

Research Paper

ROLE OF SOIL MOISTURE IN ALLELOPATHIC ACTIVITY OF *MEDICAGO SATIVA* L. RESIDUES IN *ZEA MAYS* L. FIELDS

Hoda A. Ahmed¹, Salama M. El-Darier¹, and Marwa H. Zein El-Dien²

1-Biological Science Department,
Faculty of Science, King Faisal University,
380 Al-Hofuf, Kingdom of Saudi Arabia

2- Botany and Microbiology Department,
Faculty of Science, Alexandria University, Alexandria,
Egypt.

Abstract

The allelopathic activity of *Medicago sativa* L. (alfalfa) residues on germination, growth and nutrient uptake of *Zea mays* L. (maize) grown under different soil moisture conditions were investigated. Germination percentage (GP) of maize plants was promoted by 1% *Medicago sativa* aqueous extract (MSAE) concentration under both full and half field capacity (FFC and HFC, respectively). At 8% MSAE concentration, both plumule and radicle length (PL and RL) of the examined species were significantly reduced under both water regimes. In addition, the application of *Medicago sativa* crude powder (MSCP) at 8% concentration was observed to significantly reduce the dry matter accumulation of different seedling parts upon decrease in soil moisture level. The study revealed that MSCP under both water treatment conditions have different effects (inhibitory and stimulatory) on nutrients concentration and uptake of the estimated elements like nitrogen, potassium and phosphorus (NPK).

Key words: Allelopathy, Dry weight, Germination efficiency, Nutrient uptake, *Medicago sativa* L., Phytotoxicity, Soil moisture, *Zea mays* L..

INTRODUCTION

Allelopathy is a natural phenomenon that takes place through secretion of phytochemicals from one plant which causes beneficial or harmful effect on other (Ferguson and Rathinasabapathi, 2003). From agricultural point of view, allelopathy plays an important role in biological weed management and crop productivity (Bhadoria, 2011).

M. sativa (alfalfa) has showed different mode of allelopathic effects on a variety of crop plants such as maize, tomato and others. Allelochemicals of alfalfa have been detected in all plant parts and their concentrations are in the order of leaf > seed > whole plant > soil > root > flower > stem (Singh *et al.*, 2001). Thus, the crops cultivated immediately

after harvesting of alfalfa showed poor growth and reduced yield. El-Darier and Youssef (2000) suggested that the addition of aqueous extract of alfalfa strongly affects the germination efficiency and growth characters of *Lepidium sativum*.

The degree of allelopathic effect is more dependent on the extent of some stresses, such as environmental conditions (solar radiation, temperature levels) (Putnam, 1988), soil salinity (El-Darier and Youssef, 2007) and type (El-Darier *et al.*, 2014), moisture (Blum *et al.*, 1999), than others like optimal nutrients or biological factors (insect or disease pressure) that occur during the growing season.

Soil plays an important role as the biological environment in which potential allelochemicals detoxified or toxified through the microbial action (Inderjit, 1998; Inderjit and Weiner, 2001; Ohno, 2001). The soil moisture level is the main soil physical factor which affects activity of soil microorganisms (Rizvi and Rizvi, 1992; Reinhardt *et al.*, 1996). This is due to the fact that water serves as a solvent and carrier of allelochemicals and leachates from an aerial plant parts and in the soil.

Z. mays (maize) plant is described as one of the most important crops in Egypt. The main objective of the contemporary study was to investigate the possible allelopathic effects of *M. sativa* crop residues on germination, growth and nutrient uptake of *Z. mays* under stress condition of water regime. This is in sequence will enable us to assess the relative response and validate the proper soil moisture condition in the cultivation system of this crop.

MATERIALS AND METHODS

Field observations were set up during summer 2012 - 2013 in an area of 15 feddans (6.3 hectare) cultivated with maize in traditional cropping systems after harvesting of alfalfa at El-Hammam region; about 75 km southwest of Alexandria city.

Preparation of *Medicago sativa* Aqueous Extract (MSAE)

Stock aqueous extract was obtained by soaking air-dried alfalfa plant material in 10% (w/v) distilled water at room temperature ($20 \pm 2^\circ\text{C}$) for 24 hours with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper and the purified extract was adjusted to pH 6.8 with 1M HCl. Different concentrations (1, 2, 4 and 8%) were prepared from the stock solution using distilled water.

Germination Bioassay

Twenty five seeds of maize were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from $19-22^\circ\text{C}$ and night temperature from $12-14^\circ\text{C}$. 6 and 10 cm of each level of the alfalfa extract (1, 2, 4 and 8%) representing the field capacity were added daily to three replicates. Germination percentage (GP), plumule (PL) and radicle (RL) length were recorded after 15 days at the end of the experiment. Seed germination index (SGI) was calculated according to Scott *et al.*, (1984) as follow:

$$\text{SGI} = \sum \text{Ti Ni} / \text{S}$$

Where,

Ti = is the number of days after sowing

Ni = is the number of seeds germinated on day i

S = is the total number of seeds planted

Likewise, phytotoxicity (PT) of the donor species extract was expressed as a percentage of germination of the recipient species in different concentration levels with respect to control. Higher values indicate lower toxicity (Cayuela *et al.*, 2007).

Phytotoxicity (PT) = [1- (allelopathic/control) /100]

Growth Experiment

Pot experiment was carried out to test the effect of different concentrations of *M. sativa* crude powder (MSCP) (w/w) on dry matter accumulation, nutrient concentration and uptake in sandy loam soil under different water condition. Grains of maize plant were obtained from the Breeding Program of the Agricultural Research Center, Giza, Egypt.

Soil analysis

Six soil samples from natural sites where the alleged allelopathic materials are not present were used to undergo the pot experiment. The samples were air-dried and passed through 2mm sieve to eliminate the gravels and debris, and finally analyzed for some of their chemical and physical properties according to Allen *et al.*, (1974).

Implantation

Ten seeds of maize were sown in plastic pots (diameter 20cm x height 22cm) The electrical conductivity (EC) of soil- water extract (1:1 w/v) was measured using conductivity meter. Soil pH was determined in 1:1 (w/v) soil: water suspension using glass electrode. Soluble calcium and magnesium were determined volumetrically in soil –water extract 1:1 by the varsinate method (EDTA) using ammonium purpurate as an indicator for calcium and Eriochrome black T for calcium plus magnesium. Soluble chlorides were determined by titration with 0.01 N silver nitrate solution and potassium chromate as indicator. Soluble carbonate was determined volumetrically, in the soil water extract (1:1) by titration against 0.05 N hydrochloric acid solution using methyl orange as an indicator. Soluble sulphate with sandy loam soil completely mixed with electrically crushed crude powder of alfalfa plant (w/w) (MSCP) concentrations of 1, 2, 4 and 8 % under full (FFC) and half (HFC) field capacity. The experiment was performed under normal laboratory conditions with day temperature ranging from 19 - 22°C, light duration was 12 hours and light intensity was 1350 foot-candle (F.C.). The plants were watered every two days on the average with normal tap water. Pots were carefully irrigated from the top the first time and sub-irrigated thereafter to avoid seedling disturbance. The amount of water corresponding to average soil-plant-transpiration calculated from weight loss over 24-hours for three replicates. Seedlings of maize were harvested one month after planting. One treatment was run as control without any percent of the crude powder.

Complete morphologically homogenous maize seedlings from each treatment were harvested, washed and separated into different organs which were dried at 65°C till constant weight to determine the weights of stem (SW), leaf (LW), root (RW) and total weight (TW). A part of the dried samples were ground in a Wiley Mill to pass 1.0 mm² screen. Nitrogen (N), phosphorus (P) and potassium (K) were determined according to procedures described by Allen *et al.* (1974). At the end of the experiment (one month after sowing) the total nutrient uptake (mg plant⁻¹ month⁻¹) at each MSCP concentration was calculated according to the following relation:

Total nutrient uptake = Nutrient concentration × Total dry weight

Statistical Treatment of Data

Data obtained were subjected to standard analysis of variance (ANOVA) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company (Zar, 1984). Significance of differences was accepted when $P \leq 0.05$.

RESULTS

Germination Bioassay

GP, SGI and PT of maize grains were apparently varied with MSAE concentrations under FFC (Fig.1) and HFC (Fig.2). The attained GP values at control conditions (68.3 % under FFC and 60% under HFC) were increased upon applying 1 % MSAE concentration (82% and 72.6 % under both water regimes). However, this current motivation goes to a marked reduction (42.3%, 37.7% under FFC and HFC, respectively) at 8% concentration. Comparatively, SGI values demonstrate the stimulatory effect of 1 % MSAE concentration; the control values (10.2 and 9) rose to 12.3 (under FFC) and 10.9 (under HFC). As higher MSAE concentrations were applied, PT values were increased, 38% and 37.2% were recorded at 8% under FFC and HFC, respectively. Measurements of PL after emergence from maize grains give a good indicator about the possibility of water supplement as a factor controlling the germination process. The demonstrated data pointed up that PL, RL were significantly affected ($P \leq 0.05$) by MSAE concentrations under FFC and HFC (Fig. 3 and 4, respectively). There was a noticed reduction in values of PL under both water regimes. At 8% concentration 12.7, 9.7 cm were attained under FFC and HFC, respectively. Surprisingly, the same RL was achieved at 8% concentration under both water regimes (4.77 cm). The control value of RL in sandy soil was 23 and 21cm under FFC and HFC respectively, which clarify water shortage consequence on elongation of the radicle. There was an explicable inhibitory effect of MSAE concentrations to radicle elongation among the applied concentrations. At 8% MSAE concentration, values of about 8.3 and 7.3 were obtained under FFC and HFC respectively.

Growth Experiment

Dry matter accumulation

There was a significant reduction ($P \leq 0.05$) of TW as a consequence of raising MSCP concentrations and water stress (Table 2). At 8% concentration, the values reduced to 2.5 and 2 g under FFC and HFC compared to the control values which were 11.8 and 10.9 g under both water regimes, respectively. Definitely, water stressed seedlings have demonstrated relatively lower values of SW. The values of SW were about 2.8 and 2.7 g, which reduced to 0.8 and 0.6 g at 8% concentration under FFC and HFC, respectively. The control values of LW were about 6.7 and 5.7 g under FFC and HFC, respectively which exhibit their maximum reduction at 8% MSCP concentration (1.06 and 0.9 g under FFC and HFC, respectively). Due to the allelopathic influence of MSCP, RW decreased significantly ($P \leq 0.05$) under both water regimes. Higher MSCP concentrations have illustrated more reduction. For example, at 8% concentration, RW reduced to 0.7 and 0.5 g under FFC and HFC, respectively.

Concentration and uptake of some nutrients

The response of NPK concentrations (mg g^{-1} d. wt.) in maize plant to MSCP concentrations is represented in table 2. A gradual decrease in N concentration in maize plant was significantly correlated ($P \leq 0.05$) to MSCP concentrations especially under water stress conditions. The highest N concentrations (21 and 19 mg g^{-1} d. wt. under FFC and HFC, respectively) were achieved at control level. At 8% concentrations were reduced to 12.5 and 10.5 mg g^{-1} d. wt. under both water regimes, respectively. Frequently, lower N concentrations were attained under HFC compared to that estimated under FFC ($P \leq 0.05$). P concentration suffered a gradual reduction ($P \leq 0.05$) under both water regimes. At control, the values were about 11 and 8.5 mg g^{-1} d. wt. under FFC. Continuously, the values reduced to about 8.5 and 4.5 mg g^{-1} d. wt. under FFC and HFC, respectively, at 8% concentration. On the other hand, K concentration

increased under both water regimes. At control, K concentrations were 20 and 18 mg g⁻¹ d. wt. under FFC and HFC, respectively. Under FFC, K concentration has increased gradually to 31 mg g⁻¹ d. wt. at 8% concentration level. Under HFC, K concentrations have decreased to 17.5 and 16.5 mg g⁻¹ d. wt. at 1 and 2 % concentration levels, respectively. Continuously, at 4 and 8 % concentrations, the value increased to about 21 and 24.5 mg g⁻¹ d. wt.

N uptake in maize plant decreased ($P \leq 0.05$) along with higher MSCP concentrations as well as water stress ($P \leq 0.05$) is demonstrated in Fig.5. At control, the values were about 253.7 and 207 mg plant⁻¹ month⁻¹ under FFC and HFC, respectively. At 8 % concentration, the values decreased to 31.3 and 21 mg plant⁻¹ month⁻¹ under FFC and HFC, respectively. Likewise, there was a significant reduction ($P \leq 0.05$) of P and K uptake process at higher MSCP concentration levels. At control, P uptake began with values about 129.8 and 92.7 mg plant⁻¹ month⁻¹ under FFC and HFC, respectively which reduced at 8 % concentration (21.3 and 9 mg plant⁻¹ month⁻¹) under both water regimes. At control, K uptake values were about 236 and 196.2 mg plant⁻¹ month⁻¹ under FFC and HFC, respectively, which decreased (77 and 49 mg plant⁻¹ month⁻¹ under FFC and HFC) at 8 % concentration.

DISCUSSION

In view of increasing demand on food, study of the natural factors that may influence the productivity and yield of crops become a topic of great interest (Verbruggen *et al.*, 2012). *Zea mays* as one of essential food crops faces a number of growth challenge factors like water stress, allelochemicals, salinity and others. In agreement with Singh *et al.*, (2009), the first two factors induce oxidative damage in corn and lead to generation of reactive oxygen species. These in turn have a consequence of several physiological disorders and crop productivity.

Reduction of seed germination and seedling growth is known to take place in alfalfa when it is cultivated continuously in one field (Miller, 1983). Particularly, in maize seedling, 1% MSAE concentration was promoting for germination process only under both water regimes. The physiological roles of allelochemicals have not been fully studied in plants. An allelochemical can be beneficial in one plant, or harmful in another. This action depends on allelochemical nature, concentration and treatment duration (Whittaker and Feeny, 1971; Rice, 1979; Hale and Orcutt, 1987). A similar trend was described by El-Darier (2002) during the estimation of the allelopathic effect of *Eucalyptus rostrata* on the growth and metabolite accumulation of broad bean and maize plants. The last mentioned author found that in clay soil, 1% of *Eucalyptus* leaf-litter water extract revealed a distinct stimulatory effect on the germination of the two species (maize and broad bean). Concerning MSAE concentration in the present study, this response could be explained by the presence of some allelochemicals which can be considered as promoters. Moreover, these phytochemicals indirectly enhance plant growth through reduction of aluminum toxicity in maize (Lambers *et al.*, 1998). Additionally, such stimulation was supported by data provided by other studies (El-Darier and Youssef, 2000). Besides, 1 % *Beta vulgaris* aqueous extract has encouraged wheat germination. On the other hand, application of concentrations above 1 % aqueous extract showed delayed germination (Hegab, 2008). Previously, Evenari (1949) stated that germination inhibition and stimulation appear in different concentrations, sometimes one after the other in the same concentration. All other MSAE concentrations were inhibitory to maize germination process (more Phytotoxicity was observed).

These assessments are also in a harmony with Chung and Miller (1995a) who reported that aqueous extracts of alfalfa reduce seed germination in corn and soybean.

The obtained data elucidate the negative effect of water stress on the ability of maize grains to germinate. Allelochemicals production is activated under stress conditions; consequently the potential for allelopathic interference is raised. For example, the inhibition of ferulic acid threshold concentration that affects seedling growth was reduced upon slight moisture stress or growth temperature than the highest end of the normal growth range for a species (Einhellig, 1996).

Measurements of PL after emergence from maize grains give a good indicator about the possibility of water supplement as a factor controlling the germination process. Evaluation of RL correlated with higher MSAE concentrations has demonstrated their depressing influence on maize growth process. Comparatively, PL of maize seedling suffered more reduction relative to their radicle. Evidently, Siddiqui (2007) found that the more allelopathic effect of black pepper on shoot than root growth of *Vigna mungo* upon increasing the concentrations. In a parallel work, Oyerinde *et al.* (2009) found significant reductions in radicle and plumule lengths of *Zea mays* when treated with *Tithonia diversifolia* shoot aqueous extract. Roots are more sensitive to allelopathic effect than hypocotyle and shoot growth (Tefera, 2002). Alfalfa contains the secondary steroid saponine which inhibits growth of aerial parts and roots elongation in maize in addition to its antifungal, antibacterial and antiviral actions (Shi *et al.*, 2008).

In a parallel experiment Bhowmik and Doll (1982) demonstrated greater inhibition of the donor species (maize and soybean) with the double-pot watering method than surface and surface watering methods, signifying the strong correlation between the allelopathic potential and stress conditions. However, corn does require more water right up to physiological maturity. As maize approaches physiological maturity more water can be removed from the soil profile without impacting final grain yield.

Dry weight recordings had illustrated the negative allelopathic effect of MSCP toward the recipient plant. These findings agree with that obtained by Terzi *et al.* (2003), where the root and stem dry weights of cucumber seedlings was negatively influenced by decomposed walnut leaves and juglone, depending on the concentration.

NPK uptake in maize plant decreased along with higher MSCP concentrations as well as water stress. It was suggested that N should be applied to a water-sensitive variety of maize plant to bring out its potential under drought indicating the water- N level correlation (Zhang *et al.*, 2007). More reduction in P uptake has occurred due to water stress. Plants response to water stress implied several mechanisms of adaptation. These include morphological, physiological and biochemical modifications (Anjum *et al.*, 2011). Although P exists in high concentration in many soils, it is largely in unavailable form for uptake (Bialeski, 1973; Schactman, 1998). In an earlier work, Leachates of *Chenopodium album* caused significant reduction in shoot fresh and dry weights as well as in the accumulation of N, P, K, Ca and Mg of tomato shoots (Qasem and Hill, 1989). In agreement with Singh *et al.*, (2009), water stress has impaired the different growth and physiological parameters of *Zea mays* grown under allelopathic conditions.

In conclusion, soil moisture content is a key factor that controls the allelopathic activities of *Medicago sativa* L. and consequently affects the yield of subsequent cultivated crop.

Table (1): Variation in some physical and chemical characteristics of the soil applied to carry out the growth experiment. Considering that, a: dsm^{-1} , b: % and c: mg g^{-1} .

Parameter	Value
Physical properties	
Texture	Sandy clay loam
Clay ^b	24±
Sand ^b	58
Silt ^b	18
Chemical properties	
Electrical conductivity ^a	2.72
Organic matter ^b	9.08
pH	7.82
Free carbon ^b	1.50
N ^c	1.100
P ^c	0.520
K ^c	3.50
Ca ^c	15.40
Mg ^c	1.40
Cl ^c	15.25
CO ₃ ^c	35.00
SO ₄ ^c	18.70

Table (2): Allelopathic effect of different concentrations of *Medicago sativa* crude powder (MSCP) on dry weight (g) of *Zea mays* seedlings (30 days) under full (FFC) and half field capacity (HFC). (Data are means of three replicates).

Currency (g)		Stem dry weight (SW)		Leaf dry weight (LW)		Root dry weight (RW)		Total dry weight (TW)	
Soil water capacity		FFC	HFC	FFC	HFC	FFC	HFC	FFC	HFC
Concentration (%)	C	2.8 ^d	2.7 ^d	6.7 ^d	5.7 ^d	2.3 ^d	2.5 ^d	11.8 ^e	10.9 ^e
	1	2.2 ^c	2.0 ^c	3.4 ^c	3.0 ^c	1.5 ^c	1.5 ^c	7.1 ^d	6.5 ^d
	2	1.5 ^b	1.5 ^b	2.3 ^b	2.0 ^b	1.1 ^b	1.0 ^b	4.9 ^c	4.5 ^c
	4	1.2 ^a	1.2 ^b	1.7 ^b	1.5 ^b	0.8 ^a	0.6 ^a	3.7 ^b	3.3 ^b
	8	0.8 ^a	0.6 ^a	1.06 ^a	0.9 ^a	0.7 ^a	0.5 ^a	2.5 ^a	2.0 ^a

Different letters for each column indicate significance at $p \leq 0.05$.
C: control

Table (3): Allelopathic effect of different concentrations of *Medicago sativa* crude powder (MSCP) on concentration (mg g^{-1} d. wt.) of nitrogen (N), phosphorus (P) and potassium (K) of *Zea mays* seedlings (30 days) under full (FFC) and half field capacity (HFC). (Data are means of three replicates).

Nutrient		N		P		K	
Soil water capacity		FFC	HFC	FFC	HFC	FFC	HFC
Concentration (%)	C	21.5 ^e	19.0 ^d	11.0 ^b	8.5 ^c	20.0 ^a	18.0 ^b
	1	19.5 ^d	18.5 ^c	10.5 ^b	8.5 ^c	23.0 ^b	17.5 ^a
	2	18.0 ^c	17.5 ^c	9.5 ^a	7.5 ^b	25.5 ^c	16.5 ^a
	4	15.5 ^b	13.5 ^b	10.0 ^b	6.5 ^b	29.5 ^d	21.0 ^c
	8	12.5 ^a	10.5 ^a	8.5 ^a	4.5 ^a	31.0 ^e	24.5 ^d

Different letters for each column indicate significance at $p \leq 0.05$.

C: control

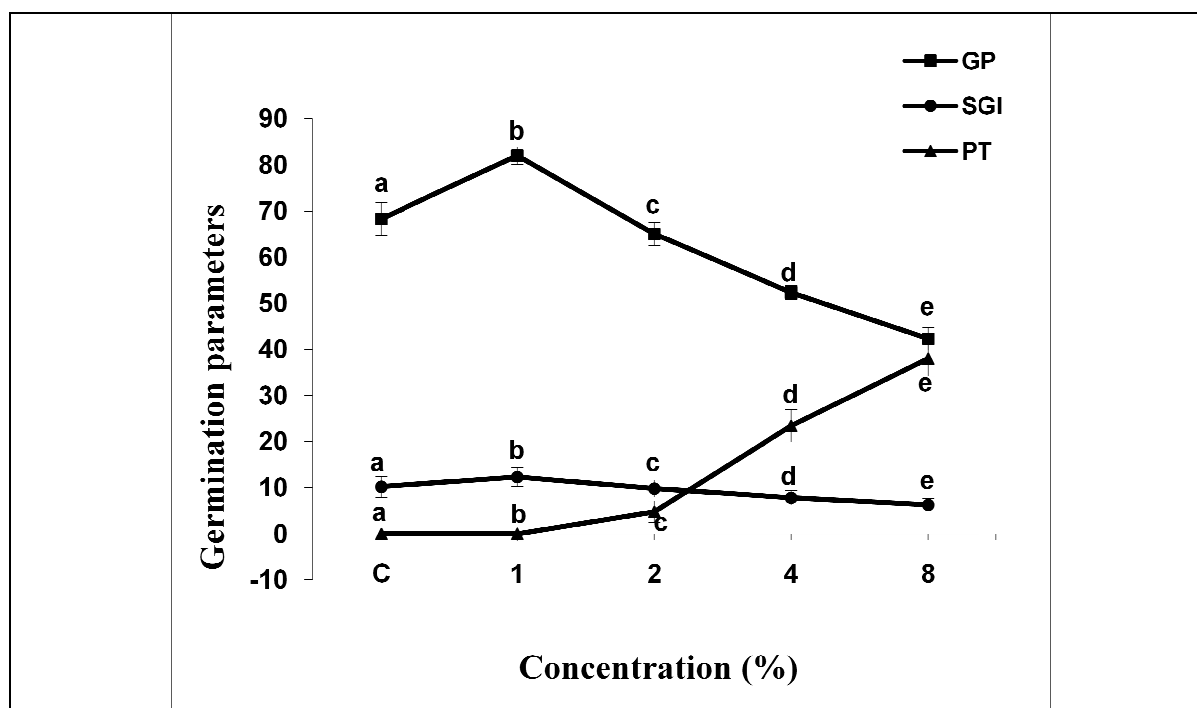


Figure (1): Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on germination percentage (GP), seed germination index (SGI) and phytotoxicity (PT) of *Zea mays* seedlings (15 days) under full field capacity (FFC). (Data are means of three replicates). Different letters for each parameter indicate a significant difference at ≤ 0.05 level of probability as evaluated by ANOVA test.

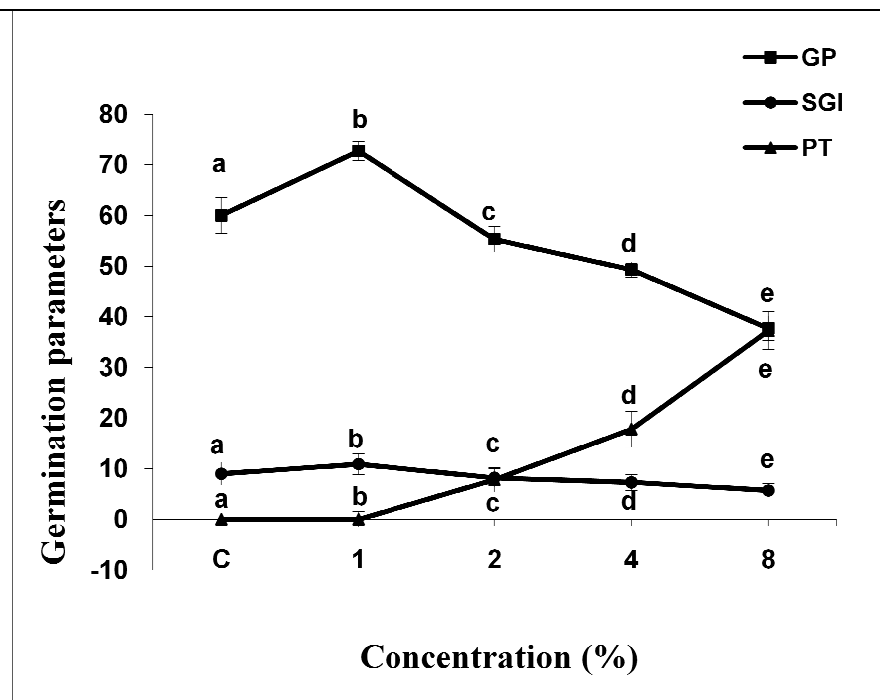


Figure (2): Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on germination percentage (GP), seed germination index (SGI) and phytotoxicity (PT) of *Zea mays* seedlings (15 days) under half field capacity (HFC). (Data are means of three replicates). Different letters for each parameter indicate a significant difference at ≤ 0.05 level of probability as evaluated by ANOVA test.

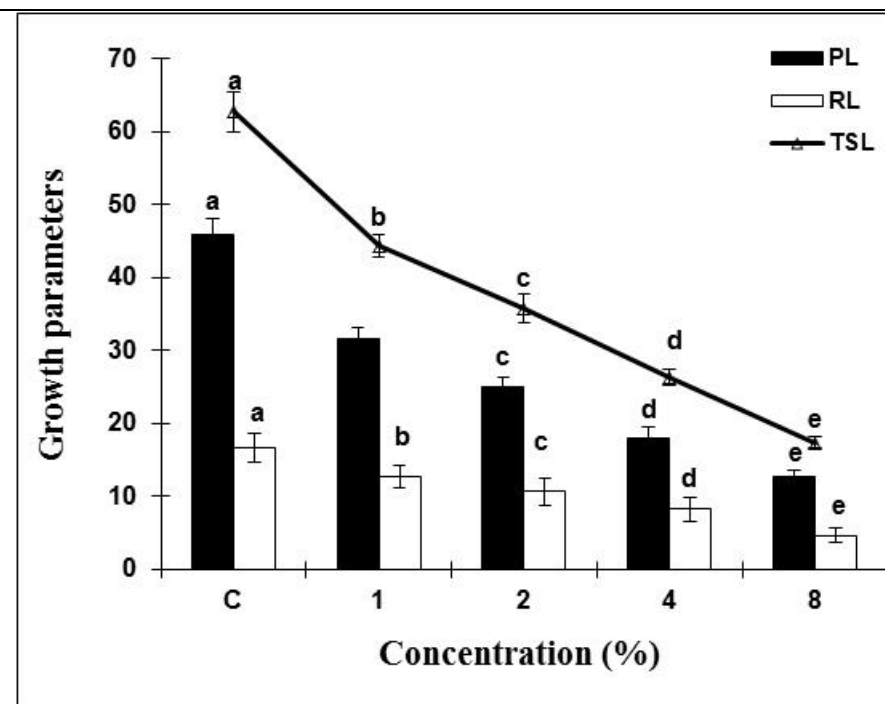


Figure (3): Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on plumule (PL) and radicle (RL) lengths (cm) as well as total seedling length (TSL) (cm) of *Zea mays* seedlings (15 days) under full field capacity (FFC). (Data are means of three replicates). Different letters for each parameter indicate a significant difference at ≤ 0.05 level of probability as evaluated by ANOVA test.

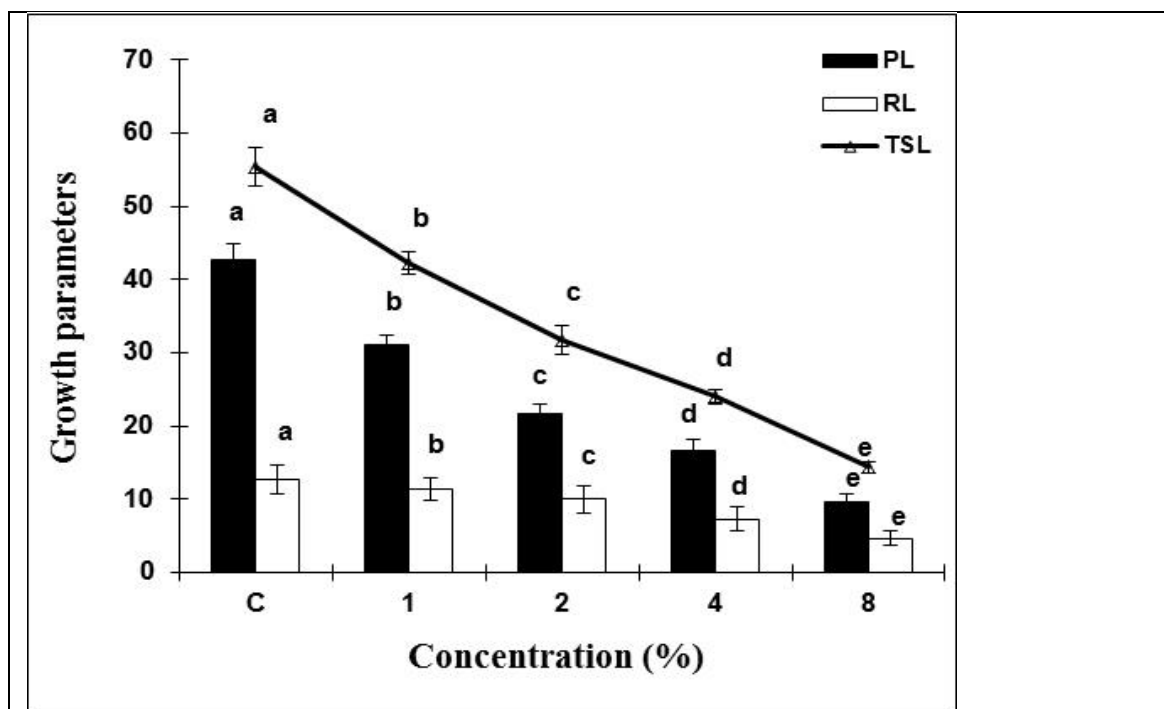
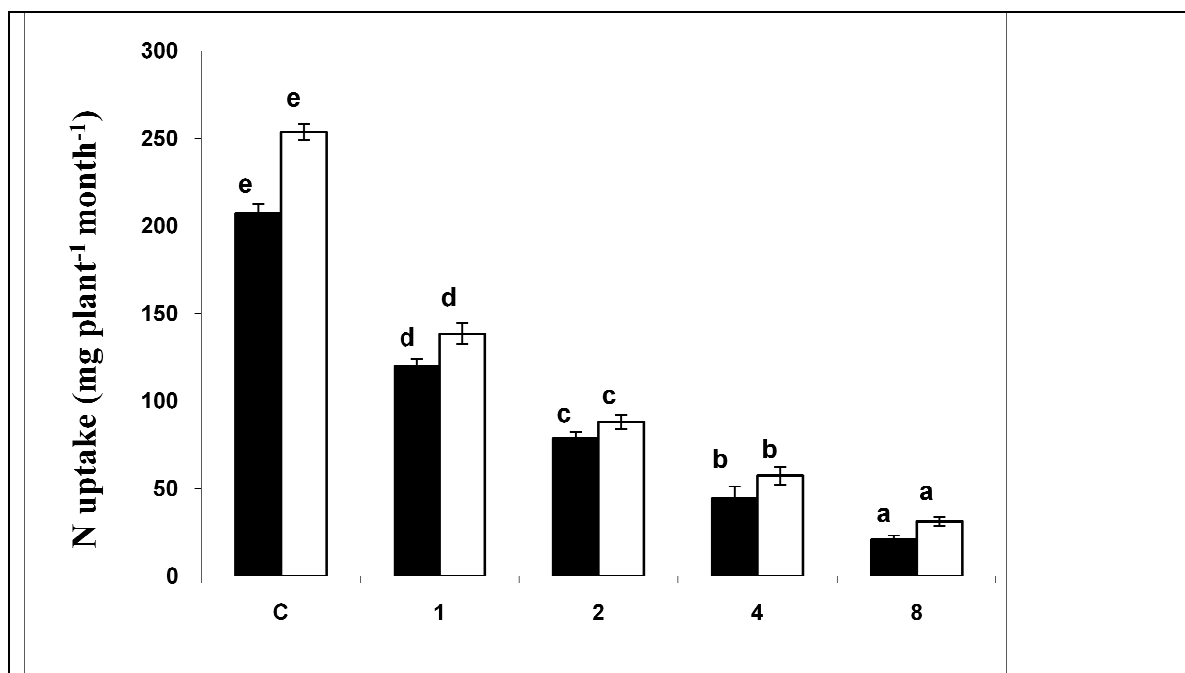


Figure (4): Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on plumule (PL) and radicle (RL) lengths (cm) as well as total seedling length (TSL) (cm) of *Zea mays* seedlings (15 days) under half field capacity (HFC). (Data are means of three replicates). Different letters for each parameter indicate a significant difference at ≤ 0.05 level of probability as evaluated by ANOVA test.



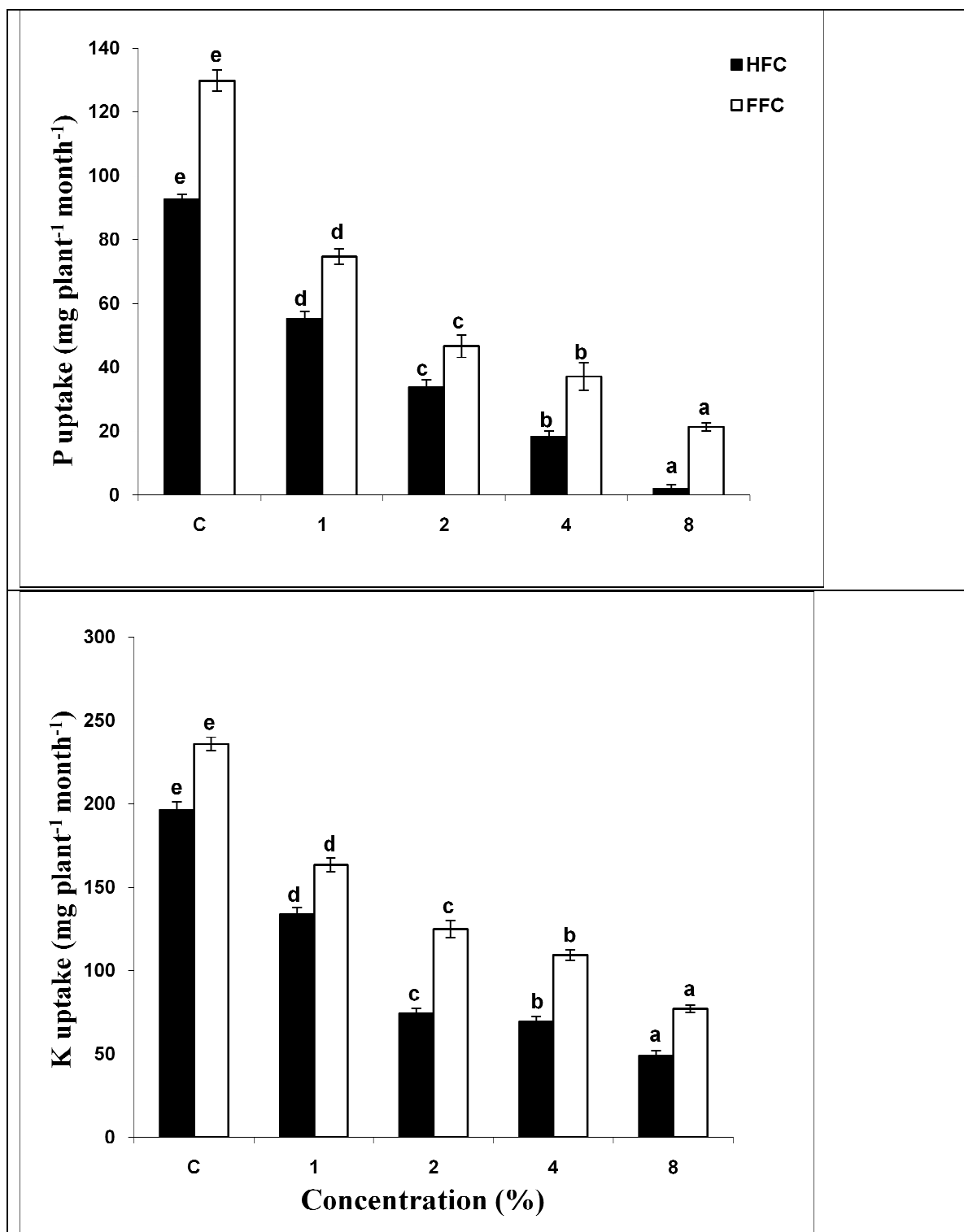


Figure (5): Allelopathic effect of different concentrations of *Medicago sativa* crude powder (MSCP) on the uptake (mg plant⁻¹ month⁻¹) of N, P and K of *Zea mays* under half (HFC) and full field capacity (FFC). (Data are means of three replicates). Different letters for each parameter indicate a significant difference at ≤ 0.05 level of probability as evaluated by ANOVA test.

REFERENCES

1. ALLEN, S.; GRIMSHAW, M.H.; PARKINSON, J.A. AND QUARMBY, C. 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific publications Osney, Oxford, London, pp. 565.
2. ANJUM, S.A.; XIE, X.; WANG, L.; SALEEM, M.F.; MAN, C. AND LEI, W. 2011. Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, Vol. 6(9): 2026-2032.
3. BHADORIA, P.B.S. 2011. Allelopathy: A Natural Way towards Weed Management. *American Journal of Experimental Agriculture*, Vol. 1(1): 7-20
4. BHOWMIK, P.C. AND DOLL, J.D. 1982. Corn and soybean response to allelopathic effects of weed and crop residues. *Agronomy Journal*, 74:601-606.
5. BIELESKI, R.L. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annual Review of Plant Physiology*. Vol. 24: 225-252.
6. CAYUELA, M.L, MILLNER P, SLOVIN J, ROIG A. 2007. Duckweed (*Lemna gibba*) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting. *Chemosphere*, Vol. 68:1985–1991.
7. CHUNG, I.M. AND MILLER, D.A. 1995a. Differences in autotoxicity among seven alfalfa cultivars. *Agronomy Journal*, Vol. 87: 596-600.
8. EINHELLIG, F.A. 1996. Interactions involving allelopathy in cropping systems. *Agronomy Journal*, Vol. 88: 886-893.
9. EL-DARIER, S.M. 2002. Allelopathic effects of *Eucalyptus rostrata* on growth, nutrient uptake and metabolite accumulation of *Vicia faba* L. and *Zea mays* L. *Pakistan Journal of Biological Sciences*, 5: 6-11.
10. EL-DARIER, S.M. AND YOUSSEF, R.S. 2000. Effect of soil type, salinity, and allelochemicals on germination and seedling growth of a medicinal plant *Lepidium sativum* L. *Annals of Applied Biology*, Vol. 136 (3): 273 – 279.
11. EL-DARIER, S.M. AND YOUSSEF, R.S. 2007. Does salinity enhance allelopathic effects of *Tribulus terrestris* L. in watermelon agro-ecosystems at Nobaria, Egypt?. *El-Minia Science Bulletin*, Vol. 18 (2): 307- 328.
12. EL-DARIER, S.M.; ABDELAZIZ, H.A. AND ZEIN EL-DEIN, M.H. 2014. Effect of soil type on the allelotoxic activity of *Medicago sativa* L. residues in *Vicia faba* L. agroecosystems. *Journal of Taibah University for Science* Vol. 8: 84–89.
13. EVENARI, M. 1949. Germination inhibitors. *The Botanical Review*, Vol. 53: 153-194.
14. FERGUSON, J.J. AND RATHINASABAPATHI, B. 2003. Allelopathy: How plants suppress other plants. EDIS (<http://edis.ifas.ufl.edu>), *University of Florida IFAS Extension*, publication number HS944.
15. HALE, M.G. AND ORCUTT, D.M. 1987. The Physiology of Plants Under Stress. Blackburg, Virginia, p. 206.
16. HEGAB, M.M.; KHODARY, S.E.A.; HAMMOUDA, O. AND GHAREIB, H. R. 2008. Autotoxicity of chard and its allelopathic potentiality on germination and some metabolic activities associated with growth of wheat seedlings. *African Journal of Biotechnology*, Vol.7 (7):884-892.
17. INDERJIT, J. 1998. Allelopathic interference of chickweed, *Stellaria media* with seedling growth of wheat (*Triticum aestivum*). *Canadian Journal of Botany*, Vol. 76:1317-1321.
18. INDERJIT, J. AND WEINER, J. 2001. Plant allelochemical interference or soil chemical ecology? *Perspectives in Plant Ecology Evolution and Systematics*, Vol. 4(1): 3-12.
19. LAMBERS, H.; CHAPIN, F.S.I. AND PONS, T.L. 1998. Plant Physiological Ecology. *Biologia Plantarum*, Vol. 42 (1): 64.

20. MILLER, D.A. 1983. Allelopathic effects of alfalfa. *Journal of Chemical Ecology*, 9:1059–1072.
21. OHNO, T. 2001. Oxidation of phenolic acid derivatives by soil and its relevance to allelopathic activity. *Journal of Environmental Quality*, Vol. 30:1631-1635.
22. OYERINDE, R.O.; OTUSANYA, O.O. AND AKPOR, O. B. 2009. Allelopathic effect of *Tithonia diversifolia* on the germination, growth and chlorophyll contents of maize (*Zea mays* L.). *Scientific Research and Essay*, Vol. 4 (12):1553-1558.
23. PUTNAM, A.R. 1988. Allelochemicals from plants as herbicides. *Weed Technology* 2: 510-518.
24. QASEM, J.R. AND HILL, T.A. 1989. Possible role of allelopathy in the competition between tomato, *Senecio vulgaris* L. and *Chenopodium album* L. *Weed Research*, Vol. 29:349–356.
25. REINHARDT, P.; CAUSA, M.; MARIAN, C.M. AND HE\S, B.A. 1996. Adsorption of CO on TiO₂ (110) studied by means of a cluster model surrounded by multipoles obtained from slab calculations. *Physical Review B*, Vol. 54:14812–14821.
26. RICE, E.L. 1979. Allelopathy: An update. *Botanical Review*, 45:17–109.
27. RIZVI, S. J. H. AND RIZVI, V. 1992. Allelopathy: basic and applied aspects. Chapman and Hall, London. pp. 480.
28. SCHACTMAN, D.P.; REID, R.J. AND AYLING, S.M. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology*, Vol. 116: 447-453.
29. SCOTT, S.J.; JONES, R.A. AND WILLIAMS, W.A. 1984. Review of data analysis methods for seed germination. *Crop Science*, Vol. 24: 1192-1199.
30. SHI, J.; ARUNASALAM, K.; YEUNG, D.; KAKUDA, Y. AND Mittal, G. 2008. Saponins from edible legumes: Chemistry, processing, and health Benefits. *Journal of Medicinal Food*, Vol. 7(1): 67-78.
31. SIDDIQUI, Z.S. 2007. Allelopathic effects of black pepper leachings on *Vigna mungo* Hepper. *Physiologia Plantarum*, Vol. 29(4): 303-308.
32. SINGH, H.P.; BATISH, D.R. AND KOHLIE, R.K. 2001. Allelopathy in agroecosystems: An overview. *Journal of Crop Production*, Vol. 4 (2):1-41.
33. SINGH, N. B. SINGH, D.; SINGH, A. 2009. Modification of physiological responses of water stressed *Zea mays* seedlings by leachate of *Nicotiana plumbaginifolia*. *General and Applied Plant Physiology*, 35 (1–2): 51–63.
34. TEFERA, T. 2002. Allelopathic effect of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef*. *Journal of Agronomy and Crop Science*, Vol., 188: 306-310.
35. TERZI, I.; KOCAÇALIŞKAN, I.; BENLİGLU, O. AND SOLAK, K. 2003. Effects of juglone on growth of cucumber seedlings with respect to physiological and anatomical parameters. *Acta Physiologia Plantarum*, Vol. 25 (4): 353-356.
36. VERBRUGGEN, E.; VAN DER HEIJDEN, M.G.A.; RILLIG, M.C. AND KIERS, E.T. 2012. Minireview: Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist*, doi: 10.1111/j.1469-8137.2012.04348.x
37. WHITTAKER, R.H. AND FEENY, P.P. 1971. Allelochemicals: Chemical interactions between Species. *Science*, 171 (3973): 757 – 770.
38. ZAR, J.H. 1984. Biostatistical Analysis Prentice-Hall: Inc. New Jersey, 718pp.
39. ZHANG, L.X.; LI, S.X.; ZHANG, H. AND LIANG, Z.S. 2007. Nitrogen rates and water stress effects on production, lipid peroxidation and antioxidative enzyme activities in two maize (*Zea mays* L.) genotypes. *Journal of Agronomy and Crop Science*, Vol. 193 (6):387-397.