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Impacts of Allelopathic Potential of Aqueous Extracts of Maize (*Zea mays* L.) and Soybean (*Glysine max* L. merril)

By

Naseer Ahmad

Department of Plant sciences

Faculty of Biological Sciences

Quaid-i-Azam University

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Impacts of Allelopatic Potential of Aqueous Extracts of Maize (Zea *mays* L.) and Soybean (*Glysine max* L. Merril)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Philosophy

In

Plant sciences

(Plant physiology)

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DEDICATION

I dedicate this humble
unpretentious and pragmatic effort
to
my late father, to whom I owe an immense
debt of his unconditional
and constant
support, encouragement and his unceasing
confidence in my
abilities

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيمِ

In The Name Of Allah The Most Beneficent & The Most Merciful

CERTIFICATE

This is to certify that this thesis entitled as **"Impacts of Allelopathic Potential of Aqueous Extracts of Maize (Zea *mays* L.) and Soybean (Glycine *max* L. Merril)"** submitted by **Naseer Ahmad** is accepted in its present form by the **Department of Plant Sciences, Faculty of Biological Sciences ,Quaid-i-Azam University, Islamabad** as satisfying the thesis requirements for the degree of

Master of Philosophy

in

Plant Sciences
(Plant Physiology)

SUPERVISOR: _____
Prof. Dr. Asghari Bano

EXTERNAL EXAMINER: _____
Dr. Zubaida Choudary

CHAIRMAN: _____
Prof. Dr. Mir Ajab Khan

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List of Abbreviations

Abbreviations	Full Name
ABA	Abscissic acid
AgNO3	Silver nitrate
APS	Ammonium per sulphate
BSA	Bovin Serum Albumen
BHT	Butylated hydroxyl toluene
^0C	Centigrade
CAT	Catalase
Cm	Centimeter
CO_2	Carbon di oxide
Ca	Calcium
$CuSO_4$	Copper sulphate
DW	Dry weight
DI	Deionize
DMSO	Di Methyl Sulf Oxide
EC	Electrical Conductivity
FW	Fresh weight
g	Grams
hrs	Hours
H_2	Hydrogen
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HPLC	High performance liquid chromatography
K	Ptassium

K2HPO4	Dipotassium hydrogen phosphate
K2CrO4	Potassium chromate
Kg	Kilogram
L	Liter
mM	Milli Molar
min	Minutes
ml	Milliliter
Mg	Magnesium
NARC	National Agriculture Research Council
NH_4NO_3	Ammonium hydroxide
NH_4HCO_3	Ammonium bicarbonate
Na	Sodium
NaCl	Sodium Chloride
$NaNO_2$	Sodium Nitrite
N_2	Nitrogen
Na_2CO_3	Sodium carbonate
nm	Nanometer
O_2	Oxygen
OD	Optical density
POD	Peroxidase
SOD	Superoxide dismutase
APX	Ascorbate peroxidase
CAT	Catalase
P	Phosphorus
PSB	Phosphate solubilizing bacteria
P_2O_5	Phosphorus pentaoxide
ppm	Parts per million
PVP	Polyvinyl pyrolidine
rpm	Revolution per minute
RCBD	Randomized Complete Block Design
CRD	Complete Randomized Design

ROS	Reactive oxygen species
RWC	Relative water content
RFE	Rotary thin film evaporator
µg	Micro gram
µl	Microliter
Wt	Weight
%	Percentage
Zn	Zinc
$ZnSO_4$	Zinc sulphate

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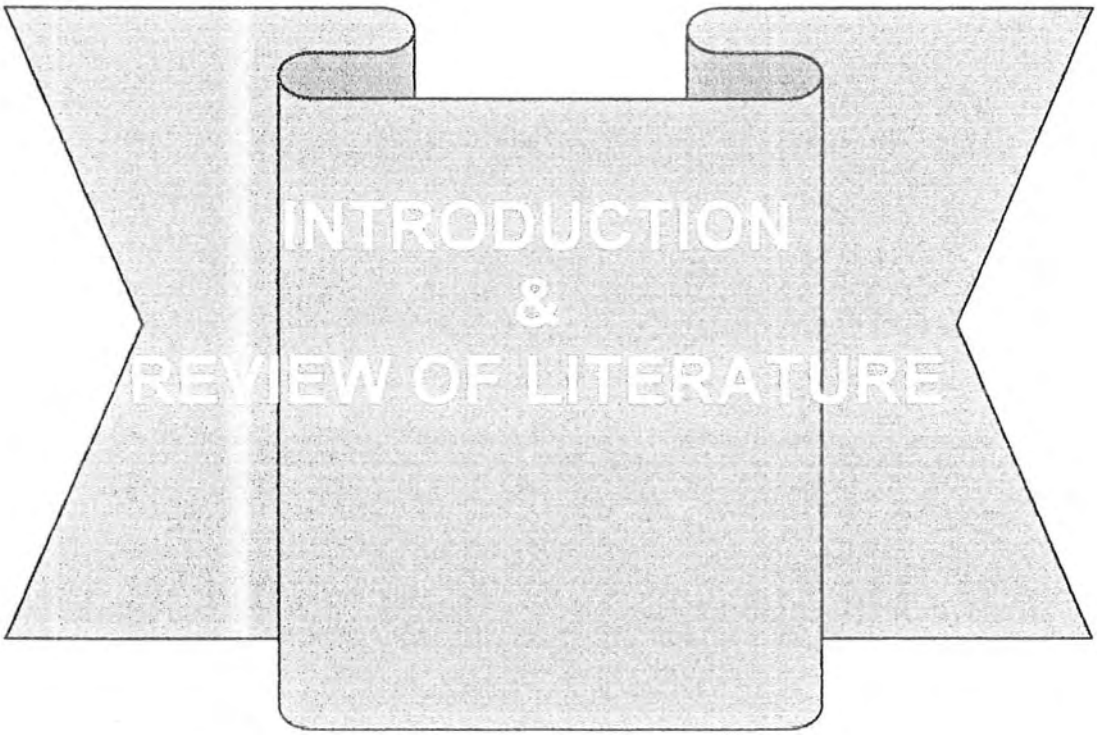
NASEER AHMAD

Abstract

The present investigation was aimed to asses the allelopathic effects of maize on soybean and vice versa under drought stress conditions. In the first experiment, the aqueous extracts from leaves and roots of the two crops were applied at the rate of 2% and 4% concentrations as seed soaking for assessing their allelopathic effects on seed germination and seedling growth. The fresh and oven dried extracts were prepared from drought treated and unstressed plants. The extracts of both maize and soybean significantly inhibited the germination (%) and seedling growth of each other. However, the extracts prepared from drought subjected soybean plants were found more inhibitory on growth of maize seedling. The 2% maize leaves extracts were found as less inhibitory to soybean seedling. The leaves extracts were found more effective than root extracts.

Second experiment comprises determining the effects of aqueous extracts of maize on physiology of soybean and vice versa in pot experiment under natural conditions. The extracts were prepared from maize and soybean plants subjected to 9d drought stress. The extracts were applied as seed soaking prior to sowing for 8h under axenic conditions. The extracts from unstressed plants applied at the same concentration were treated as control. The leaves extracts of both maize and soybean prepared from drought subjected and unstressed plants exhibited no significant effects on chlorophyll content of either of the crop but carotenoid content was significantly decreased. Maximum proline, protein and soluble sugar accumulation was found in maize and soybean plants which were supplied with leaves extracts prepared from drought treated plants. Increase in antioxidant activity (superoxide dismutase, peroxidase, ascorbate peroxidase and catalase) and endogenous abscisic acid (ABA) content occurred in response to application of leaves and root extracts of both maize on soybean and vise-versa. However the extent of increase in superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase was grater in maize plants treated with soybean leaf extracts prepared from pre-stressed plants. The soybean leaf extracts were found more effective as compared to maize leaves extracts. The pH and EC of soil cultivated with soybean was not significantly affected but availability of macro and micronutrient in soybean was significantly decreased by application of maize leaf and root extracts. The soybean leaf extracts prepared from drought treated plants significantly decreased the soil pH and EC of maize cultivated soil. On the contrary, soybean root extract prepared from unstressed plants significantly increased the soil EC as compared to control. On the average, the soybean leaves extracts prepared from drought stressed plants were found more effective in modulating the physiology of maize indicating the higher allelopathic potential of soybean as compared to maize.


INTRODUCTION
&
REVIEW OF LITERATURE

1. Introduction and Review of literature

Allelopathy is derived from two Greek words; 'Allelon' meaning each other and 'Pathos' meaning to suffer i.e. injurious effects of one upon another. Prof. Hans Molisch, a German scientist coined this term in 1937, which refers to all biochemical interactions (stimulatory and inhibitory) among the plants, including microorganisms. It represents the plant-against-plant aspect of the broader field of chemical ecology. Although the impact of Allelopathy on agriculture was recognized by Theophrastus in 300 B.C, but most research has been conducted after 1960. In the last 60 years, Allelopathy research has broadened to new areas like the plant-insect/ nematodes/ pathogens/ aquatic ecosystems interactions. Hence, in 1996, International Allelopathy Society broadened its definition, "Allelopathy refers to any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems". Allelopathy provides basis to sustainable agriculture, hence, it is priority area of research in developed countries of the world like USA, Canada, European Union countries, Russia, Japan, Korea, Australia, Mexico, Brazil, etc (Narwal *et al.* 2005). It is a multidisciplinary area of research involving agriculture like agronomy, soil science, genetics, plant breeding, agroforestry, horticulture, vegetable crops and plant protection such as weeds control, insects control, diseases control and nematodes control and fields of biosciences including biotechnology, biochemistry, microbiology, plant physiology and aquaculture (Narwal *et al.* 2005).

1.1 History of Allelopathy

Although, the impact of allelopathy on agriculture was recognized by Democritus and Theophrastus in the 5th and the 3rd Century B.C., respectively (Smith and Secoy, 1977) and by DeCandolle in 1832 but most of the progress in this field has occurred in the twentieth century (Rice, 1984). Since the 1960's allelopathy has been increasingly recognized as an important ecological mechanism which influences plant dominance, succession, formation of plant

communities and climax vegetation and crop productivity. It has been related to the problems with weed-crop interference (Bell and Koeppe, 1972), phytotoxicity in stubble mulch fanning (McCalla and Haskins, 1964) and in certain types of crop rotations (Conrad, 1927). Rice (1984) indicated that allelopathy contributed to weed seed longevity problem through two mechanisms, (a) chemical inhibitors in the seed prevented their decay by microbes and (b) the inhibitors kept the seed dormant, although viable for many years.

1.2 Proof of Allelopathy

A number of investigations have provided excellent evidences for allelopathy but only few investigators have followed a specific protocol (similar to Koch's postulates for proof of disease) to achieve convincing proof (Fuerst and Putnam, 1983). The proof of allelopathy generally involves the following sequence of studies:

- Demonstrate the interference using the suitable controls, describe the symptoms and quantify the growth reduction.
- Isolate, characterize and assay the chemical against species that were previously affected. Identification of chemicals that are not artifacts is essential.
- Obtain toxicity with similar symptoms when chemicals are added back to the system.
- Monitor the release of chemicals from the donor plant and detect them in the environment (soil, air, etc.) around the recipient and ideally, in the recipient plant.

1.3 Allelochemicals

Allelochemicals refer mostly to the secondary metabolites produced by plants and are byproducts of primary metabolic processes (Levin, 1976). They have an allelopathic effect on the growth and development of the same plant or neighbouring plants. The term allelochemicals include,

- Plant biochemicals that exert their physiological/toxicological action on plants (allelopathy, autotoxicity or phytotoxicity),
- Plant biochemicals that exert their physiological/toxicological action on microorganisms (allelopathy or phytotoxicity) and

- Microbial biochemicals that exert their physiological/toxicological action on plants (allelopathy and phytotoxicity).

1.3.1 Occurrence of allelochemicals

The existence of allelochemicals in higher plants and microorganisms has been documented. Plant parts known to contain allelochemicals according to Rice (1974) are as follow:

(i) Roots and rhizomes: In general, they contain fewer and less potent or smaller amounts of allelochemicals than leaves, but sometimes it may be the reverse also.

(ii) Stems: They contain allelochemicals and are sometimes the principal sources of toxicity.

(iii) Leaves: They are the most important sources of allelochemicals. Specific inhibitors in leaves have been demonstrated by many workers.

(iv) Flowers/inflorescence and pollen: Although studies on flowers on flowers or inflorescence are limited, but there is growing evidence that the pollen of corn and *Parthenium* have allelopathic properties.

(v) Fruits: Many fruits are known to contain toxins and have been found inhibitory to microbial growth and seed germination.

(vi) Seeds: Seeds of many plant families or species have been found to inhibit seed germination and microbial growth.

1.3.2 Modes of release of allelochemicals

A major pre-requisite of allelopathy is that an organic substance allelochemical be transferred from a donor plant to recipient plant, therefore, mode of transfer may play a great role in toxicity and persistence of allelochemicals. The donor plant generally stores these chemicals in the plant cells in a bound form, such as water-soluble glycosides, polymers including tannins, lignins and salts. Hence, these chemicals are not toxic to the donor plants. Once these chemicals from the donor plants are released into the environment, they may be either degraded or transformed into other forms, which affect the receiver plants and may also be toxic to the host plant (autotoxicity). Upon cleavage by plant enzymes or environmental stress, these

chemicals are released into the environment from special glands on the stems or leaves. First the terpenoids such as α-pinene, cineole and camphor are released to the environment through volatilization. Then the water-borne phenolics and alkaloids are moved out by rainfall through leaching. Next, phytotoxic aglycones such as phenolics are released during the decomposition of plant residues in soil. Finally, many secondary metabolites such as scopoletin and hydroquinones may be released to the surrounding soil through root exudates. Release through leachates and root exudates require water solubility and broad range of allelochemicals are involved ((Narwal *et al.* 2005).

The processes by which the allelochemicals are released from the plants to the environment have been described. Allelochemicals are released into the environment by volatilization, leaching from aboveground parts, root exudation and/or by decomposition of plant material (Rice, 1984).

1.3.2.1 Volatilization

Allelochemicals may volatilize from the plants to the atmosphere. The volatile vapours may be absorbed directly from the atmosphere by plants, the adsorbed vapours may condensate in dew and fall to ground and these volatile compounds may be absorbed on the soil particles and subsequently taken by plants from the soil solution. The camphene, camphor, cineole, dipentene, α-pinene and β-pinene are volatile inhibitors produced by several shrubs of the Southern California Chaparral (White *et al.* 1989). From the plants rich in such compounds, these may be released continuously as vapours to the atmosphere. The pulverized leaves of cruciferae species *(Brassica juncea, B. nigra, B. napus, B. rapa* and *B. oleracea)* also release volatile substances. The volatiles of *B. juncea and B. nigra* were most harmful to germinating seeds of lettuce and wheat (Oleszek, 1987).

1.3.2.2 Leaching

Leaching is the removal of substances from plants by the action of aqueous solvents such as rain, dew, mist, fog and snow. All plants seem to be leachable, but the degree depends on type of tissue, stage of maturity and type, amount and duration

of precipitation. Many allelopathic compounds both organic and inorganic are leached, such as phenolic acids, terpenoids and alkaloids.

1.3.2.3 Root exudates

The release of allelochemicals via root exudates has been documented. Many compounds are exuded form the roots, which may influence the growth of microorganisms and associated higher plants (Barnes and Putnam 1986, 1987).

In soil environment, transformations by rhizosphere microorganisms may inactivate the original exudation compounds and in other cases may create new active allelochemicals. Exudates vary according to plant species, its age and temperature, light, plant nutrition, microbial activity around the roots and the nature of the medium supporting the roots.

1.3.2.4 Decomposition of plant residues

The decomposition of plant residues adds the largest quantity of allelochemicals to the soil (Rice, 1985). At plant death, materials compartmentalized in cells are released into the environment. Important variables in this process for allelopathy are the nature of the plant residues, the soil type and the conditions of decomposition. Depending on the decomposing conditions, substances highly toxic, non-toxic or stimulatory to plants may be formed during the decomposition of similar plant residues. In general, more severe and persistent toxicity occurs in cold and wet soils.

1.3.3 Factors influencing production of allelochemicals

It is still not known whether allelochemicals are actively released by plants or if it just happens passively, independently from external factors. This could possibly be due to natural selection, becoming more adapted to the surrounding environment (Moore *et al.* 1998). However, plants growing under stressful conditions may produce a higher concentration of allelochemicals (Einhellig, 1996). Causes for these stressful conditions can be one of the following:

1.3.3.1 Light

The plants growing in glasshouses have been noted to not produce as large quantities of inhibitors as some kinds of plants growing outdoors. This suggested the important role of light quality on the production of inhibitors (Rice, 1984). The following light quality has been proved to play a role in the release of some compounds:

1.3.3.2 Ionizing radiation

It strongly increases the amount of various phenolic inhibitors in tobacco and sunflower plants (Fomenko, 1968).

1.3.3.3 Ultraviolet radiation:

It has been found by Frey-Wyssling and Babler (1957) that adding UV light to greenhouse light improved the growth of greenhouse tobacco and increased the chlorogenic acid content.

1.3.3.4 Red and far red light

It was demonstrated by Jaffe and Isenberg (1969) that concentration of several phenolic compound, between which just ferulic and p-cumaric acids were identified, increased in potato tuber disks at a faster rate with red light than in disks irradiated with an equivalent dose of far-red light.

1.3.3.5 Visible light

Zucker found (1963) that visible light stimulates the synthesis of chlorogenic acid in potato tuber disks in water and it stimulates synthesis of p-cumaryl esters in similar disks in the phenylalanine culture.

1.3.3.6 Water stress

It's one of the most obvious abiotic stress but very little is known about it. Del Moral in 1972 used NaCl in the culture solution to induce water stress on sunflower plants. The osmotic potential, in order to change the pressure, caused a drought stress and resulted in substantial increases in the concentration of chlorogenic and

isochlorogenic acids in roots, stems and leaves over amounts in control plants (Rice, 1984).

1.3.3.7 Temperature

Plants that are going through temperature stress tend to produce more allelochemicals and are more susceptible to allelochemicals, i.e., they have a lower inhibition threshold. Koeppe (1970) did an experiment with tobacco plants and concluded that chilled plants (at 8° to 9°C) produced more scopoletin compared with plants grown at 32°C. This was true for all organs except for roots that showed a decreased concentration of these phenolic compounds. Einhellig and Eckrich (1988) noted that crops grown under the higher end of normal conditions had a lower concentration threshold by ferulic acid. For example, grain sorghum was inhibited by half the amount of ferulic acid when it was grown at 37° C compared to 29° C. Soybean grown at 23° C was not affected by 100μM ferulic acid whereas the same concentration inhibited soybean grown at 34° C.

1.3.4 Allelopathic agents

Allelopathic agents and phytocides have been proved to play an important role in the production of compounds in sunflower and tobacco plants by Dieterman (1964) and Einhellig (1970). Both crops were sprayed with 2, 4 D and showed an increase in the concentration of scopolin in various plant parts. When these crops were grown in a solution of scopoletin, tobacco plants showed a significant increase in scopoletin and scopolin but no increase in chlorogenic acid. The concentration of total phenols in carrots was tested by Sarkar and Phan (1974) when these were exposed to ethylene and it was found that at least four new phenols were produced, which do not occur normally in carrot tissues (Rice, 1984).

1.3.4.1 Pathogens and Predators

Woodhead (1981) found that sorghum plants that were infected with sorghum downy mildew (*Sclerospora sorghi*) or rust (probably *Puccinia purpurea*) and other sorghum plants that were infested with shootfly (*Atherigona soccata*) had increased concentrations of phenolics. There is good evidence that such increase in

allelochemicals enhance the resistance of at least some plants to pathogens and predators, but nobody has investigated the possibility that such increases in allelochemics may increase the allelopathic effect of the infected or infested plants (Rice, 1984).

1.3.4.2 Age of plant organs

Many authors found age of plant organs to be a relevant factor involved in the production of compounds. Experiments done by Koeppe (1969) showed that the concentration of Scopolin and chlorogenic acids in leaves of tobacco plants varied with the age of the leaves, even in control plants. The results of his experiments proved the increase of total amount of these compounds with the age of leaves. Similar tests done by Woodhead (1981) showed that Phenolic acid concentration in Sorghum leaves decreased in all cultivars with age in healthy plants, particularly after 28 days of age, but increased again in the flowering stage, reaching about the same level found in the young plants.

1.3.4.3 Day length

It's one of the many factors involved in the compounds production. Its influence has got different effects on long-day and short-day plants. The highest concentration of allelochemicals has been noted to be the period just before the changes of meristem from the vegetative to the flowering shape in most of cases. However there are some exceptions in which the compounds release is stimulated by the opposite day length in which the plant will be flowering (e.g. short-day plant produces more compounds when receiving a long irradiation) (Rice, 1984).

1.3.5 Mode of action of allelochemicals

Allelopathic agents influence the plant growth (Rice, 1984) through the following physiological processes viz., (i) cell division and cell elongation, (ii) phytohormone induced growth, (iii) membrane permeability, (iv) mineral uptake, (v) availability of soil phosphorus and potash, (vi) stomatal opening and photosynthesis, (vii) respiration, (viii) protein synthesis and (ix) changes in lipid and organic acid metabolism, (x) inhibition of porphyrin synthesis, (xi) inhibition or stimulation of

specific enzymes, (xii) corking and clogging of xylem elements, (xiii) stem conductance of water (xiv) internal water relations.

1.3.6 Fate of allelochemicals

Except the volatile allelochemicals, which are absorbed by plants directly from the air or as leachates (after dissolution in rain, dew, mist or snow), the soil mediates all allelopathic responses. Potential allelochemicals must remain active in the soil to have an allelopathic effect. The biological activity, persistence, movement and fate of natural products in the soil depend upon their interaction with the soil adsorption complex, soil microbial population and chemical environment of the soil. Adsorbed allelochemicals may be biologically active or rendered inactive, depending on nature of the adsorbing surface, but adsorbed molecules are less available to soil microbes. Some natural products/allelochemicals may be irreversibly bound in soil humic substances. Thus allelopathic effects in soil depend on the relative rates of allelochemicals, addition and decomposition or fixation in the soil (Norwal *et al.* 2005).

1.3.7 Crop residues

In monocropping, the crop and weed residues do not pose any management problems. Because residues are incorporated into the soil sufficiently ahead of planting time, to allow their complete decomposition and thus toxins released during the decay become harmless to the succeeding crop. However, since 1960's Multiple Cropping Systems have been introduced (owing to availability of short duration and high yielding varieties of crops) in areas where climate and irrigation facilities are favorable for crop production throughout the year. The adoption of multiple cropping systems in subtropical and tropical countries under irrigated conditions have firstly, led to a greater production of crop residues

1.4 Interaction with biotic and abiotic factors

Biotic and abiotic factors can influence both the production of Allelochemicals by the donor species (the species from which the Allelochemicals originate) and modify the effect of an allelochemical on the receiver plant. The influence of factors such as light, nutrient availability, water availability, pesticide treatment and disease can affect the amount of allelochemicals in a plant (e.g. Inderjit and Del Moral 1997; Reigosa *et al.* 1999). Even though the production of allelochemicals in a plant can increase in response to stress, it is not clear whether a corresponding release of allelochemicals to the environment also occur (Einhellig 1996; Inderjit and Del Moral 1997). In general the sensitivity of target plants to allelochemicals is affected by stress and typically it is increased (Einhellig 1996, Reigosa *et al.* 1999). On the basis of several examples discussed by Einhellig (1996) and Inderjit and Del Moral (1997) the authors conclude that allelopathy and stresses interact under natural conditions. This implies that the result of an experiment designed to investigate allelopathic activity will be strongly influenced by the test conditions. Under laboratory conditions, which is typically less stressful than field conditions, the allelopathic effect might be reduced (Romeo and Weidenhamer, 1998).

1.4.1 Changes in chemical characteristics of the soil

It has been hypothesised that allelopathic plants in addition to qualitative and quantitative changes in the soil content of allelochemicals also may cause changes in soil chemical characteristics (Inderjit 1998). In one study, the presence of *Pluchea lanceolata*, an aggressive evergreen asteracean weed, apparently influence certain soil properties. In addition to the higher phenolic content of soils in the vicinity of *P. lanceolata* compared to soils between 10 and 40 m away, pH, electrical conductivity, potassium (K+) and soluble chloride (Cl-) were influenced in the soil in contact with *P. lanceolata*. However, it was not established that the observed nutrient alterations resulted from phenolics excreted from *P. lanceolata* (Inderjit 1998). As the *P. lanceolata*- infested soils had significant negative effects on seedling growth of various crop plants compared to non-infested soils, it is possible that the effect of allelopathic plants can be due to the allelochemicals in the soil and/or to altered soil nutrients. Generally, phenolic acids are considered to have important influence on nutrient cycling in terrestrial ecosystems. Phenolic monomers and phenolic acids can form complexes with nutrients and thereby influence the nutrient availability and nutrients turn over in soil (Apple 1993; Kuiters 1991).

1.4.2 Nitrification

Investigations by Rice and Pancholy (cf Rice 1984) have indicated that phenolic compounds can inhibit the oxidation of $NH4^+$ to $NO3^-$ through toxicity towards nitrifiers (Rice 1984). These results have been much discussed by Bremner and McCarty (1993) who found no inhibitory effects using pure phenolic compounds on soil and reported that phenolics and terpenoids enhanced the immobilization of $NH4^+$ by soil organisms rather than the inhibition of nitrifying bacteria.

1.4.3 Nutrition

Concerning the nutritional effects of allelochemicals, the effects are sometimes indirect, and we enter the border zone of allelopathy to that of plant nutrition and plant – microorganism interactions. As an example of the direct effects, it can be mentioned that some phenolic acids, which form complexes with plant nutrients (Kruse, 2000), interfere with the nutrient uptake, thus causing a lower concentration of nutrients in plant tissues (Einhellig, 1996). Ferulic acid has been found to increase the uptake of certain ions, but the effects are dependent on the age of the acceptor. This is not uncommon. (Einhellig, 1984) Some plants, if not all, exude certain compounds into their rhizosphere, which changes the availability of several nutrients of the surrounding soil. These are, among others, organic acids such as citric acid, fumaric acid etc, amino acids and phenolics or phytosiderophores. Most of these compounds work by changing the pH of the soil and/or function as chelating agents for the nutrients (Marschner, 1998).

This, of course, affects the plant itself, but also other plants with roots entering this rhizosphere are affected, as well as plants which establish themselves where another plant was before. In most cases this effect will be positive, as the purpose of these chemicals is to increase the availability of the nutrients most needed, but the effect can be negative if leaching or depletion is caused, or if two succeeding plants, or two plants with overlapping roots are in need of different nutrients, as the compounds exuded to make one mineral more available, can make another mineral less available, for example through changes in the pH value. One example is *Pluchea*

lanceolata, which presence has been noted to influence soil characteristics such as pH, electrical conductivity and contents of potassium and chloride, of the soil in the vicinity. (Kruse, 2000)

An example of increased nutrient uptake is that dried leaves of ground-ivy (*Glechoma hederacea*) had a stimulating effect on shoot and root growth of radish (*Raphanus sativus*) and downy brome (*Bromus tectorum*), but the effect was much higher, when nutrients (N, P and K) were not abundant, indicating that the positive effect was caused by a higher uptake of nutrients (Rice, 1986).

Some plants exude allelochemicals, such as volatile terpenoids, which may inhibit the oxidation of ammonium to nitrate, by affecting the nitrifying microorganisms, and this leads to a higher NH4 +/NO3 - -ratio. (Courtney et al.. 1991) This theory has been rejected by Bremner and McCarty (1988). This will of course lead to a change in N-availability, as most plants prefer to take up N either as NH4 + or as NO3 -, although some can take it up in either form. As mentioned in the part "activities and effects", some allelochemicals can change the water relations of other plants, as well as inhibit the root hair formation. This will also lead to changes in the uptake and transport of mineral nutrients. The lack of root hairs can impede the formation of root nodules in leguminous plants, as the nitrogen-fixing bacteria enter through the root hairs. (Kruse, 2000)

Such reduction of nodulation and N-fixation caused by quackgrass has been observed in a number of legumes by Weston and Putnam (1985) and Putnam and Tang (1986).
This reduces the production of ammonium and nitrate and can lead to nitrogen deficiency in the plant.

One could argue that the recognition mechanism between the N-fixing bacteria and the specific host plant is allelopathic, as a complex exchange of chemical signals takes place, before the bacteria becomes established in nodules. (Hopkins, 1999)
Some allelopathic plants inhibit mychorrizal development. One example is that water extracts of the evergreen dwarf shrub *Empetrum hermaphroditum* hagerup, which

caused inhibition of the mycorrhizae *Paxillus involutus* Batsch (Fr.) growing with Scots pine (*Pinus silvestris* L.). (Nilsson *et al.* 1992) As mycorrhizae tend to increase the nutrient uptake of infected plants, this inhibition does have a negative effect on the nutrition of the affected plant.

1.5 Effects of allelopathic plants in natural ecosystems

1.5.1 Effects on population and community structure

In Spanish scrublands, the floristic diversity, richness and evenness of herbs found beneath the Mediterranean scrub Crimson spot rockrose (*Cistus ladanifer*) is significantly lower than in adjacent plots without this plant. The allelopathic activity of *C. ladanifer* is thought to play an important role as leaf exudates of this plant inhibit the germination of seeds of species that are absent from - but found growing adjacent to *C. ladanifer* scrublands. The distribution of these species is apparently limited by the allelopathic action of *C. ladanifer* (Chaves and Escudero, 1997). Also some species growing close to *C. ladanifer* are affected by the exudates by delayed seed germination and reduced seedling growth. On the basis of these results, it is suggested that the allelopathic activity of a plant may reduce both number and population size of other species by reducing their competitive ability (Chaves and Escudero, 1997). In interpreting the results, it must be considered that the exudates were extracted in ethanol and that the seed germination tests were carried out only under laboratory conditions and without osmotic controls. Allelopathic plants may induce genetic changes within associated plant populations. The release of allelochemicals from *Ailanthus altissima*, tree-of-heaven, seems to be responsible for altering the genetic pool of susceptible neighbouring plant species. Both close (<1 m) and distant populations (> 10 m) of *Tridens flavus* are inhibited by Ailanthus toxins. But, the distal population includes a class of highly susceptible individuals not present in the proximal population. It is suggested that the genotypes sensitive to the allelochemicals have been removed from the gene pool of the proximal population by selection (Lawrence et al.. 1991).

1.5.2 Invasion and dominance

Allelopathy has been discussed as one of several factors affecting the ability of a plant to invade and establish in a new ecosystem. A few examples demonstrating the importance of allelopathy for successful invasion are presented below. The release of allelochemicals from plants known as aggressive colonisers e.g. *Elytrigia repens* (quackgrass) and*Vulpia myuros* (silvergrass), have lead some to suggest that allelopathy is involved in successful invasions (e.g. Friebe *et al.* 1995, An *et al.* 1997). However, the actual importance of the release of allelochemicals by these coloniser plants has rarely been demonstrated under naturalconditions. For some aggressive coloniser species previously reported to be allelopathic, further investigations have not confirmed that release of allelochemicals was essential for their ability to establish in new habitats.

By now it is known that several organic compounds interfere with various important processes, and that they cause a variety of responses in the plants affected - wilting and chlorosis, as observed in some cases (Carroll, 1994), are just two examples. An array of the processes that allelochemicals interfere with, and the results of such interferences are:

1.5.3 Respiration

Several allelochemicals have been found to perturb respiratory metabolism, among others

sorgoleone, juglone, quercetin, umbelliferon, ferulic acid, gramine and cineole. Some of these affect the mitochondria at very low concentrations. Stenlid (1970) found that the production of ATP in mitochondria was inhibited by a variety of flavonoids. Hadasova and Plhak (1963) found quackgrass *(Elytrigia repens,* (previously *Agropyron repens))* to inhibit the respiration of wheat plants, grown in the same pot. In 1965 and 1967, Plhák studied the effects of quackgrass on sugarbeet, (*Beta vulgaris* ssp. *vulgaris*) and found that sugarbeet was inhibited, both in mixtures with quackgrass and when succeeding quackgrass. The inhibition was correlated with changes in respiratory patterns at the beginning of the growth, the respiration was inhibited, but later it was moderately stimulated. (Vicherkova, 1999)

1.5.4 Photosynthesis

Some cinnamic and benzoic acids and scopoletin and chlorogenic acid, inhibits photosynthesis in whole plants (Einhellig *et al.* 1970; Patterson, 1981). Artemisinin was shown to reduce photosynthesis in *Lemna minor* at a concentration of only 1 μm (Stiles *et al.* 1994). In enzymatically isolated leaf cells of velvetleaf (*Abutilon theophrasti*), ferulic, p-coumaric, chlorogenic and vanillic acids inhibited photosynthesis from 33 to 65% (Mersie and Singh (1993).

1.5.5 Water balance and stomatal functions

In 1969 and 1970, Vicherková studied the effects of quackgrass on the water relations of flax (*Linum*) and sunflower (*Helianthus*) in both mixed and succeeding pot cultures. Quackgrass was found to be inhibitory to the growth of the other species, and the inhibition was accompanied by changes in the water relations of the plants. In the early stages the quackgrass decreased the transpiration rates, but later the transpiration became balanced for a short time, and then water loss increased. The diurnal changes in transpiration matched stomatal apertures in the later stages stomata remained open, even during acute water deficits, indicating that quackgrass effected disorders of the stomatal regulatory capacity. Also the size and density of stomata was changed. Substances extracted from quackgrass rhizomes caused decreased transpiration, water content and cell sap osmotic pressure as well as degree of stomatal opening, when applied to flax growing in pots. These effects were caused by a water deficit – possibly due to restricted water uptake by the roots. (Vicherkova *et al.* 1999)

1.5.6 Stem conductance of water

Van Alfen and Turner (1975) found out that, after 4 hours in a 200 g/ml solution of water-soluble glycopeptide toxins from cultures of *Ceratocystis ulmi*, stem conductance of water in *Ulmus Americana* seedlings was reduced by 79% and leaf water potential was reduced by 3 bars to the point in which the seedlings wilted.

1.5.7 Xylem element flux

Different allelopathic compounds have been noted to produce modification in the corking and clogging of xylem elements. Researchers observed that aqueous extracts of many allelopathic plant species caused browning, corking and clogging of xylem vessels in numerous species. The brown substance was a mixture of pectin, lignin, suberin, melanins and many unidentified substances (Bogdan and Grodzinsky, 1971, 1974).

1.5.8 Membrane permeability

Several allelopathic agents change membrane permeability. It is a common damage caused by marasmin (Ownes, 1969). Aescin, a triterpeneglycoside, affects *Ophiobolus graminis* by inducing leakage of ribonucleotide material and nucleosides (Carroll, 1994).

1.5.9 Cell division, development and organization

Cell elongation is inhibited by slowing mitosis of the root cells, which is an effect of e.g. coumarin and scopoletin (Einhellig, 1995b), or by making the root cells expand radially rather than vertically. This was for example observed in cucumber (*Cucumis sativus* L.), caused by volatiles from *Salvia Leucophylla* L. (Muller, 1965). Volatile monoterpenes, such as cineole and camphor cause reduced cell division, changes in the shape of root cells and their nuclei. Gramine and hordenine is assumed to damage cell walls and disorganize cell organelles. Lipid globules have been observed in cells of root tips, which had been subjected to these alkaloids, indicating a slowing of metabolism of food reserves. (Einhellig, 1995b) On the whole plant level these interferences can affect the elongation of the radicle (Liu and Lovett, 1993), or cause morphological changes in plant organs.

In the roots of cereals in close contact with quackgrass rhizomes (Vicherková et al., 1999), changes like lack of root hair formation, damage of root tips and damage to cell walls of rhizodermis and root cortex, and sometimes necrosis were observed.

These effects were also caused by volatile substances on wheat roots when placed over ether extract of quackgrass rhizome. (Vicherkova *et al.* 1999).

1.5.10 Changes in protein synthesis

It has been noted that potatoes (*Solanum tuberosum*) produce toxic substances that inhibit tree growth when the potatoes are cultivated between rows of young apple (*Malus domestica*) trees. The toxins also decrease the total nitrogen content in the branches and roots of apple trees, change the composition of proteins in the bark of the branches, increase the amount of soluble albumins and decrease the amount of residual proteins (Krylov, 1970). Cinnamic acid has been found to interfere with the mechanism of protein synthesis (Carroll, 1994).

1.5.11 Inhibition or stimulation of specific enzymes

The changes in enzymatic activity caused by allelochemicals lead to changes in the production and degradation of various compounds within the plant. Among the changes detected by Vicherková and colleagues (1999) are N-content, saccharide content and the phosphoester fraction in plant tissues as well as the level of chlorophyll and other plastids. Inhibition of cellulites by wattle tannins was demonstrated (Benoit and Starkey, 1968) Also hemicellulases appear to be inhibited by tannin.

Some of the allelochemicals affect susceptible organisms in more than one way. Sorgoleone from *Sorghum* species, and juglone from walnut (*Juglans regia*) inhibit chloroplast CO_2-dependent oxygen evolution, but they also inhibit the mitochondrial functions, in the case of sorgloeone, by blocking the electron flow. (Einhellig, 1995b) Also, different species react differently to the same allelopathic compound. Different concentrations of the same compound can have opposite effects. Low concentration of an inhibitory allelochemical can stimulate growth rather than inhibit it. Compounds in the same chemical class have different levels of toxicity.

One example is the effect on germination of wheat (*Triticum aestivum*) caused by water extract from quackgrass rhizomes, as well as the soil and nutrient solution in

which quackgrass was grown (Vicherkova et al., 1999). In this experiment, seed germination was positively affected at lower concentrations and inhibited at higher concentrations.

Some compounds will only delay the germination of susceptible plants, but this is still giving them a disadvantage in competition and leaving them vulnerable to infections by soil microorganisms for a longer time (Liu and Lovett, 1993).

The strength of the effect, or the threshold concentration, depends not only on the species and the compound, but also on environmental factors like light, water and temperature, as well as biological stresses such as pest attacks. (Einhellig, 1996) Some allelochemicals are not directly harmful to the host plant but to the microbes on which the plant's health depends on (e.g. *Rhizobium* species are affected by some allelochemicals and this leads to poor nodulation).

1.6 Relationships between plant hormones and allelopathic agents

Plant hormones are commonly divided into six different groups: auxins, gibberellins, cytokinins, abscissic acid, ethylene and brassinosteroids. Several allelopathic compounds are structurally similar to plant hormones, presenting the slight differences from them (Olofsdotter, 1998). Some mechanisms of action of allelopathic agents seem to resemble those of synthesis of plant hormones. Effects on hormones activity are clearly showed by experiments done on phenolic growth inhibitors from *Salix rubra* and apple trees which prove to suppress the activity of IAA and gibberellin (GA) (Kefeli and Turetskaya, 1967). Other examples are the ferulic acid that activates the synthesis of ABA (Hollapa and Blum, 1991) and the antiauxin and antigibberlin activity played by some terpenes (Komai *et al.* 1981, Watanabe *et al.* 1982).

1.7 Effect of allelochemical on antioxidant

ROS (Reactive Oxygen Species) mediated allelopathic mechanisms Redox transformations that ultimately result in the formation of reactive oxygen species (ROS) play an important role in interactions between plants and their pathogens,

mutualists, and competitors (Appel *et al.* 1993). Controversially, not only have ROS been implicated in signal transduction and in plant defense mechanisms, such as the hypersensitive response, but also ROS accumulate in plant cells in response to compatible pathogen infections and are known to damage cells, often leading to cell death (Huckelhoven *et al.* 2003). The toxicity of many quinones and phenols can largely be attributed to the formation of semiquinone radicals that donate electrons to molecular oxygen, forming superoxide anions (O2) (Testa et al.. 1995). These can undergo a series of further reactions to become the more reactive hydroxyl (OH^-) or hydroperoxyl (HO_2) radicals (Hammondkosak *et al.* 1996).Subsequently, these radicals can affect membrane permeability, cause damage to DNA and proteins, and generate lipid peroxide signaling molecules. Some allelochemicals rapidly depolarize the cell membrane, increasing membrane permeability, inducing lipid peroxidation, and causing a generalized cellular disruption that ultimately leads to cell death (Yu *et al.* 2003). Conversely, H_2O_2 is thought to be directly toxic to microbes, to contribute to the structural reinforcement of plant cell walls, and to coordinate the activation of defense genes and phytoalexin production (Grant *et al.* 2000).

Allelopathic cucumber root exudates/extracts and phenolic acids have an autotoxic effect on the roots of cucumbers (Yu *et al.* 2003). Exposure to these allelopathic agents reduced stomatal conductance, leaf transpiration and net photosynthesis, and significantly increased root peroxidase (POD) and superoxide dismutase (SOD) activities.

SOD activity increased gradually with exposure to increasing concentrations of root extracts/exudates from cucumber, suggesting that the presence of superoxide anions also increased in treated roots as the concentrations of root extracts/exudates increased. SOD converts superoxide anions into hydrogen peroxide (H_2O_2), which can then be converted into harmless H2O molecules in a reaction catalyzed by catalase or POD. Conversely, it has also been reported that some allelochemicals reduce SOD and POD activity. The phytotoxic allelochemical secalonic acid from the fungus *Aspergillus japonicus*, significantly reduced SOD and POD activity in several plants Zeng (RS *et al.* 2001). Likewise, aqueous extracts from rice blocked SOD and catalase activity in *Echinochloa crus-galli* (Lin *et al.* 2000). These results suggest

that, in some cases, an allelochemical may be directly involved in the production of ROS whereas the increase in oxidizing enzymes is a secondary response to the increase in free radicals. In other cases, the allelochemical might directly inhibit oxidizing enzymes in some way, leaving the plant vulnerable to oxidative damage.

A transient oxidative burst in plant cells in response to elicitation by pathogens has been reported many times, but a correlation between allelopathic chemicals and a transient increase in ROS (has been elucidated recently (Bais *et al.* 2003). Using the ROS-sensitive fluorescent dye dichlorofluorescein (DCF), ROS generation was visualized in roots of *Arabidopsis thaliana* that were in direct contact with catechin, an allelochemical from the invasive weed C. maculosa (Bais *et al.* 2003). Subsequent experiments showed that the addition of ascorbic acid along with catechin blocked the ROS response, supporting the hypothesis that increased activity of antioxidants and antioxidant Enzymes are probably 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.

1.8 Maize

Maize being the highest yielding cereal crop in the world, is of significant importance for countries like Pakistan, where rapidly increasing population has already out stripped the available food supplies.In Pakistan maize is third important cereal after wheat and rice. Maize accounts for 4.8% of the total cropped area and 3.5% of the value of agricultural output. It is planted on an estimated area of 0.9 million hectare with an annual production of 1.3 million tonnes. The bulk (97%) of the total production come from two major provinces, NWFP, accounting for 57% of the total area and 68% of total production. Punjab contribute 38% acreage with 30% of total maize grain production. Very little maize 2-3% is produced in the province of Sindh and Balochistan. Though not included in Pakistan official statistics maize is an important crop of AJK with about 0.122 million hectare of maize being planted during kharif. Similarly a very growing and high yielding sector of maize, the spring maize area and production in Punjab is not accounted for , which covers around 0.070

million ha with about 050 million tonnes of maize grain being produced (Anon, 2007).

1.9 Soybean

Soybean Glycine max (L.) Merril is one of the important oil and protein crop of the world. Jimenez *et al.* (1991) reported that the supplies of oils and protein especially from animal sources (meat and fish) are becoming scarce and expensive particularly in developing nations. As a logical source of oils and proteins, soybean can play a major role in elevating nutritional standards of foods in developing nations, where human beings are facing protein deficiencies (1985). Beg (1995) stated that soybean is capable in narrowing the gap between the production and consumption of oils in Pakistan, provided it can be fitted into the cropping pattern. One of the reasons for lesser area, lower yield, smaller returns and nominal production of soybean in Pakistan is the erratic and poor emergence of the crop in the field partly due to poor seedling establishment and due to higher temperature. Seed quality is determined by its viability and vigor, which depends upon the conditions under which the seed has been produced.

1.10 Maize and soybean intercropping

Maize and soybean are two crops, normally grown in association. Increased productivity of intercropping over sole cropping has been attributed to better use of solar radiation, nutrients and water (Willey, 1990; Keating and Carberry, 1993; Morris and Garrity, 1993). The availability of nutrients and water enhances exploitation of available solar radiation for greater crop productivity. There is potential for higher productivity of intercrops when interspecific competition is less than intraspecific competition for a limiting resource (Francis, 1989). The inclusion of legumes in intercrops has been reported to reduce interspecific competition for N due to symbiotic N fixation (Clement *et al.* 1992a). Reduced competition for N due to greater N fixation efficiency of the intercropped legume compared to sole legume (Rerkasem and Rerkasem, 1988; Sangakkara, 1994) and transfer of the fixed N to the associated non-legume component (Fujita *et al.* 1990; Clark and Myers, 1994) have also been reported.

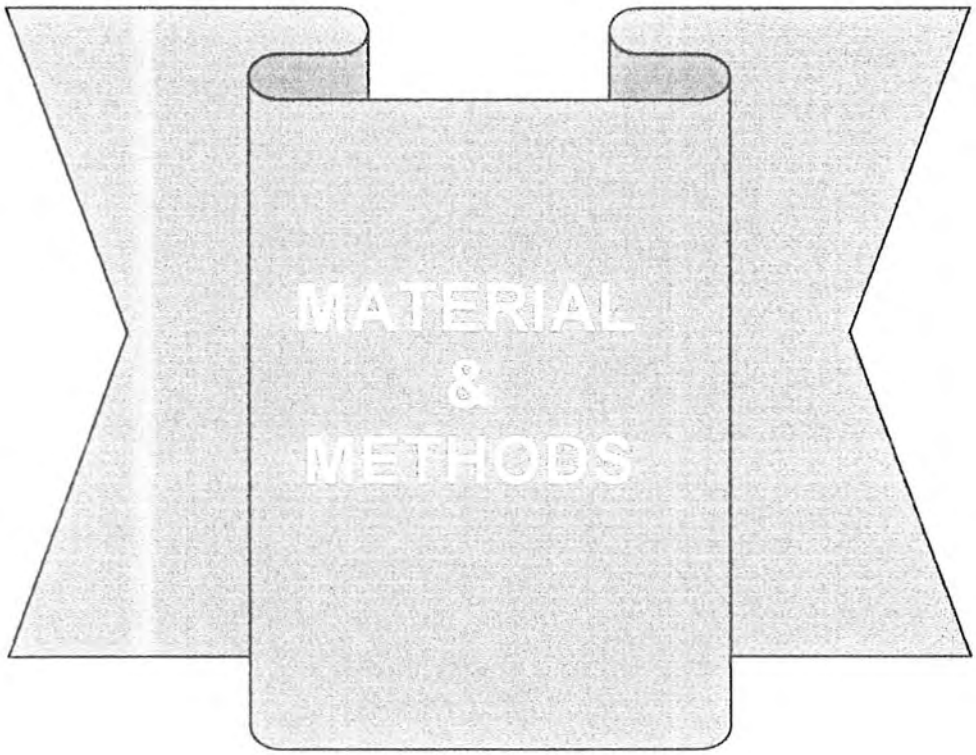
Allelopathy has been related to the problems with crop production as Crop monocultures or sole crops (Patrick *et al.* 1963; Kozel and Tukey, 1968; Patrick, 1971), certain crop rotations (Schreiner and Reed, 1907; Patrick *et al.* 1963; Patrick., 1971) and Crop mixtures or intercropping systems (Rakhteenko *et al.* 1973b; Tsuzuki, 1980; Nielson *et al.* 1981; Cruz *et al.* 1988). The field crops generally add phytotoxins or allelochemicals to the soils mainly through crop residues and partially through root exudates, therefore, their allelopathic effects have been studied

However, there are view research results indicating the allelopathic effects of maize (Minorisky, 2002) and soybean (Jimenez *et al.*1983). Moreover, to the best our knowledge, the physiological and biochemical basis of allelopathic interference of maize and soybean and also within maize and soybean have not been studied.

Aims and Objectives

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:

- The allelopathic potential of maize on soybean and vise versa were determined to evaluate the physiological and biochemical basis of allelopathic interaction in these two crops
- To evaluate which part of the plant has more inhibitory effects
- Aqueous extracts of different plant parts (fresh and oven dried) were assessed for the allelopathic potential under drought stress and unstressed conditions.


MATERIAL
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METHODS

Materials and methods

Experiment.No.1

2. Effect of Maize and Soybean fresh and oven dried extracts on Germination (%) and seedling growth of each other

2.1 Plant material and growing conditions

The experiment was carried out at Department of Plant Sciences, Quaid-i-Azam University, Islamabad in Randomized Complete block Design (RCBD) with three replications under natural conditions. Seeds of maize (*Zea mays* L.) cv. Islamabad Gold and Soybean (*Glyciene max* L.) cv. NARC1 were obtained from National Agriculture Research Council, Islamabad. The seeds were surface sterilized with 95% ethanol for 3 min and then with 10 % chlorox solution for five min with shaking and thoroughly washed with sterile water, were sown in earthen pots measuring (23 x 24 $cm^{2)}$ filled with clay and sand in ratio of 7:1 and kept under natural environmental conditions during mid of April.

2.1.1 Induction of drought

The drought was induced 15 days after sowing by withholding water supply for nine days. Control plants received water on regular basis. All the plants were uprooted washed with distilled water and carried to laboratory for preparation of plant extracts.

2.2. Preparation of plant extracts

Plants were separated into roots and leaves and cut into small pieces less than 2cm. Half of the plant materials were utilized for preparation of fresh extracts while half were oven dried.

2.2.1 Preparation of fresh extracts

Fresh extracts from roots and leaves of both maize and soybean were prepared separately. 10gm plant material (leaves and roots separately) was added into 100ml distilled water (1:10 w/v) and kept in shaker for 1h. After shaking for an hour, extracts were placed at room temperature for 48 h as according to Wardle *et al.* (1992). The extracts were then filtered with muslin cloth followed by Whatsman filter paper No.1 and stored at 4°C for further use.

2.2.2 Preparation of Oven dried extracts

For preparation of oven dried extracts, plant materials (root or leaves) were kept in an oven at 70 C for three days and ground finely with help of electric grinder. 10gm oven dried plant powder was immersed into 100 ml distilled water giving concentration of 10 % or 100 gm/L. The mixture was stirred mechanically for ten minutes and kept at room temperature for 48h. The extracts were filtered according to same method as described for filtration of fresh extracts and stored at 4°C in refrigerator for future use.

2.3 Germination and seedling growth bioassay

The seeds of both maize (*Zea mays* L.) cv. Islamabad Gold and soybean (*Glycine max* L. Merril) cv. NARC1 were surface sterilized with 95% ethanol and 10% chlorox for 5 min and thoroughly washed with sterile water several times. Petri plates were also sterilized by autoclaving them for one hour. Ten seeds based on treatments were placed evenly in Petri plates (9cm) lined with three layers of filter paper. For germination experiment, 10 % stock solution was further diluted to 2% and 4% solutions respectively. 10ml of fresh or oven dried extract (leaf or root extract of either maize or soybean) was added to each Petri plate and sterile water was used as a control. The experiment was conducted in growth chamber with minimum exposure to light during data collection. After six days, germination was determined by counting the number of seeds germinated in each petri plate and expressed in percentage. The seeds with 5mm radical lengths were considered as germinated.

% seed germination = Total seeds- germinated seeds / total seeds x 100

After 14d, plant seedlings (both maize and soybean) were removed carefully from petri plates and number of roots/seedling, total root length and shoot length (cm), shoot and root fresh weight (gm) and shoot and root dry weight (mg) were determined randomly from five selected seedlings.

2.4 Statistical analysis

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, 1961).

Experiment No.2

2.4 Effect of Maize and soybean fresh and oven dried extracts on physiology of each other

2.4.1 Plant Material and growing conditions

The experiment was carried out in green house at Department of Plant Sciences, Quaid-i-Azam University, Islamabad in Complete Randomized Design (CRD) with three replications. Seeds of maize (*Zea mays* L.) cv. Islamabad Gold and Soybean (Glyciene max L.) cv. NARC1 were obtained from National Agriculture Research Council, Islamabad. The seeds were surface sterilized with 95% ethanol for 3 min and then with 10 % chlorox solution for five min with shaking and thoroughly washed with sterile water.

2.4.1.1 Application of maize and soybean root and shoot extracts

Maize and soybean fresh and oven dried extracts (10 %w/v) were applied to each other as seed soaking prior to sowing for 8h. The seeds were sown in earthen pots measuring (23 x 24 $cm^{2)}$ filled with clay, and sand in ratio of 7:1 and kept under natural environmental conditions during mid of April.

2.4.1.2 Collection of plant sample for physiological and biochemical analysis

Maize and soybean plants were harvested 40 DAS and samples were freeze dried for physiological and biochemical analysis. The following traits were taken into account.

2.4.2 Physiological Traits

The following physiological traits were determined for assessment of allelopathic effects of maize and soybean on each other.

2.4.2.1 Leaf protein contents

Protein content of leaves was determined following the method of Lowery *et al.* (1951) using BSA as standard.

Phosphate Buffer (Stock Solution)

a Monobasic sodium phosphate:

27.6g was dissolved in distill water (1000ml)

b Dibasic sodium phosphate:

53.6g was dissolved in 1000ml

Monobasic sodium phosphate (16ml) and dibasic sodium phosphate (84ml) were mix together to get the desire pH (7.5) of phosphate buffer.

Reagent A: 2g sodium carbonate (Na_2CO_3)

0.4g NaOH (0.1 N) and 1g Na-K tartrate was dissolved in100 ml of distilled water

Reagent B: $CuSO_4.5H_2O$ (0.5g) was dissolved in 100ml of distill water.

Reagent C: Solution A (50ml) and Solution B (1ml) were mixed together.

Reagent D: Folin phenol reagent was diluted with distill water in 1:1 ratio.

Procedure:

Fresh leaves 0.1g were ground with the help of mortar and pestle in 1ml of phosphate buffer pH 7.5 and, centrifuged for 10 min at 3000rpm. The supernatant (0.1ml) of given sample containing unknown amount of protein was poured in the test tubes and total volume of 1ml was made by distilled water. 1ml of reagent C was added. After shaking for 10min, 0.1ml of reagent D was added. The absorbance of each sample was recorded at 650nm after 30 min incubation. The BSA (Bovine Serum Albumen) of different concentration viz 20,40,60,80,320, and 640mg was prepared. The absorbance of BSA was recorded at 650 nm.The concentration of protein was determined by using the following formula

Protein content mg/g = K value ×dilution factor × Absorbance /Weight of the sample

2.4.2.2 Sugar estimation

Sugar estimation of fresh leaves was done following method of Dubois *et al.* (1956).

Procedure:

Fresh plant material (0.5g) was homogenized with 10ml of distilled water in a clean mortar and then centrifuged at 3000 rpm for 5 min. In 0.1ml of supernatant 1ml of 80% (w/v) phenol was added, after incubation at room temperature, 5ml concentrated sulphuric acid was added. The sample was incubated for 4 hrs and then absorbance of each sample was recorded at 420 nm. The concentration of unknown sample was calculated by using the following formula

Sugar content mg/g = K value ×dilution factor × Absorbance /Weight of the sample

2.4.2.3 Chlorophyll (a and b) and carotenoid content of leaves

Chlorophyll and caroteniod content of leaves were determined by the method of Aron (1968).

Procedure:

Crude preparation 1ml was mixed with 4ml of 80% (w/v) acetone and allowed to stand in dark at room temperature. It was centrifuged at 2000 rpm for 5 min to clear the suspension. Supernatant was used for chlorophyll determination. Absorbance of solution was read at 645 nm (chlorophyll a) and at 663nm (chlorophyll b) and 480nm (caroteniod) on spectrophotometer against 80% (v/v) acetone blank. The Chl. *a* and *b* were calculated by the following formula.

Chl. 'a' (mg/g) = [12.7 (OD663)-2.69(OD645)] ×V/1000×W

Chl. 'b' (mg/g) = [22.9 (OD645)-4.68(OD663)] ×V/1000×W

Where

V = Volume of the extract (ml)

W = Weight of fresh leaf tissue (g)

2.4.2.4 Proline content of leaves

The proline contents of leaves were measured by the method of Bates *et al.*, (1973). Fully expanded fresh leaves were sampled. Purified praline was used to standardize the procedure for quantifying sample values. Reagent acid Ninhydrin was prepared by warming 1.25g ninhydrin in 30ml glacial acetic acid and 20ml 6 M phosphoric acid, with agitation, until dissolved. Kept cool (store at 4°C), the reagent remain stable for 24 hrs. Approximately 0.5g of plant material was homogenized in 10ml of 3% aqueous sulphosalicylic acid and homogenate filtered with Whatman # 42 filter paper. 2ml of filtrate was reacted with 2ml acid ninhydrin and 2ml of glacial acetic acid in a test tube for 1 hr at 100°C and reaction is terminated in ice bath. The reaction mixture was extracted with 4ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warm to room temperature and absorbance read at 520nm against toluene as blank. The proline concentration was determined by the formula given as;

Proline content mg/g = K value ×dilution factor × Absorbance /Weight of the sample

2.4.2.5 Determination of Antioxidants activity

The following antioxidants activity was determined.

2.4.2.5.1 Assay for Superoxide Dismutase Activity (SOD)

SOD activity was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using method of Beauchamp and Fridovich (1971). The reaction mixture (3ml) was composed of 13mM methionine, 0.075 mM NBT, 0.1 mM EDTA, 0.002mM riboflavin and 0.1ml of enzyme extract in 50mM phosphate buffer (pH 7.8). The mixture in tube was placed below light chamber for 15 min. The absorbance was read at 560nm with spectrophotometer. 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.

2.4.2.5.2 Assay for Peroxidase activity (OD min/gm f.w).

POD activity was measured by the method of Vetter *et al.* (1958) as modified by Gorin and Heidema (1976). The assay mixture contained 0.1 ml enzyme extract, 1.35 ml 100 mM MES buffer (pH 5.5), 0.05% H_2O_2 and 0.1% p-phenylenediamine. 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.

2.4.2.5.3 Assay for Ascorbate peroxidase

Ascorbate peroxidase activity was determined according to Asada and Takahashi (1987).

The reaction mixture (1ml) contained 50 mM of potassium phosphate buffer(pH 7.0),0.5 mM of ascorbic acid , 0.1 mM of H_2O_2, and 200 µl of enzyme extract.

The absorbance was read as the decrease at 290 nm against the blank. The enzyme activity was expressed in U mg $^{-1}$ protein (U=change of 0.1 absorbance min^{-1} mg^{-1} of protein)

2.4.2.5.4 Assay for Catalase (CAT)

Catalase (CAT) was measured according to Chandlee and Scandalios. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H2O2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in the absorbance at 240 nm. The enzyme activity was expressed in U mg^{-1} protein (U=1mM of H_2O_2 reduction min^{-1} mg^{-1} of protein).

2.4.2.5.5 Determination of endogenous ABA content

The extraction and purification was made following the method of Ketter and Doerhung, (1995)

Procedure

The plant leaves (0.5g) were grounded in 80% methanol at 4C° with an antioxidant, Butyrate hydroxyl toluene (BHT). The leaf was extracted at 4C° in dark for 72 hrs with subsequent change of solvent. The extracted sample was

centrifuged and the supernatant was reduced to aqueous phase using rotary thin film evaporator (RFE) the pH of aqueous phase was adjusted to 2.5-3.0 and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate phase was dried down completely using rotary thin film evaporator (RFE). The dried sample was re-dissolved in 1 ml of methanol (100%) and was analyzed on HPLC (Shimadzu, C-R4A Cgrommatopac; SCL-6B system controller) using UV detector and C-18 column (39*300mm) for identification of hormones. 50µl samples filtered through 0.45 Millipore filter were injected in column. Pure ABA (Sigma) was used as standard for identification and quantification of hormone. ABA was identified on the basis of retention time and peak height of the standard. Methanol, and acetic acid and water (30:1:70) were used as mobile phase .The flow rate was adjusted at 0.5 ml/min with an average time for 10 min. /sample. The wavelength used for detection of ABA was 254nm.

2.4.2.6 Chemical analysis of rhizospheric soil

2.4.2.6.1 Soil pH and Soil Electrical Conductivity (EC)

The pH of rhizospheric soil was measured by preparing 1:1(soil:water) suspension (McKeague, 1978; Mclean, 1982). Air dried soil sample (10g) was mixed in 10ml distilled water and stirred for 1 hour on magnetic stirrer for homogenous mixing. Then the suspension was filtered with Whatman No. 42 filter paper. The pH of filtrate was determined with pH meter while the EC of extract was recorded by electrical conductivity meter. Readings were measured in microsiemens per centimeter (µS/cm).

2.4.2.6.2 Nutrients analysis of Rhizospheric Soil

The rhizospheric soil was analyzed for macro and micronutrients (Na, Ca, Mg, K, P, NO_3-N, Fe, Cu, Cr, Co,Ni, Zn and Mn) following the Ammonium Bicarbonate-DTPA method developed by Soltanpour and Schwab (1977). Method for preparation of reagents, stock solutions, working solution and standards solutions is given in appendix.

Extraction solution preparation

The 0.005M solution was obtained by adding 1.97g DTPA to 800ml distilled water. Approximately 2ml (1:1) ammonium hydroxide (NH_4OH) was added to facilitate dissolution and to prevent effervescence when bicarbonate was added. When most of DPTA was dissolved, 79.06g ammonium bicarbonate (NH_4HCO_3) was added and stirred gently until dissolved. The pH was adjusted to 7.6 with ammonium hydroxide. The solution was diluted to 1L with distilled water.

Extraction method

The 10ml extracting solution was added into 10g air dried rhizospheric soil, and shaken on a reciprocal shaker for 15minutes at 180 cycles/minute. The rhizospheric soil extract was then filtered through filter paper whatmann No. 42. This rhizospheric soil extract was used to analyze following macro and micronutrients.

2.4.2.7 Macronutrients analysis of soil

2.4.2.7.1 Phosphorus (P)

1ml aliquot of the soil extract was diluted to 10ml with distill water. The color developing reagent for phosphorus (2.5ml) was added carefully to prevent loss of sample due to excessive foaming. Stirred and allowed to stand for 30minutes. The color intensity was measured at 880nm wavelength using a spectrophotometer. The standards were developed in the same way and a standard calibration curve was obtained using absorbance for standards.

2.4.2.7.2 Sodium (Na), Potassium (K), Calcium(Ca) and Magnesium(Mg)

1ml of rhizospheric soil extract and 9ml of distill water was taken in a test tube and analyzed for Na, Ca, Mg and K on atomic absorption Spectrophotometer.

2.4.2.8 Micronutrients analysis of rhizospheric soil

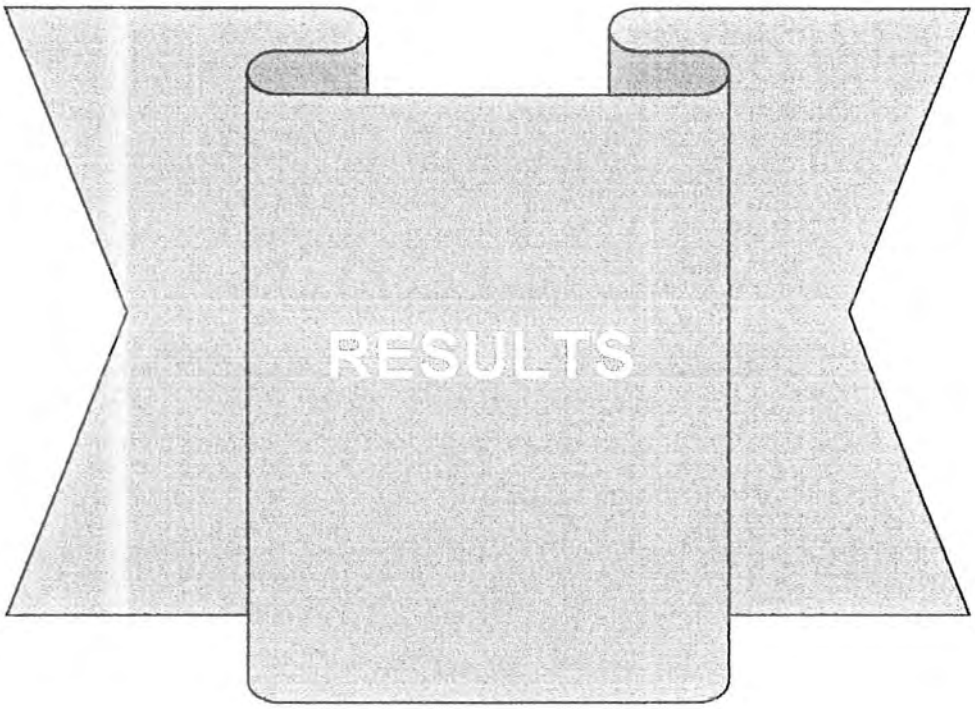
For micronutrient: Fe, Cu, Cr, Co, Zn, and Mn analysis 1ml of soil extract and 9ml of distill water was taken in a test tube and analyzed on Atomic Absorption Spectrophotometer. Measurement of micro and micronutrients of rhizospheric soil was done by the formula.

Nutrients concentration (µg/g) = metal con. (ppm)× Final vol. of solution/ sample weight

A = Total volume of extract (ml)
W = Weight of dry rhizospheric soil

2.4.2.9 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, 1961).


RESULTS

Results

Experiment NO.1

3. Effect of Maize and Soybean fresh and oven dried extracts on Germination (%) and seedling growth of each other

3.1 Effect of maize extracts on soybean germination (%) and seedling growth

3.1.1 Effect of maize extracts (2 and 4%) on soybean germination percentage

Both the leaves as well as root extracts (fresh and oven dried) significantly inhibited the germination percentage of soybean at 2 % as well 4 % aqueous extract. The treatment MDL (F) at 2 % showed 33 % inhibition while at 4% it showed 46 % inhibition of germination as compared to control. Similarly, the treatment MDL (OD) exhibited 50 % inhibition in germination at 2 % and 54 % significant inhibition of germination at 4 % of extract as compared to control. The treatments MCL (F) at 2 % exhibited no significant effect on soybean germination but at 4 % showed significant decrease in germination percentage of soybean as compared to control.

Like leaves extracts, the root extracts of maize plants also exhibited significant effects on germination of soybean. The treatment MDR (F) at 2 % concentration showed 42 % decrease in germination percentage of soybean while at 4 % concentration; it showed 46 % decrease in soybean germination as compared to control. Likely, the treatment MCR (F) also showed significant (25 %) decrease in soybean germination percentage at 2 % concentration but at 4 % concentration, it showed 29 % decrease in germination as compared to control. The treatment MCR (OD) at 2 % concentration showed 29 % while at 4 % concentration; it exhibited 37 % significant decrease in germination percentage of soybean as compared to control (Table.1).

It was found from the results that higher concentration of both leaves as well as root extracts (fresh and oven dried) of maize significantly decreased the germination potential of soybean seeds. However, the extracts prepared from drought

subjected maize plants were more effective in decreasing the germination percentage of soybean as compared to that which were obtained from unstressed maize plants

3.1.2 Effect of maize extracts (2 and 4%) on soybean root length

Results presented in Table-1 revealed that both the concentration (2 % and 4 %) of maize leaves as well as root extracts significantly inhibited the elongation of soybean root. The treatment MDL (F) at 2% concentration showed 50 % significant decrease in root length while at 4 % concentration exhibited 49 % decrease in root length as compared to control. The treatment MDL (OD) was found with 48 % significant decrease in root length at 2 % extract concentration and 46 % significant decrease in root length at 4 % concentration as compared to control. The 2 % maize leaves extract was found as more effective in the decreasing of root elongation as compared to 4 % concentration.

Like leaves extracts, maize root extracts also significantly inhibited the elongation of soybean root at both levels of concentration (2 and 4%) except for treatment MDR (OD) which at 2 % concentration exhibited no significant effect on root length of soybean. Interestingly, the root extracts prepared from non-stressed maize plants showed more pronounced effects on root elongation of soybean as compared to the extracts which were prepared from roots of drought stressed plants. In case of 4 % concentrations, all the treatments including MDR (F), MCL (F) and MCL (OD) were non-significantly different from each other but significantly different from control (Table-2).

3.1.3 Effect of maize extracts on soybean shoot length (cm)

Results presented in Table- 2 showed that fresh as well as oven dried extracts of maize leaves and root at 2% concentration exhibited no significant effects on shoot length of soybean as compared to control. However, 4% concentration of both leaves and roots extracts significantly affected the shoot length of soybean. All the treatments were non-significantly different from each other but significantly different from control. The treatment MDL (F) showed 37% decrease in shoot length as compared to control while treatment MDL (OD) showed 36% significant decrease in shoot length as control which was non-significantly different from MDL (F) at

P>0.05. Both the treatment MCL (F) and MCL (OD) were found with 27 % significant decrease in shoot length as compared to control. Among 4 % leaves extracts of maize, both fresh and oven dried extracts prepared from maize plants subjected to drought were more effective in decreasing soybean shoot length as compared to extracts obtained from unstressed maize plants. All the 4 % root extracts (both fresh and oven dried) of maize also significantly decreased the soybean shoot length when applied as seed pre-soaking treatment. However, the treatments involving the fresh extracts prepared from maize plant roots which were not subjected to drought were found as more effective in the inhibition of soybean seedling length as compared to those which were extracted from roots of maize plants subjected to drought. The treatment MCR (OD) showed 41% significant decrease in shoot length as compared to control. The treatment MDR (OD) exhibited 29 % decrease in the leaves length which was also significantly different from control but non-significantly different from treatment MCR (OD). The treatments MCR (F) and MDR (F) were found with 25 % decrease in shoot length as compared to control. The results revealed that just like leaves extracts, the 4 % concentration of root extracts also significantly decreased the soybean leaves length.

3.1.4 Effect of maize extracts on soybean root fresh weight

The result revealed that aqueous extracts (both fresh and oven dried) of maize leaves and root at 2% concentration non-significantly decreased soybean seedling root fresh weight as compared to control. However, maize leaves and root extract at 4% concentration significantly decrease soybean root fresh weight at P>0.05. The treatment MDL (F) showed 58% decrease as compared to control while treatment MDL (OD) exhibited 49% significant decrease in root fresh weight. The treatment MCL (F) also showed 52% significant decrease in root fresh weight which was non-significantly different from MCL (OD) showing 42% decrease in root fresh weight as compared to control. The results showed that both fresh and oven dried leaves extract prepared from maize plants subjected to drought were more effective than leaves extract prepared from unstressed plants. All the 4% root extracts (fresh and oven dried) also significantly decreased the soybean seedling root fresh weight at P>0.05. Maximum (71%) decrease in root fresh weight was found in treatment MCR (OD) which was significantly different from control as well as treatments MCR (F) and

MDR (OD) respectively. The treatment MDR(F) showed 57% decrease in root fresh weight which was non-significantly different from treatment MCR(OD) but significantly different from control (Table.3).

The results revealed that oven dried extract prepared from unstressed maize plant roots was more effective in decreasing soybean seedling root fresh weight

3.1.5 Effect of Maize extracts on soybean shoot fresh weight

It was found that aqueous extracts (fresh and oven dried) of maize leaves and root significantly decreased soybean shoot fresh weight when applied as seed presoaking. The 4 % maize leaves extracts were more effective in decreasing shoot fresh weight of soybean seedling as compared to 2% extracts. The treatment MCL (F) at 2 % concentration showed 18 % decrease in shoot fresh weight while at 4% it showed 36% decrease in shoot fresh weight as compared to control. Similarly, the treatment MDL (F) showed 27% decrease in shoot fresh weight at 2% but at 4%, it showed 40% decrease in leaves fresh weight as compared to control. The 2 % maize leaves extract which was extracted from drought subjected plants was more effective than unstressed plant extracts. However, in case of 4% extract, there were no such visible differences as for as effect on soybean leaves fresh weight was concerned. The treatment MDL (OD) at 2% concentration showed 35 % decrease in leaves fresh weight as compared to control. At 4% concentration of maize leaves extract, treatment MDL (OD) showed 40 % decrease in shoot fresh weight as compared to control. Overall, 4 % fresh as well as oven dried extracts were more effective in decreasing soybean shoot fresh weight as compared to 2% maize leaves extracts. This showed that increase in concentration of maize leaves extracts posse's inhibitory effects on soybean shoot fresh weight. (Table.3)

The fresh as well as oven dried extracts of maize root at both concentrations (2% and 4%) negatively influenced the soybean leaves growth which resulted decrease in shoot fresh weight. The 2% as well as 4% maize extracts significantly decreased the shoot fresh weight as compared to control ($P>0.05$). However, the 2% root extracts prepared from drought subjected maize plants were more provident in decreasing the soybean seedling fresh weight as compared to that extracted from

unstressed plants. The treatment MDR (F) showed 18 % while MDR (OD) as 25 % significant decrease in shoot fresh weight as compared to control. The treatment MDR (OD) was significantly different from all other treatments at P>0.05. Similarly, the 4 % maize extracts prepared from drought subjected as well as unstressed maize plants were nearly equally effective in decreasing the soybean fresh weight. The treatment MCR (F) showed 34 %, MDR (F) as 29 %, MCR (OD) as 16 % and MDR (OD) as 28 % significant decrease in shoot fresh weight as compared to control. The results further revealed that root extracts were also effective in decreasing soybean fresh weight indicating allelopathic effect of maize roots on soybean growth.

3.1.6 Effect of Maize extracts on soybean root dry weight

The results presented in Table.4 revealed that 2% aqueous extracts (fresh and oven dried) of maize leaf and root significantly decreased soybean seedling root dry weight as compared to control at P>0.05. The treatment MDL (F) showed 97% significant decrease in root dry weight as compared to control. The treatments MCL (F), MCL (OD) and MDL (OD) showed 95%, 93% and 50% respectively significant decrease in root dry weight. The root extracts also significantly decreased the soybean seedling root dry weight. The treatment MDR (F) showed 96% decreased in root dry weight which was significantly different from control but non- significantly different from treatments MCR (F), MCR (OD) and MDR (OD) respectively. The treatment MDR (OD) showed 95% decrease in root dry weight as compared to control.

The application of 4 % aqueous extracts of maize leaf and root possessed no significant effect on soybean root dry weight. The results further showed that 2% fresh extract prepared from leaf of drought treated maize plant was more effective in decreasing soybean root dry weight as compared to that prepared from unstressed plants.

3.1.7 Effect of maize extracts on soybean shoot dry weight

The application of maize leaves and root aqueous extracts as seed soaking prior to sowing in soybean resulted a significant decrease in shoot dry weight (P>0.05). Both 2% and 4% maize extracts were found effective in decreasing soybean

shoot dry weight (Table.4). The treatment MCL (F) showed 64% decrease in shoot dry weight at 2% concentration and 82% decrease in shoot dry weight at 4% concentration as compared to control. At 2 % concentration, treatment MDL (F) showed 74% decrease while at 4% concentration, it showed 83% decrease in shoot dry weight as compared to control. The plants raised from seeds pre-soaked in 2% oven dried leaves extract prepared from unstressed maize plants showed 60 % decrease in shoot dry weight while that of stressed plant extracts decreased soybean shoot dry weight by 73 % as compared to control. The treatment MCL (OD) at 4% concentration showed 77 % significant decrease in shoot dry weight as compared to control. Similarly, the treatment MDL (OD) was found with 85% decrease in shoot dry weight as compared to control. The results also revealed that the maize leaves extracts (both 2% and 4%) prepared from drought subjected plants were more effective in decreasing soybean shoot dry weight as compared to that extracted from unstressed plants.

Like leaves extracts, maize root extracts also significantly decreased soybean shoot dry weight at both levels of concentrations as compared to control (Table-9). The treatment MCR (F) showed 70% decrease at 2% concentration while 83% decrease at 4% level in leaves dry weight as compared to control. The treatment MDR (F) was found with 62% decrease in shoot dry weight at 2% concentration and with 81% decrease in shoot dry weight at 4% level. The treatment MCR (OD) was found with 66% decrease in shoot dry weight at 2% level of extract while 81% at 4% level. Similarly, the treatment MDR (OD) showed 70% decrease in shoot dry weight at 2% level while 80% decrease in shoot dry weight at 4% level. It was also found from the results that in case of 2% root extracts, oven dried extracts were more effective in decreasing soybean shoot dry weight when extracted from drought subjected maize plants. In case of 4 % level, the extracts prepared from roots of unstressed plants were more effective in decreasing shoot dry weight as compared to extracts prepared from roots of drought subjected maize plants.

3.1.8 Effect of maize extracts (2 and 4%) on soybean root number per seedling

The results presented in Table.5 revealed that application of maize aqueous extracts of both leaves and root as seed soaking prior to sowing significantly decreased the number of roots per seedling of soybean at P>0.05. However, the 4%

maize extract of both leaves and root were found as more effective in decreasing the root number per seedling of soybean as compared to 2% maize extracts. The treatment MDL (F) at both 2% as well as 4% level showed 32% significant decrease in root number per seedling as compared to control. The treatment MDL (OD) exhibited 26 % decrease at 2% concentration in number of roots per seedling while at 4% level; it showed 32% decrease as compared to control. Similarly, Treatments MCL (F) and MCL (OD) were found with 16% and 5% decrease in root number per seedling at 2% concentration but at 4% level , it exhibited 32% and 26 % as compared to their respective control. The results showed that at 2% concentration, maize extracts prepared from drought subjected plants were more effectives as compared to those prepared from unstressed plants. While at 4% level, there were found no such variations among the effectiveness of extracts on the inhibition of rooting in soybean as compared to control.

Like the leaves extracts, maize root extracts also significantly decreased the rooting in soybean seedling. Here again, it was found that 4% maize extract was more effective than 2% in decreasing the rooting in soybean seedling. The treatment MDR (F) showed 21% decrease in roots per seedling at 2% concentration while at 4% concentration it exhibited 37% decrease as compared to control. Similarly, the treatment MDR (OD) was found with 16% decrease in rooting at 2% concentration of maize root extract while at 4% level, it showed 26% decrease as compared to control. The treatment MCR (OD) at 2% concentration of root extract showed 16% decrease in soybean rooting while at 4% level; it exhibited 31% decrease as compared to control. The results further revealed that root extracts (fresh and oven dried) both 2% as well as 4% were more effective in decreasing the number of roots per seedling as compared to leaves extracts.

3.2 Effect of soybean extracts on maize germination (%) and seedling growth

3.2.1 Effect of soybean extracts (2 and 4%) on maize germination percentage

The results presented in Table.6 showed that soybean leaf as well as root extracts both at 2 % and 4 % concentration significantly inhibited the germination in maize as compared to control ($P>0.05$). It was investigated that 4 % maize extracts were more effective in decreasing seed germination percentage. The treatment SCL

(F) at 2 % concentration of maize leaves extract showed 28% decrease in germination of maize while at 4% level, it exhibited 44% decrease as compared to control. The treatment SDL (F) at 2% concentration was found with 52% decrease in germination while at 4% level, it showed 60% decrease in germination % of maize as compared to control. Similarly, the treatment SDL (OD) showed 64% decrease at 2% concentration of maize leaves extract while at 4% concentration, it was found with 60% decrease in seed germination as compared to control. The results revealed that both fresh and oven dried extracts prepared from drought subjected maize plants were more effective in decreasing the germination percentage of maize as compared to those extracted from non-stressed plants.

Like leaf extracts, root extracts of soybean also significantly decreased the germination % in maize when applied as seed pre-soaking. However, 4% root extracts were more effective as compared to 2% soybean root extracts. The treatment SCR (F) showed 52% decrease in germination % while at 4% level; it exhibited 44 % decrease in germination % as compared to control. The treatment SDR (F) showed 64% decrease at 2% concentration of soybean root extract while at 4% concentration; it exhibited 72% decrease as compared to control. Similarly, the treatment SDR (OD) showed 56% decrease at 2% concentration of soybean root extract while at 4% level, it showed 52% decrease in germination % of maize. It was investigated that root extracts of soybean both fresh as well as oven dried were as effective as leaf extracts in the inhibition of seed germination in maize.

3.2.2 Effect of soybean extracts (2 and 4%) on maize root length

Table-7 shows effect of soybean leaf as well root extracts on root length of maize seedling. It was found that Oven dried extracts at 2% as ell as 4% concentrations were more effective in the inhibition of root length in maize as compared to fresh extracts. At 2% concentration, the soybean leaves aqueous extracts significantly decreased the root length in maize when applied as seed pre soaking. However, the effects of leaves extracts on root length in maize at two different concentrations were variable. The treatment SCL (F) at 2% concentration showed 28% significant decrease in root length while at 4% concentration; it exhibited 19% non-significant decrease in root length as compared to respective control. Similarly,

the treatments SDL (F) at both concentrations (2% and 4%) showed no significant effects on seedling root length as compared to control. However, the treatment SDL (OD) showed 34% decrease at 2% concentration while at 4% level it exhibited 46% significant decrease in maize seedling root length as compared to control. It was investigated that soybean leaves extracts prepared from drought treated plants were effective in decreasing maize seedling root length as compared to those prepared from unstressed plants.

Like leaves extracts, soybean roots extracts were also effective in decreasing the maize seedling root length. The treatment SCR (F) showed 56% decrease in root length at 2% concentration while at 4% level, 25% decrease as compared to control, indicating that fresh extract prepared from unstressed soybean roots was more effective at 2% concentration rather than at 4% concentration. The treatment SDR (F) showed 28% decrease in root length at 2% concentration of soybean root extract while at 4% level; it exhibited 47% decrease as compared to control. The treatment SDR (OD) was found with 35% decrease in root length at 2% concentration of root extract while showed 34% decrease at 4% level as compared to control. The results showed that at 2% soybean root extracts were more effective when prepared from unstressed plants while at 4% level, they were more effective when extracted from drought treated plants. It was found that root extracts were as effective as soybean leaves extracts in the inhibition of root length in maize. However, leaves extracts were more effective that root extracts when applied as seed pre-soaking to maize.

3.2.3 Effect of soybean extracts (2 and 4%) on maize shoot length

The results as described in Table. 7 showed that leaf length in maize was significantly inhibited by soybean extracts when applied as seed pre soaking. However, the 4% soybean extract was more effective than 2% extracts particularly that ones which were prepared from drought treated plants. The leaves extracts were found as more effective in decreasing shoot length in maize as compared to root extracts. The treatment SDL (F) at 2% concentration showed 36% decrease while at 4% level it exhibited 30% decrease in shoot length indicating that fresh extract at 2% concentration was more effective than 4% concentration. The treatment SDL (OD) at 2% concentration of soybean leaves extract showed 33% decrease while at 4% level, it showed 43% decrease in shoot length which indicated that oven dried leaf extract at

4% concentration was more effective than at 2% level. The extracts prepared from unstressed soybean plants were less effective as compared to those prepared from drought treated plants at both levels of concentrations.

The root extracts were also effective in decreasing the maize shoot length as both levels of concentration. The treatment SCR (F) showed 36% significant decrease in shoot length at 2% concentration in leaf length while at 4% level; it exhibited 44% decrease as compared to control. The treatment SDR (F) was found with 25% decrease in shoot length at 2% concentration of root extract while at 4% level it showed 36% decrease as compared to control indicating that 2% oven dried extract was more effective than 4% extract. The treatment SDR (OD) showed 20% decrease in shoot length at 2% concentration while at 4% level it showed 26% decrease in shoot length of maize as compared to control. It was investigated that oven dried extracts were more effective at 4% concentration as compared to 2% root extracts. The results further revealed that 4% extracts of unstressed plant roots were more effective than 2% extracts.

3.2.4 Effect of soybean extracts (2 and 4%) on maize root fresh weight

It was found that aqueous extracts of soybean leaf as well as root significantly decreased the maize seedling root dry weight as compared to control ($P>0.05$). It was also investigated that fresh and oven dried extracts prepared from drought subjected soybean plants were more effective as compared to those prepared from unstressed plants. The results further revealed that 2 % fresh extract was more effective in decreasing maize seedling leaf weight as compared to 4% fresh extract. The treatment SDL (F) at 2% concentration showed 46% decrease in root fresh weight while at 4% concentration it showed 32% decrease as compared to control. Similarly, the treatment SDL (OD) showed 22% decrease at 2% level while at 4% level it exhibited 24% significant decrease in root fresh weight as compared to control indicating that 4% oven dried extract was more effective rather than 2% extract. The treatment SCL (F) was found with 100% decrease in root fresh weight at 2% concentration while at 4% level, it was found with 19% decrease as compared to respective control. Similarly, the treatment SCL (OD) showed 30% decrease at 2% concentration while

at 4% level, it showed 31% decrease in maize seedling root fresh weight as compared to control (Table: 8)

The application of soybean root extracts as seed pre-soaking also caused the decrease in maize seedling root dry weight except for treatment SCR (OD) which at both 2% and 4% concentration of extract showed no significant effect on root dry weight. The treatment SDR (F) at 2% concentration showed 38% decrease in root fresh weight while at 4% level of soybean root extract, it showed 36% decrease as compared to control. The treatment SDR (OD) at 2% concentration of soybean root extract showed 41% decrease while at 4% level it showed 26% decrease in root dry weight as compared to control. The results revealed that soybean root extracts were more effective in decreasing the maize seedling root fresh weight at 2% concentration as compared to 4% concentration. It was also found that extracts of drought treated plants were more effective than extracts of unstressed plants (Table: 6)

3.2.5 Effect of soybean extracts (2 and 4%) on maize shoot fresh weight

The results presented in Table.8 revealed that soybean leaves and root aqueous extracts significantly decreased the maize seedling fresh weight as compared to control when applied as seed pre-soaking. Interestingly, the 4% soybean leaves extract prepared from unstressed soybean plants was more effective in decreasing maize shoot fresh weight as compared to extracts prepared from drought treated plants. However, at 2% concentration, it was more effective when prepared from drought subjected plants. At both concentrations, oven dried extracts were more inhibitory to shoot fresh growth as compared to fresh ones. The treatment SDL (F) showed 47% decrease in leaves fresh weight at 2% concentration of extract while at 4% level it showed 32% decrease as compared to control. Similarly, the treatment SDL (OD) was also found with 47% decrease in shoot fresh weight at 2% concentration of extract while at 4% concentration it was found with 37% decrease as compared to control. The treatment SCL (F) showed 43% decrease in shoot fresh weight at 2% concentration of extract while at 4% level it exhibited 32% decrease as compared to control.

Like leaf extracts, the root extracts of soybean were also effective in decreasing maize shoot fresh weight. However, again it was found that 2% extracts both fresh and oven dried were more effective than 4%. The treatment SDR (F) showed 45% decrease in shoot fresh weight at 2% concentration of extract while at 4% concentration it was found with 43% decrease as compared to control. Similarly, the treatment SDR (OD) showed 43% decrease at 2% concentration while at 4% level it showed 33% decrease in shoot fresh weight as compared to control. The results further revealed that soybean root extracts prepared from roots drought treated plants were more effective in decreasing maize seedling leaf fresh weight as compared to extracts prepared from unstressed plants

3.2.6 Effect of soybean extracts (2 and 4%) on maize root dry weight

The results described in Table.9 showed that aqueous extracts of soybean leaf and root significantly decreased the maize root dry weight. However, the leaves extract both fresh as well as oven dried were more effective than root extracts. The results further revealed that 2% soybean leaf extracts were more effective in decreasing maize seedling root dry weight as compared to 4% extracts. Among fresh and oven dried leaves extracts, the extracts prepared from drought treated plants were more effective than extracts prepared from unstressed plants. The treatment SDL (F) showed 45% decrease in root dry weight at 2% concentration while it exhibited 41% decrease at 4% level as compared to control. Similarly, the treatment SDL (OD) was found with 28% decrease in root dry weight at 2% concentration extract while at 4% level it was found 30% decrease as compared to control. The treatment SCL (F) showed 38% decrease in root dry weight at 2% concentration while at 4% level it showed 16% decrease as compared to control. Similarly, the treatment SCL (OD) showed 30% decrease at 2% concentration while at 4% concentration it exhibited 23% decrease in root dry weight as compared to control. It was investigated that soybean leaf fresh extracts prepared from drought plants were more effective at both levels of concentrations than oven dried extracts.

Like leaf extracts, root extracts also resulted a decrease in maize seedling root dry weight. However, significant decrease in root dry weight was found at 4% concentration of extract ($P>0.05$). Only treatment SCR (OD) at 4% concentration

showed non-significant decrease in root dry weight. Only the treatment SDR (OD) at 2% concentration showed significant decrease (40%) in root dry weight as compared to control. The results further revealed that soybean extracts prepared from roots of drought treated plants were more effective in decreasing the maize seedling root dry weight as compared to extracts prepared from roots of unstressed soybean plants.

3.2.7 Effect of soybean extracts (2 and 4%) on maize shoot dry weight

It was found that like shoot fresh weight, aqueous extracts of soybean leaves and root significantly decreased the shoot dry weight of maize seedling at both levels of concentration i.e. 2% and 4%. However, the leaves extracts were found as more effective than root extracts. The extracts prepared from leaves of drought treated plants were more effective in decreasing seedling shoot dry weight. The treatment SDL (F) was found with 46% decrease in shoot dry weight at 2% concentration while at 4% level it exhibited 39% decrease as compared to control. Similarly, the treatment SDL (OD) showed 15% decrease at 2% concentration while at 4% level it showed 45% decrease in shoot dry weight as compared to control indicating that oven dried extracts prepared from drought subjected plants were more effective at 4% concentration. However, it was found that fresh extracts prepared unstressed plants were more effective at 2% concentration. The treatment SCL (F) was found with 41% decrease at 2% concentration of extract while at 4% level it was found with 34% decrease as compared to control.

The root extracts were also effective in decreasing maize shoot dry weight at both levels of concentration. However, the fresh extracts prepared from drought subjected plants were found as more effective. The treatment SDR (F) showed 41% decrease at 2% concentration while at 4% level it showed 53% decrease in shoot dry weight as compared to control. The results further revealed that soybean fresh root extracts were more effective than oven dried extracts at both levels of concentrations. (Table: 9)

3.2.8 Effect of soybean extracts (2 and 4%) on number of roots per seedling of maize

The results presented in Table: 10 showed that aqueous extracts of soybean leaves and root significantly decreased the number of roots per seedling of maize. It

was found that 4% extracts were more effective than 2% extracts. The results also further revealed that extracts of unstressed plants were more effective than drought treated plants. The treatment SDL (F) showed 22% decrease in root number per seedling at 2% concentration while at 4% concentration it showed 32% decrease as compared to control. Similarly, the treatment SDL (OD) showed 35% decrease in root number per seedling at 2% concentration while 4% concentration it showed 52% decrease as compared to control. It was found that at 2% concentration, the fresh extracts of unstressed plants were more effective than oven dried extracts while at 4% concentration; the oven dried extracts of drought treated plants were more effective.

Like soybean leaves fresh and oven dried extracts, root extracts were also effective in decreasing number of roots per seedling as compared to control. Both concentrations of soybean root extracts significantly decreased the root number per seedling in maize at $P>0.05$. The oven dried extracts of drought subjected plants were more effective in decreasing the root number as compared to fresh extracts. The treatment SDR (OD) showed 35% decrease in maize rooting at 2% concentration while at 4% concentration it showed 52% decrease as compared to control. Similarly, the treatment SDR (F) showed 30% decrease at 2% concentration while at 4% concentration it showed 48% decrease in root number per seedling as compared to control.

Table: 1 Effects of aqueous leaf and root extract (2% and 4%) of maize on germination percentage of soybean seedling

Treatments	Germination %age after 6 days	
	Level 2%	Level 4%
C	8.000 A	8.000 A
MCL (F)	6.333 AB	5.333 BC
MDL (F)	5.333 BC	4.333 BC
MCL (OD)	5.667 BC	4.667 BC
MDL (OD)	4.000 C	3.667 C
MCR (F)	6.000 BC	5.667 B
MDR (F)	4.667 BC	4.333 BC
MCR (OD)	5.667 BC	5.000 BC
MDR (OD)	5.667 BC	4.333 BC
	LSD: 1.808	LSD: 1.651

Table: 2 Effects of aqueous leaf and root extract (2% and 4%) of maize on Shoot and Root length of soybean seedling

Treatments	Level 2%		Level 4%	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
C	21.333	15.33 A	18.67 A	13.00A
MCL (F)	18.667	14.67 A	13.67 B	9.000B
MDL (F)	15.000	7.667 C	11.67 B	6.667B
MCL (OD)	15.000	11.00 BC	13.67 B	8.667B
MDL (OD)	13.000	8.00 C	12.00 B	7.000B
MCR (F)	14.667	8.00 C	14.00 B	7.000B
MDR (F)	15.333	10.00 BC	14.33 B	6.500B
MCR (OD)	15.333	10.00 BC	11.00 B	9.167B
MDR (OD)	18.000	12.00 AB	13.33 B	9.333B
	NS	LSD: 3.334	LSD: 4.002	LSD: 2.732

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

Table: 3 Effects of aqueous leaf and root extract (2% and 4%) of Maize on Shoot and Root Fresh Weight of soybean seedling

Treatments	Level 2%		Level 4%	
	Shoot Fresh Wt. (g)	Root Fresh Wt. (g)	Shoot Fresh Wt. (g)	Root Fresh Wt. (g)
C	0.8900A	0.310	0.9733A	0.3333A
MCL(F)	0.7300B	0.243	0.6200DE	0.1600B
MDL(F)	0.6533C	0.163	0.5633 E	0.1400BC
MCL(OD)	0.8633A	0.220	0.5800 E	0.1933 B
MDL(OD)	0.5767D	0.217	0.5833 E	0.1700 B
MCR(F)	0.7667B	0.177	0.6433CD	0.1667 B
MDR(F)	0.7333B	0.190	0.6900 C	0.1433BC
MCR(OD)	0.860A	0.183	0.8200 B	0.09667 C
MDR(OD)	0.6633C	0.183	0.6967 C	0.1900 B
	LSD: 0.05425	NS	LSD: 0.05425	LSD: 0.05425

Table: 4. Effects of aqueous leaf and root extract (2% and 4%) of maize on Shoot and Root Dry Weight of soybean seedling

Treatments	Level 2%		Level 4%	
	Shoot Dry Wt (mg)	Root Dry Wt. (mg)	Shoot Dry Wt (mg)	Root Dry Wt. (mg)
C	0.3800A	0.04000A	0.6667 A	0.033 0
MCL(F)	0.1367B	0.02000B	0.1200 B	0.010
MDL(F)	0.09667B	0.01333B	0.1100 B	0.010
MCL(OD)	0.1533 B	0.02667B	0.1533 B	0.017
MDL(OD)	0.1033 B	0.02000B	0.1000 B	0.013
MCR(F)	0.1133 B	0.02000B	0.1133 B	0.017
MDR(F)	0.1433 B	0.01667B	0.1267 B	0.013
MCR(OD)	0.1300 B	0.02000B	0.1233 B	0.027
MDR(OD)	0.1133 B	0.02000B	0.1300 B	0.017
	LSD: 0.05425	LSD: 0.05425	LSD: 0.05425	NS

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

Table: 5. Effects of aqueous leaf and root extract (2% and 4%) of maize on Number of roots per seedling of soybean

Treatments	Level 2%	Level 4%
	No of roots per seedling	
C	6.333A	6.333 A
MCL(F)	5.333ABC	4.333 B
MDL(F)	4.333 C	4.333 B
MCL(OD)	6.000 AB	4.667 B
MDL(OD)	4.667 C	4.333 B
MCR(F)	5.000 BC	5.000 B
MDR(F)	5.000 BC	4.000 B
MCR(OD)	5.333ABC	4.667 B
MDR(OD)	5.333ABC	4.667 B
	LSD: 1.143	LSD: 1.190

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

Table: 6 Effects of aqueous leaf and root extract (2% and 4%) of Soybean on germination percentage of Maize seedling

Treatments	Germination %age after 6 days	
	Level 2%	Level 4%
C	8.33A	8.333 A
SCL(F)	6.00ABC	4.667BC
SDL(F)	4.00BCD	3.333BC
SCL(OD)	6.33AB	5.000BC
SDL(OD)	3.00D	3.333BC
SCR(F)	4.00BCD	4.667BC
SDR(F)	3.00D	2.333 C
SCR(OD)	8.00A	6.333AB
SDR(OD)	3.66CD	4.000BC
	LSD: 2.358	LSD: 2.762

Table: 7 Effects of aqueous leaf and root extracts (2% and 4%) of Soybean on Shoot and Root length of Maize seedling

Treatments	Level 2%		Level 4%	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
C	24.00A	22.667A	23.33A	22.67 A
SCL(F)	16.66BC	16.333B	21.33A	18.33AB
SDL(F)	15.33C	19.00AB	16.33B	17.67AB
SCL(OD)	18.00BC	14.33BC	15.67B	12.33 B
SDL(OD)	16.00C	15.00BC	13.33B	12.33 B
SCR(F)	15.33C	10.33C	13.00B	17.00AB
SDR(F)	18.00BC	16.33B	15.00B	12.00 B
SCR(OD)	22.00A	19.33AB	16.00B	12.67 B
SDR(OD)	19.00B	14.66BC	17.33B	15.00 B
	LSD: 2.557	LSD: 5.188	LSD: 4.002	LSD: 6.078

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

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

Table: 8 Effects of aqueous leaf and root extract (2% and 4%) of Soybean on Shoot and Root Fresh weight of Maize seedling

Treatments	Level 2%		Level 4%	
	Fresh Shoot Wt. (g)	Fresh Root Wt. (g)	Fresh Shoot Wt. (g)	Fresh Root Wt. (g)
C	0.990A	0.667A	0.7667 A	0.5900 A
SCL(F)	0.567C	0.48BCD	0.5233BC	0.4600BC
SDL(F)	0.520C	0.363 D	0.5233BC	0.4033 C
SCL(OD)	0.543C	0.47BCD	0.4800BC	0.4100 C
SDL(OD)	0.527C	0.52BC	0.4833BC	0.4500BC
SCR(F)	0.587BC	0.42CD	0.4233 C	0.4300BC
SDR(F)	0.543C	0.41CD	0.4367C	0.3800 C
SCR(OD)	0.683B	0.58AB	0.6233B	0.5200AB
SDR(OD)	0.563C	0.39D	0.5167BC	0.4367BC
	LSD: 0.1085	LSD: 0.1085	LSD: 0.1329	LSD: 0.09396

All such mean which share a common English letter are non significantly different other wise they differ significantly at P=0.05

Table: 9 Effects of aqueous leaf and root extract (2 % and 4%) of Soybean on Shoot and Root Dry weight of Maize seedling

Treatments	Level 2%		Level 4%	
	Shoot Dry Wt (mg)	Root Dry Wt. (mg)	Shoot Dry Wt (mg)	Root Dry Wt. (mg)
C	0.793A	0.530AB	0.6800 A	0.4667 A
SCL (F)	0.470BC	0.330C	0.4500 BC	0.3900ABC
SDL (F)	0.427C	0.293C	0.4133BCD	0.2767 D
SCL (OD)	0.453BC	0.370C	0.3667 CD	0.3600BCD
SDL (OD)	0.467BC	0.370C	0.3733 CD	0.3267 CD
SCR (F)	0.517BC	0.533AB	0.3367 CD	0.3500BCD
SDR (F)	0.467BC	0.403BC	0.3167D	0.3433CD
SCR (OD)	0.573B	0.600A	0.4933B	0.4300AB
SDR (OD)	0.477BC	0.317C	0.4200BCD	0.3633BC
	LSD: 0.1213	LSD: 0.1435	LSD: 0.1085	LSD: 0.07671

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

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

Table: 10 Effects of aqueous leaf and root extract (2% and 4%) of Soybean on Number of roots per seedling of Maize.

Treatments	Level 2%	Level 4%
	No of roots per seedling	
C	7.667A	8.333A
SCL (F)	5.000C	4.333B
SDL (F)	6.00ABC	5.667B
SCL (OD)	7.333AB	4.333B
SDL (OD)	5.000C	4.000B
SCR (F)	5.333BC	4.333B
SDR (F)	5.333BC	4.333B
SCR (OD)	5.667ABC	5.000B
SDR (OD)	5.000C	4.000B
	LSD: 1.953	LSD: 1.896

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

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

Experiment NO.2

3.3 Effect of Maize and Soybean fresh and oven dried extracts on physiology of each other

3.3.1 Effect of Maize fresh and oven dried extracts on physiology of soybean

3.3.1.1 Effect of Maize leaves and root aqueous extracts (fresh and oven dried) on proline content of Soybean seedling

The results revealed that all the treatments resulted in accumulation of proline in soybean leaves. However, maximum increase in proline content occurred in plants raised from seeds pre-soaked in fresh and oven dried leaves extract obtained from maize plants subjected to drought. Treatments MDL (F) and MDL (OD) increased significantly the proline accumulation by 81 % and 78 % respectively as compared to control. Treatment MCL (OD) resulted in 76 % significant increase in proline content of leaves as compared to control (Fig.1)

Maize root extracts also showed stimulatory effects on proline accumulation in soybean leaves. However, significant increase (65 %) in proline accumulation occurred in soybean plants raised from seeds pre-soaked in maize roots oven dried extract obtained from maize plants which were not subjected to drought (Fig.1).

3.3.1.2 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on protein content of Soybean seedling

All the treatments decreased the protein content of soybean leaves except for treatment MDL (F) which significantly increased protein content by 3 % as compared to control. Significant decrease (37 %) in protein content was found in soybean plants raised from seeds pre-soaked in maize leaf oven dried extract obtained from plants which were not exposed to drought i.e. treatment MCL (OD). Similarly, the treatment MDL (OD) also caused 16 % non-significant decrease in protein content as compared to control.

All the root fresh as well as oven dried extracts significantly decreased the soybean leaf protein content. The treatment MCR (F) resulted in 30 %, treatment MDR (F) in 39 %, treatment MCR (F) in 45 % and treatment MDR (OD) in 23 % significant reduction in protein content as compared to control. Maximum reduction in leaf protein was found in soybean plants raised from seeds pre-soaked in oven dried maize roots extract obtained from plants which were not subjected to drought (Fig.2)

3.3.1.3 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on sugar content of Soybean seedling

Results showed that maize fresh as well as oven dried aqueous extracts significantly increased the sugar content of soybean leaves as compared to control. Maximum increase (48 %) in sugar content was observed in treatment MDL (F) which was non-significantly different from treatment MDL (OD) which showed 46% increase in sugar content as compared to control. The treatment MCL (F) was found to posses 36% more sugar as compared to control which was non-significantly different from treatment MCL (OD) which showed 39% increase in sugar content as compared to control. Overall, maximum increase i.e. 48% was observed by treatment MDL (F).

Among root extracts, all the treatments significantly increased the soybean leaf sugar content except for treatment MDR (F) which showed non-significant increase in sugar content as compared to control. The treatment MDR (OD) was found to posses maximum and significant (35%) increase in soybean leaf sugar content as compared to control. The treatment MCR (F) showed 23% while the treatment MCR (OD) showed 25% significant increase in sugar content as compared to control (Fig.3).

Overall, the both fresh as well as oven dried extracts of maize leaves collected from plants subjected to drought were more effective in increasing the soybean leaf sugar content as compared to root extracts.

3.3.1.4 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on chlorophyll "a" and "b" content of Soybean seedling

Results shown in Fig.4 revealed that maize leaf as well as root extracts (fresh and oven dried) exhibited no significant effects on soybean leaf chlorophyll content. However, chlorophyll 'b' was more affected as compared to 'a' (Fig.5).

3.3.1.5 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on Carotenoid content of Soybean seedling

The aqueous extracts of maize leaf as well as root significantly decreased the soybean seedling carotenoid content when applied as seed pre-soaking. The treatment MDL (F) showed 49 % while treatment MDL (OD) was found with 63 % significant decrease in carotenoid content as compared to control. Both the treatments MCL (F) and MCL (OD) exhibited 40 % decrease in carotenoid content as compared to control. The results suggested that maize leaf extracts both fresh as well as oven dried prepared from drought subjected plants were more effective in decreasing carotenoid content of soybean seedling as compared to control (Fig.6).

The root extracts of maize also significantly decreased the carotenoid content of soybean seedling. The treatment MDR (F) showed 39 %, while the treatment MDR (OD) showed 49 % significant decrease in carotenoid content as compared to control. The oven dried root extract prepared from drought subjected maize plants was found as more effective in decreasing soybean seedling carotenoid content (Fig.6).

3.3.1.6 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on Superoxide dismutase (SOD) activity of Soybean seedling.

Results showed that aqueous extracts of both fresh and oven dried leaves had maximum and significant (79 %) increase in SOD activity of soybean leaves as compared to control. Oven dried leaf extracts showed no effects on SOD activity of soybean seedling as compared to control. However, treatment MCL (OD) showed 18 % non-significant increase in SOD activity as compared to control.

The aqueous extracts both of fresh and oven dried root also showed no significant effect on SOD activity of soybean leaves. However, treatment MCR (OD) resulted in significant decrease (89 %) in SOD activity of soybean leaves as compared to control (Fig.7).

3.3.1.7 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on peroxidase activity (POD) of Soybean seedling

Significant increase in POD activity of soybean leaves was caused by both fresh as well as oven dried aqueous extracts obtained from maize leaves subjected to drought (Fig.8). Treatment MDL (F) showed 49 % significant increase in POD activity as compared to control. Treatment MDL (OD) also significantly increased POD activity by 41% as compared to control. The treatments MCL (F) as well as MCL (OD) resulted in 22 % and 4 % non-significant increase in POD activity as compared to control respectively.

Root extracts both fresh and oven dried extracted from unstressed as well drought treated maize plants exhibited no significant effects on POD activity of soybean leave. Only treatments MCR (F) and MDR (F) resulted in 22 % and 16 % non-significant increase in POD activity of soybean leaves as compared to control.

3.3.1.8 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on Ascorbate peroxidase (APX) activity of Soybean seedling

From the results presented in Fig.9 it was found that among fresh and oven dried leaves extracts; significant increase (74 %) in ascorbate peroxidase activity (APX) of soybeans leaves was caused only by fresh leaves extract obtained from maize plants subjected to drought. Oven dried extract i.e. treatment MDL (OD) showed 61 % non-significant increase in ascorbate peroxidase (APX) activity as compared to control. Fresh as well as oven dried leaves extracts obtained from maize plants not subjected to drought exhibited no significant effect on ascorbate peroxidase (APX) activity of soybean leaves when applied as seed pre-soaking.

Fresh and oven dried extracts obtained from maize roots prepared from unstressed whether unstressed or drought subjected plants showed no significant effects on ascorbate peroxidase(APX) activity of soybean leaves except for treatment MDR (OD) which resulted in 67% significant increase in ascorbate peroxidase (APX) activity as compared to control. Treatment MCR (OD) increased non-significantly the ascorbate peroxidase (APX) activity of soybean leaves by 10 % as compared to control.

3.3.1.9 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on catalase (CAT) activity of Soybean seedling

Among different extracts prepared from maize leaves, only that were found as more effective in increasing the catalase (CAT) activity of soybean seedling which were extracted from drought subjected maize plants. The treatment MDL (OD) showed significant and maximum (56%) increase in catalase (CAT) activity as compared to control which was statistically non-significantly different from treatment MDL (F) showing 51 % increase in catalase (CAT) activity as compared to control. All the other treatments exhibited no significant increase in catalase (CAT) activity as compared to control (Fig.10).

The root extracts of maize exhibited no significant effects on catalase (CAT) activity of soybean seedling. Maximum increase (39 %) in catalase (CAT) activity was found in treatment MDR (F).

The results showed that plants raised from seeds presoaked in aqueous extracts of maize leaves obtained from plants subjected to drought were found with more catalase (CAT) activity as compared to control and other treatments (Fig.10).

3.3.1.10 Effect on maize aqueous extract (leaf and root) on endogenous ABA content of soybean

The results showed that the aqueous extract of maize plant significantly increased the ABA content of soybean. All the treatments were significantly different from that of control. The treatments MDL (F) and MCL (F) were found with higher ABA content as compared to control, indicating that leaves extracts were more

effective as compared to root extracts. However the extracts prepared from drought subjected plants were significantly more effective as compared to unstressed plant. Similarly the extracts prepared from plant roots were also found effective as compared to control. However the extracts prepared from roots of drought treated maize plants were more effective as compared to extracts prepared from roots of unstressed plants. The treatment MDR (F) was significantly different from MCR (F) and as well as from control (Fig.11).

3.3.1.11 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on soil pH of soybean cultivated soil

It was found that both leaf as well as root aqueous extracts of maize exhibited no significant effects on soil pH of soybean cultivated soil. However, it was investigated that maize extracts non-significantly decreased the alkalinity of soybean grown soil as compared to control (Table-1).

3.3.1.12 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on soybean cultivated soil electrical conductivity

Results presented in Table-1 showed that maize fresh as well as oven dried aqueous extracts significantly decreased the soil electrical conductivity (EC) as compared to control. Among the leaf aqueous extracts the highest decrease in EC occur in treatment MCL (OD) which is 45 %. While MDL (OD), MCL (F) and MDL (OD) caused 31 %, 30 % and 10 % decrease in soil electrical conductivity (EC) respectively.

Similarly root extracts showed decrease in the soil electrical conductivity (EC). MCR (F) caused 55% decrease while treatments like MDR (OD), MCR (OD) and MDR (F) decrease 48%, 21% and 19% decrease in soil electrical conductivity (EC) respectfully. It is clear from the table that maize root extracts have greater effect on the soil electrical conductivity (EC) as compare to leaf extracts

3.3.1.13 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on macronutrients of soybean planted soil

The result presented in Table. 2 showed that aqueous extract prepared from leaf and root (fresh and oven dried) has significant effect on macronutrient availability.
In case of P, maximum treatments have significant effect, which decreased the P, availability to the plant as compared to control. However the treatment MCL (F) highly significant that reduce the availability of P, to the soybean plant as compared to other treatments and control.

Similarly Na, availability to soybean plant is also affected by aqueous extract prepared from leaf and root (fresh and oven dried) of maize plant. All the treatments significantly different from each other. However the treatments MCR (OD), MDL (F), MCL (OD) and MDR (F) are highly significant (P>005) from control. It's clear from the table that both leaf and root have grater effect on decreasing the availability of P, to the soybean plant
In the same way F, availability is also effected by the by aqueous extract prepared from leaf and root (fresh and oven dried) of maize plant. All the treatments have significantly decreased the availability of K, to the soybean plat as compared to control. However the treatments MDL (OD) and MDL (F) was found highly significant as compared from other as well as from control. Its clear from the statistical data that leaf extract have significant effect on the availability of K, to the soybean plant.

In case of Ca, all the treatments have significant effect as compared to control. The treatments like MDL (OD) and MDL (F) was found highly significant as compared to other treatments and also from control. The leaf extract prepared from the plant subjected to drought was found highly effective as compared to the root extracts
Likewise in case of Mg, treatments MDL (OD) and MDL (F) was found significantly different from the other treatments and also from the control. It's clear from the table that leaf extract prepared from drought-subjected plant have greater effect on the availability on Mg, to the soybean plant.

3.3.1.14 Effect of Maize leaf and root aqueous extract (fresh and oven dried) on micronutrient of soybean planted soil

Results presented in Table-3 showed that application of different maize leaves aqueous extracts including both fresh and oven dried significantly decreased the availability of micronutrients to the soybean plant as compare to control. The Treatments MDL (F) and MDL (OD) showed highly significant decrease in availability of Fe as compared to control and other treatments. It's clear from the table that leaf extracts prepared from drought subjected maize plants significantly decreases Fe availability as compared to root extracts. The Cu availability to soybean plants was also significantly decreased by application of maize leaves aqueous extracts. The treatments MDL (F) and MDL (OD) are highly significant as compared to control. Both the leaf and root have the same effect on Cu availability.

Likewise Cr availability maximum treatments are non significant while treatment MDL (OD) is highly significant as compared to control. Similarly in case of Co all the treatment reduce its availability as compared to control. All the treatments are highly significant from control but they are non- significant from each other.

Similarly in case of Zn availability most of the treatments are non-significant but however treatments like MDL (OD) and MDL (F) are highly significant from control. Similarly in case of Mn availability all the treatment are significantly different from control however treatments like MDL(OD), MDL(F) and MCL(OD) are highly significant from control. Which reduce Fe availability to plant.

Likewise Ni all the treatments are significantly reduces its availability as compared to control. The treatments like MDL (OD), MDLF) and MDR (F) are highly significantly and reduce Ni availability to plant as compared to control (Table.5).

3.3.2 Effect of Soybean leaves and root aqueous extracts on physiology of maize

3.3.2.1 Effect of Soybean leaves and root aqueous extracts on proline content of maize

It was found that soybean leaves extracts significantly increased the proline content of maize flag leaves as compared to control. However, the root extracts were

found with no significant effects on proline content (Fig.12). The treatment SDL (F) was found with maximum increase (57 %) in proline content as compared to control. Similarly, the treatment SDL (OD) showed 49% significant increase in proline content. The treatment SCL (F) also showed significant increase in proline content but treatment SCL (OD) was found with no significant increase. The results further revealed that leaves extracts prepared from drought treated soybean plants were more effective in increasing the proline content of maize flag leaves as compared to extracts of unstressed plants.

3.3.2.2 Effect of Soybean leaves and root aqueous extracts on protein content of maize

The results presented in Fig.13 showed that application of soybean leaves and root extracts exhibited no significant effects on protein content of maize leaves except for treatment SDL (F) which significantly increased (24%) the protein content as compared to control. The results further revealed that soybean leaves extracts prepared from drought treated plants were more effective in increasing the maize leaves protein content as compared to extracts prepared from unstressed plants.

3.3.2.3 Effect of Soybean leaves and root aqueous extracts on sugar content of maize

All the treatments significantly different from control. It was found that soybean leaves extracts significantly increased the sugar content of maize flag leaves as compared to control. The treatment SDL (F) was highly significant from control as well as from maximum treatments at $P>0.05$. The leaf extract was found significant as compared that of root in increasing the sugar content of the maize plants ((Fig.14).

3.3.2.4 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on Chlorophyll 'a' and 'b' content of maize seedling

Soybeans leaves as well as root extracts exhibited no significant effect on chlorophyll 'a' content of maize leaves when applied as seed pre-soaking (Fig.15). However, chlorophyll 'b' content of maize seedling was significantly decreased by all kinds of soybean leaves and root extracts. The treatment SDL (F) was found with 89 % significant decrease in chlorophyll 'b' as compared to control. The treatment SDL

(F) was non-significantly different from treatment SDL (OD) with 87 % decrease in chlorophyll 'b' content as compared to control. The treatment SCL (F) showed 76% decrease in chlorophyll 'b' as compared to control. The extracts prepared from leaves of drought subjected soybean plants were investigated as more effective in decreasing maize seedling chlorophyll 'b' content (Fig.16).

The soybean root extracts also significantly decreased the maize seedling chlorophyll 'b' content. The treatment SDR (F) showed 76 % significant decrease in chlorophyll 'b' content of maize seedling as compared to control. The treatment SDR (F) was non-significantly different from treatment SDR (OD) with 88 % decrease in chlorophyll 'b' content as compared to control. It was found that root extracts of µcontent of maize seedling (Fig.16).

3.3.2.5 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on carotenoid content of maize seedling

There was found significant decrease in carotenoid content of flag leaf in maize plants raised from seeds pre-soaked in soybean leaves fresh as well as oven dried extracts. The treatment SDL (F) showed 51% decrease in carotenoid content as compared to control. The treatment SDL (F) was non-significantly different from treatment SDL (OD) which also showed 51% decrease in carotenoid content as compared to control. However, maximum decrease (52%) in carotenoid content was found in plants raised from seeds pre-soaked in oven dried leaves extract prepared from control soybean plants (Fig.17).

Root extracts also significantly decreased the carotenoid content of maize seedling. However, the oven dried root extract of drought subjected soybean plants was found as more effective in decreasing the maize seedling carotenoid content.

Overall, leaves extracts were found as more effective in decreasing the carotenoid content of maize leaves as compared to root extracts, particularly that ones which were prepared from drought subjected soybean plants (Fig.17).

3.3.2.6 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on Superoxide dismutase activity of maize seedling.

From the results described in Fig.18 revealed that seed pre-soaking of maize seeds in fresh and oven dried leaves extract of soybean resulted in an increase in the SOD activity of maize flag leaves. Treatments SCL (F), SDL (F) and SDL (OD) resulted in 50 %, 54 % and 51 % significant increase in SOD activity of maize as compared to control. The treatment SCL (OD) showed 39% increased in SOD activity as compared to control.

Application of soybean root fresh as well as oven dried extracts also increased the SOD activity of maize flag leaves. However, significant increase (37 %) in SOD activity of maize flag leaves was found in treatment SCR (OD) as compared to control. The treatment SDR (OD) showed 28 % non-significant increase in SOD activity as compared to control.

3.3.2.7 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on peroxidase activity (POD) activity of maize seedling

The results showed that application of both fresh as well as oven dried soybean leaves extracts as seed soaking prior to sowing increased the POD activity in maize flag leaves (Fig.19). The treatment SDL (F) resulted in 61 % significant increase in POD activity which was significantly different from control as well as treatment SCL (F) respectively. Treatment SDL (OD) showed 60 % significant increase in POD activity as compared to control but was non-significantly different from SDL (F) and SCL (OD) treatments.

Root extracts also showed an increase in POD activity of maize. The treatment SDR (F) showed 34 % significant increase in POD activity as compared to control. The treatment SDR (OD) exhibited 45 % increase in POD activity which was significantly different from control but non-significantly different from treatment SDR (F). It was found that maximum increase in POD activity was found in maize plants which were raised from seeds pre-soaked in oven dried root extract obtained from soybean plants which were subjected to drought.

3.3.2.8 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on ascorbate peroxidase activity (APX) activity of maize seedling.

Application of both fresh as well as oven dried soybean leaf extracts as seed soaking before sowing showed an increase in ascorbate activity of maize leaves (Fig.20) Treatment SDL (F) showed 90 % significant increase in ascorbate activity as compared to control while treatment SDL (OD) showed 91 % increase in ascorbate activity of maize which was significantly different from control but non-significantly different from treatment SDL (F) and SCL (F) respectively. The treatment SCL (OD) also resulted in significant increase (81%) in ascorbate activity as compared to control. However, maximum increase in ascorbate activity was found in maize plants which were raised from seeds treated with soybean leaves oven dried extract obtained from plants subjected to drought.

Root extracts application as seed soaking to maize prior to sowing also showed an increase in ascorbate activity. Maximum and significant increase (85 %) was found in treatment SCL (OD) which was significantly different from control but non-significantly different from treatments SCR (F), SDR (F) as well as SDR (OD) respectively. Overall, leaf extracts were found as more effective in increasing the ascorbate activity of maize plants (Fig.20).

3.3.2.9 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Catalase activity activity of maize seedling.

Results presented in Fig.21. showed that significant increase in Catalase activity was found when soybean leaf extracts were applied as seed soaking prior to sowing of maize. The treatment SDL (F) showed 70 %, SCL (OD) 69 % and SDL (OD) 71 % significant increase in Catalase activity as compared to control. The treatment SCL (F) exhibited 41 % increase in Catalase activity which was non-significantly different from all treatments as well as control. Maximum increase in Catalase activity was found in maize plants raised from seeds which were pre-soaked in soybean leaves extract obtained from plants subjected to drought.

Application of soybean root extracts both fresh as well as oven dried exhibited no significant effect on catalase activity of maize. However, maximum increase in

catalase activity was found in maize plants which were raised from seeds presoaked in aqueous extract of soybean root fresh extract obtained unstressed plants

3.3.2.10 Effect of soybean fresh aqueous extract (leaf and root) on ABA content of maize

The results presented in table revealed that all the treatments were found significantly different from that of control. All the treatments significantly increased the ABA content as compared to control. However the treatments SCR (F) and SDR (F) were found with significant increased in ABA content as compared to control. It was found that soybean leaves extracts were more effective in increasing the maize leaves ABA content as compared to root extracts. The extract prepared from drought treated plants significantly increased the ABA content as compared to unstressed plant extract. The treatment SDL (F) was found with maximum increase in ABA content which was highly significant as compared to control (Fig.22).

3.3.2.11 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on soil pH of maize cultivated soil

The soil pH was significantly decreased by leaves extracts prepared from drought subjected soybean plants. The treatment SDL (F) significantly decreased the soil pH by 10% as compared to control which was non-significantly different from SDL (OD) which showed 13 % decrease in soil pH as compared to control. The SCL (F) and SCL (OD) were found with no significant variations in soil pH (Table.4).

The root extracts showed no significant effects on soil pH of maize rhizosphere except for oven dried extract which was prepared from drought subjected soybean plant roots. The treatment SDR (OD) showed 10 % significant decrease in soil pH as compared to control (Table.4).

3.3.2.12 Effect of Soybean leaves and root aqueous extracts (fresh and oven dried) on soil electrical conductivity (dSm^{-1}) of maize cultivated soil

Results indicated in Table.4 showed that soybean fresh as well as oven oven dried aqueous extract effect the soil electrical conductivity (EC). Treatment SDL (F) showed highest decrease (27%) in soil electrical conductivity (EC) while treatments

like SCL(OD) and SCL(OD) have the same effect 13% .Treatment SCL (F) have no significant effect on the soil electrical conductivity (EC).

Likewise root fresh as well as oven dried aqueous extract has the same effect on the soil electrical conductivity (EC). Treatment SCR(OD) have the highest effect 25% while treatment SCR(F) and SCR(OD) represent 13% ,7% decrease in the soil electrical conductivity (EC) whereas treatment SDR(OD) have no significant effect on the soil electrical conductivity.

3.3.2.13 Effect of soybean leaves and root aqueous extract (fresh and oven dried) on macronutrient of maize planted soil

From the results presented in Table-5 showed that application of soybean leaves and root extracts as seed soaking prior to sowing significantly decreased the availability of macronutrients to maize plants. However, aqueous extracts which were prepared from drought subjected soybean plants were more effective as compared to extracts of unstressed plants. The treatments SDL (F) and SDL (OD) were found with significant decrease in phosphorous availability as compared to control. It was found that aqueous extracts of drought subjected soybean plants were more effective in decreasing the phosphorous availability as compared to extracts of unstressed plants.

It was found that sodium availability was also significantly decreased by soybean leaves aqueous extracts. All the treatments were statistically non-significant from each other indicating that extracts prepared from unstressed and drought treated plants were equally effective in decreasing Na availability to maize plants. The K availability was also significantly decreased by soybean leaves extracts but root extracts showed no significant effects. Maximum decrease in K availability was found in treatments MDL (F) and MDL (OD) as compared to other treatments. This showed that extracts prepared from drought subjected plants were more effective as compared to extracts of unstressed plants.

The Ca availability was also significantly decreased by application of soybean fresh and oven dried extracts as seed soaking prior to sowing in maize. However, highly significant decrease in Ca availability to maize plants was found in treatments MDL (F) and MDL (OD) as compared to control. The results showed that soybean leaves and root extracts exhibited no significant effects on Mg availability as

compared to control. It was found that the Mg was the only macronutrient, which availability to maize plants was not affected by application of soybean leaves and root extracts.

3.3.2.14 Effect of soybean leaves and root aqueous extract (fresh and oven dried) on micronutrient of maize planted soil

The results showed that application of soybean leaves and root extracts as seed soaking prior to sowing significantly decreased the availability of micronutrients to maize plants (Table-6).

Soybean leaves decreased the availability of Fe to maize plants and root extracts. However, maximum decrease was observed only in treatments SDL (F) and SCL (OD) which were non-significantly different from each other but significantly different from control. The root extracts were found with no significant effects on the availability of Fe to maize plants. The results showed that soybean leaves also significantly decreased the availability of Cu and root extracts. However, the leaves extracts were found as more effective as compared to root extracts.

It was found that maize aqueous extracts of both maize leaves and roots showed no significant effects on the Cr availability to soybean plants. The results showed that Co availability was significantly decreased by the application of maize leaves and root extracts to soybean plants. However, all the treatments were non-significantly different from each other. The leaves and root extracts of maize exhibited no significant effects on Zn availability to soybean plants except for extracts prepared from drought treated plants which significantly decreased the Zn availability.

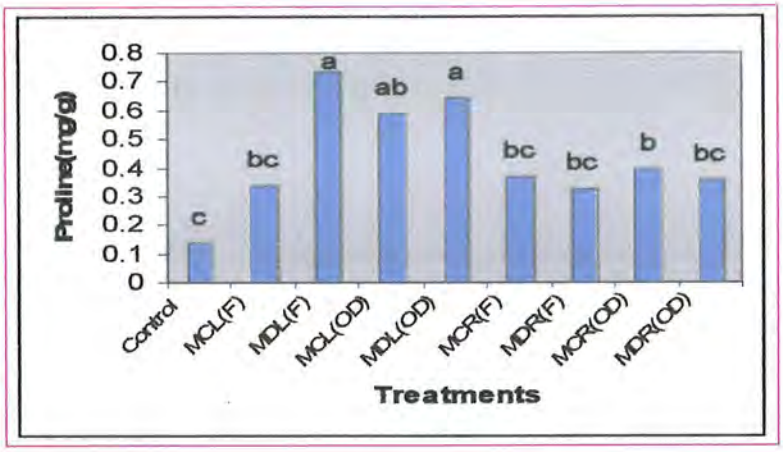


Figure: 1 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on Proline (mg/g) content of Soybean seedling

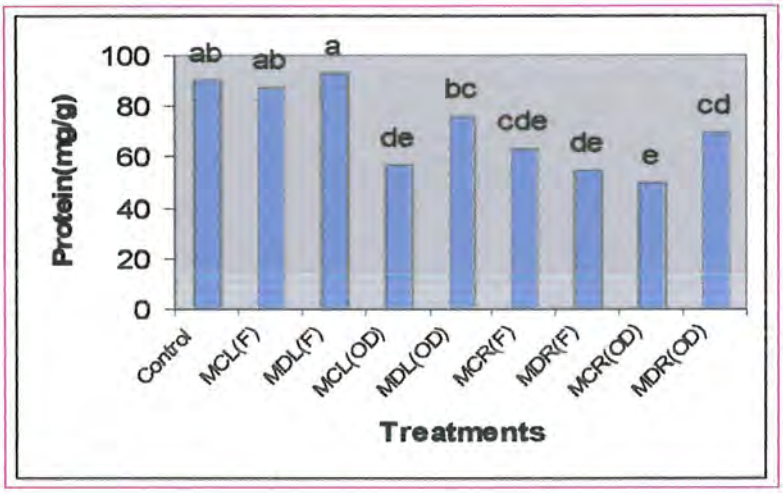


Figure: 2 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on protein (mg/g) content of Soybean seedling

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

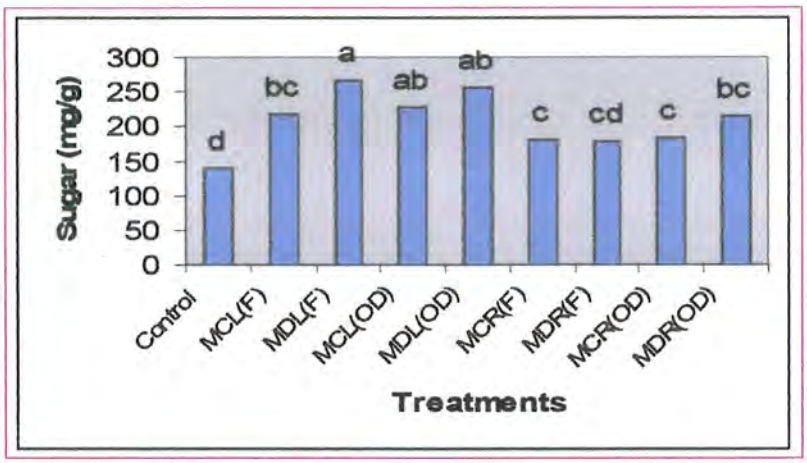


Figure: 3 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on sugar (mg/g) content of Soybean seedling

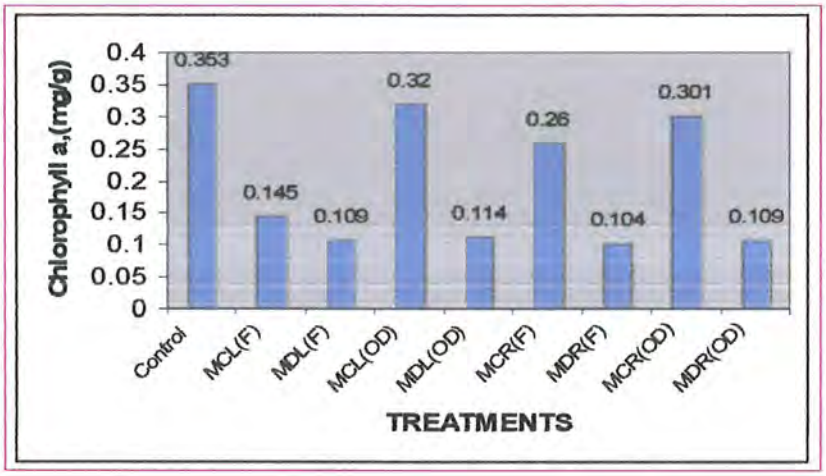


Figure: 4 Effect of maize leaf and root aqueous extracts (fresh and oven dried) on Chlorophyll 'a' content of soybean seedling

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

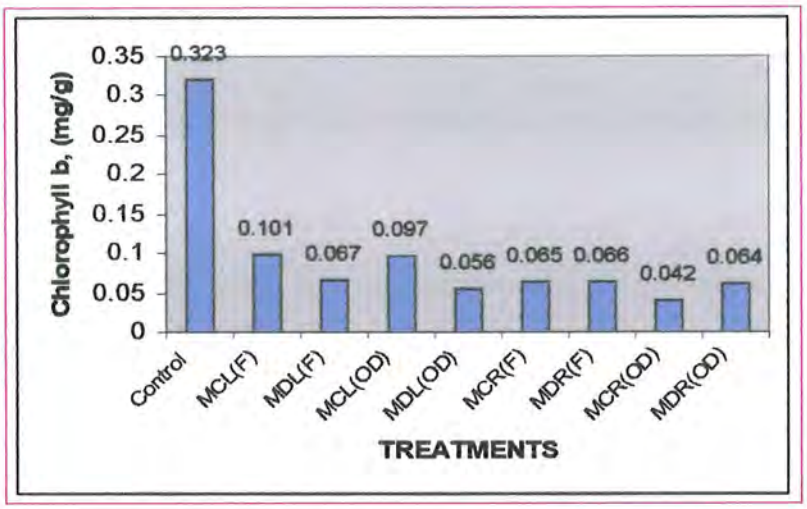


Figure: 5 Effect of maize leaf and root aqueous extracts (fresh and oven dried) on Chlorophyll 'b' content of soybean seedling

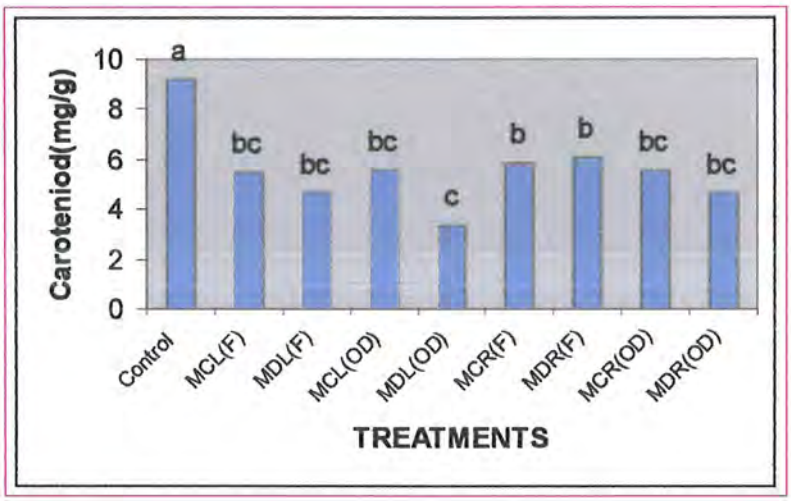


Figure: 6 Effect of maize leaf and root aqueous extracts (fresh and oven dried) on caroteniod content of soybean seedling

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

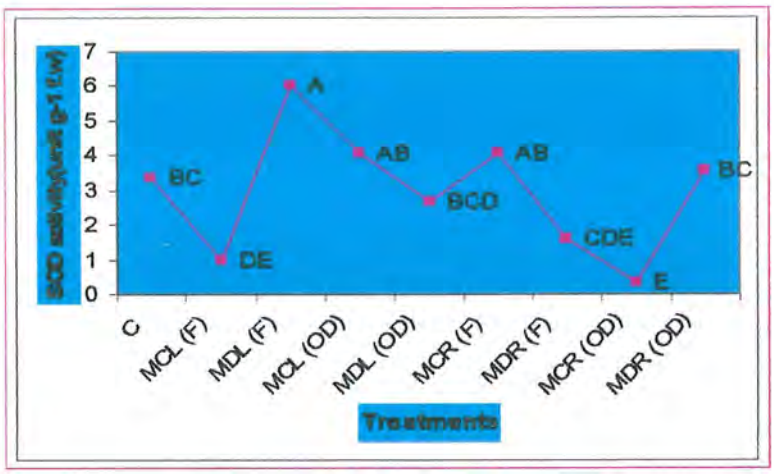


Figure: 7 Effect of Maize shoot and root aqueous extracts (fresh and oven dried) on Superoxide dismutase (SOD) (unit's g^{-1} f.w) of Soybean seedling

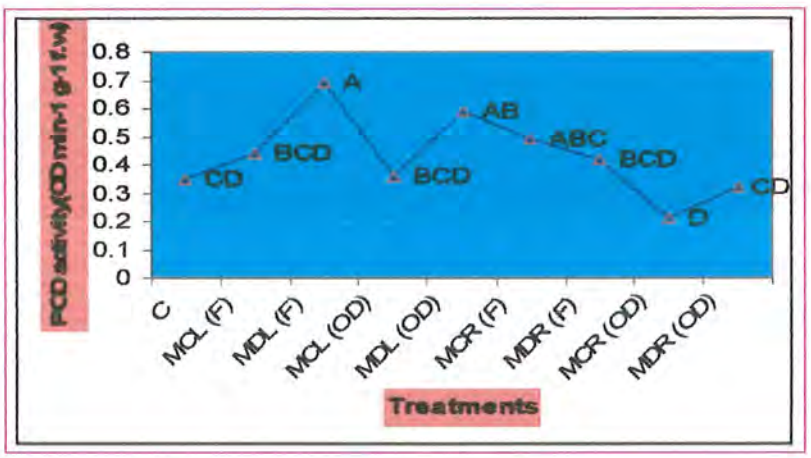


Figure: 8 Effect of Maize shoot and root aqueous extracts (fresh and oven dried) on Peroxidase activity (POD) (O.D $min^{-1}g^{-1}$ f.w) of Soybean seedling

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

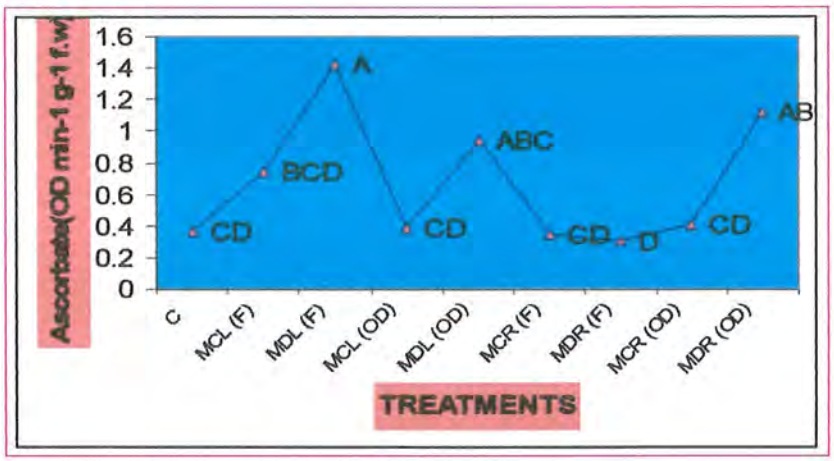


Table: 9 Effect of Soybean shoot and root aqueous extracts (fresh and oven dried) on Ascorbate peroxidase (APX) (U mg^{-1}protien) activity of Maize seedling

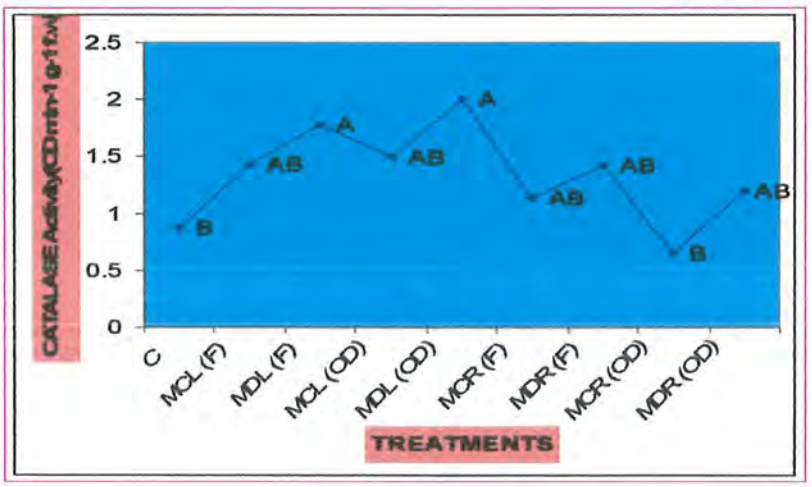


Figure: 10 Effect of maize shoot and root aqueous extracts (fresh and oven dried) on Catalase (CAT) (O.D $min^{-1}g^{-1}$ f.w) activity of soybean seedling

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

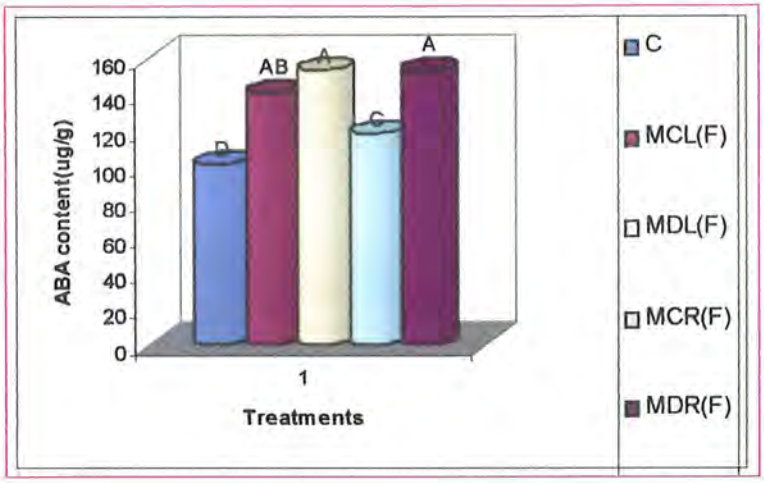


Figure: 11 Effect of maize extract on endogenous ABA (ug/g) content of soybean plant

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

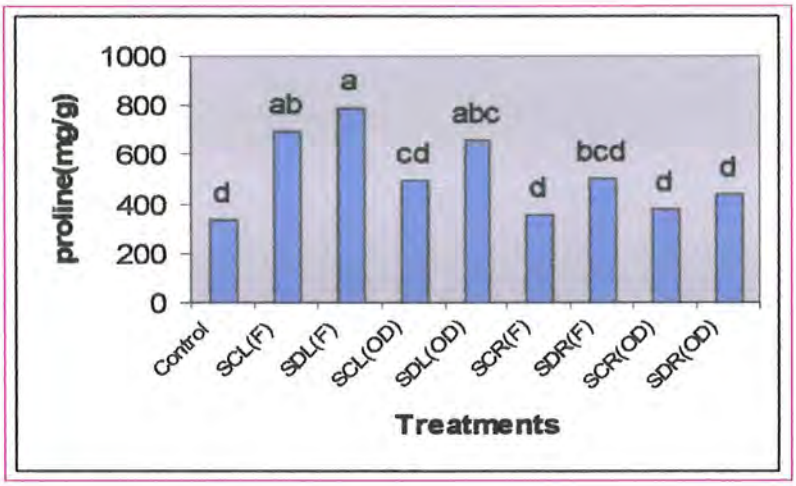


Figure: 12 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Proline (mg/g) content of Maize seedling

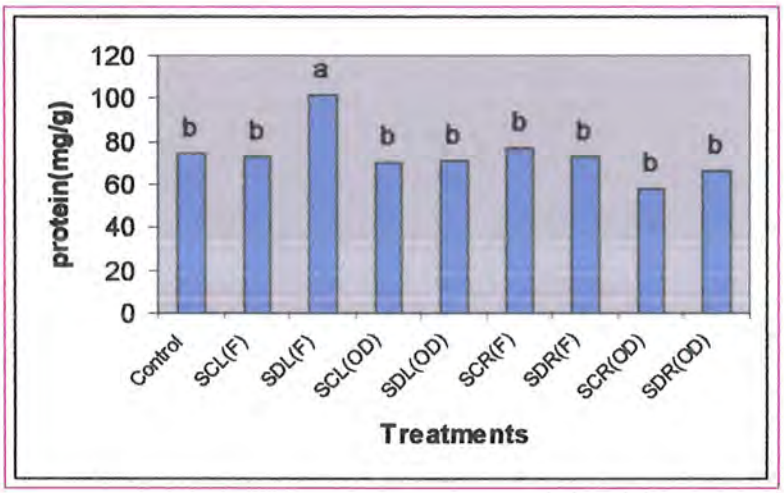


Figure: 13 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on protein (mg/g) content of Maize seedling

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

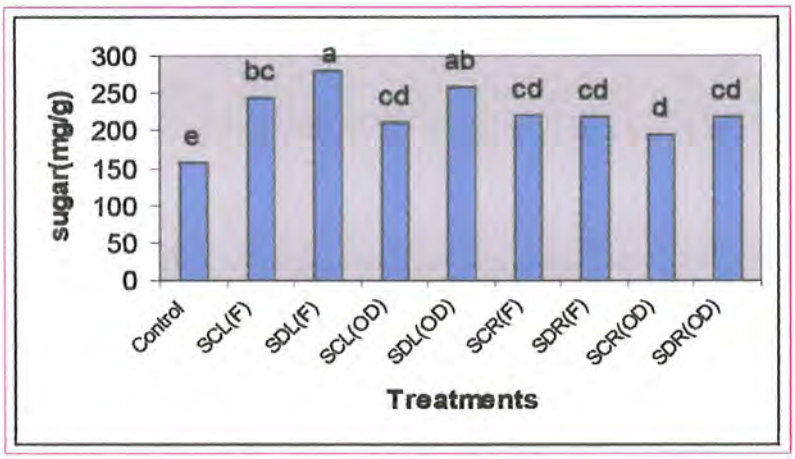


Figure: 14 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Sugar content of Maize seedling

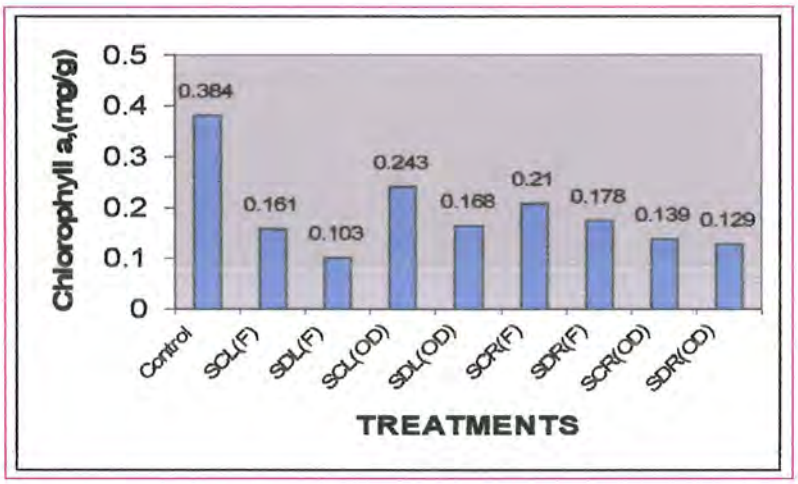


Figure: 15 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Chlorophyll 'a' content of Maize seedling

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

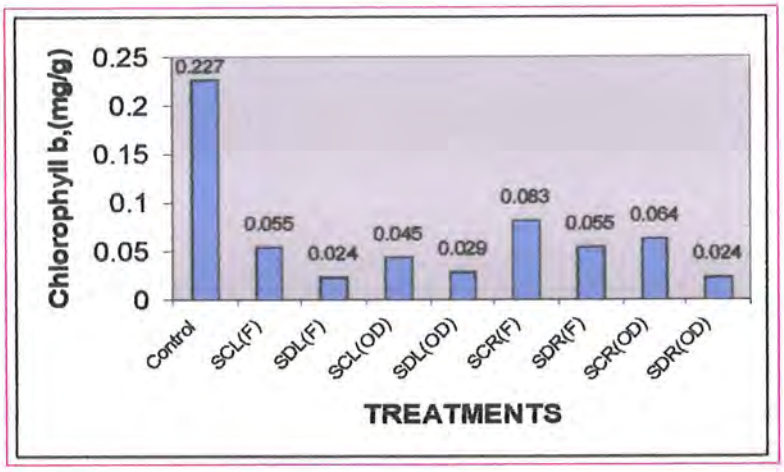


Figure: 16 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Chlorophyll 'b' content of Maize seedling

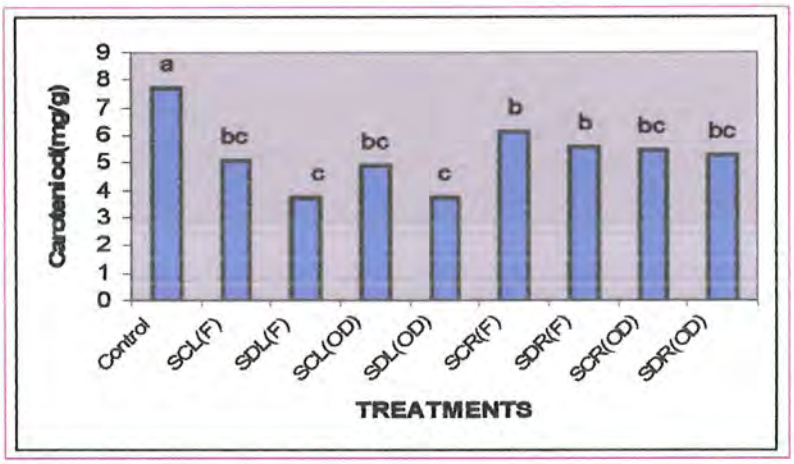


Figure: 17 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on and carotenoid content of Maize seedling

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

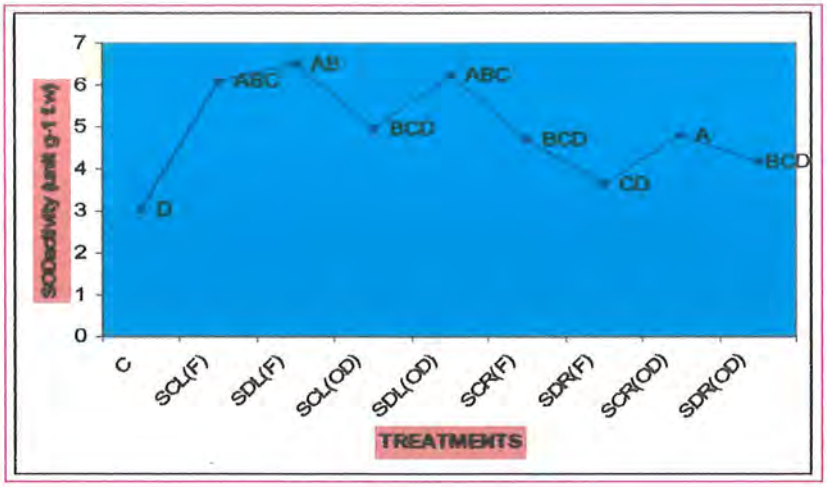


Figure: 18 Effect of Soybean shoot and root aqueous extracts (fresh and oven dried) on Superoxide dismutase (SOD) (unit's g^{-1} f.w) of Maize seedling.

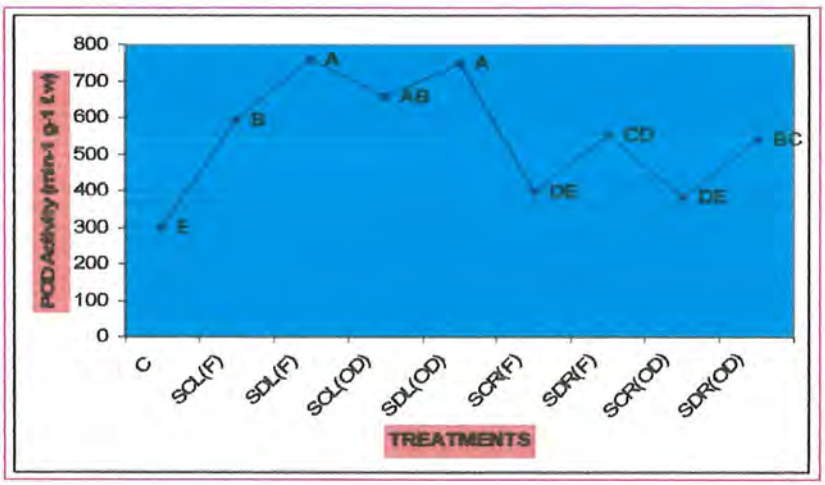


Figure: 19 Effect of Soybean shoot and root aqueous extracts (fresh and oven dried) on Peroxidase activity (POD) (O.D $min^{-1}g^{-1}$ f.w) of Maize seedling

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

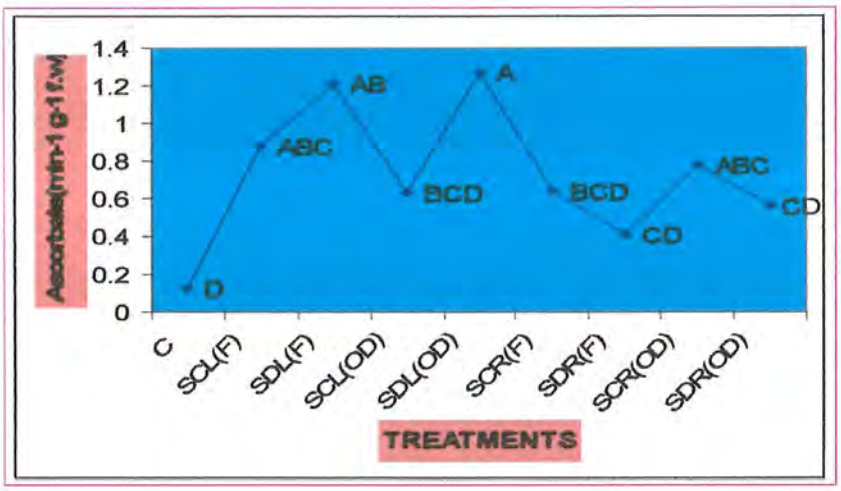


Figure: 20 Effect of Soybean shoot and root aqueous extracts (fresh and oven dried) on Ascorbate peroxidase (APX) (U mg^{-1}protien) activity of Maize seedling

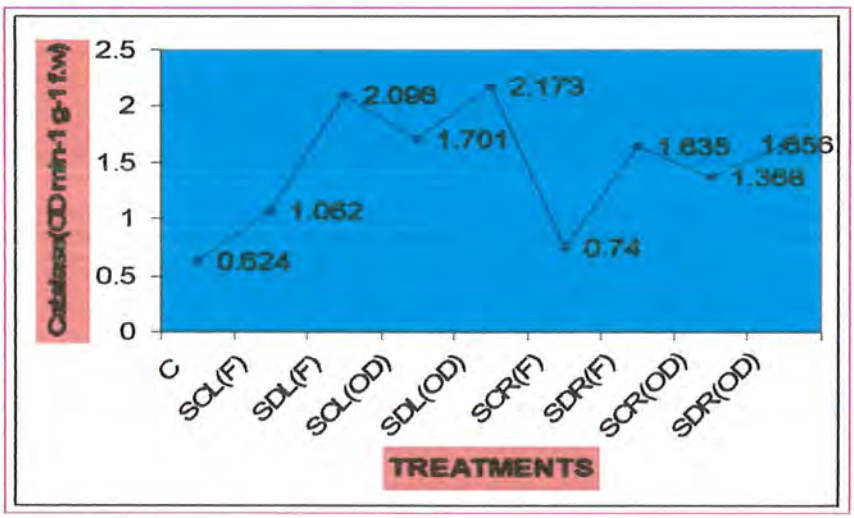


Figure: 21 Effect of Soybean shoot and root aqueous extracts (fresh and oven dried) on Ctalase (CAT) (O.D $min^{-1}g^{-1}$ f.w) activity of Maize seedling

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

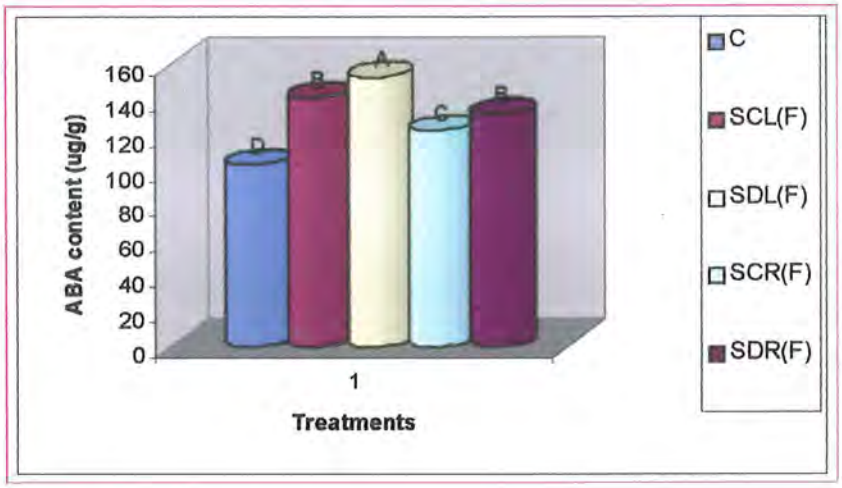


Figure: 22 Effect of soybean extract on endogenous ABA (ug/g) content of maize plant

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

Table: 1 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on Soil pH and soil EC of Soybean cultivated soil

Treatment	Soil PH	Soil EC (dsm^{-1})
Control	7.333	290.0 A
MCL(F)	6.167	203.3 ABC
MDL(F)	6.467	260.0 A
MCL(OD)	6.933	160.0 BC
MDL(OD)	6.000	200.0 ABC
MCR(F)	6.767	130.0 C
MDR(F)	6.000	236.7 AB
MCR(OD)	6.400	230.0 AB
MDR(OD)	6.167	150.0 BC
	NS	LSD: 88.15

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

Table: 2.Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on macronutrient of Soybean planted soil

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

Table: 3 Effect of Maize leaf and root aqueous extracts (fresh and oven dried) on micronutrient of Soybean planted soil

	Micronutrients						
Treatment	Fe (ppm)	Cu (ppm)	Cr (ppm)	Co (ppm)	Zn (ppm)	Mn (ppm)	Ni (ppm)
C	0.4915cd	0.049cd	0.005 b	0.051 b	0.216 b	0.419 c	0.0295 c
MCL (F)	0.753abc	0.062abcd	0.017 b	0.075 a	0.243 b	0.745abc	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)	0.691abcd	0.068abcd	0.015 b	0.078 a	0.2885ab	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	0.063abcd	0.006 b	0.075 a	0.282 ab	0.529 bc	0.079 ab
MDR (F)	0.673abcd	0.073 abc	0.009 b	0.0805 a	0.2405 b	0.673abc	0.068 ab
MCR(OD)	0.5275bcd	0.045 d	0.007 b	0.079 a	0.2215 b	0.639abc	0.06 ab
MDR(OD)	0.65abcd	0.056 bcd	0.007 b	0.069 ab	0.2225 b	0.639abc	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

Table: 4 Effect of Soybean leaf and root aqueous extracts (fresh and oven dried) on Soil pH and soil EC of maize seedling

Treatment	Soil PH	Soil EC (dsm^{-1})
Control	7.233 A	50.00 AB
SCL(F)	6.933 AB	50.00 AB
SDL(F)	6.500 BC	36.67 B
SCL(OD)	7.133 A	43.33 B
SDL(OD)	6.233 C	43.33 B
SCR(F)	6.867 AB	43.33 B
SDR(F)	6.900 AB	53.33 AB
SCR(OD)	7.100 A	66.67 A
SDR(OD)	6.467 BC	50.00 AB
	LSD: 0.4571	LSD: 17.47

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

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant

Table: 5 Effect of soybean leaf and root aqueous extracts (fresh and oven dried) on macronutrient of maize planted soil

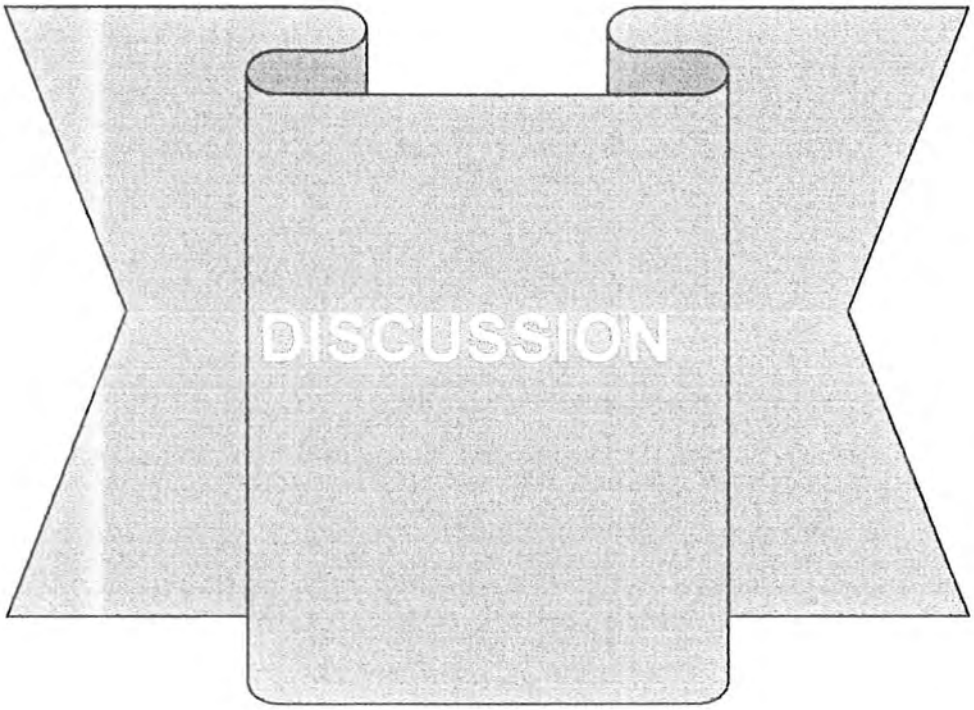
	Macronutrients				
Treatment	P (ppm)	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
C	0.241 b	5.7765 c	6.0665 c	36.087 c	0.059 abc
SCL (F)	0.4225 ab	7.7525 ab	7.7595ab	38.294 bc	0.067 abc
SDL (F)	0.564 a	8.628 ab	8.745 ab	43.332 a	0.011 c
SCL(OD)	0.291 b	8.123 ab	8.304 ab	41.084 ab	0.058 abc
SDL(OD)	0.5625 a	9.305 a	9.276 a	43.036 a	0.082 ab
SCR (F)	0.4175ab	7.2065 bc	7.5225 bc	40.816 ab	0.079 ab
SDR (F)	0.355 ab	7.992 ab	7.7735ab	41.521 ab	0.108 a
SCR(OD)	0.254 b	7.0705 bc	7.62 abc	37.679 bc	0.035 bc
SDR(OD)	0.3065 b	7.5695abc	8.2715 ab	38.675 bc	0.086 ab
	LSD: 0.19	LSD: 0.0532	LSD: 0.0376	LSD: 3.656	LSD:1.548

Table: 6 Effect of soybean leaf and root aqueous extracts (fresh and oven dried) on micronutrient of maize planted soil

	Micronutrients						
Treatment	Fe (ppm)	Cu (ppm)	Cr (ppm)	Co (ppm)	Zn (ppm)	Mn (ppm)	Ni (ppm)
C	0.417 c	0.011 c	0.046 d	0.065 bcd	0.209 c	0.059 abc	0.025 b
SCL (F)	0.488 bc	0.051 b	0.069 abc	0.065 bcd	0.3525 c	0.067 abc	0.066 ab
SDL (F)	0.7625 ab	0.081 a	0.087 a	0.079 abc	0.746 ab	0.011 c	0.062 ab
SCL(OD)	0.846 a	0.041 b	0.077 abc	0.075 bc	0.484 bc	0.058 abc	0.075 a
SDL(OD)	0.745 ab	0.067 ab	0.079 ab	0.078 abc	0.7945 a	0.082 ab	0.061 ab
SCR (F)	0.589 abc	0.05 b	0.061bcd	0.076 bc	0.435 c	0.079 ab	0.047 ab
SDR (F)	0.583 abc	0.057 ab	0.0655bcd	0.085 ab	0.2655 c	0.108 a	0.067 a
SCR(OD)	0.632abc	0.05 b	0.057 cd	0.1 a	0.3115 c	0.035 bc	0.044 ab
SDR(OD)	0.63abc	0.06 ab	0.063bcd	0.059 cd	0.2915 c	0.086 ab	0.052 ab
	LSD: 0.283	LSD:0.027	LSD:0.0196	LSD: 0.0217	LSD: 0.297	LSD: 0.0532	LSD:1.71248

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

C-Control
SCL (F) – Fresh leaf extract from control soybean plant
SDL (F) – Fresh leaf extract from drought stressed soybean plant
SCL (OD) – Dried leaf extract from control soybean plant
SDL (OD) – Dried leaf extract from drought stressed soybean plant
SCR (F) – Fresh root extract from control soybean plant
SDR (F) – Fresh root extract from drought stressed soybean plant
SCR (OD) – Dried root extract from control soybean plant
SDR (OD) – Dried root extract from drought stressed soybean plant


DISCUSSION

DISCUSSION

Experiment No.1

4. Effect of Maize and Soybean fresh and oven dried extracts on Germination (%) and seedling growth of each other

4.1 Effect of maize extracts on soybean germination (%) and seedling growth

4.1.1 Effect of maize and soybean extracts on seed germination (%)

The results indicate that aqueous extracts from leaves and roots of maize and soybean show inhibitory influence on each other. It was found that maximum inhibition of seed germination was caused by leaves extracts of both maize and soybean on each other. The results found in this study are in consistent with those of Turk and Tawaha (2003), who reported that leaf extracts of black mustard (*Brassica nigra* L.) exhibited the greatest inhibition as compared to other plant parts.

The extracts prepared from drought subjected plants were inhibitorier to seed germination as compared to extracts prepared from unstressed plants. It has been reported earlier that plants growing under stress conditions produce higher concentrations of allelochemicals (Einhelling 1996). This may be due to increased sensitivity of target plants to allelochemicals under stressful conditions (Reigosa et al., 1999). However, the extracts at higher concentrations were more effective in the inhibition of seed germination in both crop species as compared to lower concentrations. This indicated that higher concentrations of extracts of both maize and soybean were inhibitory to each other. This finding is congruent with the results of Chung and Miller (1995) who found that the degree of inhibition in seed germination increased with increasing extract concentration.

It was found that soybean leaves oven dried extract was more effective in inhibiting the seed germination in maize. These results are in agreement with previous

findings of Iman et al., (2006) who reported that soybean posses more allelochemicals as compared to maize. The results showed that 4% extracts prepared from roots of drought subjected maize and soybean plants were also effective in the inhibition of seed germination in both crop plants. The roots are generally reported to posses lesser allelopathic effects as compared to leaves but sometime it may be the reverse also (Rice, 1984). The release of allelochemicals via root exudates has been documented. Many compounds are exudated from the roots which may influence the growth of associated plants (Barnes and Putnam., 1986). Recently, the Batish et al (2007) have reported that nettle-leaved goosefoot roots and their exudates exerted allelopathic effects on wheat by releasing water soluble phenolic acids as a putative allelochemicals in the soil.

4.1.2 Effect of maize and soybean extracts on shoot and root length

The results revealed that application of 4 % extracts of both maize and soybean significantly decreased the shoot length of each other. However, the extracts prepared from drought treated plants of both maize and soybean were effective as compared to extracts of unstressed plants. However, the 4% extracts of soybean plants were more effective in decreasing shoot length of maize as compared to that of maize extracts in decreasing shoot length of soybean. The results were in agreement with previous findings of Iman et al., (2006) who found maximum reduction in shoot length of maize when supplied with leaves extracts of vegetable soybean. However, it was found that soybean leaves extracts were more effective in reducing shoot length in maize as compared to root extracts. This showed that leaves of soybean posses more allelochemicals as compared to roots (Terzi 2008).

The aqueous extracts of both maize and soybean significantly inhibited the root elongation of each other. However, maize extracts at higher concentrations were less effective in the inhibition of root length in soybean as compared to effects of soybean extracts on root length in maize. This finding is congruent with the results of Chung and Miller (1995) who found that the degree of inhibition in seed germination increased with increasing extract concentration. The negative effects of allelochemicals on root length have been reported by many workers (Turk et al, 2003; Chung et al., 1995). The

mechanism of root growth inhibition by allelopathic substances might be as a result of reducing cell division and elongation. The radical has been reported as more sensitive to allelochemicals (Turk et al. 2005).

4.1.3 Effect of maize and soybean extracts on shoot and root fresh weight

It was found that leaves extracts of both maize as well as soybean significantly decreased the shoot fresh weight in both crop plants. However, higher concentrations (4%) of extracts were more effective as compared to extracts with lower (2%) concentration. The reduction in shoot fresh weight with increasing concentrations of leaves extracts have been reported in other plants such as findings of Oyun (2006) who reported that shoot fresh weight significantly decreased in maize with application of higher concentrations of *Gliricidia* and *Acacia* leaf leachates as compared to lower concentrations. The results further revealed that extracts prepared from drought subjected plants were more effective in decreasing shoot fresh weight as compared to extracts of unstressed plants in maize and soybean. The results were in agreement with findings that plants generally produce more allelochemicals under stress conditions as compared to unstressed conditions (Einhelling 1996). The leaves extracts of soybean were more effective in decreasing shoot fresh weight in maize as compared to that of maize extracts. This showed that aqueous extracts of soybean are inhibitorier to maize as compared to that of soybean. Perhaps the fact that soybean posses more phenolics as compared to maize (Iman et al., 2006). Root extracts of soybean were also effective in decreasing shoot fresh weight in maize. However, the root extracts were less effective as compared to leaf extracts. This shows that soybean leaves posses more allelochemicals as compared to roots. Abenavoli et al (2001) have documented concentration dependant effect of coumarin on wheat root histology.

The results showed that aqueous extracts of maize and soybean significantly decreased root fresh weight of each other. However, the 4% maize leaves extracts were found as more effective in decreasing root dry weight in soybean as compared to decrease in root dry weight of maize by soybean extracts. The maize leaves extracts were found as more inhibitory than root, other workers have also confirmed higher allelopathic effect of

leaves. Higher allelopathic effect of corn shoots as compared to root indicates greater concentration of allelochemicals in corn leaves. Economou et al (2002) noticed that leaves of *Conyza albida* had more allelopathic effect than stems and roots additionally and it was reported that leaves of congress weed (Tefera, 2002) and alfalfa (Chon and Kim, 2002) had more allelopathic effect than stem and roots. Sensitivity of soybean roots to allelopathic effect could be attributed to its direct contact with the extract during bioassay. The mechanism of root growth inhibition by allelopathic substances might be as a result of reducing cell division and elongation (Iman et al., 2006).

4.1.4 Effect of maize and soybean extracts on shoot and root dry weight

The application of maize and soybean aqueous extracts prior to sowing as seed soaking significantly decreased shoot dry weight of either of the crop. However, the maize leaves extracts were more effective in decreasing the soybean shoot dry weight at 4 % concentration while the soybean leaves extracts were more effective at lower level of concentration i.e. 2 % concentration to decrease shoot dry weight of maize. The decrease in soybean shoot dry weight might be due to presence of allelochemicals in maize leaves. The occurrences of different allelochemicals which may inhibit the growth of neighboring plants at higher concentrations have been reported in maize (Noguchi et al., 2000). The soybean leaves extract at lower concentration (2%) showed more decrease in maize shoot dry weight while at higher concentration it showed lesser effects on shoot growth. The allelopathic effect is concentration and genotype specific. The lower concentration of soybean leaves extracts may be highly inhibitory to maize shoot dry weight while at higher concentrations it may be less inhibitory. The root extracts of both maize and soybean were less inhibitory to each other as compared to leaves extracts. The roots are generally reported to posses lesser allelopathic effects as compared to leaves (Rice, 1984).

It was investigated that the aqueous extracts of maize and soybean significantly decreased the root dry weights of each other. However, the leaves extracts were more effective as compared to root extracts. A number of studies have revealed that allelochemicals significantly decrease the growth of roots and ultimately the root dry

weight (Nazir et al, 2007). The soybean has been reported with five allelochemicals in leaves while maize is reported to posses three allelochemicals (Iman et al., 2006; Noguchi et al, 2000). The decrease in root dry weight of these plants may be due to presence of these allelochemicals.

4.1.5 Effect of maize and soybean extracts on root number per seedling

It was found that aqueous extracts of both maize and soybean significantly inhibited root formation in each other. However, the extracts prepared from drought treated maize and soybean plants were more effective in the inhibition of rooting. The increased secretion of allelochemicals in response to stressed conditions has been reported (Einhelling 1996). The soybean extracts were found as more effective in the inhibition of rooting in maize. The soybean has been reported with more allelochemicals as compared to maize (Iman et al., 2006). It was found that leaves extracts were more effective as compared to root extracts. The maximum decrease in rooting of maize due leaves extracts might be due more concentration of allelochemicals in leaves as compared to roots (Rice 1984). The mechanism of root growth inhibition by allelopathic substances might be as a result of reducing cell division and elongation.

Experiment No.2

4.2 Effect of Maize and Soybean fresh and oven dried extracts on physiology of each other

4.2.1 Effect of maize and soybean extracts on proline content of leaves

The results showed that application of maize and soybean leaves extracts significantly increased the proline content of each other. However, the soybean extracts were lees effective as compared to maize extracts. It was also found that maize root extracts were also effective in the accumulation of proline in soybean. However the root extracts of soybean exhibited no significant effects on proline content in maize. The results showed that extracts prepared from drought treated plants were effective as compared to extracts of unstressed plants. This indicated that maize produce allelochemicals in higher concentrations under drought stress conditions. It has been reported earlier that plants growing under stressful conditions may produce a higher concentration of allelochemicals (Einhellig, 1996). The increase in proline content in response to allelochemicals has been reported. Abdulghadar et al. (2008) reported that proline content was significantly increased in leaves of Dodder by application of heliotrope leaves extracts. The increased accumulation in proline content in response to drought and salt stresses has been reported (Shao et al. 2006; Erdei et al., 2002). But the role of proline in chemical stress is not known and needs more study.

4.2.2 Effect of maize and soybean extracts on protein content of leaves

It was found that maize leaves extracts significantly decreased the protein content of soybean leaves while the soybean extracts were not effective in decreasing the protein content of maize leaves. However, it was investigated that soybean leaves extracts which were prepared from drought treated plants were effective in decreasing soluble protein content in maize leaves. Overall, maize extracts were found as more effective than soybean extracts. These results resemble with the previous findings of Duhan et al. (1995) who revealed significant decrease in the level of soluble proteins in legume crops in response to *Acaccia nilotica* extracts. Baziramakenga et al.,(1997) concluded 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 posses three 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(Einhellig 1995; Hopkins 1999). The non-significant decrease in protein content of maize leaves in response to soybean extracts may be due to higher tolerance of maize plants to soybean extracts. Maize has been found to accumulate amino acids in response to Eucalyptus extracts (El-Darier asnd S.M 1999) which might be considered as an adaptive mechanism to increase stress tolerance induced by soybean extracts. Allelochemicals can cause growth inhibition by affecting physiological processes such as respiration, cell division, and water and nutrient uptake (Einhellig 1986). Buchholtz (1971) found deficient levels of nitrogen and potassium in corn watered with quackgrass solutions. He suggested that inhibition of nutrient uptake and, to a lesser degree, water uptake, are important allelopathic modes of action.

4.2.3 Effect of maize and soybean extracts on soluble sugars of leaves

From the results it was found that soluble sugars were significantly increased by application of soybean and maize extracts to each other. However, the soybean extracts were found as more effective in increasing the sugar content of maize, indicating that soybean posses more chemicals as compared to maize. Soybean has been reported to contain five allelochemicals while maize is reported to posses three allelochemicals (Iman et al., 2006). The higher concentration of allelochemicals present in soybean might be responsible for inducing stress in maize plants which in response secreted more soluble sugars to alleviate the adverse effects of stress. In radish increased concentration of soluble sugars in response to leaf extracts of heliotrope has been reported (Abdulghader 2008). Present findings for increased sugar concentration in leaves of maize suggests for an imposed respiratory metabolism stress as an action mechanism by soybean allelochemicals. This indicates that some respiratory enzymes probably are blocked by allelochemicals existing in soybean leaves. Similar results in the increase of soluble sugars of maize in response to leaf extracts of Acacia and Eucalyptus have been reported (Sahar et al., 2005).

4.2.4 Effect of maize and soybean extracts on chlorophyll and carotenoid content of leaves

The results showed that maize leaves extracts exhibited no significant effects on chlorophyll content of soybean. However, the soybean extracts both fresh as well oven dried significantly decreased the chlorophyll b content of maize leaves. It was found that the extracts prepared from drought subjected plants were found as more effective in decreasing the chlorophyll b content as compared to extracts of unstressed plants. This indicated that soybean produce allelochemicals in higher concentrations under drought stress conditions. It has been reported earlier that plants growing under stressful conditions may produce a higher concentration of allelochemicals (Einhellig, 1996). There are several reports that chlorophyll content of leaves decrease under stressful conditions. According to Pefiuelas & Filella (1998) chlorophylls generally decrease under stress and during senescence. The reduced chlorophyll 'b' concentration may be due to increased chlorophyllase activity (Sudhakar et al., 1997). On the whole chlorophylls of both maize and soybean were not significantly affected by extracts of each other.

It was found that carotenoid content was significantly decreased in both maize and soybean by application of aqueous extracts of each other. However, the extracts prepared from drought subjected plants were more effective in both maize and soybean as compared to extracts prepared from unstressed plants. The results further showed that soybean extracts were more effective in decreasing maize leaves carotenoid content as compared to maize extracts. Carotenoids are responsible for quenching of singlet oxygen (Knox and Dodge, 1985) and thus help in overcoming oxidative stress. The decrease in carotenoid content under stresses has been reported. El-Tayeb (2005) found that carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants. Water stress has also been reported to significantly decrease carotenoid content (Chernyad, ev 2004). It can be inferred that soybean extracts were more effective in decreasing maize leaves carotenoid content.

4.2.5 Effect of maize and soybean extracts on antioxidants activity

The results showed that application of aqueous extracts of both maize and soybean as seed soaking prior to sowing increased the antioxidant activity of each other. However, the higher extract concentrations prepared from drought subjected plants showed more profound effects on antioxidant systems of both maize and soybean. The increase in the activity of antioxidants in response to stresses has been reported in many plants. Both biotic and aboitic stresses are known to induce plants to produce reactive oxygen species (Dat et al., 2000). One of the earliest responses of plants to pathogens, wounding, drought, extremes of temperature or physical and chemical shocks is the accumulation of reactive oxygen species (ROS) such as superoxide(SOD), hydroxyl radicals, hydrogen peroxide and singlet oxygen (Jiang and Zhang 2004). It was found that leaves extracts of both the crop plants showed phytotoxity to each other. Therefore, both showed tremendous increase in antioxidants activity in response to aqueous extracts of each other. A transient oxidative burst in plant cells in response to elicitation by pathogens has been reported many times, but a correlation between allelopathic chemicals and a transient increase in ROS has been elucidated only recently (Bais et al., 2003) Using the ROS-sensitive fluorescent dye dichlorofluorescein (DCF), ROS generation was visualized in roots of *Arabidopsis thaliana* that were in direct contact with catechin, an allelochemical from the invasive weed C. maculosa (Bais et al., 2002). Within 10 s of contact with catechin (an allelochemicals), the roots of *A. thaliana* generated a wave of ROS that moved backwards from the root meristematic region, into the central elongation zone, and finally into the mature region of the root. Subsequent experiments showed that the addition of ascorbic acid along with catechin blocked the ROS response, supporting the hypothesis that increased activity of antioxidants and antioxidant enzymes is probably 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.

Application of aqueous extracts of both maize and soybean significantly increased superoxide dismutase activity (SOD) of each other. However, the leaves extracts of maize prepared from drought subjected plants were found as more effective in increasing SOD activity of soybean leaves as compared to the effect of soybean leaves extracts on maize. This indicated that soybean showed higher response to maize extracts which showed that maize extracts are more toxic to soybean. A constitutively high antioxidant capacity of soybean was seen to that particular stress of maize leaves extracts. Consequently, the mechanism that reduces reactive oxygen species (ROS) and increased SOD activity was found in soybean as compared to maize. The increase in SOD activity in response to different stresses has been reported (Tuna et al., 2008; Meloni et al., 2003; Bor et al., 2003).

The POD activity was significantly increased in both crops in response to leaves and root extracts of both soybean and maize to each other. However, the maize leaves showed more POD activity in response to soybean extracts. The increase in POD activity in response to stresses has been reported by different workers. The POD activity was increased four times as compared to control in response to salinity stress (Manchandia et al., 1999). In tolerant plant species, POD activity was found to be higher, enabling plants to protect themselves against the oxidative stress (Scalet et al., 1995) whereas, such activity was not observed in sensitive plants (peters et al.,1989). The POD has been reported to oxidize phenolic compounds (Sheen and Calvert 1969). Therefore, there occurred increased POD activity in maize leaves in response to soybean extracts which has been reported to contain five phenolic compounds (Iman et al., 2006). The exposure to these allelopathic agents significantly increased leaf peroxidase and superoxide dismutase activities in maize leaves.

The aqueous extracts of both maize and soybean prepared from drought subjected plants significantly increased Ascorbate peroxidase (APX) activity of each other. However, the Ascorbate peroxidase (APX) activity was found as higher in maize than soybean which indicated that soybean extracts are more allelopathic to maize. It showed that soybean leaves extracts created chemical stress which resulted in increased Ascorbate peroxidase (APX) activity in maize leaves. The increased activity of Ascorbate

peroxidase (APX) activity in maize leaves may be due to higher phytotoxisity of soybean on maize.

The results showed that catalase activity was significantly increased by aqueous extracts of both soybean and maize. However, maximum increase in Ascorbate peroxidase (APX) activity was found in the flag leaves of maize indicating that maize plants were more responsive to the application of soybean leaves extracts.

The increase in catalase (CAT) activity in response to both biotic and abiotic stresses has been reported. Bandursk (2002) showed that catalase (CAT) and guaiacol peroxidase increased in two oats varieties (Aramir and R567) under water stress, while Jiang and Zhang (2002) reported that in maize seedlings exposed to water stress, the activity of catalase(CAT) and other antioxidant enzymes increased and was correlated with low levels of H_2O_2. The increased activity of catlase (CAT) in maize flag leaves may be due to higher concentration of allelochemicals present in the soybean leaves which exhibited chemical stress on maize leading to increased activity of catalase (CAT). An increase in CAT activity has also been observed in other studies on allelochemical modes of action, that is, ferulic acid increased CAT activity in maize seedlings (Devi & Prasad 1996), and benzoic acid in cucumber cotyledons (Maffei *et al.* 1999). From the results it can be inferred that soybean posses some allelochemicals which might have increased the catalase (CAT) activity of maize. The maize is resistant to soybean to allelochemicals extracted by soybean.

4.2.6 Effect of maize and soybean extracts on soil pH, EC and nutrients

The results showed that maize and soybean leaves aqueous extracts significantly decreased the soil macro and micro nutrients availability of each other. The root extracts were found as less effective. The results further revealed that soybean extracts prepared from drought treated plants were more effective. These results resemble with previous findings of Einhellig (1996) who reported that plants growing under stressful conditions produce a higher concentration of allelochemicals as compared to unstressed conditions. The effect of allelochemicals on nutrient uptake can be found in many studies. Kamal and Bano (2008) reported that sunflower aqueous extracts significantly reduced the soil

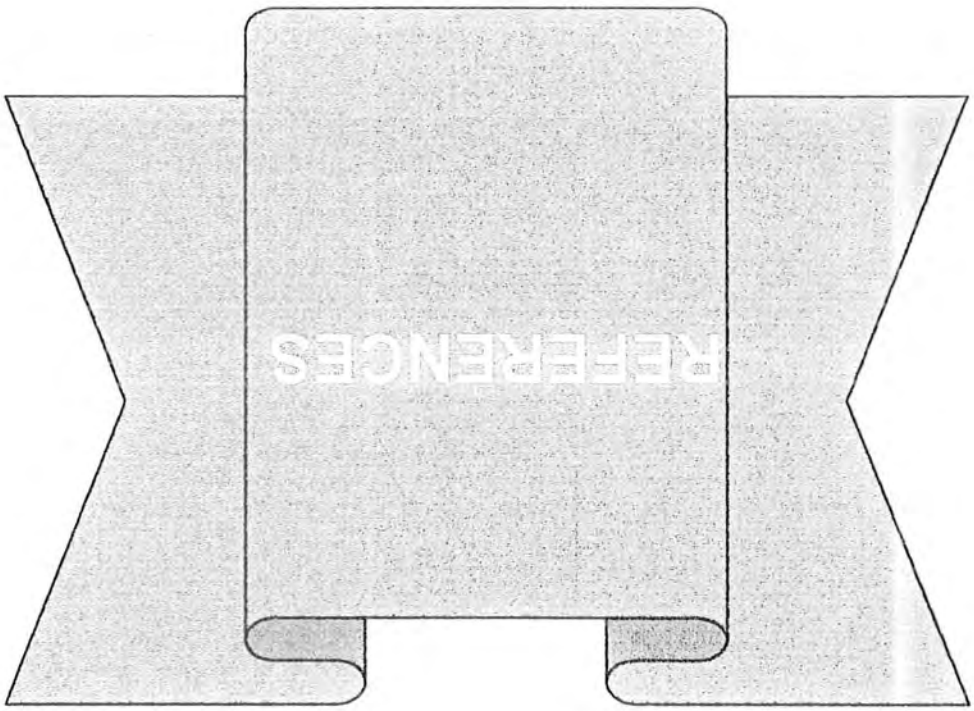
macro and micro nutrients availability to wheat plants. Mineral uptake of allelochemicals can alter the rate at which ions are absorbed by the plants. Soybean has been found to posses more allelochemicals as compared to maize (Iman et al., 2006). The efficient decrease in the availability of soil nutrients by soybean leaves extracts to maize might be due to this higher concentration of phenolics present in the soybean leaves. 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). It was found that soil pH and electric conductivity (EC) was decreased by aqueous extracts of both maize and soybean. However, the soybean leaves extracts were found as more effective in decreasing soil pH and EC. Kamal and Bano (2008) have reported that pH and E.C of wheat cultivated soil was significantly decreased when aqueous extracts of sunflower were applied.

4.2.7 Effect of maize and soybean extracts on endogenous ABA content

It was found that ABA content was increased by aqueous extracts of both maize and soybean. However, the ABA content of maize was found as more pronounced in response to application of soybean leaves aqueous extracts. The results further revealed that soybean leaves extracts prepared from drought treated plants were more effective in increasing the maize leaves ABA content. The increase in ABA content in response to allelochemicals has been reported by many workers. The Yang et al. (2008) found that ABA content of rice was significantly increased in response to application of *Ageratin adenophora* aqueous extracts. The soybean has been reported to posses ferulic acid an allelochemical (Iman et al., 2006). It has been found that ferulic acid activates the synthesis of ABA in plants (Hollapa and Blum 1991). The increase in ABA content of maize leaves might be due to presence of ferulic acid in the leaves of soybean.

Conclusion

It is inferred form the results that both maize and soybean have allelopathic effects on each other. However, the soybean leaves extracts prepared from drought subjected plants significantly increased the proline accumulation, endogenous ABA content and antioxidant activity of maize indicating that allelopathic potential of soybean is more pronounced under drought stress than maize. The allelopathic potential of roots was found lower than leaves. It is inferred that allelopathic potential of soybean is greater than maize, which is further augmented under drought stress conditions. The extracts of both plants affected the soil pH, EC and nutrient availability when applied on each other. Further research must be carried out to account the allelopathic potential of these plants on rhizosphere microbes also as well as other succeeding crops e g. wheat etc. Identification of allelochemicals of these crops must be evaluated.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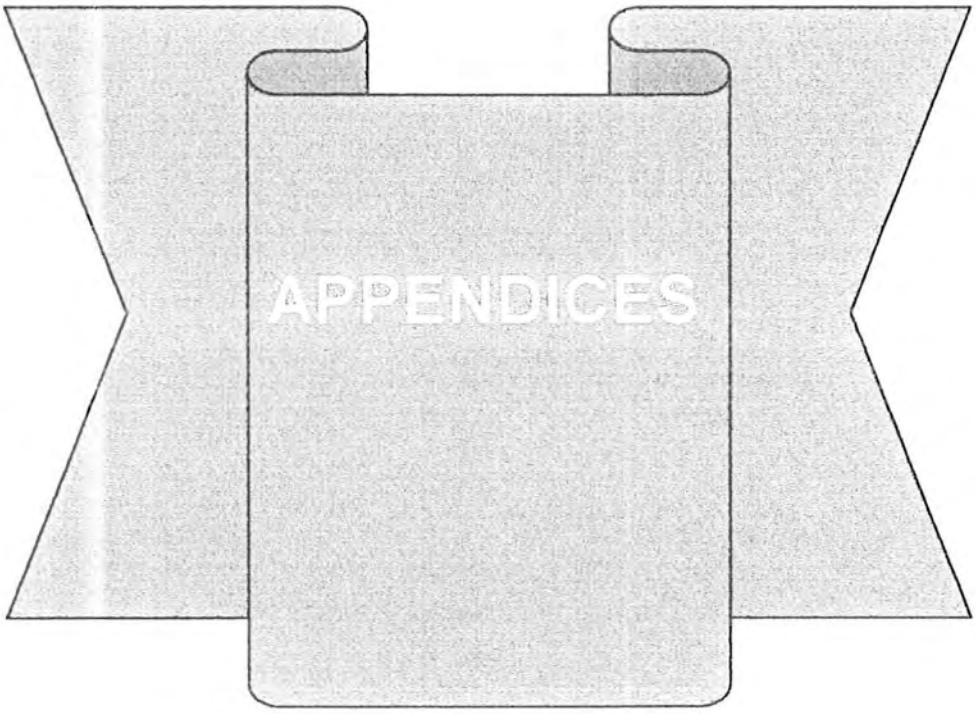
APPENDICES

Soil Analysis Reagents

The soil nitrogen content and total extractable P was determined by the method described by Soltanpour (1985).

Metal con.µg/g= metal con.(ppm)×final vol.of solution/sample wt

Determination of P content

Preparation of mixed reagents

Mixed reagent was prepared by mixing 1L of 5 NH_2SO_4 with 1L distilled water containing potassium nitrate (0.2908 g).

Working color reagent

For the preparation of working color reagent, 0.74 of ascorbic acid was dissolved in 140 ml of mixed reagent.

Preparation of standard solutions

KH2PO4 solution (100 ppm) was prepared by dilution stock (100 ppm) solution. From this (100 ppm), 0, 0.5, 1, 1.5, 2, 2.5, and 3.0 ppm solutions were prepared.

Sample preparation

Samples along with standard were prepared as follows: 1 ml of sample (or standards solution), 9.0 ml of distilled water and 2.5 ml of working color reagent (color reagent + ascorbic acid) were mixed and analyzed after 15 to 20 minutes on Spectronic 21 at 880 nm.

Determination of Na^+, K^+, Ca^{++} and Mg^{++} ions

REAGENTS

i. Lanthanum diluting solution

Lanthanum oxide (La_2O_3; 5, 9 g) was dissolved in 20 ml distilled water in 500 ml flask and placed in cold water bath. Concentrated HCl (10.5 ml) and HNO_3, (14 ml) were added to 100 ml flask containing lanthanum oxide. The final volume was diluted with 200 ml distilled water.

ii. High stock solutions

a. K^+ = (2000 ppm): 3.815 g KCL dissolved in 1L distilled water
b. Ca^{++} = (10.000 ppm) 24.97 g $CaCO_3$ dissolved in 1L distilled water.
c. The suspension was filtered through a medium pore size filter in 30 ml beaker.
d. Concentration of Fe^{++}, Mn^{++} and Zn^{++} were determined with Atomic Absorption Spectrophotometer (Shimadzu, AA670).

All the solutions were prepared according to Whitney (1988).

Determination of Cl^{-1} ions

Reagents

A. Potassium chromate, 5 percent solution.

Dissolved 5 g of potassium chromate in 50 ml of distilled water and added 1 N Silver nitrate drop wise until a slight permanent red precipitate was produced. Filtered and diluted to 100 ml.

B. Silver nitrate, 0.005 N solutions.

O.8495 g silver nitrate was dissolved in water and diluted to 1L with distilled water. The solution was kept in brown bottle away from light.

Protein Analysis Reagents

Following reagents were prepared for the determination of protein content determination.

Phosphate Buffer (Stock solution)

Monobasic sodium phosphate

27.6 was dissolved in distilled water (1000ml)

Dibasic sodium phosphate

53.6g was dissolved in 1000ml distilled water.

Monobasic sodium phosphate (16ml) and dibasic sodium phosphate (84ml) was mixed together to obtain the desired pH (7.5).

Reagent A

2.0g sodium carbonate (Na_2CO_3)

0.4 g NaOH (0.1N) and 1g Na-K tart rate was dissolved in 100 ml of distilled water.

Reagent B

The Cu SO_4.5H2O (0.5) dissolved in 100ml of distilled water.

Reagent C

Solution A (50) and solution B (1ml) were mixed together.

Reagent D

Folin phenol reagent was diluted with distilled water in the ratio 1:1.

Reagents Required for SOD

Required Reagents

i. Phosphate buffer 0.05M (pH 7.0) made by dissolving 0.1M sodium dihydrogen Phosphate and Disodium hydrogen phosphate final volume being 200ml and a net molar concentration of 0.05M.

ii. Phosphate buffer 0.05M (pH 7.8) made by the same reagents with a net volume of 200ml and molarity being 0.05M.

iii. Added 1gm of PVP in 100ml of Phosphate buffer pH 7.0

iv. 27.92mg Na2EDTA + 1.4921gm Methionine (Sigma) + 49.06mg NBT dissolved all these in 100ml of pH 7.8 buffer.

v. Aliquot 10ml solution 4* was taken and diluted it to 50ml with buffer pH 7.8

vi. 2.0ml of step 5* was used in 3.0ml of buffer pH 7.0 for blank

vii. 12 micromolar Riboflavin (sigma) in buffer pH 7.8 (prepare freshly). For it weigh 1.13mg Riboflavin and dissolved in 100ml of buffer having pH 7.8

viii. 20ml of solution of step 6* was diluted to 50ml with buffer pH 7.8

ix. 0.5ml of step 8* was taken and dissolved it again in 3ml of buffer pH 7.8 + 0.5ml Enzyme extract.

Appendix

Appendix.1. Analysis of variance for Germination % at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	29.630	3.704	3.333	0.0161
Within	18	20.000	1.111		
Total	26	49.630			

Coefficient of Variation = 18.48%

Appendix.2 Analysis of variance for Germination % at Level 4% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	38.296	4.787	5.170	0.0018
Within	18	16.667	0.926		
Total	26	54.963			

Coefficient of Variation = 19.10%

Appendix.3 Analysis of variance for shoot length at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	157.852	19.731	1.818	0.1392
Within	18	195.333	10.852		
Total	26	353.185			

Coefficient of Variation = 20.26%

Appendix.4 Analysis of variance for root length at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	191.185	23.898	6.326	0.0006
Within	18	68.000	3.778		
Total	26	259.185			

Coefficient of Variation = 18.10%

Appendix.5 Analysis of variance for shoot length at Level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	118.519	14.815	2.721	0.0370
Within	18	98.000	5.444		
Total	26	216.519			

Coefficient of Variation = 17.17%

Appendix.6 Analysis of variance for root length at Level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	100.574	12.572	4.955	0.0023
Within	18	45.667	2.537		
Total	26	146.241			

Coefficient of Variation = 18.78%

Appendix.7 Analysis of variance for shoot fresh weight at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.277	0.035	61.134	0.0000
Within	18	0.010	0.001		
Total	26	0.287			

Coefficient of Variation = 3.18%

Appendix.8 Analysis of variance for root fresh weight at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.049	0.006	23.014	0.0000
Within	18	0.005	0.000		
Total	26	0.054			

Coefficient of Variation = 7.79%

Appendix.9 Analysis of variance for shoot fresh weight at Level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.431	0.054	39.202	0.0000
Within	18	0.025	0.001		
Total	26	0.456			

Coefficient of Variation = 5.41%

Appendix.10. Analysis of variance for root fresh weight at Level 4%extract in soybean subjected to different treatments of maize extract

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.103	0.013	16.847	0.0000
Within	18	0.014	0.001		
Total	26	0.117			

Coefficient of Variation = 15.60%

Appendix.11. Analysis of variance for shoot dry weight at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.184	0.023	25.087	0.0000
Within	18	0.016	0.023		
Total	26	0.200			

Coefficient of Variation = 19.87%

Appendix.12. Analysis of variance for root dry weight at Level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.386	0.048	40.989	0.0000
Within	18	0.021	0.001		
Total	26	0.407			

Coefficient of Variation = 55.49%

Appendix.13. Analysis of variance for shoot dry weight at Level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.796	0.100	124.983	0.0000
Within	18	0.014	0.001		
Total	26	0.811			

Coefficient of Variation = 15.45%

Appendix.14. Analysis of variance for root dry weight at Level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.001	0.000	2.579	0.0453
Within	18	0.001	0.000		
Total	26	0.003			

Coefficient of Variation = 48.19%

Appendix.15. Analysis of variance for number of roots per plant at level 2% extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	9.185	1.148	2.583	0.0450
Within	18	8.000	0.444		
Total	26	17.185			

Coefficient of Variation = 12.68%

Appendix.16. Analysis of variance for number of roots per plant at level 4%extract in soybean subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	11.333	1.417	2.942	0.0272
Within	18	8.667	0.481		
Total	26	20.000			

Coefficient of Variation = 14.87%

Appendix.17. Analysis of variance for Germination % at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	103.407	12.926	6.843	0.0004
Within	18	34.000	1.889		
Total	26	137.407			

Coefficient of Variation = 26.70%

Appendix.18. Analysis of variance for Germination % at Level 4% extract in maize subjected to different treatments of soybean extract

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	77.333	9.667	3.729	0.0097
Within	18	46.667	2.593		
Total	26	124.000			

Coefficient of Variation = 34.50%

Appendix.19. Analysis of variance for shoot length at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	217.185	27.148	12.217	0.0000
Within	18	40.000	2.222		
Total	26	257.185			

Coefficient of Variation = 8.16%

Appendix.20. Analysis of variance for root length at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	302.000	37.750	4.127	0.0060
Within	18	164.667	9.148		
Total	26	466.667			

Coefficient of Variation = 18.39%

Appendix.21. Analysis of variance for shoot length at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	286.074	35.759	6.568	0.0005
Within	18	98.000	5.444		
Total	26	384.074			

Coefficient of Variation = 13.88%

Appendix.22. Analysis of variance for root length at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	320.667	40.083	3.192	0.0194
Within	18	226.000	12.556		
Total	26	546.667			

Coefficient of Variation = 22.78%

Appendix.23. Analysis of variance for shoot fresh weight at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.535	0.067	17.314	0.0000
Within	18	0.069	0.004		
Total	26	0.604			

Coefficient of Variation = 10.12%

Appendix.24. Analysis of variance for root fresh weight at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.229	0.029	6.514	0.0005
Within	18	0.079	0.004		
Total	26	0.307			

Coefficient of Variation = 13.86%

Appendix.25. Analysis of variance for shoot fresh weight at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.269	0.034	5.244	0.0017
Within	18	0.116	0.006		
Total	26	0.385			

Coefficient of Variation = 15.10%

Appendix.26. Analysis of variance for root fresh weight at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.101	0.013	4.045	0.0066
Within	18	0.056	0.003		
Total	26	0.158			

Coefficient of Variation = 12.34%

Appendix.27. Analysis of variance for shoot dry weight at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.302	0.038	6.894	0.0003
Within	18	0.099	0.005		
Total	26	0.400			

Coefficient of Variation = 14.34%

Appendix.28. Analysis of variance for root dry weight at Level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.294	0.037	5.233	0.0017
Within	18	0.127	0.007		
Total	26	0.421			

Coefficient of Variation = 20.12%

Appendix.29. Analysis of variance for shoot dry weight at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.288	0.036	8.806	0.0001
Within	18	0.074	0.004		
Total	26	0.362			

Coefficient of Variation = 14.95%

Appendix.30. Analysis of variance for root dry weight at Level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.075	0.009	4.519	0.0038
Within	18	0.038	0.002		
Total	26	0.113			

Coefficient of Variation = 12.43%

Appendix.31. Analysis of variance for number of roots per plant at level 2% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	24.741	3.093	2.386	0.0599
Within	18	23.333	1.296		
Total	26	48.074			

Coefficient of Variation = 19.58%

Appendix.32. Analysis of variance for number of roots per plant at level 4% extract in maize subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	45.852	5.731	4.689	0.0031
Within	18	22.000	1.222		
Total	26	67.852			

Coefficient of Variation = 22.44%

Appendix.32. Analysis of variance for Prolin content of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.842	0.105	5.920	0.0009
Within	18	0.320	0.018		
Total	26	1.162			

Coefficient of Variation = 30.24%

Appendix.34. Analysis of variance for Protein content of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	6324.905	790.613	10.616	0.0000
Within	18	1340.560	74.476		
Total	26	7665.464			

Coefficient of Variation = 12.04%

Appendix.35. Analysis of variance for Sugar content of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	41055.566	5131.946	9.614	0.0000
Within	18	9607.897	533.772		
Total	26	50663.463			

Coefficient of Variation = 11.07%

Appendix.36. Analysis of variance for chlorophyll "a" of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.263	0.033	1.199	0.3531
Within	18	0.493	0.027		
Total	26	0.756			

Coefficient of Variation = 81.99%

Appendix.37. Analysis of variance for chlorophyll "b" of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.179	0.022	2.497	0.0509
Within	18	0.161	0.009		
Total	26	0.341			

Coefficient of Variation = 96.66%

Appendix.38. Analysis of variance for caroteniod content of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	60.045	7.506	4.476	0.0040
Within	18	30.186	1.677		
Total	26	90.231			

Coefficient of Variation = 22.97%

Appendix.39. Analysis of variance for POD of soybean plant subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.505	0.063	4.325	0.0047
Within	18	0.263	0.015		
Total	26	0.767			

Coefficient of Variation = 27.77%

Table: 43. Analysis of variance for ABA content of Soybean planted subjected to different treatments of maize extract

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	3308.797621	827.19941	19.599687	.0029
Within	5	211.0236221	42.204724		
Total	9	3519.821243			

Coefficient of Variation = 5.0382639%

Appendix.44. Analysis of variance for pH of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	2.821	0.353	4.958	0.0023
Within	18	1.280	0.071		
Total	26	4.101			

Coefficient of Variation = 3.91%

Appendix.45. Analysis of variance for EC of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	2340.741	292.593	2.821	0.0322
Within	18	1866.667	103.704		
Total	26	4207.407			

Coefficient of Variation = 19.64%

Appendix.46. Analysis of variance for P of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.0083586	0.0083586	4.2936988	.0217
Within	9	0.0175205	0.0019467		
Total	17	0.084389611			

Coefficient of Variation 16.002219%

Appendix.47. Analysis of variance for Na of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	19.912278	2.4890348	5.3901416	.0105
Within	9	4.1559785	0.4617754		
Total	17	24.0682565			

Coefficient of Variation = 8.0499979%

Appendix.48. Analysis of variance for K of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	15.41370778	1.9267135	3.8072037	.0313
Within	9	4.5546345	0.5060705		
Total	17	19.96834228			

Coefficient of Variation = 9.6818723%

Appendix.49. Analysis of variance for Ca of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sumof Squares	Mean Square	F-value	Prob.
Between	8	136.2926751	17.036584	5.3201202	.0109
Within	9	28.820638	3.2022931		
Total	17	165.1133131			

Coefficient of Variation = 5.0107982%

Appendix.50. Analysis of variance for Mg of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.079237618	0.0099047	4.2902592	.0218
Within	9	0.02077784	0.0023086		
Total	17	0.100015458			

Coefficient of Variation = 10.429182%

Appendix.51. Analysis of variance for Fe of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.327934778	0.0409918	3.3675476	.0445
Within	9	0.1095535	0.0121726		
Total	17	0.437488278			

Coefficient of Variation = 16.828506%

Appendix.52. Analysis of variance for Cu of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.003261778	4.0772	3.3851476	.0439
Within	9	0.001084	1.2044		
Total	17	0.004345778			

Coefficient of Variation = 16.85537%

Appendix.53. Analysis of variance for Cr of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.002541778	3.1772	3.7040155	3.7040155
Within	9	7.72	8.5778		
Total	17	0.003313778			

Coefficient of Variation = 62.204973%

Appendix.54. Analysis of variance for Co of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.001706778	2.1335e	3.1711396	.0526
Within	9	6.055e	6.7278e		
Total	17	0.002312278			

Coefficient of Variation = 10.87999%

Appendix.55. Analysis of variance for Zn of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.046557111	0.0058196	4.626717	.0172
Within	9	0.0113205	0.0012578		
Total	17	0.057877611			

Coefficient of Variation = 13.197995%

Appendix.56. Analysis of variance for Mn of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.366599111	0.0458249	2.6052329	.0877
Within	9	0.158306	0.0175896		
Total	17	0.524905111			

Coefficient of Variation = 19.355126%

Appendix.57. Analysis of variance for Ni of soybean plant soil subjected to different treatments of maize extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.005317	6.6463e	3.9850933	.0273
Within	9	0.001501	1.6678e		
Total	17	0.006818			

Coefficient of Variation = 19.371371%

Appendix.58. Analysis of variance for Prolin content of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	618394.236	77299.280	7.071	0.0003
Within	18	196772.937	10931.830		
Total	26	815167.173			

Coefficient of Variation = 20.10%

Appendix59. Analysis of variance for Protein content of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	3432.725	429.091	3.599	0.0114
Within	18	2146.182	119.232		
Total	26	5578.907			

Coefficient of Variation = 14.71%

Appendix.60. Analysis of variance for Sugar content of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	31498.839	3937.355	11.221	0.0000
Within	18	6315.968	350.887		
Total	26	37814.807			

Coefficient of Variation = 8.37%

Appendix.61. Analysis of variance for chlorophyll "a" of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.168	0.021	1.686	0.1700
Within	18	0.225	0.012		
Total	26	0.393			

Coefficient of Variation = 58.64%

Appendix.62. Analysis of variance for chlorophyll "b" of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.095	0.012	10.355	0.0000
Within	18	0.021	0.001		
Total	26	0.116			

Coefficient of Variation = 50.38%

Appendix.63. Analysis of variance for caroteniod content of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	34.754	4.344	5.489	0.0013
Within	18	14.247	0.791		
Total	26	49.001			

Coefficient of Variation = 16.87%

Appendix.67. Analysis of variance for CAT of maize plant subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	7.270	0.909	2.354	0.0627
Within	18	6.950	0.386		
Total	26	14.220			

Coefficient of Variation = 42.84%

Table: 68. Analysis of variance ABA content of maize planted subjected to different treatments of soybean extract

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	4	2839.911972	709.97799	54.824062	.0003
Within	5	64.7505825	12.950117		
Total	9	2904.662554			

Coefficient of Variation = 2.7593384%

Appendix.69. Analysis of variance for pH of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	5.036	0.630	1.846	0.1335
Within	18	6.140	0.341		
Total	26	11.176			

Coefficient of Variation = 9.03%

Appendix.70. Analysis of variance for EC of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	67666.667	8458.333	3.203	0.0192
Within	18	47533.333	2640.741		
Total	26	115200.000			

Coefficient of Variation = 24.87%

Appendix.71. Analysis of variance for P of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.239032	0.029879	3.9042773	.0290
Within	9	0.068876	0.0076529		
Total	17	0.307908			

Coefficient of Variation = 23.06172%

Appendix.72. Analysis of variance for Na of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	16.11831711	2.0147896	3.5157988	.0394
Within	9	5.1576065	0.5730674		
Total	17	21.27592361			

Coefficient of Variation = 9.8138373%

Appendix.73. Analysis of variance for K of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	13.04136	1.63017	3.4778984	.0406
Within	9	4.2185045	0.4687227		
Total	17	17.2598645			

Coefficient of Variation = 8.6372709%

Appendix.74. Analysis of variance for Ca of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	99.61607111	12.452009	4.7666233	.0157
Within	9	23.511	2.6123333		
Total	17	123.1270711			

Coefficient of Variation = 4.0348057%

Appendix.75. Analysis of variance for Mg of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.01336	0.00167	3.0144404	.0603
Within	9	0.004986	5.54e		
Total	17	0.018346			

Coefficient of Variation = 36.211084%

Appendix.76. Analysis of variance for Fe of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.31096	0.03887	2.467336	.1002
Within	9	0.1417845	0.0157538		
Total	17	0.4527445			

Coefficient of Variation = 20.184552%

Appendix.77. Analysis of variance for Cu of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.005932	7.415e	5.1652477	.0121
Within	9	0.001292	1.4356e		
Total	17	0.007224			

Coefficient of Variation = 23.041283%

Appendix.78. Analysis of variance for Cr of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.002486	3.1075e	4.0978022	.0251
Within	9	6.825e	7.5833e		
Total	17	0.0031685			

Coefficient of Variation = 12.965112%

Appendix.79. Analysis of variance for Co of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.003649778	4.5622e	4.9232614	.0141
Within	9	8.34e	9.2667e		
Total	17	0.004483778			

Coefficient of Variation = 13.028147%

Appendix.80. Analysis of variance for Zn of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.701494	0.0876867	5.0657839	.0129
Within	9	0.1557865	0.0173096		
Total	17	0.8572805			

Coefficient of Variation = 30.443346%

Appendix.81. Analysis of variance for Mn of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.192653	0.0240816	4.6986499	.0164
Within	9	0.046127	0.0051252		
Total	17	0.23878			

Coefficient of Variation = 13.000725%

Appendix.82. Analysis of variance for Ni of maize plant soil subjected to different treatments of soybean extract.

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	0.003684444	4.6056e	1.6646586	.0164
Within	9	0.00249	2.7667e		
Total	17	0.006174444			

Coefficient of Variation = 29.99994%