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IMPACT OF ALLELOPATHIC POTENTIAL OF MAIZE (*ZEA MAYS* L.) ON PHYSIOLOGY AND GROWTH OF SOYBEAN [*GLYCINE MAX* (L.) MERR.]

NASEER AHMAD AND ASGHARI BANO[1]

[1]*Department of Plant Sciences, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan*
[2]*Department of Biotechnology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan.*
Corresponding author's e-mail: banoasghari@gmail.com

Abstract

The aim of the present investigation was to determine the allelopathic effect of aqueous extract from either fresh or oven dried leaves and root of unstressed or water stressed maize plants on soybean growth and its effects on rhizosphere soil treated with maize plant extract. The extracts were applied for 10h to soybean [*Glycine max* (L.) Merr.] as seed soaking treatment prior to sowing. The application of extract prepared from fresh leaves of unstressed maize plants significantly increased micronutrients contents of soil. Higher concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} contents were found in rhizosphere of soybean plants treated with fresh and oven dried leaf extracts of drought stressed maize plants. Both fresh and oven dried leaf extracts significantly increased the Fe^+, Cu^+, Cr^{3+}, Zn^{2+} and Co^+ content of soil. The extract prepared from fresh leaves of drought stressed maize plants significantly increased the accumulation of proline, sugar and endogenous abscisic acid content of soybean leaves. The protein content was decreased by these treatments. Significant increases were recorded in the activities of superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APOX), catalase (CAT) and endogenous abscisic acid in response to application of fresh as well as oven dried leaf extracts prepared from drought stressed maize plants. The leaf extracts were more effective than root extracts and oven drying further augmented its stimulatory effect on the accumulation of Na^+ content and micronutrients eg. Co, Zn^{2+}, Mn^{3+} and Cu^+ etc. It can be inferred that aqueous extracts possess allelopathic effects which alter the physiology of soybean plants.

Introduction

Plants secrete different types of secondary metabolites, which influence the growth and development of the surrounding plants and microbes by a process called Allelopathy which plays a significant role in agroecosystems, and affects the growth, quality and quantity of the produce (Kohli *et al*., 1998; Singh *et al*., 2001). The allelochemicals can be present in any part (leaf, stem and root) of the plant. In Rhizosperic soil the concentration of allelochemicals is high as compared to bulk soil. Allelochemicals produced by one crop species can influence the growth, productivity, and yield of other crops or the same crop (Batish *et al*., 2001). These noxious chemicals influence target species in different ways; affecting shoot/root growth, they may interfere nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source. Allelopathy is an interference mechanism in which living or dead plants release allelochemicals exerting an effect (mostly negative) on the associated plants, and can play an important role in natural ecosystems (Fitter, 2003; Inderjit & Duke, 2003).

Allelochemicals can show different modes of action on plants. The delay and reduction of seeds germination and/or inhibition of root and shoot growth are the first, visible symptoms of allelopathy stress. Research on maize allelopathy has been undertaken to study the allelopathic potential of various parts of maize. Maize and soybean are tow crops, normally grown in association. However, there view research results indicating the allelopathic effect of maize (Minorisky, 2002) and soybean (Jimenez *et al*., 1983). Moreover, to the best of our knowledge allelopathic interference of maize and soybean have not been studied. Hence, since allelopathy is part of crop-crop interference, it is time to investigate, whether there is allelopathic effect of maize and soybean on each other for the better under standing of the system, bearing this in the mind this research was undertaken using water as extracting medium because alleloshemicals are often water soluble and escape into the environment by means of root exudation (Tawaha & Turk, 2003).

Keeping in view the importance of maize and soybean as crops of economic importance and their cultivation in inter-cropping, the present research work was aimed.Most of the exotic plant effects reported have been identified as caused by allelopathic interaction which resulted in interference with physiological and biochemical processes in plants, due to chemicals released by the neighbor plants(Bughio *et al*., 2013) Among the allelochemicals which take part in plant to plant interactions are phenols, terpenes, glucosides, alkaloids, amino acids and sugars(Zouheir & Mohamed, 2011)

Materials and Methods

The experiment was carried out under natural conditions at the Department of Plant Sciences, Quaid-i-Azam University, Islamabad in Randomized Complete Block Design (RCBD) with three replications. Seeds of maize (*Zea mays* L.) cv. Islamabad Gold and Soybean (*Glyciene max* L.) cv. NARC1 obtained from National Agricultural Research Council, Islamabad, were surface sterilized with 95% ethanol for 3 min followed by shaking in 10% chlorox for 5 min and subsequently rinsed with autoclaved distilled water. Seeds were sown in earthen pots measuring 23 x 24 cm^2 filled with clay and sand (3:1).

Induction of drought to maize plant: The drought was induced 15 days after sowing (three leaf stages of maize plants) by withholding water supply for 9 days. Control plants received water as and when required. At 40 DAS (days after sowing) all the plants were uprooted and leaves and roots were used separately for extraction.

Preparation of plant extracts: Plants were separated into roots and leaves and cut into 2cm pieces. Half of the plant materials were utilized for preparation of fresh extracts while half were oven dried. Plant material (leaves and roots separately) was extracted in100ml distilled water (1:10 w/v) and kept in shaker (Excel E 24) at 2000 rpm, for 1h. thereafter the extracts were incubated at room temperature for 48 h according to Wardle *et al*., (1992), filtered with muslin cloth followed by filtration with Whatman No.1 filter paper and stored at 4˚C till further use. For preparation of oven dried extracts, plant materials (root or leaves) were kept in an oven at 70˚C for 72h till constant weight and ground finely. The oven dried plant powder (10g) was suspended in 100 ml distilled water. The mixture was stirred for 10 min and incubated at room temperature for 48h; the extracts were filtered as described previously for fresh extracts and stored at 4˚C till future use.

Application of maize shoot and root extracts on soybean: Soybean seeds were soaked for 10 h in fresh and oven dried extracts of leaves and root of maize plant. The seeds were sown in earthen pots measuring (23 x 24 cm^2) filled with clay and sand (3:1).

Determination of biochemical contents: Leaf samples of soybean were collected (40 DAS) and utilized for physiological and biochemical analysis. The proline contents of leaves were measured by the method of Bates *et al*., (1973). Soluble protein content of leaves was determined following the method of Lowry *et al*., (1951) using BSA as standard. Sugar estimation of fresh leaves was done following method of Dubois *et al*., (1956).

Determination of Antioxidants activity: The SOD activity was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using method of Beauchamp & Fridovich, (1971).One unit of enzyme activity was taken as that quantity of enzyme which reduced the absorbance reading to 50 in comparison with tube lacking enzyme. POD activity was measured by the method of Vetter *et al*., (1958) as modified by Gorin & Heidema (1976). Changes in absorbance were recorded at 485 nm for 3 min with the spectrophotometer. The activity of POD was presented as $OD_{485\ nm}$ /min /mg protein.

Ascorbate peroxidase activity was determined according to Asada & Takahashi (1987). The enzyme activity was expressed in U mg $^{-1}$ protein (U=change of 0.1 absorbance min^{-1} mg^{-1} of protein). Catalase activities (CAT) was measured according to Chandlee & Scandalous (1984) .The enzyme activity was expressed in U mg^{-1} protein (U=1mM of H_2O_2 reduction min^{-1} mg^{-1} of protein).

Determination of endogenous ABA content: Endogenous ABA content was determined by the method of Kettner & Doerffling, (1995).

Nutrients analysis of Rhizospheric soil: The rhizospheric soil was analyzed for macro and micronutrients (Na, Ca, Mg, K, P, NO3-N, Fe, Cu, Cr, Co, Ni, Zn and Mn) following the Ammonium Bicarbonate-DTPA method developed by Soltanpour & Schwab (1977).

Statistical analyses: The data was analyzed statistically by Analysis of Variance technique and comparison among treatment means was made by Duncan's Multiple Range Test (DMRT) using MSTAT-C version 1.4.2 (Duncan's, 1955).

Results

Effect of leaves and root extracts of maize (fresh and oven dried) on soil nutrient content: The fresh and oven dried aqueous extracts were prepared from leaves and roots of unstressed (control) and drought stressed maize plants and their impact on growth and biochemical contents of soybean and the rhizosphere soil was investigated. The extracts prepared from fresh leaves of control (unstressed) maize plants significantly increased the P content of rhizospheric soil of soybean. Where as, the impact of all other treatments on P content were non-significant at $p<0.05$. The oven dried leaf extracts prepared from leaves and root of both control as well as drought stressed maize plants significantly increased the Na content of soybean rhizospheric soil (Table 1). Significant increases in K, Ca and Mg contents were observed in rhizosphere of soybean plants treated with fresh and oven dried leaf extracts prepared from drought stressed maize plants. Both fresh and oven dried leaf extracts significantly increased the Fe, Cu, Cr and Zn content of rhizosphere soil. The Application of maize extracts of all the treatments significantly increased the Co content except the oven dried extract prepared from roots of drought stressed maize plants (Table 2).

Table 1. Effect of maize leaf and root extracts (fresh and oven dried) on macronutrient content of soil.

Treatment	Macronutrients				
	P (ppm)	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
C	0.2495 bc	6.6205 d	6.4045 c	32.049 d	0.3689 c
MCL (F)	0.419 a	7.289 cd	7.5 bc	35.443 bcd	0.468 bc
MDL (F)	0.2825 bc	9.363 ab	8.196 ab	38.004 ab	0.549 ab
MCL (OD)	0.241 bc	8.6145 abc	7.6855 bc	37.468 abc	0.4569 bc
MDL (OD)	0.313 b	9.764 a	9.4205 a	41.429 a	0.5864 a
MCR (F)	0.2925 bc	8.0945 bcd	7.0495 bc	33.5 cd	0.4512 bc
MDR (F)	0.1875 c	8.5 abc	6.661 bc	35.046 bcd	0.4165 c
MCR (OD)	0.247 bc	9.9135 a	6.625 bc	35.644 bcd	0.3867 c
MDR (OD)	0.2495 bc	7.8145 bcd	6.586 bc	32.832 d	0.4626 bc
	LSD: 0.099	**LSD: 1.537**	**LSD: 1.609**	**LSD: 4.048**	**LSD: 0.108**

Table 2. Effect of maize leaf and root extracts (fresh and oven dried) on micronutrient content of soil.

Treatment	Micronutrients						
	Fe (ppm)	Cu (ppm)	Cr (ppm)	Co (ppm)	Zn (ppm)	Mn (ppm)	Ni (ppm)
C	0.4915 cd	0.049 cd	0.005 b	0.051 b	0.216 b	0.419 c	0.0295 c
MCL (F)	0.753 abc	0.062 abcd	0.017 b	0.075 a	0.243 b	0.745 abc	0.062 ab
MDL (F)	0.875 a	0.083 ab	0.024 ab	0.0775 a	0.3435 a	0.837 ab	0.087 ab
MCL (OD)	0.691 abcd	0.068 abcd	0.015 b	0.078 a	0.2885 ab	0.783 ab	0.058 bc
MDL (OD)	0.7895 ab	0.087 a	0.044 a	0.0865 a	0.361a	0.903 a	0.0905 a
MCR (F)	0.447 d	0.063 abcd	0.006 b	0.075 a	0.282 ab	0.529 bc	0.079 ab
MDR (F)	0.673 abcd	0.073 abc	0.009 b	0.0805 a	0.2405 b	0.673 abc	0.068 ab
MCR (OD)	0.5275 bcd	0.045 d	0.007 b	0.079 a	0.2215 b	0.639 abc	0.06 ab
MDR (OD)	0.65 abcd	0.056 bcd	0.007 b	0.069 ab	0.2225 b	0.639 abc	0.066ab
	LSD: 0.249	**LSD:0.0248**	**LSD:0.0209**	**LSD:0.0185**	**LSD:0.0802**	**LSD: 0.300**	**LSD:0.029**

All such mean which share a common English letter are non significantly different from each other at P=0.05

C- Control

MCL (F) = Fresh leaf extract from control maize plant, MDL (F) = Fresh leaf extract from drought stressed maize plant

MCL (OD) = Dried leaf extract from control maize plant, MDL (OD) = Dried leaf extract from drought stressed maize plant

MCR (F) = Fresh root extract from control maize plant, MDR (F) = Fresh root extract from drought stressed maize plant

MCR (OD) = Dried root extract from control maize plant, MDR (OD) = Dried root extract from drought stressed maize plant

Proline content of soybean leaves: The results revealed that MDL (F), MCL (OD), MDL (OD) and MCR (OD) resulted in significantly higher accumulation of proline in soybean leaves. However, maximum increase in proline content occurred in soybean plants treated with fresh and oven dried extract from leaves of drought stressed maize plants. However, the shoot extract was more effective than the root extract in increasing the proline content of soybean seedling (Fig. 1).

Protein content of soybean leaves: No significant effect of fresh leaf extract prepared from unstressed or drought stressed condition. The oven dried leaf extract from control (unstressed) maize seedling as well as fresh or oven dried root extract from control (unstressed) or drought stressed maize seedling significantly decreased protein content of soybean seedlings. The magnitude of decrease was similar in these treatments (Fig. 2).

Sugar content of soybean leaves: Fresh as well as oven dried extracts from unstressed or drought stressed maize leaves or roots significantly increased the sugar content of soybean leaves as compared to control. Maximum increase (48%) in sugar content was observed in treatment MDL (F) that differed non-significantly with treatment MDL (OD) showing 46% increase in sugar content over control. The treatment MDR (OD) showed significant (35%) increase in leaf sugar of soybean as compared with unstressed (control), (Fig. 3).

Superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APOX) and catalase (CAT) activities of soybean leaves: The fresh and oven dried extracts from leaves of drought stressed maize plants and oven dried extract from roots of untreated maize plants significantly increased the SOD activity of soybean leaves. However, the fresh leaves extract and oven dried root extract significantly decreased the SOD activity (Figs. 4-7). Fresh leaf extract from drought stressed maize plants showed maximum increase in APOX followed by oven dried root extract from drought stressed maize. Significant increase in POD activity of soybean leaves was recorded by both fresh as well as oven dried aqueous extracts obtained from drought stressed maize leaves. Fresh and oven dried leaf extract from drought stressed maize plants significantly increased the POD activity; the maximum increase was due to fresh leaf extract (Fig. 8). The extracts prepared from drought stressed plants significantly increased the catalase activity of soybean. However, significantly higher (56%) increase in catalase (CAT) activity was recorded in treatment MDL (OD) that was at par to treatment MDL (F) when compared with the control.

ABA content of soybean leaves: The fresh extracts of drought stressed as well unstressed maize leaves and roots significantly increased the accumulation of ABA in soybean. However, the magnitude of ABA accumulation was higher in soybean plants treated with leaf and root extracts of drought stressed maize plants. However, the effect of fresh root extract from drought stressed plants was at par with that fresh extract from unstressed maize plants (Fig. 8).

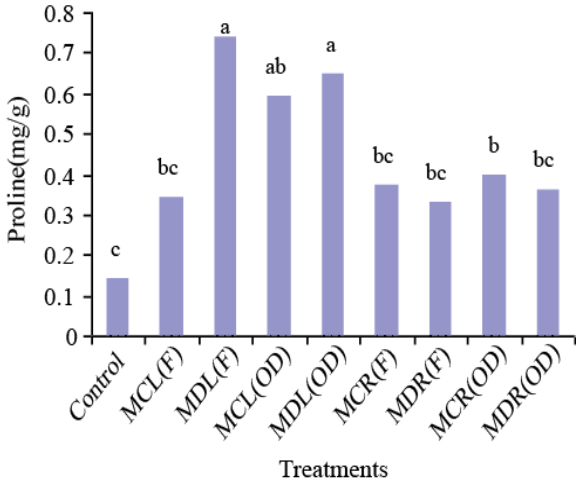

Fig. 1. Effect of maize leaf and root extracts (fresh and oven dried) on Proline (mg/g) content of soybean leaves.

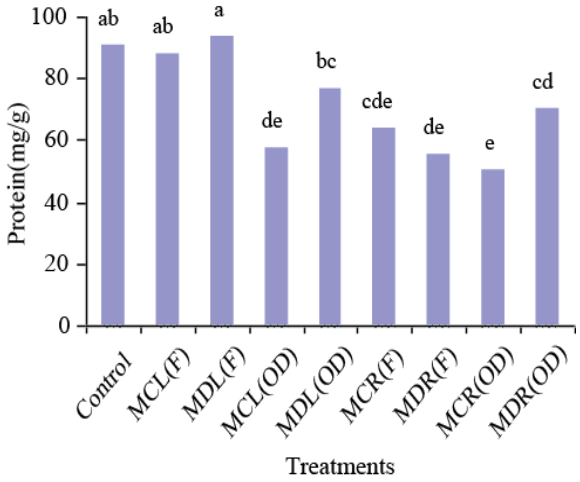

Fig. 2. Effect of maize leaf and root extracts (fresh and oven dried) on protein (mg/g) content of soybean leaves.

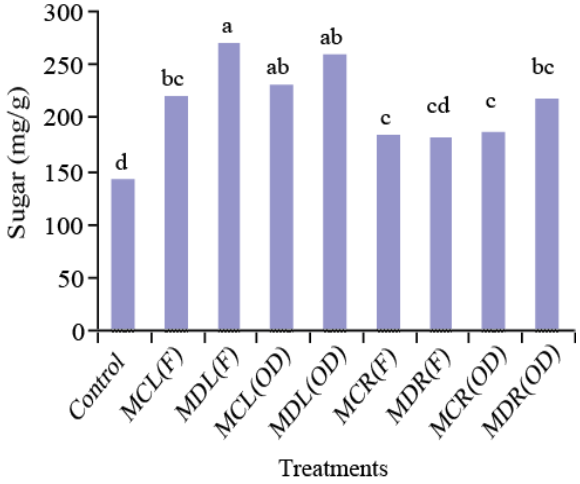

Fig. 3. Effect of leaf and root extracts of maize (fresh and oven dried) on sugar (mg/g) content of soybean leaves.

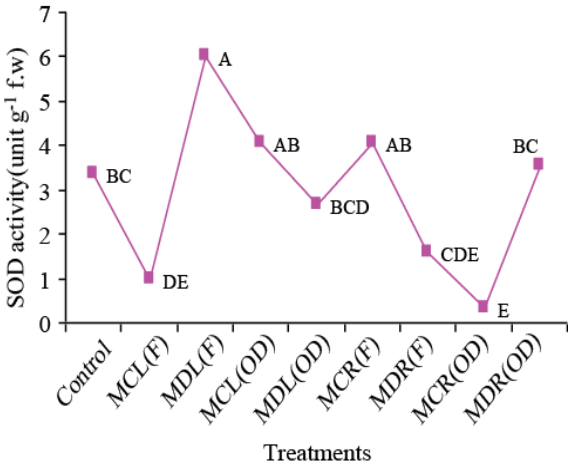

Fig. 4. Effect of shoot and root extracts of maize (fresh and oven dried) of Superoxide dismutase (SOD) (unit's g^{-1} f.w) of soybean leaves.

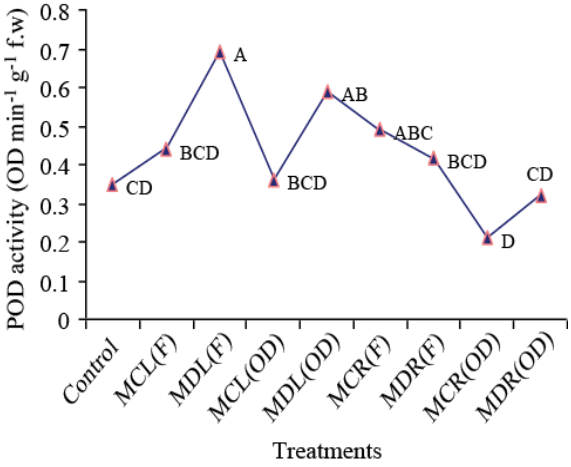

Fig. 5. Effect of shoot and root extracts of maize (fresh and oven dried) on Peroxidase activity (POD) (O.D $min^{-1}g^{-1}$ f.w) of soybean leaves.

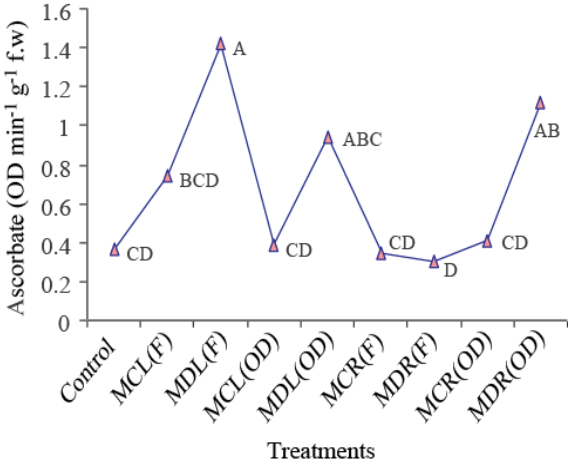

Fig. 6. Effect of shoot and root extracts of maize (fresh and oven dried) on Ascorbate peroxidase (APOX) (U mg^{-1}protien) activity of soybean leaves.

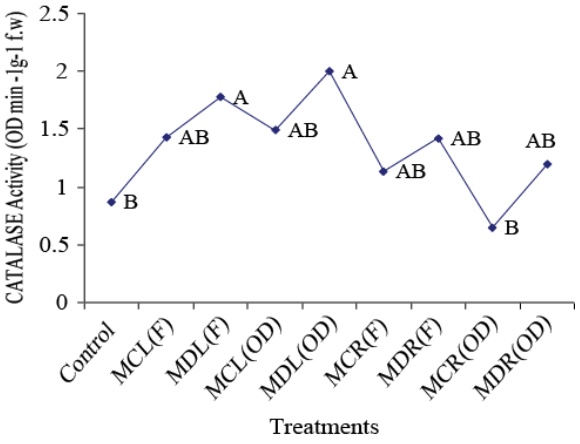

Fig. 7. Effect of shoot and root extracts of maize (fresh and oven dried) on Catalase (CAT) (O.D $min^{-1}g^{-1}$ f.w) activity of soybean leaves.

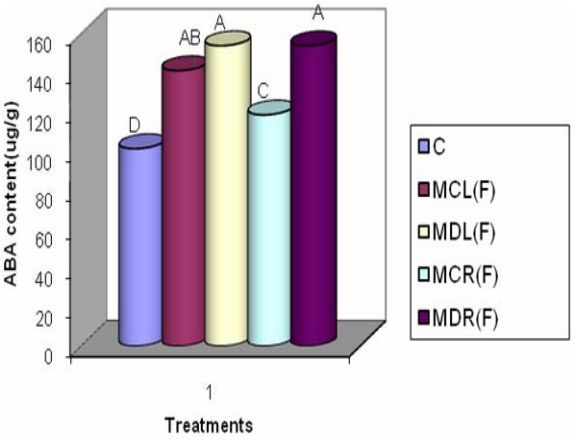

Fig. 8. Effect of maize extract on endogenous ABA (ug/g) content of soybean leaves.

Discussion

The maize extracts prepared from fresh and drought stressed maize leaves were more effective in altering the micronutrient content of the rhizosphere soil of soybean plants raised from seeds treated with these extracts. The oven dried leaf extracts either from control (unstressed) or drought stressed maize plants increased Na content and from the fresh root of drought stressed maize. The oven dried leaf extract of drought stressed plants insistently increased heavy metals (Cr, Cu, Ni, Zn and Co) over control. Drought stress increased the potency efficiency of leaf and root extract of soybean seedling growth Einhellig (1996) reported that plants growing under stressful conditions produce higher concentration of allelochemicals as compared to unstressed conditions. Allelochemicals can alter the rate at which ions are absorbed by the plants. Reduction in both macro and micronutrients are encountered in the presence of phenolic acids (Rice, 1974). The phenolics have been reported to produce complexes with plant nutrients (Kruse, 2000), which interfere with nutrient uptake of the plant. Most of these compounds work by changing the pH of soil and /or function as chelating agents for soil nutrients (Marschner, 1998).

The higher concentration of allelochemicals present in the extracts prepared from leaves of drought treated maize plants caused higher accumulation of proline in soybean seedling. Whereas, the root extracts also caused increase but of lower magnitude than that of leaves. The accumulation of proline in response to application of plant extracts has been reported previously (Abdulghadar *et al*., 2008). Proline is an osmoregulant, which accumulate under stress conditions in water and salt stresses (Shao *et al*., 2006; Erdei *et al*., 2002).

It was found that maize leaves extract significantly decreased the protein content of soybean leaves. Duhan *et al*., (1995) demonstrated significant decrease in the level of soluble proteins in legume crops in response to *Acaccia nilotica* extracts. Baziramakenga *et al*., (1997) demonstrated that phenolic acids reduced the incorporation of certain amino acids into proteins and thus reduce the rate of protein synthesis. Maize has been reported to contain 3 Phenolic acids (Iman *et al*., 2006), which might have resulted in decreasing the protein content of soybean leaves. The phenolic acids have been shown to be toxic to activities of many enzymes (Hopkins. 1999).

Soluble sugars content were significantly increased by the application of maize extracts on soybean. In radish increased concentration of soluble sugars in response to leaf extracts of heliotrope (*Heliotropium foertherianum*) has been reported (Abdulghader, 2008). Similar increase in soluble sugars of maize in response to leaf extracts of *Acacia* and *Eucalyptus* has been reported (Sahar *et al*., 2005).

The increase in the activity of antioxidants in response to stresses has been previously reported. Both biotic and aboitic stresses are known to induce plants to produce reactive oxygen species (Dat *et al*., 2000). The increased activity of antioxidants and antioxidant enzymes is perhaps a secondary effect of many allelochemicals. It seems that the receiving plant increases the activities of these enzymes in an attempt to counteract the harmful effects of ROS generated either by the various oxidative states of allelochemicals themselves or by a plant signaling cascade that is induced by the allelochemical. (Rocio *et al*., 2007).

The application of maize extracts to soybean prior to sowing increased the endogenous ABA content of soybean seedling. The increase was greater due to application of fresh leaf and root extracts from drought stressed plants. Many workers have reported similar increase in ABA content in response to allelochemicals. Yang *et al*., (2008) found that ABA content of rice was significantly increased in response to application of *Ageratin adenophora* aqueous extracts.

Conclusion

Both leaves and root extracts are effective, however leaf extract are more effective the magnitude of inhibitory effective is pronounced with extracts prepared from the drought stressed plants. Drought resulted in increased ABA biosynthesis and addition of extract from leaves of either unstressed or by drought stressed maize increase ABA contents of soybean leaves. Future research is needed to evaluate the effects of maize leaves extract in the amelioration of salt and drought stress.

The enhanced production of osmoregulant (praline), sugar content, stimulation of the activities of antioxidant enzymes by soybean leaves treated with extract from leaves of drought stresses maize constitute an important strategy for inducing tolerance to soybean under stress.

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