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INFLUENCE OF LEAF EXTRACT OF NETTLE LEAF GOOSEFOOT (*CHENOPODIUM MURALE* L.) AND NACL SALINITY ON GERMINATION AND SEEDLING GROWTH OF RICE (*ORYZA SATIVA* L.)

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Abstract

Aqueous leaf extract of nettle leaf goosefoot (*Chenopodium murale* L.) was evaluated alone or in combination with NaCl salinity for its influence on germination and seedling growth of rice. The leaf extract and NaCl alone or in combination did not have any significant effect on germination, but the shoot and root lengths decreased significantly by the treatments compared to control. Root growth was affected more than the shoot. The combination of leaf extract and NaCl drastically reduced the shoot and root growth more than the separate effects of these stress treatments. The results suggest that the leaf extract of *Chenopodium murale may* release some toxic phenolic allelochemicals which deleteriously affected the seedling growth of rice plant.

Introduction

Weeds are undesirable plants which compete with main crops in the growth media for nutrients, moisture, space, light and hamper the healthy growth ultimately reducing the growth and yield both qualitatively and quantitatively. Weeds are known to exhibit allelopathy by releasing water- soluble allelochemicals from leaves, stems, roots, rhizomes, flowers, fruits and seeds (Alam *et al*., 1990; Ahn & Chung, 2000; Batish *et al*., 2007a, 2007b; Duke *et al*., 2007; Le Tourneau *et al*., 1956; Rice, 1984). Weeds which complete with main crop plants for nutrients and environmental variables normally appear to be toxic to the germination and seedling growth of the plants. The allelopathic potential of several weeds have been studied in the laboratory (Azmi & Alam, 1989; Bhowmik & Doll, 1984; Kato-Noguchi *et al*., 2002). Batish *et al*., (2007b) conducted experiment using residue of *Chenopodium murale* on the growth of chickpea and pea and found that their root and shoot length significantly decreased. Le Tourneau *et al*., (1956) found that water extracts from 23 common weeds and crop residues inhibited germination and growth of wheat seedling. The phototoxic effects of 14 aqueous root extracts upon germination and seedling growth of 15 plant species has been recorded (Lawrence & Kilcher, 1962). The phytotoxic effects can be attributed either to allelochemicals present in the plant or weed residues or to microbial toxins produced during decomposition (Rice, 1984).

Chenopodium murale L., commonly known as nettle leaf goosefoot is an important annual weed. It is distributed throughout the temperate and tropical regions and found in Pakistan in almost every field in winter season. Earlier studies on allelopathic effect of weeds have been carried out normally under normal growth condition, but there seem to be no report on crop plants growing under saline conditions. The objective of this study was to ascertain the effects of aqueous leaf extract of nettle leaf goosefoot (*Chenopodium murale* L.) in the presence or absence of NaCl on the germination and seedling growth of rice.

Materials and Methods

The fresh leaves of nettle leaf goosefoot (*Chenopodium murale* L.) were collected, washed thoroughly with distilled water and dried in an oven at 70ºC for 72 hours. The dried leaf samples were ground in a Wiley mill to pass through a 20mesh screen. The aqueous leaf extract was prepared by soaking 5 g of the powdered leaf sample in 100 ml distilled water for 24 hours. Afterwards, the extract was filtered, using Whatman filter paper no 42 and then kept in a refrigerator in a reagent bottle. Five ml of aqueous leaf extract was added to 0.8% sterilized agar-gel (Ames Chemical Co. Iowa, USA) prepared in distilled water and supplemented with 0.0, 0.2 and 0.4% Sodium chloride (NaCl) solution. Fifty ml of the agar media of each treatment was poured into a series of 100 ml capacity of glass bowls. A similar set, but without leaf extract was also prepared to determine the NaCl effect and bowl with only 0.8% agar gel was considered as control. Good healthy rice (*Oryza sativa* L.) seeds (cv IR-85) were manually selected and surface sterilized with 2% Sodium hypochlorite (NaOCl) solution for five minutes and then rinsed and washed thoroughly with distilled water several times and briefly blotted into fine quality filter paper. Ten healthy rice seeds per bowl were placed on the surface of the solidified agar gel contained in each bowl in a circle with the embryo side up and pointing inwards. The bowls were then covered with sterilized Petri dishes and incubated at 28ºC in an incubator. The bowls were kept in a randomized design with four replications. The experiment was terminated after 120 hours of growth. Seeds were considered germinated when the emergent radical reached 2 mm in length. The germinated seeds were counted. Their shoot and root lengths were measured. The Duncan Multiple Range Test was used to assess the treatment effects.

Results and Discussion

Germination: The aqueous leaf extract of nettle leaf goosefoot (*Chenopodium murale* L.) and Sodium chloride alone or in combination with leaf extract had no significant effect on rice seed germination (Table 1). However, the seedling growth of both shoot and root were significantly reduced in all treatments compared to control. It has been estimated that seed germination is considered to be the most critical stage especially under stress conditions. The first requirement of seed germination is water for hydrolysis of reserves as a medium of translocation and hydrolysis of enzymes. During germination, biochemical changes take place, which provides the basic framework for subsequent growth and development. From the interference viewpoint, allelopathy is an important phenomenon in seed germination and seedling emergence through weed-crop interaction in the growth medium. It has been reported that the extract of plant leaves, stems, roots, fruits and seeds are known to exhibit water soluble phytotoxins which affect the general growth of the test plants (Alam *et al*., 1997; Batish *et al*., 2007b; Rice, 1984).

In the field of allelopathy, the difficulty in using seed germination as a bioassay has been appreciated. Leather & Einhellig (1985) have reviewed various techniques used for seed germination tests and concluded that many factors like temperature light/ dark cycle, oxygen availability, toxic products, volume of solution, osmotic potential and other interference of the growth media may modify the results. In the present study, every effort was made to perform the experiment under controlled conditions, but the results suggest that neither the salinity nor the combination of leaf aqueous extract had any inhibitory effect on the seed germination. However, the reports on inhibition of

germination by plant extracts are not lacking. One interesting phenomenon, which has the effect on the final result, is the sterilization of seeds on growth media. This has clearly been demonstrated by the study of Martin *et al*., (1990), who found no effect of crop residues on corn seed germination under sterile conditions, but under non-sterile conditions a significant inhibition in germination was recorded. Another factor, which has been shown to affect the results, is the osmotic potential of the medium. Leather & Einhellig (1985) considered 150 millimoles to be germination inhibitory and extract of 4 g of dried sunflower tissue in 100 ml of water was found to have the osmotic potential of 150 millimoles. It seems that in their studies either the osmotic value did not reach the expected high value or the test plants used were tolerant to high osmotic potential.

Hicks *et al*., (1989) found that wheat straw at 1g/100 ml of water had no effect on germination of resistance genotypes of cotton (*Gossypium hirsutum*). Le Tourneau *et al*., (1956) using 2g/100 ml of water of common lambs quarters found reduction in seed germination of wheat. In their experiment, Mallik & Tesfai (1988) reported that water extract of shoot of common lambsquarters at 1% level reduced soybean seed germination. Castro *et al*., (1984) found that a concentration of 4 g of bermuda grass (*Cynodon dactylon* L.)/100ml of water reduced rice seed germination. In the present study, a concentration of only 0.25g /50ml of agar media was used, which was much lower than the threshold required for inhibition of seed germination. It was perhaps for this reason that generally no inhibitory effect on rice seed germination was found. But it did have an allelopathic effect which was observed in seedling growth in most of the treatments.

Shoot and root length: Both shoot and root lengths of rice seedling were significantly reduced in all treatments compared to control (Table 1). The leaf extract of nettle leaf goosefoot alone significantly reduced the shoot length by 45%. Similarly, the extract in combination with 0.2 and 0.4% NaCl levels reduced the shoot lengths by 71 and 75%, respectively compared to control. The NaCl salinity levels (0.2 and 0.4%) alone reduced the shoot length by 25 and 53%, respectively compared to control. The aqueous leaf extract of nettle leaf goosefoot had severe inhibitory effects on root length and reduced the root length by 78%. In a similar way, the aqueous leaf extract in combination with salinity levels, reduced the root lengths by 90 and 97% compared to control. Biomolecules specially termed allelochemicals are produced by a plant through leachates that escape into the growing media and subsequently influences the growth and development of rice plants.

It has been found that leaf extract of *Chenopodium murale* reduced the growth of mustard crop (Datta & Ghosh, 1982). Bhowmik & Doll (1984) found that water extract of residues of common lambs quarters inhibited the shoot and root growth of corn. Similar results have been reported by Anaya *et al*., (1987) and Satoh *et al*., (1989), who have stated that seedling growth of soybean, corn, squash, bean and cucumber decreased by using water extract of *Chenopodium mural* residue.

It was also found that root growth was affected more than the shoot. This is because of the fact that root remained touched with the aqueous extract. This may be due to the fact that roots were in direct contact with the allelochemicals.

Salinity level of 0.2% alone slightly increased the root length (1.14%) while the higher salinity level (0.4%) alone reduced the root length by 42% and their effects were lower than the effects of leaf extract alone or in combination (Table 1). Higher salinity levels have normally been found to decrease the seedling growth of agricultural crops (Alam *et al*., 1997; Grattan *et al*., 2002.).

Table 1. Effect of aqueous leaf extract of nettle leaf goosefoot (*Chenopodium murale* L.) and sodium chloride (NaCl) on germination and seedling growth of rice.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)
Control (no leaf extract, no NaCl)	93a**	4.39a (-)	9.67a (-)
Leaf extract alone	92a	2.43b** (-44.64)***	2.09c (-78.38)
0.2 % NaCl alone	90a	3.25b (-25.96)	9.78a (+1.14)
Leaf extract + 0.2 % NaCl	93a	1.27c (-71.11)	1.00d (-89.65)
0.4 % NaCl alone	93a	2.04b (-53.53)	5.63b (-41.77)
Leaf extract + 0.4 % NaCl	90a	1.09c (-75.17)	0.26e (-97.31)

*250mg leaf extract / 50ml of 0.8 % agar gel

**Means in a column followed by same letters do not differ significantly as 5 % level by DMRT.

***Values in parentheses indicate percent increase (+) or decrease (-) over control.

The present studies clearly indicates that allelolpathic agent from *Chenopodium murale* under saline conditions was more injurious and intensified the effects of salinity on the growth of rice.

It is therefore concluded that leaf extract of nettle leaf goosefoot alone or in combination with NaCl, significantly reduced the growth of rice seedlings under our experimental conditions. Root growth was affected more than the shoot. It is therefore very important to uproot this weed, manually from the experimental fields at very early stage of its emergence.

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