation of phenolics released from the root leachate of *Verbisina encelioides* in the soil was also reported by Inderjit *et al*. (1999). Productions of toxins via degradation might be considered to be adaptive because it reduces potential autotoxicity (Williamson, 1990).

It is necessary to develop mechanism to reduce autotoxicity, because chemicals at the time of leachate are more concentrated and most damaging for the source plants. Richardson & Williamson (1988), bioassayed the fresh foliage and litter of *Ceratiola ericoides* and found greater toxicity in the litter than in the fresh foliage. Autotoxicity can be removed by degradation of compounds with time. Sometimes harmless compounds, undergo degradation but do not decline with time. For

instance, *Ceratiola ericoides* released certiolin, which is inactive but it undergoes degradation to produce hydroxycinnamic acid, which inhibits seed germination and radical growth of grasses (Tanrisever *et al*., 1987). The most effective way of minimizing autotoxicity is the production of extensive root system, where compounds become more diluted and also degraded by the rhizosphere microorganisms (Williamson, 1990).

D. muricata is a desert annual, which appears only after rains at high temperatures. When plant matures and dies down, the leachates enter the soil, which with the passage of time undergo degradation and level of phenolics declines. Thus allowing the germination of other plants in the field next year after monsoon showers. The distribution and amount of allelochemical phytotoxic substances depend upon the number of donor plants. Dudai *et al*. (1999), extracted essential oils from 32 aromatic plants, which are used as bioherbicides. Similarly, *Croton bonplandianum* also produced harmful effects on weedy associates (Datta & Sinha-Roy, 1975). Czarnota *et al*. (2003), reported seven sorghum species as allelopathic and weed suppressive.

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