United Journal of Agricultural Science and Research

Research Article

Foliar Application of AllelopathicExtract to Study Morpho-Physiological Traits and Antioxidative Response of *Zea mays* and *Viganaradiataunder* Saline Soil

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Volume 1 Issue 2 - 2019

Received Date: 02 Aug 2019

Accepted Date: 23 Aug 2018

Published Date: 31 Aug 2018

2. Keywords

Allelopathic extract; Salinity; *Zea mays*; *Viganaradiata*; Antioxidative enzymes

1. Abstract

Allelopathy is a naturally occurring ecological phenomenon of interference among organisms that may be employed for managing weeds, insect pests and diseases in field crops. In field crops, allelopathy can be used following rotation, using cover crops, mulching and plant extracts for natural pest management. Application of allelopathic plant extracts can effectively control weeds and insect pests. However, mixtures of allelopathic water extracts are more effective than the application of single-plant extract in this regard under stress conditions. Therefore, the present study was conducted to observe the effect of different concentrations i.e. 0, 3, 5 and 10%of allelopathic extract on *Zea mays* and *Viganaradiata* under saline soil. The results revealed that the concentration of allelopathic extract significant improve plumule length, radicle length, total fresh and dry biomass, total chlorophyll and carotenoid contents. However, *Zea mays* showed better growth, biomass and chlorophyll contents than *Viganaradiata*. Moreover, high concentration of salinity causes oxidative damage to the tissues of *Zea mays* and *Viganaradiata*. It was noticed that the contents of malondialdehyde (MDA), proline and activity of superoxidase dismutase (SOD) and peroxidase (POD) were significantly increased in the plants grown under saline conditions but applications of allelopathy reduced the antioxidative activity of enzymes. These results suggested that *Zea mays*showed better growth and development than *Viganaradiata* when grown under saline condition with the application of allelopathic extract. Hence the application of allelopathic extract might be a useful indicator for the plants grown under salinity.

3. Introduction

Allelopathy has been broadly defined as the direct or indirect negative effect of one plant or microorganism on another, although beneficial interactions have also been considered allelopathic[1]. Allelopathy is a phenomenon whereby secondary metabolites synthesized by fungi, viruses, microorganisms and plants influence biological and agricultural systems, which may be either stimulatory or inhibitory [2]. The word allelopathy is derived from two Greek words: 'allelon', meaning 'of each other', and 'pathos', meaning 'to suffer'[3]. This ancient concept was known to classical researchers in the Greek and Roman era [4]. Detrimental effects of crop plants on other plants were observed by Theophrastus and by Pliny II while De Candolle considered allelopathy to be soil sickness. The term 'allelopathy' was first used by Austrian plant physiologist Molisch, who defined it as the chemical interaction among plants and microorganisms [5]. With respect to phytoplankton, the term allelopathy is specifically applied to the inhibitory effects of secondary metabolites produced by one species on the growth or physiological function of another species. The exudation of secondary metabolites allows the pro-

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Citation: Rasool SJ, Foliar Application of AllelopathicExtract to Study Morpho-Physiological Traits and Antioxidative Response of *Zea mays* and *Viganaradiataunder* Saline Soil. United Journal of Agricultural Science and Research. 2019; 1(2): 1-9.

ducer of the allelopathic chemical(s) to monopolize limiting resources (e.g., nutrients, light) for its own population growth [6].

Salinity is one of the most serious problems limiting plant growth and productivity. Approximately 6% of the earth's land area (800 million hectares) is affected by either salinity or the associated condition of sodicity[7]. Salinity is one of the limiting factors in agricultural production of all crops in the world specifically in cereals crops. Salinity is a serious abiotic stress factor limiting crop growth and agricultural productivity [8]. According to UNEP, approximately 50% of world area is affected by salinity [9]. Increasing level of salt stress reduced the plant fresh and dry weights, germination rate plant length, root dry weight, rate of photosynthesis, lipids and energy production [10]. Though the melatonin was discovered in earlier 1993-94 but a potential role in plants of various metabolic processes as a hormone was confirmed much later [11]. In plants, it is formed in very complicated way from N-acetyl serotonin [12]. Salinity is an event where soluble salts are washed and mixed into groundwater and come to the surface of the soil with high base water followed by the accumulation of these salts on the soil surface as a result of water being reduced through evaporation [13]. Soil salinity [14] or water salinity is one of the leading stress factors and can adversely affects plant production [15]. Salinity may occur as a result of natural factors and unsuitable agricultural practices [16] and although it causes significant product losses every year, stress factors cause about 25% loss of products per year [17].

High salt concentrations in soil cause ionic stress, hyperosmotic stress, and secondary stresses such as oxidative stress by increasing reactive oxygen species (ROS) including superoxide radicals (O– 2), hydrogen peroxides (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) [18]. Overproduction of ROS in mitochondria and chloroplasts under stress conditions has been suggested as the major contributor to oxidative damage in plant. In mitochondria, electron leakage and release of O^{-2} and H_2O_2 in respiration have been proposed. In chloroplast, many stress factors that limit CO_2 assimilation due to stomatal closure lead to a decrease in carbon reduction by the Calvin cycle and to a decrease in oxidized $NADPH^+$, which can serve as an electron acceptor in photosynthesis. Under these conditions that the availability of electron acceptors are limited to form photosystem I, oxygen can accept electrons to form O^{-2} by the Mehler reaction, which triggers chain reactions that generate H_2O_2 and $^{\cdot}OH$ [19]. These ROS are highly reactive and can alter normal cellular metabolism through the oxidation of lipids, proteins and nucleic acids [20]. To mitigate the oxidative damage induced by ROS, plants employ a variety of enzymatic and non-enzymatic antioxidant defenses [21]. Enzymatic antioxidant systems typically consist of several antioxidant enzymes that participate in the detoxification of ROS. Superoxide dismutase (SOD) is a major scavenge rof O^{-2}, and its enzymatic action results in the formation of H_2O_2 and O_2[22]. Peroxidase (POD) converts H_2O_2 to H_2O via the ascorbate-glutathione pathway. The ascorbate-glutathione pathway provides protection against oxidative stress by a series of coupled redox reactions in photosynthetic tissues, mitochondria and peroxisomes [23]. POD is the first enzyme in this pathway involved with the elimination of H_2O_2 using ascorbate as an electron donor in an oxidation-reduction reaction [24].

Zea mays more commonly referred to as maize, is a member of the grass family Poaceae, or true grasses. *Zea mays* is rich in genetic diversity at both inter and intra-varietal levels, and the genetic structure of several local maize germplasm collections are well characterized [25]. *Zea mays* is thought to have originated 55–70 million years ago in what is now central or South America and has since diversified into nearly 10,000 nondomestic relatives [26]. There exists no direct ancestor for *Zea mays*; however, to date, the closest relative to maize are the teosintes [27]. Prehistoric selection has resulted in ears lacking seed cases called glumes and seeds that adhere to the cob until manual removal. These alterations limit the ability of *Zea mays* to survive without human intervention. *Zea mays* is an annual plant with C4 metabolism, making it very efficient at carbon fixation. It has the greatest global production of any crop species around 800 million tonnes was produced worldwide in 2013, accounting for 32% of the total cereal production. The top three producers include the United States, China, and Brazil. *Zea mays* is grown on more areas of the planet than any other crop and is grown on every continent except Antarctica. The grain of *Zea mays* is used for food, feed, and industrial products including biodegradable foams, plastics, and adhesives [28]. Additionally, maize stover, the leaves and stalk of the *Zea mays* plant, is used for forage, biofuel production, and chemical production.

Vigana radiate(also known as moong bean, green gram and mung bean) is a fast-growing warm-season legume and has a diploid chromosome number of 2n=22 [29]. *Vigana radiate* is mainly cultivated today in China, India and Southeast Asia but can be found in dry regions within Southern Europe and United States [30]. *Vigana radiate* are a good source of dietary protein, folate and iron. This legume species was moved from the genus Phaseolus to Vigna and is correctly cites as *Vigana radiate*[31]. *Vigana radiate* [L.] R. Wilczek (mungbean or green gram) is an ancient legume crop that was domesticated in India some 3.5 million years ago. *Vigana radiate* is a versatile crop that only takes 60-65 days to harvest and one of the major edible pulse crops of India, China other countries in South and South East-Asia. It is also cultivated

and eaten in Southern Europe, the Southern USA and in semi-arid countries in Africa e.g. Kenya. The mature seeds provide an invaluable source of digestible protein, fiber, B vitamins and minerals, particularly iron, potassium, magnesium and zinc for humans (including infant supplements) in places where meat is lacking or where the population is mostly vegetarian [32]. *Vigana radiate* is not only grown for seeds but also as forage (fodder for cattle). Mungbean is a self-pollinated diploid (2n = 22) plant with the estimated genome size of 494 to 579 Mb depending on the analysed genotype [33].

The uniqueness of *Zea mays* and *Vigana radiate* due to its high biomass production and tolerance towards salinity stress environment can be valuable traits to study; however, sufficient information is not available regarding salinity stress on growth and antioxidative defense system with the application of allelopathy extract in *Zea mays* and *Vigana radiate*. Therefore, the present study was planned to investigate the effects of different concentrations of allelopathy extract on growth, lipid peroxidation, and antioxidant enzymatic activities in *Zea mays* and *Vigana radiate*. According to best of our knowledge, this study is among the few studies which focus on allelopathy applications in saline stress environment in order to investigate their suitability for stress conditions. Findings from the present study will add to our understanding the mechanism of allelopathy extract under saline condition in *Zea mays* and *Vigana radiate*.

4. Materials and Methods

4.1. Experimental Design and Growth Conditions

A pot experiment was conducted in Agriculture Department of BahauddinZakariya University, Punjab, Pakistan. Five seeds of *Zea mays* and *Vigana radiate* were used for in each pot (30-cm-tall × 40-cm-wide) on March 2018. Each treatment was arranged in a completely randomized design (CRD) with three replications. De-ionized water was added to maintain the soil moisture content at 60% (w/w) of water-holding capacity, after every 2 days. Each pot contained 10 kg soil with one plant in each pot. Natural soil used for pot experiment was taken from agricultural land of BahauddinZakariya University. The physio-chemical properties of natural soil are as follow: pH 6.2, EC 2.5 dS cm^{-1}, 24 g kg^{-1} organic matter, 26.16 mg kg^{-1} exchangeable K, 0.29 g kg^{-1} total P and 30 g kg^{-1} total N. The natural soil with non-uniform of total salinity of 0.3% to >5% that mainly consisted of chlorides and other inorganic compounds from seawater. In the soil-improved, low-salinity patch lands, some crops such as *Zea mays* and *Vigana radiate* are cultivated. The allelopathy extract was prepared by extracting the plants of Melilotusindicus from the different experimental stations of Bahawalpur. Than these samples were prepared the liquid extract of Melilotusindicus after harvesting of plants and freezed it for 24 hours. Then milled it and centrifuged it for 10 minutes at 15000 rev/minute. Different concentration of extract were prepared 1%, 3%, 5%, and 10%by diluting with water. Pots were placed in a glasshouse, where plants received natural light, with day/night temperature of 40/35 °C and day/night humidity of 50/60%. All plants were harvested in April 2018 for different morphological traits and enzymatic study.

4.2. Sampling and Data Collection

Functional leaf in each treatment was picked at a rapid growth stage before the second harvest during 09:00–10:30 a.m. The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen, and stored in a freezer at low temperature (−80 °C) for further analysis. Total fresh biomass was measured by weighing stems and leaves. Later, all plants were dried in an oven at 105 ˚C for 1 h, then at 70 ˚C for 72 h to determine their dry weight.

4.3. Determination of Chlorophyll

For the analysis of chlorophyll contents, 0.1 g of fresh leaf sample was extracted with 8 mL 95% acetone for 24 h at 4 ˚C in darkness. The absorbance was measured by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll contents were calculated by the standard method of [34] and expressed in mg g-1 fresh weight (FW).

4.4. Determination of Chlorophyll, Malondialdehyde, Proline and Antioxidant Enzyme Activities

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. 0.1 g of frozen leaves was ground at 4 ˚C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1%polyethene pyrrole (PVP). The homogenate was centrifuged at 10,000 × g at 4 ˚C for 15 min. The mixtures were heated at 100 ˚C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using spectrophotometer (xMark™ microplate absorbance spectrophotometer, BIO-RAD, USA) at the wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l $molg^{-1}$ using the following formula: 6.45 (A532 - A600) - 0.56 A450. The method was followed by [35] and expressed in mg g-1 fresh weight (FW).

Proline contents were measured by using (0.1 g) homogenate in 3% of aqueous sulphosalicylic acid and distilled water. The proline content was assessed by the technique described by [36] and expressed in mg g^{-1} fresh weight (FW).

In order to check enzymes activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0) including 0.5 mmol EDTA and 0.15 molNaCl. The homogenate was centrifuged at 12000 ×g for 10

min at 4°C and the supernatant was used for measurement of SOD and POD activities.

The SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mMnitroblue tetrazolium (NBT), 1.17 mM riboflavin, 10 mM methionine, and 100 µL enzyme extract. Finally, reading was taken by using spectrophotometer (xMarkTM micro plate absorbance spectrophotometer, BIO-RAD, USA). The method was followed by [37] and expressed in mg g^{-1} fresh weight (FW).

POD activity in leaves was estimated using the method of [38] and was assayed using guaiacol as the substrate. The reaction mixture (3 mL) contained 0.05 mL of enzyme extract, 2.75 ml of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1 % H_2O^2, and 0.1 mL of 4 %guaiacol solution. The increase in the absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme causing a change in absorbance of 0.01 per minute. The specific POD activity was expressed as U g^{-1} FW min^{-1}.

5. Statistical Analysis

All results were presented as arithmetic means with standard deviation. The data were tested with one-way ANOVA followed by HSD test to compare treatments means at ($P \leq 0.05$ or $P \leq 0.01$). Graphical work was carried out using SigmaPlot 14 software.

6. Results

6.1. Effect of AllelopathicExtract on Morphological Traits of *Zea mays* and *Viganaradiata*

Results regarding different morphological traits of *Zea mays* and *Viganaradiata* are shown in (Table 1). It was noticed that the application of allelopathic extract significantly improved plant growth and development. However, high amount of salinity in the soil is dangerous for the plants and reduced plant growth and development. 10%allelopathic extract in saline soil significant improved 44% and 37%plumule length in *Zea mays* and *Viganaradiata* respectively and 28% and 24% radicle length in *Zea mays* and *Viganaradiata* respectively when compared with the plants grown without allelopathic extract in saline soil. Fresh and dry biomass showed the same trend and noticed that fresh and dry biomass increased by 54% and 42% in *Zea mays* respectively however increased by 46% and 31% in *Viganaradiata* respectively compared with the plants grown without allelopathic extract in saline soil. These results depicted that allelopathic significantly improved plant growth and biomass under saline soil. Moreover, in comparison with two plants *Zea mays* significant showed better growth and development than *Viganaradiata* under salinity stress.

6.2. Effect of AllelopathicExtract on Chlorophyll and Carotenoid Contents of *Zea mays* and *Viganaradiata*

Results regarding with total chlorophyll and carotenoid contents are given in (Table 2). Allelopathic extract significant promote the high contents of chlorophyll and carotenoid contents i.e. photosynthetic pigments. Our results depicted that 10%allelopathic extract significantly improved chlorophyll contents by 34% and 21% in *Zea mays* and *Viganaradiata* respectively when compared with the plants grown without allelopathic extract in saline soil. In the same way 10%allelopathic extract significantly increased carotenoid contents by 19% and 14% when compared with the plants grown without allelopathic extract in saline soil. These results suggested that allelopathic extract significantly increased photosynthetic pigments under salinity stress.

6.3. Effect of AllelopathicExtract on Malondialdehyde, Proline Contents and AntioxidativeEnzymes Contents of *Zea mays* and *Viganaradiata*

Malondialdehyde (MDA), proline contents and antioxidant enzyme activities, i.e., superoxidase dismutase (SOD) and peroxidase (POD) were significantly influenced with and without allelopathic extract under salinity stress (Figure 1). Compared with 10%allelopathic extract, MDA contents were enhanced by 38% and 29% in *Zea mays* and *Viganaradiata* respectively at no allelopathic extract which suggested that salinity stress induced oxidative damage to the leaves of *Zea mays* and *Viganaradiata*. The contents of proline comes in to play to reduced salinity stress and highest contents of proline were observed in the plants where no allelopathic extract were added and increased by 64% and 50% in *Zea mays* and *Viganaradiata* respectively compared with the plants grown at 10%allelopathic extract. It was also noticed that allelopathic extract significantly reduced the antioxidative activities of SOD and POD. The enzymatic activity of SOD increased by 29% and 14% in the *Zea mays* and *Viganaradiata* respectively while increased by 64% and 42%*Zea mays* and *Viganaradiata* respectively when grown without allelopathic extract compared with the plants grown at 10%allelopathic extract in saline soil. These results depicted that the contents of MDA and proline and enzymatic activities of SOD and POD significantly increased in the plants grown without allelopathic extract in saline soil. However, application of allelopathic extract reduced salinity stress by increased the enzymatic activities.

Table1: Influence of different concentrations of allelopathy on plumule length, radicle length, total fresh weight and total dry weight under salinity stress.
Relative radiance of plastic filter used: S1A0 (Salinity level with no allelopathy), S1A0 (Salinity level with 1% allelopathy), S1A1 (Salinity level with 1% allelopathy), S1A3 (Salinity level with 3% allelopathy), S1A5 (Salinity level with 5% allelopathy) and S1A10 (Salinity level with 10% allelopathy). Letters indicate statistical differences ($P \leq 0.05$) or ($P \leq 0.01$) according to an LSD test. n = 3

Treatment		Plumule length (cm)	Radicle length (cm)	Total fresh weight (g)	Total dry weight (g)
S_1A_0	*Zea mays*	3.9±0.4 gh	3.1±0.6 f	7.2±1.6 h	4.5±0.8 e
S_1A_1		4.9±0.8 f	3.9±0.8 e	9.9±1 f	6.9±0.4 d
S_1A_3		5.9±0.8 d	4.6±1.2 cd	12.6±0.3 d	8.4±0.3 c
S_1A_5		6.7±1.1 c	5.1±0.4 b	14.8±1.3 b	10.8±0.8 b
S_1A_{10}		8.9±1.3 a	5.8±0.3 a	17.5±2.1 a	12.4±1.9 a
S_1A_0	*Vigana radiate*	3.4±0.1 hi	2.4±1 g	6.1±0.5 i	4.5±0.1 e
S_1A_1		4.2±0.6 fg	3.1±0.5 f	8.2±0.9 g	6.9±0.8 de
S_1A_3		5.1±0.9 e	3.9±0.4 e	9.7±1.3 f	8.4±0.4 c
S_1A_5		6.3±1 cd	4.6±0.3 cd	11.5±0.2 e	10.8±1.6 b
S_1A_{10}		7.5±1.3 b	5.2±1 b	13.6±1.9 c	12.4±1.9 a

Table 2: Influence of different concentrations of allelopathy on total chlorophyll and carotenoid contents under salinity stress.
Relative radiance of plastic filter used: S1A0 (Salinity level with no allelopathy), S1A0 (Salinity level with 1% allelopathy), S1A1 (Salinity level with 1% allelopathy), S1A3 (Salinity level with 3% allelopathy), S1A5 (Salinity level with 5% allelopathy) and S1A10 (Salinity level with 10% allelopathy). Letters indicate statistical differences ($P \leq 0.05$) or ($P \leq 0.01$) according to an LSD test. n = 3

Treatment		Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
S_1A_0	*Zea mays*	2.11±0.3 e	0.51±0.3 e
S_1A_1		2.29±0.5 f	0.62±0.2 f
S_1A_3		2.61±0.4 d	0.81±0.2 d
S_1A_5		2.80±0.9 bc	1.01±0.8 bc
S_1A_{10}		2.98±0.8 a	1.14±0.2 a
S_1A_0	*Vigana radiate*	1.94±0.9 f	0.47±0.4 g
S_1A_1		2.21±0.4 fe	0.58±0.4 fe
S_1A_3		2.41±0.3 e	0.74±0.4 e
S_1A_5		2.75± 0.9 c	0.95±0.5 c
S_1A_{10}		2.84±0.9 bc	1.05±0.1 b

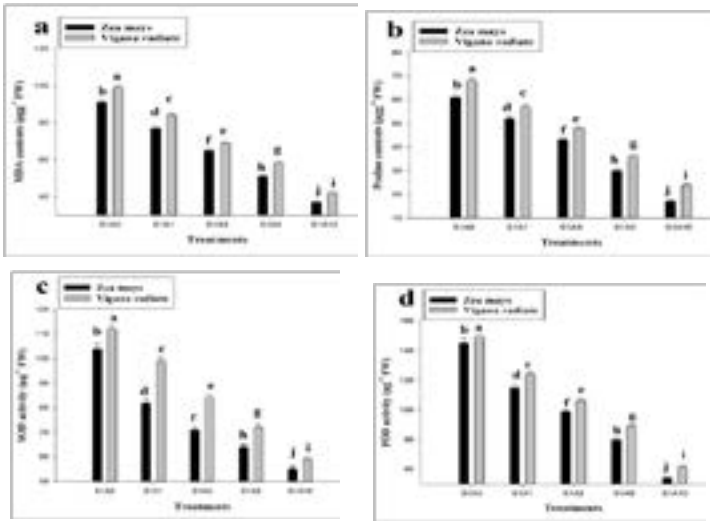

Figure 1: Influence of different concentrations of allelopathy on MDA (a), Proline (b), SOD (c) and POD (d) in *Zea mays* and *Viganaradiata*. Relative radiance of plastic filter used: S1A0 (Salinity level with no allelopathy), S1A0 (Salinity level with 1% allelopathy), S1A1 (Salinity level with 1% allelopathy), S1A3 (Salinity level with 3% allelopathy), S1A5 (Salinity level with 5% allelopathy) and S1A10 (Salinity level with 10% allelopathy). Letters indicate statistical differences ($P \leq 0.05$) or ($P \leq 0.01$) according to an LSD test. n = 3

7. Discussions

Allelopathy is the biochemical interaction of inhibition and promotion within plants or microorganisms [39]. There are three basic features of allelopathy: firstly, the object of interaction is the plants, while the interaction between plants and animals or the interaction between plants and organisms is not included. Secondly, the material of interaction is the secondary metabolites of plants, and must have the suitable way getting into the environment, but not the secondary metabolites had changes within plants. Thirdly, allelochemicals is used for influencing the growth and the development of its own or neighboring plants [40]. If it is used in chemical communication of plants (such as giving an alarm) or for polluting environment (such as volatiles and NO of some trees forming the smog), we will also not see it as the scope of allelopathy [41]. The key of allelopathy research is the release mechanism of allelochemicals, namely, why and under what conditions do the plants release allelochemicals. Allelochemicals, the secretions of plants, is the chemical substances which can affect the growth, behavior and population biology of other live beings [42], including chemical substances between plants, as well as plants and animals. In recent years, with the continuous exploration and the advances in science and technology, especially the development of the chemistry and biology, the study of allelochemicals has also been developed substantially [43]. There are many known allelochemicals: water soluble organic acid, straight-chain alcohols, aliphatic series, aldehydes, ketones, simple unsaturated lactone, long-chain fatty acids, multi-alkyne, naphthoquinone, anthraquinone acid, quinone compound, simple phenols, benzoic acid and its derivatives, cinnamic acid and its derivatives, coumarin, flavonoids, tannins, terpenoids, steroids, amino acids, peptides, alkaloids, cyanohydrin, sulfide, glucosinolates, nucleotides [44]. While phenolic acids and the terpenoid compounds are the more common types. Allelopathy has two forms, self-toxicity and allelopathy [45]. The studies have shown that rice, wheat, corn, sugar cane and other grasses, soybeans, broad beans and other legminosae, and planted forests and tea plantations have the obvious self-poisoning phenomenon. One of fully studied crops is the rice. [44]. and other researchers have found that rice stubble and straw can produce some toxic substances in the process of their decomposition, which will inhibit the growth of rice seedlings. Strongest inhibition happens at the temperature of 20-25, while when the temperature is °C >30 °C inhibition will decrease significantly over time [46]. As a result, it will reduce the productive tillers, effective heads, 1000-seed weight and output of the rice [47].

The application of allelopathic extract is focus on the interaction between higher plants and higher plants, between higher plants and microorganisms, between microbes and higher plants, and

between microbes and microorganisms. Allelopathy has a broad application prospects in increasing crop production, forest tending, plant protection, biological control, etc. The research and application of allelopathy have the great significance on the prevention of exotic invasive noxious weeds [48]. In the present study, it was noticed that application of allelopathic extract significantly improved plant growth and development under saline conditions (Table 1). However, salinity stress reduced the plant growth and development in *Zea mays* and *Viganaradiata*. Soil salinity [14] is one of the leading stress factors and can adversely affects plant production [15]. Salinity may occur as a result of natural factors and unsuitable agricultural practices [16] and although it causes significant product losses every year, stress factors cause about 25% loss of products per year [17]. In plants with salt tolerance, salt is not taken into the plant, stored in intracellular spaces without being inserted into physiological events, deported from the plant, so the tissue tolerance and antioxidant substances are provided, and furthermore, some of the ions such as K^+, Na^+, Cl^- and SO_4 are not carried into the plants or scapus[49,50]. However, allelopathy, occurs when one plant, through its living or decaying tissue, interferes with growth of another plant via a chemical inhibitor and therefore the influence, usually detrimental (the pathos), of one plant on another, by toxic chemical substances from living plant parts, through their release when a plant dies, or their production from decaying tissue [51].

Increasing levels of salinity stress in the soil affects photosynthesis adversely. The main effects of salinity on photosynthesis are related to changes in pigment compositions and ultrastructure of chloroplast, decreased net photosynthesis rate, reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) efficiency, and inhibition of electron transport and PSII activities [52]. Inhibition of the plant growth and the photosynthesis under salt stress was believed to be the result of osmotic and ionic stress components [53,54]. In the present study, photosynthetic pigments significantly reduced by salinity stress while the application of allelopathic extract increased the chlorophyll and carotenoid contents in *Zea mays* and *Viganaradiata*. Some previous studies have indicated that stomatal closure, caused by low osmotic potential, is typically the first mechanism involved in photosynthesis inhibition and is termed as 'osmotic effect'[55-59]. Osmotic stress caused by the inhibition of water absorption in highly saline environments has been frequently observed by researchers.

Salt in excess causes generation of ROS such as superoxide radical (O^-), H_2O_2, singlet oxygen ($_1O2$), and hydroxyl radicals (OH). Antioxidant enzymes such as SOD, peroxidase (POX), and catalase (CAT) are involved in the scavenging of ROS. The SOD catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen. CAT dismutases H_2O_2 into H_2O_2 and O_2, whereas POD decomposes H_2O_2 by oxidation of cosubstrates, such as phenolic compounds and or antioxidants [60]. Excess salt adversely affects architecture, lipid, and pigment composition of thylakoid membranes and causes decreased photochemical activity of PSII [61]. Plants exposed to excess salt have been shown to accumulate proline in their tissues. Accumulation of proline is an adaptive response of plants against stresses. Proline is believed to be regulatory or signal molecule activating some physiological and molecular responses [62]. Mechanism of proline accumulation is related to increased synthesis, decreased catabolism, or increased degradation of proteins [63]. In the present study, it was noticed that high contents of MDA were found in the leaves of *Zea mays* and *Viganaradiata* which showed that excess salt causes oxidative damage to the leaves while contents of proline and antioxidative activities of SOD and POD comes into play to reduce salt stress (Figure 1). It was observed that application of allelopathic extract significant reduced the enzymatic activities of SOD and POD and the contents of MDA and proline in the leaves of *Zea mays* and *Viganaradiata*. In addition, expression of proteins related with ROS scavenging is enhanced in salt tolerant mutant or wild plants than in salt sensitive mutants [64]. These studies indicated that salt tolerant species had greater levels of proteins related with reactive oxygen species (ROS) scavenging, ion transport, stress signaling and photosynthesis than those in salt sensitive species. The accumulation of ROS is harmful to plant structure and function; thereby, to restore cellular redox balance antioxidants are activated to detoxify the toxic levels of ROS [65]. Enzyme activities are enhanced in leaves of both *Zea mays* and *Viganaradiata*, suggesting that these activates its antioxidant system under saline condition. Foliar application of allelopathy maintained the SOD and POD activities under saline conditions. It is reported that melatonin activates antioxidant defense system in plants to reduce oxidative damage [66]. Exogenous application of allelopathic extract reduced MDA content, accounting for enhanced antioxidant response and the protective role of membranes thus increasing the plant tolerance to damage. A reduction in *Zea mays* and *Viganaradiata* yield was observed in our study which might be due to reduction in yield contributing traits like fertile spikelets $spike^{-1}$, 1000 grain weight and biomass $plant^{-1}$ under saline conditions, attributed to reduced photosynthetic activity of plants [67].

8. Conclusions

Based on the findings of the present study, it can be concluded that *Zea mays* and *Viganaradiata* has a considerable potential to cope with salinity stress due to an antioxidative defense mechanism. However, the application of allelopathic extract plays a significant role in altering growth, antioxidant enzymatic activities, and

MDA and proline contents in leaves of *Zea mays* and *Viganaradiata*. The allelopathic extract i.e. 10% significantly improved plant growth, biomass and photosynthetic pigments under saline soil. However, it was also noticed that *Zea mays* has significant high potential to growth in saline soil than *Viganaradiata* with the foliar application of allelopathic extract. High contents of MDA in the leaves of *Zea mays* and *Viganaradiata* under saline soil showed that salt stress induced oxidative damage while the contents of proline and enzymatic activities of SOD and POD comes into play to reduce environment toxicity. These results depicted that foliar application of allelopathic extract significant improved plant growth and development in *Zea mays* and *Viganaradiata*. However, future research is needed on the effects of allelopathic extract on growth and development of *Zea mays* and *Viganaradiata*. Moreover, potential for salinity stress with the foliar application of allelopathy should be tested under field conditions.

9. Acknowledgements

The financial support from Higher Education Commission, Islamabad for the support of Bachelor degree in the Agricultural Department University of Bahauddin Zakariya University is acknowledged.

References

1. Bittencourt-Oliveira MDC, Chia MAde, Oliveira HSB,Araújo MKC,Molica RJR, Dias CTS.Allelopathic interactions between microcystin-producing and non-microcystin-producing cyanobacteria and green microalgae: implications for microcystins production. J Appl Ecol. 2015; 27(1): 275-284.

2. Torres A, Oliva RM, Castellano D, Cross P. Proc First World Congress on Allelopathy—A Science for the Future: 16–20 September.1996.Cádiz. Spain.

3. Rizvi SJH,Haque H, Singh VK, Rizvi VA. Discipline called allelopathy, in Allelopathy: Basic and Applied Aspects. Chapman and Hall. London. UK.1992; 1–10.

4. Lux-Endrich A, Hock B. Allelopathy in Plant Toxicology. CRC Press. New York. NY. 2004.

5. Wills RJ. The History of Allelopathy. Springer. Dordrecht. The Netherlands. 2007.

6. Felpeto AB, Roy S,Vasconcelos VM. Allelopathy prevents competitive exclusion and promotes phytoplankton biodiversity.Oikos.2018; 127(1), 85-98.

7. Food and Agriculture Organization (FAO). Global Network on Integrated Soil Management for Sustainable Use of Salt-affected Soils. FAO Land and Plant Nutrition Management Service. Rome. 2016. (vertified on September 20, 2011). Available online at http://www.fao.org/ag/agl/agll/spush.

8. Zafar S, Ashraf MY,Niaz M,Kausar A, Hussain J. Evaluation of wheat genotypes for salinity tolerance using physiological indices as screening tool. Pak J Bot. 2015; 47(2): 397-405.

9. YokoiS, Quintero FJ,Cubero B, Ruiz MT,Bressan RA, Hasegawa PM et al. Differential expression and function of Arabidopsis thaliana NHX Na+/H+ antiporters in the salt stress response. Plant J.2002; 30(5): 529-539.

10. Li C, Wang P, Wei Z, Liang D, Liu C, Yin L et al. The mitigation effects of exogenous melatonin on salinity induced stress in Malus hupehensis. J Pineal Res. 2012; 53(3): 298-306.

11. Hardeland R,Cardinali DP, Srinivasan V, Spence DW, Brown GM,Pandi-Perumal SR. Melatonin a pleiotropic, orchestrating regulator molecule. J Progress Neurobiol. 2011; 93(3): 350-384.

12. Arnao MB, Hernández-Ruiz J. Melatonin: plant growth regulator and/or bio stimulator during tress: Trends Pl Sci. 2014; 19(12): 789-797.

13. Bayat RA,Kuşvuran Ş,Ellialtıoğlu Ş,Üstün AS. Effects of proline applic ation on antioxidative enzymes activities in the young pumpkin plants (Cucurbita pepo L. and C. moschataPoir.) under salt stress. Turk JAgri& Nat Sci. 2014; 1(1): 25-33.

14. Sha HJ, HL Liu, BW Hu, JJ Gu, WC Hu, Y Jia et al. Effect of salicylic acid on the dry matter and nitrogen accumulation, partitioning and translocation in two contrasting rice genotypes under salt stress. Pak J Bot. 2019; 51(5): 1541-1550.

15. Shannon MC. Adaptation of plants to salinity: AdvAgron. 1998; 60: 75-119.

16. Kalaji MH,Pietkiewicz S. Salinity effects on plant growth and other physiological processe. ActaPhysiologiaePlantarum.1993; 15(2): 89-124.

17. Gill BS, Appels R, Botha-Oberholster A-M, Buell CR,Bennetzen JL,Chalhoub B et al. A workshop report on wheat genome sequencing: International genome research on wheat consortium. Genetics.2004; 168: 1087-1096.

18. Botella MA, Rosado A,Bressan RA, Hasegawa, PM. Plant adaptive responses to salinity stress. In Jenks. Plant Abiotic Stress: Blackwell Publishing. Malden. MA. 2005; 37–70.

19. Perl-Treves R, Perl A. Oxidative stress: an introduction. In Inz´e, D. and Van Montagu, M. (eds.) Oxidative Stress in Plants. Taylor & Francis Group. London and New York. 2002; 1–32.

20. Imlay JA. Pathways of oxidative damage.Annu Rev Microbiol. 2003; 57: 395–418.

21. Apel K. Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction: Annu. Rev. Plant Biol. 2004; 55: 373-99.

22. Bowler C, Montagu MV, Inze D. Superoxide dismutase and stress tolerance: Ann. Rev. Plant Physiol. 1992; 43: 83-116.

23. Jimenez A, Hernandez JA, Del Rio LA, Sevilla F. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves: Plant Physiol. 1997; 114: 275-84.

24. DeazevedoNeto AD, Prisco JT, Eneas-Filho J, deabrau CEB, Gomes-Filho E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes: Environ. Exp. Bot. 2006; 56: 87-94.

25. Clark RB. Effects of aluminum and mineral elements of Al-intolerant corn: Plant Soil. 1997; 47: 653-62.

26. Liu DH, Jiang WS, Hou WQ. Uptake and accumulation of copper by roots and shoots of maize (Zea mays L.). J. Environ. Sci. 2001; 13: 228-32.

27. Lagriffoul A, Mocquot B, Mench M, Vangronsveld J. Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (Zea mays L.). Plant Soil. 1998; 200: 241-50.

28. Ju GC, Li XZ, Rauser WE, Oaks A. Influence of cadmium on the production of γ glutamylcysteine peptides and enzymes of nitrogen assimilation in Zea mays seedlings: Physiol Plant. 1997; 101: 793-9.

29. Manivasagaperumal R. Vijayarengan P, Balamurugan S, Thiyagarajan G. Effect of copper on growth, dry matter yield and nutriant content of (Vignaradiata): J Phytol. 2011; 3(3): 53-62.

30. Verma JP, Singh V, Yadav J. Effect of copper sulphate on seed germination, plant growth and peroxidase activity of Mung Bean (Vignaradiata). Intl J Botany. 2011; 7(2): 200-4.

31. Manchanda JS, Bansal RL, Nayyar VK. Mode of copper fertilization in a TypicUstipassements under soybean-wheat cropping sequence: J Res Punjab Agric Univ. 2006; 43(4): 303-7.

32. Heitholt JJ, Sloan JJ, MacKownn CT. Copper, Manganese and Zinc fertilization effects on growth of soybean on calcareous soil: J Pl Nutr. 2002; 25(8): 1727-40.

33. Fageria NK. Micronutrients influence on root growth of upland rice, common bean, corn, wheat and soybean. J. Pl. Nutr. 2002; 25(3): 613-22.

34. Arnon DT. Copper enzyme in isolated chloroplasts polyphenoloxidase in Beta vulgaris: Plant Physiol. 1949; 24: 1-15.

35. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation: Arch. Biochem. Biophys. 1968; 125: 180-98.

36. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies: Plant Soil. 1973; 39: 205-7.

37. Chen CN, Pan SM. Assay of superoxide dismutase activity by combining electrophoresis and densitometry: Bot. Bull. Acad. Sin. 1996; 37, 107-11.

38. Sakharov IY, Aridilla GB. Variation of peroxidase activity in cacao beans during their ripening, fermentation and drying: Food Chem. 1999; 65: 51-4.

39. Sharmam GP, Singh JS, Raghubanshi AS. Plant invasions: Emerging trends and future implications: Current science. 2005; 88: 726-33.

40. Kong CH. Problems needed attention on plant allelopathy research: Chinese Journal of Appled Ecology. 1998; 9(3): 332-6.

41. Yan F, Yanm Z, Han L. Review on research methods for allelopathy and allelochemicals in plants. ActaEcologicaSinica. 2000; 20: 692-6.

42. Song J. Allelopathy among Plants. Chinese Journal of Ecology. 1990; 9: 43-7.

43. Sun WH, Yu SW. Allelopathy and Its Potential Application. Plant Physiology Communications. 1992; 28: 81-7.

44. Chon CH. Allelopathy in relation to agricultural productivity in Taiwan: problems and prospects. Allelopathy: Basic and Applied Aspects. London: Chapman and Hall.1992; 179-203.

45. Lu W, Zhang G, Yuan CL. Mechanism of Allelochemicals Inhibiting Continuous Cropping Cucumber Growth. Scientia AgriculturaSinica. 2002; 35: 106-9.

46. Qiang S. The History And Status of The Study on Crofton Weed (Eupatorium adenophorum) a Worst Worldwide Weed. Wuhan Botanical Research. 1998; 16: 366-72.

47. He YB, Zhang MX, He TY. Studies on the Allelopathic Effects of Chromolaenaodoratum. Journal of South China Agricultural University. 2002; 23: 60-2.

48. Nie CR, Zeng RS, Li HS. Physiological and Biochemical Mechanism of Allelopathy of Wedeliatrilobata L. Peanut Science and Technology. 2002; 31: 1-5.

49. Parida AK, Das AB. Salt tolerance and salinity effects on plants: A review. Ecotoxicol Environ Safety. 2005; 60(3): 324-49.

50. Iqbal MS, Naseem A, Mahmood K, Akhtar J. Comparative performance of wheat (Triticumaestivum L.) genotypes under salinity stres II: Ionic composition. J Biol Sci. 2001; 1(2), 43-45.

51. Zhou K, Guom WM. Allelopathic Effects of Aquatic Extracts from Solidagocanadensis's Roots and Rhizospheric Soil on the Seed Germination and Early Seedling Growth of Chinese cabbage and radish. Acta Botanica Boreali-OccidentaliaSinica. 2005; 25(1): 174-178.

52. Aggarwal A, Sharma I, Tripathi BN, Munjal AK, Baunthiyal M, Sharma V. Metal toxicity and photosynthesis. In: Itoh, S., Mohanty, P., Guruprasad, K.N. (Eds.), Photosynthesis: Overviews on Recent Progress and Future Perspectives. International Publishing House Limited, India. 2012.

53. Munns R. Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008; 59, 651-681.

54. Siddiqui MH, Mohammad F, Khan MM, Al-Whaibi MH. Cumulative effect of nitrogen and sulphur on Brassica juncea L. genotypes under NaCl stress. Protoplasma. 2012; 249, 139-153.

55. Chaves MM, Costa JM, Saibo NJM. Recent advances in photosynthesis under drought and salinity. In: Turkan, I. (Ed.), Plant Responses to Drought and Salinity Stress: Developments in a Post-Genomic Era. Academic Press Ltd-Elsevier Science Ltd. London. 2011; 57: 49-104.

56. Nebauer SG, Sanchez M, Martinez L, Lluch Y, Renau-Morata B, Molina RV. Differences in photosynthetic performance and its correlation

with growth among tomato cultivars in response to different salts. Plant PhysiolBiochem. 2013; 63: 61-69.

57. Min W, Guo H, Zhou G, Zhang W, Ma L, Ye J et al. Root distribution and growth of cotton as affected by drip irrigation with saline water. Field Crops Res. 2014; 169: 1-10.

58. Win KT, Oo AZ, Ookawa T, Kanekatsu M, Hirasawa T. Changes in hydraulic conductance cause the difference in growth response to short-term salt stress between salt-tolerant and -sensitive black gram (Vigna mungo) varieties. J Plant Physiol. 2016; 193: 71-78.

59. Hernandez-Santana V, Fernández JE, Cuevas MV, Perez-Martin A, Diaz-Espejo A. Photosynthetic limitations by water deficit: effect on fruit and olive oil yield, leaf area and trunk diameter and its potential use to control vegetative growth of super-high density olive orchards. Agric Water Manag. 2017; 184: 9-18.

60. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford University Press. NewYork. 2007; p. 888.

61. Maksymiec W. Signaling responses in plants to heavy metal stress. ActaPhysiol Plant. 2007; 29: 177-187.

62. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends Plant Sci. 2010; 15: 89-97.

63. KaviKishor PB, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KRSS et al. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr Sci. 2005; 88: 424-438.

64. Ghaffari A, Gharechahi J, Nakhoda B, Salekdeh GH. Physiology and proteome responses of two contrasting rice mutants and their wild type parent under salt stress conditions at the vegetative stage. J Plant Physiol. 2014; 171: 31-44.

65. Ahmad P, Jaleel CA, Azooz MM, Nabi G. Generation of ROS and non-enzymatic antioxidants during abiotic stress in plants: Bot Res Int. 2009; 2(1): 11-20.

66. Zhang N, Zhang HJ, Zhao B, Sun QQ, Cao YY, Li R. The RNA sequence approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. J Pineal Res. 2014; 56: 9-5.

67. Plaut Z, Butow JBS, Blumenthal C, Wrigley WC. Transport of dry matter into developing wheat kernels and its contribution to grain yield under post- anthesis water deficit and evaluated temperature. Field Crops Res. 2004; 86(2-3): 185-198.