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EDITORIAL PREFACE

It is my pleasure to present the ninth volume of the *Jordan Journal of Biological Sciences* (JJBS) to the audience. JJBS is a refereed, peer reviewed quarterly international journal issued by the Jordanian Ministry of Higher Education and Scientific Research Support Fund in cooperation with The Hashemite University, Zarqa, Jordan. This journal publishes papers in Biological Sciences encompassing all the branches at molecular, cellular and organismal levels.

A group of distinguished scholars have agreed to serve on the Editorial Board. Without the service and dedication of these eminent scholars, JJBS would have never existed. Now, the Editorial Board is encouraged by the continuous growth of the journal and its formation into a true multidisciplinary publication. I am also honored to have the privilege of working with all members of the international advisory board served by a team of highly reputable researchers from different countries across the globe. I am also delighted with our team of national and international reviewers who are actively involved in research in different biological sciences and who provide authors with high quality reviews and helpful comments to improve their manuscripts.

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At the end of this preface, I would like to thank our readers and authors for their continuing interest in JJBS, and each member of our editorial and review boards for their continued hard work, support and dedication, which made it possible to bring another new issue of JJBS to the multidisciplinary international audience. My thanks are also extended to the Hashemite University and Jordanian Scientific Research Support Fund for their continuous support to Jordan Journal of Biological Sciences. I very much appreciate your support as we strive to make JJBS one of the most leading and authoritative journals in the field of Biological Sciences.

March, 2016

Prof. Ali Z. Elkarmi
Editor-in-Chief
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Original Articles

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Effects of Plant Growth Promoting Rhizobacteria on the Performance of Greengram under Field Conditions

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Abstract

Despite reports on effects of single Plant Growth Promoting Rhizobacteria (PGPR) inoculation on legumes, response of greengram to combined inoculation with phosphate solubilizing asymbiotic *Azotobacter* and ACC deaminase positive symbiotic *Bradyrhizobium* sp. (vigna) under field conditions remains unexplored. The present study aims at identifying ACC deaminase producing and phosphate solubilizing bacterial strains and to assess their impact on greengram plants in order to find efficient and friendly co-cultures for developing effective bioinoculants for increasing sustainable production of legumes. Additionally, plant growth promoting activities of *Azotobacter* and *Bradyrhizobium* sp. (vigna) were determined using standard methods. The isolated bacterial cultures were characterized morphologically, culturally and biochemically and were identified as *Bradyrhizobium* sp. (vigna) and *Azotobacter chroococcum*. Dry matter accumulation in whole plants, symbiotic attributes, nutrient uptake and grain yields were significantly enhanced following co-inoculation of *A. chroococcum* and *Bradyrhizobium* sp. (vigna).The inoculation of *Azotobacter* with *Bradyrhizobium* increased seed yield by two fold and produced the highest grain protein. A- 75% and 52% increase in P concentration in root and shoots, respectively was observed for *A. chroococcum*, while P uptake was highest (0.52 mg/g) in shoots following combined inoculation of *A. chroococcum* with *Bradyrhizobium* at harvest. The highest N concentration in roots and shoot at harvest were observed with co-culture of *A. chroococcum* and *Bradyrhizobium* sp. (vigna). Gram negative *Azotobacter* and *Bradyrhizobium* solubilized insoluble phosphate, synthesized indole acetic acid, ammonia, cyanogenic compounds and exopolysaccharides while only *Bradyrhizobium* showed ACC deaminase activity. The results suggest that two unrelated bacteria belonging to symbiotic and asymbiotic group and capable of facilitating greengram production under field conditions and expressing multiple plant growth promoting activity can be used to produce composite bioinoculants for enhancing greengram production while saving the use of fertilizers.

Keywords: *Azotobacter*,ACC Deaminase,*Bradyrhizobium*,Greengram, Nutrient Uptake, Nodule,Seed Yield.

1. Introduction

In high input agricultural practices, chemical fertilizers are frequently used to optimize crop production. These expensive chemicals, however, when used injudiciously, have resulted in loss of soil fertility and consequently, the crop production (Lemanski and Scheu, 2014). Due to these reasons, focus in recent times has been shifted towards the use of inexpensive natural resources such as Plant Growth Promoting Rhizobacteria (PGPR): soil bacteria that colonize the roots of plants following inoculation onto seeds and that enhance plant growth (Kloepper and Schroth, 1978). The PGPR involving free living (asymbiotic) growth promoting rhizobacteria (Lugtenberg and Kamilova, 2009; Bhattacharya and Jha, 2012), symbiotic rhizobia (Ahmad *et al.,* 2013; Peix *et al.,* 2015) and phosphate solubilizers (Zaidi *et al.,* 2009; Nosrati *et al.,* 2014) have been used for enhancing the production of different crops (Mohite, 2013; Viruel *et al.,* 2104) including legumes (Noreen *et al.,* 2012; Singh *et al.,* 2013). Among non-nodule forming diazotrophs, *Azotobacter*, a free living nitrogen fixer, discovered and described in 1901 by the Dutch Microbiologist and botanist Martinus Beijerinck, play an important role in crop improvement by supplying mainly nitrogen (N) to plants. However, apart from providing N to plants, *Azotobacter* promotes plant growth directly by secreting considerable amounts of biologically active substances like B vitamins, nicotinic acid, pentothenic acid, biotin, gibberellic acid, Indole-3 Acetic Acid (IAA) and cytokinin (Ahmad *et al.,* 2005; Lenin and Jayanthi, 2012; Oskar *et al.,* 2014) and ammonia (Narula and Gupta, 1986) or indirectly by protecting the plant from diseases (Saini, 2012). Also, the secretion of 1-aminocyclopropane-1-carboxylate (ACC) deaminase by PGPR including nitrogen fixers have been found to reduce the level of plant stress hormone ethylene and consequently to enhance plant growth (Akhgar *et al.,* 2014; Hassan *et al.,* 2014; Magnucka and Pietr, 2015).The

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PGPR endowed with variable plant growth promoting activities have been used singly or in combination for enhancing legume production (Ahmad *et al.,* 2013). For example, Ara et al.(2009) conducted a field experiment to study the effects of *Bradyrhizobium* and *Azotobacter* inoculation on growth and yield of mungbean varieties viz. BARI mung-3 and BARI mung-4, using five bacterial and chemical fertilizers (20 kg N ha^{-1}). Application of *Bradyrhizobium* or *Azotobacter* or their combination significantly increased nodulation, and root and shoot weights at 35 days after sowing. Similarly, the number of seeds per pod and seed yield was significantly influenced by the bacterial biofertilizer. Also, seed inoculation with free living PGPR or symbiotic rhizobia have been shown to increase legume growth and productivity (Wani *et al.,* 2007) suggesting that use of mixed inoculants could be an effective method for sustainable production of greengram.

Greengram [*Vigna radiata* (L.) wilczek] is a popular grain legume cultivated in the tropics and forms a highly specific symbiosis with *Bradyrhizobium* sp. (vigna). Greengram contains 24% protein with excellent digestibility, 1-3% fats, 50.4% carbohydrates, 3.5-4.5% fibers and 5.5% ash, while Ca and P are 132 and 367 mg per 100 grams of seed, respectively (Frauque *et al.*,2000). Greengram is a short duration legume (maturing in 55 to 70 days), and hence, it fits well into many cropping systems, including rice and sugarcane under both rain fed and irrigated conditions. Moreover, greengram fixes about 40-50 kg N ha^{-1}. In India, it is grown in an area of 3 x 106 ha with an annual production of 1 x 106 t of grains (Sharma, 2000). Since greengram is one of the most important grain legumes, improving its production using low cost technology such as biofertilizer is urgently required to fulfill the increasing demands of pulses for ever growing population over a limited land. Even though there are no direct symbiotic connections between *Azotobacter* and greengram plants as observed between rhizobia and greengram plants, Perveen *et al.* (2002) observed a substantial increase in the yield of *Azotobacter* inoculated greengram plants which were attributed to some plant growth regulators secreted by *Azotobacter*. However, little is known about the response of greengram to combined inoculation with phosphate solubilizing asymbiotic nitrogen fixing bacteria and nodule forming *Bradyrhizobium* under field conditions. Considering the importance of symbiotic rhizobia and phosphate solubilizing *Azotobacter* and lack of information on the use of mixed cultures in greengram production, the present study was aimed at identifying ACC deaminase producing and phosphate solubilizing bacterial strains and to evaluate the effect of single and combined inoculations of notable plant growth promoting rhizobacteria belonging to asymbiotic N_2 fixer (*A.chroococcum*) and symbiotic N_2 fixer (*Bradyrhizobium* sp. (vigna) group on growth, symbiotic attributes, nutrient uptake and yield attributes of greengram, grown under field soils fertilized with/without nitrogenous (urea) and phosphatic (diammonium phosphate, DAP) fertilizers in order to find a suitable composite bacterial culture for producing effective bioinoculants for enhancing the production of greengram.

2. Materials and Methods

2.1. Isolation and Identification of Bacterial Cultures

Bradyrhizobial strains were isolated from nodules of greengram plants grown in the fields of Agricultural Sciences, Aligarh Muslim University, Aligarh, ($27^0 29^1$ latitude and 72^0 29^1 longitude) using Yeast Extract Mannitol (YEM) medium (Somasegaran and Hoben, 1985). For this, healthy and undamaged nodules were surface sterilized with sodium hypochlorite (2.5 % for 2 min.). Then, sodium hypochlorite treated nodules were rinsed in 95 % ethanol (v/v) and washed several times with distilled water. Surface sterilized nodules were then crushed gently in Normal Saline Solutions (NSS). Nodule suspensions were serially diluted in NSS and 100 µl of each diluent was spread plated on YEM agar medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India) containing 2.5% Congo red indicator dye. The inoculated plates were incubated at 28±2 °C for 5 days. A- single colony was picked and streaked 4 times on the same medium to ascertain the purity of the cultures and each chosen strain was maintained on YEM (g/l: mannitol 10; K_2HPO_4 0.5; $MgSO_4$. $7H_2O$ 0.2; NaCl 0.1; yeast extract 1; $CaCO_3$ 1 and pH was adjusted to 7) agar medium at 4 °C until use. Bradyrhizobial strain was subjected to plant infection test using greengram as a host plant in order to validate its host specificity. For *Azotobacter* isolation, soils were collected from chilli (*Capsicum annuum*) rhizosphere in sterilized polythene bags (15×12 cm2). Soil samples were serially diluted in sterile NSS and 100 µl of diluted suspension was spread plated (Buck and Cleverdon, 1960) on Ashby's mannitol agar medium (g/l: mannitol 20.0; Dipotassium hydrogen orthophosphate 0.2; Potassium sulphate 0.1; Mg SO_4 0.2; $CaCO_3$ 5.0; NaCl 0.2) (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Plates were then incubated at 28±2 °C for 5 days and colonies producing brown to black pigment (melanin) were picked and used for further experiment. Both *Bradyrhizobium* and *Azotobacter* were subjected first to Gram reaction which is an important step in the identification of bacteria to species level. The bacterial isolates were then subjected to further identification using various morphological, cultural and biochemical tests (Holt *et al.,* 1994) that included indole reaction, citrate utilization test, methyl red test, Voges-Proskauer, catalase, oxidase test, starch and gelatin hydrolysis etc. which led to their identification.

2.2. Bacterial Preparation, Seed Treatments and Planting

Azotobacter chroococcum isolated from chilli rhizosphere and *Bradyrhizobium* sp. (vigna) recovered from greengram nodules were grown in Ashby and YEM broth, respectively, to a cell density of 3x105 cells/ml (*A. chroococcum*) and 6x108 (*Bradyrhizobium*). Healthy seeds of greengram var. K-851 were purchased from Prakash Agrochemicals and seeds, Aligarh, Uttar Pradesh, India. Seeds were surface sterilized with 70% ethanol for 3 min. and 3% sodium hypochlorite, 3 min. (Vincent, 1970). Surface sterilized seeds were rinsed 6 times with sterile water and dried. Surface sterilized seeds were bacterized by soaking seeds in liquid culture medium for 2h using 10% Arabic gum as sticker to deliver

approximately 10^8 and10^5 cells ml-1 each of *Bradyrhizobium* sp. (vigna) and *A. chroococcum*, respectively. For co-inoculations, the liquid culture of each organism was mixed in equal proportion to soak the sterile seeds (100 g seeds in 200 ml broth solution for 2h). The non-coated (without inoculant) sterilized seeds soaked in sterile water served as control. The non-inoculated and inoculated seeds were sown in soil (sandy clay loam; 0.4% organic carbon, 0.75 g/kg Kjeldahl N, 16 mg/kg Olsen P, pH 7.2 and 0.44 ml/g water holding capacity (WHC), 11.7 cmol kg-1 cation exchange capacity, 5.1 cmol/kg Anion exchange capacity, 59.1 µSm-1 salt conductivity) using seed-drill method at a soil depth of 10–12cm in 5m×5m plots at 45 cm row distance (RxR) and 5-10 cm plant distance (PxP) within the rows. The treatments were:

T1= control (neither fertilized nor inoculated:NFNI);

T2= recommended dose of urea (25 kg/ha);

T3= recommended dose of diammonium phosphate (DAP) (85 kg/ha);

T4= *A. chroococcum*;

T5=*Bradyrhizobium* sp. (vigna);

T6=*A. chroococcum* with 25 kg/ha urea;

T7=*Bradyrhizobium* sp. (vigna) with 85 kg/ha DAP;

T8=*A. chroococcum* with *Bradyrhizobium* sp. (vigna);

T9=urea (25 kg/ha) with DAP (85 kg/ha).

The plots receiving inoculation treatments remained unfertilized. All nine treatments were replicated six times and experiment was laid out in a completely randomized design. The field experiments were repeated for two successive years under identical environmental conditions using the same treatments to ensure the reproducibility of the results.

2.3. Measurement of Biological and Chemical Characteristics of Plants

All plants in three replicates for each treatment were removed 50 Days After Sowing (DAS) and remaining plants in three replicates for each treatment were maintained until harvest (80 DAS). Randomly selected plants removed at 50 DAS were used to observe the extent of nodulation. Nodules collected randomly from each treatment were counted, oven dried at 80 °C and weighed. The leghaemoglobin (Lb) content in fresh nodules recovered from the root systems of greengram plants were quantified by the method of Sadasivam and Manickam (1992).For this, fresh nodules were crushed using mortar and pestle in 5 ml sodium phosphate buffer (pH 7.4) and filtered through two layers of cheese cloth. The nodule debris was discarded. The turbid reddish brown filtrate was clarified by centrifugation at 10000 g for 30 min. The supernatant was diluted to 10 ml with sodium phosphate buffer (pH 7.4). The extract was divided equally into two glass tubes (5 ml /tube) and equal amount of alkaline pyridine reagent was added to each tube. The haemochrome formed was read at 556 and 539 nm after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively. The leghaemoglobin content was calculated using the formula–

$$\text{Lb content (mM)} = \frac{[A_{556} - A_{539}] \times 2D}{23.4}$$

where, D= initial dilution.

The dry matter accumulation in whole plants (with intact roots, shoots and leaves) was measured both at 50 and 80 DAS. Total N content in roots and shoots was measured at harvest by micro-Kjeldahl method of Iswaran and Marwah (1980), while P content in roots and shoots was measured by the method of Jackson (1967). Seed yield and grain protein (Lowery *et al.*, 1951) were measured at harvest.

2.4. Statistical Analysis

The resulting data of the measured variables were pooled together and subjected to analysis of variance by applying two-way ANOVA [for two-factors (inoculation and fertilizer)] at 5% probability level using software, Mini-Tab 10.

2.5. In Vitro Bioassay of Plant Growth Promoting Activities

2.5.1. ACC Deaminase Activity, P Solubilization and Indole Acetic Acid Content

Using the spot inoculation method, 5 µl of each bacterial inoculum was placed on a section of plate (marked in 16 equal parts) containing Dworkin and Foster (DF) salt minimal medium (g.l-1: KH2PO4 4; Na_2HPO_4 6; $MgSO_4.7H_2O$ 0.2, glucose 2.0; gluconic acid 2.0; citric acid 2.0; $FeSO_4.7H_2O$ 0.001; H_3BO_3 0.01, $MnSO_4.H_2O$ 0.011; $ZnSO_4.7H_2O$ 0.124; $CuSO_4.5H_2O$ .078; MoO_3 0.01; $(NH_4)_2SO_4$ 2.0; pH 7.2) (Dworkin and Foster,1958) supplemented with 3 mM ACC (instead of $(NH_4)_2SO_4$) and incubated at 28±2 °C for 72 h and bacterial growth was checked daily. The ACC deaminase activity (EC 4.1.99.4) of *A. chroococcum* and *Bradyrhizobium* sp. (vigna) was measured by the method of Penrose and Glick (2003). The ACC deaminase activity was expressed as the amount of α-ketobutyrate produced per milligram of protein per hour. A positive control [*Mesorhizobium* LMS-1 containing pRKACC plasmid was also run along the experiment. All samples were tested in duplicate and each individual experiment was repeated three times.For phosphate solubilizing activity, culture suspensions of both *Bradyrhizobium* and *Azotobacter* weres pread plated on Pikovskaya agar (Pikovskaya, 1948) medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India). Plates were incubated at 28±2 °C for 7 days. The bacterial cultures exhibiting clear halo around growth following incubation were considered as P-solubilizers. The halo forming bacterial strains were further used to determine the extent of P-solubilization in Pikovskaya broth ($g.l^{-1}$): glucose 10; $Ca_3(PO_4)_2$ 5; $(NH_4)_2SO_4$ 0.5; NaCl 0.2; $MgSO_4.7H_2O$ 0.1; KCl 0.1; yeast extract 0.5; $MnSO_4$ and $FeSO_4$ trace; pH 7) by the chlorostannous-reduced molybdophosphoric acid blue method (King, 1932). Briefly, 100 ml of Pikovskaya broth was inoculated with 1 ml of 10^8 cells ml^1-of *A. chroococcum* and *Bradyrhizobium* sp. (vigna). The inoculated flasks were incubated for 7 days with shaking (200 r/min.) at 28±2 °C. A 20 ml culture broth from each flask was removed and centrifuged (9000g) for 30 min. To 10 ml of supernatant, 10 ml chloromolybdic acid and 5 drops of chlorostannous acid was added and volume was adjusted to 50 ml with distilled water. The absorbance of developing blue color was read at 600 nm using spectrophotometer Spectronic 20 D+. The amount of

solubilized P was calculated using the calibration curve of KH2PO4. IndoleAcetic Acid (IAA) was determined by the method described by Brick et al. (1991). Bacterial cultures were inoculated at 108 cells ml-1 and were incubated for 24 h at 28±2 °C with shaking at 125 r/min. for 72 h for *Bradyrhizobium* and 48 h for *Azotobacter* on Luria Bertani (LB) broth (g. l^{-1}: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5) supplemented with different concentrations of tryptophan (0, 100, 200, 400 and 500 µg/ml) at 28 °C. Fully grown cultures were centrifuged at 9000g for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink color indicated IAA production. Optical density was taken at 530 nm using spectrophotometer Spectronic 20 D+. The IAA concentration in the supernatant was determined using a calibration curve of pure IAA as a standard.

2.5.2. Siderophores, Ammonia, Cyanide and Exo-Polysaccharides

The release of siderophores by *Bradyrhizobium* and *Azotobacter* was assayed using Chrome Azurol S (CAS) agar medium following the method of Alexander and Zuberer (1991). Chrome Azurol S agar plates were prepared separately and divided into equal sectors and spot inoculated with 10 µl of 10^8 cells/ml of *Bradyrhizobium* and 10^5 cells/ml of *Azotobacter* and incubated at 28±2 °C for five days. Development of yellow orange halo around the bacterial growth was considered as positive for siderophore synthesis. Each individual experiment was repeated three times. Siderophore was also detected quantitatively using Modi medium (K_2HPO_4 0.05%; $MgSO_4$ 0.04%; NaCl 0.01%; mannitol 1%; glutamine 0.1%; NH_4NO_3 0.1%). Modi medium was inoculated with 108 cells/ml of *Bradyrhizobium* and 10^5 cells/ml of *Azotobacter* and incubated at 28±2 °C for 5 days. Catechol type phenolates were measured on ethyl acetate extracts of the culture supernatant using a modification of the ferric chloride-ferricyanide reagent of Hathway. Ethyl acetate extracts was prepared by extracting 20 ml of supernatant twice with an equal volume of solvent at pH 2. Hathway's reagent was prepared by adding one milliliter of 0.1 M ferric chloride in 0.1 N HCl to 100 ml of distilled water, and to this, was added one milliliter of 0.1 M potassium ferricyanide (Reeves *et al.,* 1983). For the assay, one volume of the reagent was added to one volume of sample and absorbance was determined at 560 nm for salicylates with sodium salicylate as standard and at 700 nm for dihydroxy phenols with 2, 3- Dihydroxy Benzoic Acid (DHBA) as standard.

The HCN synthesized by bacterial strains was determined following the method of Bakker and Schipper (1987). The synthesis of ammonia by the bacterial strains was detected using peptone water. Freshly grown bacterial strains (200 µl of 10^8 cells ml^{-1}) were inoculated in 20 ml peptone water in tubes and incubated at 28±2 0C for 4 days. One millilitre of Nessler reagent was added to each tube. Development of yellow colour indicated a positive test for ammonia production (Dye, 1962). Exo-Polysaccharides (EPS) secreted by *Bradyrhizobium* sp. (vigna) and *A. chroococcum* was quantitatively detected by allowing each organism to grow in 100 ml basal medium containing 5% sucrose and incubated for five days at 28±2 °C on rotary shaker (100 r/min.). After incubation, culture broth was centrifuged (5433g) for 30 min. and EPS was extracted by adding three volumes of chilled acetone to one volume of supernatant. The precipitated EPS was repeatedly washed three times alternately with distilled water and acetone, transferred to a filter paper and weighed after overnight drying at 80 °C.

3. Results

The present work was undertaken to identify symbiotic and asymbiotic nitrogen fixing bacteria from greengram nodules and chilli rhizospheres, respectively. The selected bacterial cultures were used to assess their impact on the performance of greengram under field soils. Also, the plant growth promoting activities of the symbiotic and asymbiotic nitrogen fixing bacteria were determined.

3.1. Characterization and Identification

In the present study, a total of 20 *Azotobacter* strains were isolated from chilli rhizosphere and primarily identified by morphological, cultural and biochemical tests. Of these, strain AZ19 was selected due to its ability to exhibit greater P- solubilizing efficiency and IAA synthesis and was characterized and identified (Table 1). Moreover, isolate AZ19 produced brown to black pigment called melanin on Ashby medium after 5 days incubation. Strain AZ19 was found Gram negative and showed positive reaction to methyl red, nitrate reduction, Vogues Proskauer, and catalase. Strain AZ19 could further hydrolyze starch and gelatin and displayed variable carbohydrate utilization efficiency. Comparing these properties with those given in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994), strain AZ19 was presumptively identified as Azotobacter. Of the 20 bradyrhizobial isolates, strain RB6 was especially chosen due to its better P solubilizing activity and ACC deaminase producing ability. The RB6 was Gram negative, short rods and produced circular mucoid colony on YEMA plates. The isolate RB6 showed a variable biochemical reaction (Table 1). In order to ensure the identity and specificity strain RB6 was used to inoculate greengram plants (infection test) for nodulation in sterile soil and subsequently following nodulation, it was identified as *Bradyrhizobium* specific to greengram.

Table 1. Morphological and biochemical properties of bacterial cultures

Characteristics	Bacterial Strains	
	BR6	AZ19
Morphological		
Colony	Transparent, circular and mucoid	Serrated, muciliginous, Dark pigmented
Cell Shape	Short rods	Rod
Gram reaction	-ve	-ve
Biochemical Properties		
Citrate utilization	-	-
Indole	+	-
Methyl red	+	+
Nitrate reduction	+	+
VogesProskauer	+	+
Catalase	+	+
Oxidase	-	-
Hydrolysis		
Starch	+	+
Gelatin	+	+
Carbohydrate utilization		
Glucose	+	+
Lactose	-	+
Mannitol	+	-
Sucrose	+	+
Arabinose	+	+
Xylose	-	+
Inositol	-	-
Mannose	+	+
Melanin production	-	+
Identification	*Azotobacter*	
Nodulation test	*Bradyrhizobium* sp. (vigna)	

3.2. Field Experiments

3.2.1. Total Dry Biomass and Symbiotic Characteristics

Dry matter accumulation in greengram plants measured at 50 and 80 DAS varied among treatments (Figure 1). The recommended rates of urea and DAP and mixture of both fertilizers enhanced dry biomass accumulation from 1.77 g/plant (control) to 2.16 g/plant (31%), 2.27 g/plant (38%) and 2.78 g/plant (67%), respectively at 50 DAS. Similarly, at 80 DAS, the total dry matter accumulation in greengram plants varied significantly among treatments. Furthermore, the sole application of *A. chroococcum* and *Bradyrhizobium* improved dry matter accumulation in plants by 32 and 37%, respectively at 50 DAS compared to control plants. In comparison, the composite application of fertilizers and microbial inocula for example, urea with *A. chroococcum* enhanced the total dry biomass by 42% at 80 DAS compared to un-inoculated and untreated control plants. Total dry biomass was increased further by 72%, at 50 DAS following *Bradyrhizobium* inoculation with *A. chroococcum*. In general, the bacterial cultures used either alone or in combination with fertilizers consistently increased the dry biomass of whole greengram plants (with intact roots, shoots and foliage) grown in fields.

The nodule formation and leghaemoglobin accumulation in fresh nodules following bacterial inoculations and fertilizer application varied among treatments (Table 2).Among single-inoculations, *Bradyrhizobium* maximally enhanced nodule numbers, nodule mass and leghaemoglobin significantly ($P \leq 0.05$) by 162% (55 nodules/plant), 155% (191 mg/plant) and 143% [0.17mM $(gfm)^{-1}$], respectively above control at 50 DAS. The composite application of A. chroococcum and *Bradyrhizobium* further enhanced the nodule numbers, nodule mass and leghaemoglobin significantly by 210% (65 nodules/plant), 199% (224 mg/plant) and 200% compared to control plants. Among fertilizers, mixture of urea (25 kg/ha) and DAP (85 kg/ha) showed maximum increase in nodule numbers (143%) and Lb content (143%) compared to control.

3.2.2. Nutrient Uptake, Seed Yield and Grain Protein

Among single inoculations, *A. chroococcum* showed maximum increase in P contents in roots (0.35 mg/g) while *Bradyrhizobium* resulted in maximum increase in shoots (0.45 mg/g). Generally, bacterial cultures in the presence of urea and DAP further enhanced N and P concentrations in plant organs. For example, *Bradyrhizobium* inoculated plants grown in soil fertilized with DAP had the maximum N contents in shoots (68 mg/g) and P contents in shoots (0.53 mg/g) whereas *A. chroococcum* inoculated plants had maximum shoot N (65 mg/g) and shoot P (0.50 mg/g) when grown in fields fertilized with urea. Similarly, the mixture of *A.chroococcum* and *Bradyrhizobium* sp. (vigna) showed maximum increase in the measured parameters compared to other single bacterial treatments, fertilizer application, or uninoculated and unfertilized plants (Table 2). For example, dual inoculation of *A.chroococcum* and *Bradyrhizobium* sp. (vigna) augmented the root and shoot P by 120% and 79%, respectively over control. Among bacterial treatments, the sole inoculation of *A.chroococcum* and *Bradyrhizobium* significantly ($P \leq 0.05$) increased the seed yield by 65% (5.6g/plant) and 74% (5.9 g/plant), respectively relative to control (3.4 g/plant).While comparing the effect of fertilizers and mono bacterial cultures, *Bradyrhizobium* sp. (vigna)showed maximum increase in seed yield over urea and DAP. Moreover, in the presence of 85 kg DAP/ha, *Bradyrhizobium* sp. (vigna)gave maximum seed yield (103% increase) while *A.chroococcum* in the presence of 25 kg urea/ha increased the seed yield by 94% compared to control. The dual inoculation of *A.chroococcum* and *Bradyrhizobium* increased the grain yield maximally by 109% (7.1 g/plant). The increase in grain yield following mixture of urea and DAP (7.1 g/plant) was however, comparable to those observed for combined inoculation of *A.chroococcum* and *Bradyrhizobium*(7.0 g/plant). Protein content in seeds even-though were similar among treatments yet it was greater in inoculated plants compared to control ones. The maximum increase in grain protein was observed for dual inoculations of *A. chroococcum* and *Bradyrhizobium* sp. (vigna). Two-way ANOVA in general revealed that the individual effect was significant ($P \leq 0.05$) for all the measured parameters.

Table 2. Single and co-inoculation effects of asymbiotic *Azotobacter chroococcum* and ACC deaminase positive *Bradyrhizobium* sp. (vigna) on symbiotic characteristics, nutrient uptake and seed attributes of field grown greengram.

Treatments	Symbiotic attributes			Nutrient uptake				Seed yield (g/plant)	Seed protein (mg/g)
	[a]Nodules no./plant	[a]Nodule biomass (mg/plant)	[a]Leghaemoglobin mM (g .f m)$^{-1}$	N content (mg/g)		P content (mg/g)			
				Root	Shoot	Root	Shoot		
Control	21^{e}±4	75^{f}±8	0.07^{d}±0.02	28^{e}±3	44^{d}±3	0.20^{d}±0.03	0.29^{d}±0.02	3.4^{d}±0.3	254^{b}±11
Urea (25 kg/ha)	33^{de}±4	124^{ef}±11	0.12^{c}±0.03	40^{c}±4	58^{c}±5	0.27^{c}±0.04	0.37^{c}±0.03	4.7^{c}±0.3	266^{a}±12
DAP (85 kg/ha)	35^{d}±5	138^{e}±13	0.12^{c}±0.03	39^{d}±3	56^{cd}±4	0.33^{bc}±0.03	0.46^{b}±0.03	4.9^{c}±0.4	265^{ab}±12
[1]*Azotobacter*	44^{c}±5	161^{d}±12	0.14^{bc}±0.02	43^{c}±3	60^{c}±5	0.35^{b}±0.04	0.44^{b}±0.04	5.6^{b}±0.4	265^{a}±13
[2]*Bradyrhizobium*	55^{b}±4	191^{c}±15	0.17^{b}±0.03	47^{b}±5	67^{a}±4	0.34^{b}±0.03	0.45^{b}±0.03	5.9^{b}±0.4	267^{a}±11
Azotobacter +Urea	44^{c}±6	181^{cd}±17	0.14^{bc}±0.03	50^{b}±4	65^{b}±5	0.40^{ab}±0.03	0.50^{a}±0.03	6.6^{ab}±0.5	270^{a}±10
Bradyrhizobium +DAP	56^{b}±4	211^{b}±19	0.18^{ab}±0.03	53^{a}±4	68^{a}±6	0.42^{a}±0.04	0.53^{a}±0.04	6.9^{a}±0.6	267^{a}±11
Azotobacter + *Bradyrhizobium*	65^{a}±6	224^{a}±21	0.21^{a}±0.03	54^{a}±5	70^{a}±5	0.44^{a}±0.04	0.52^{a}±0.04	7.0^{a}±0.5	270^{a}±12
Urea+ DAP	51^{c}±5	201^{bc}±19	0.17^{b}±0.03	54^{a}±4	69^{a}±4	0.46^{a}±0.04	0.54^{a}±0.04	7.1^{a}±0.5	272^{a}±11
LSD	4.2	11.2	0.03	2.7	3.4	0.04	0.06	0.5	3.1

[a]Nodule number, [a]Nodule dry biomass and [a]Leghaemoglobin content in fresh nodule was determined at 50 DAS of greengram growth; [1]*Azotobacter* indicates *A. chroococcum*;[2]*Bradyrhizobium*represents *Bradyrhizobium* sp. (vigna); DAP-diammonium phosphate; ± indicates mean value± SD; In this table each value is a mean of six replicates where each replicate constituted three plants/plot. Mean values are significant at P ≤0.05. Means followed by similar alphabets are not significantly different from each other according to post hoc Tukey HSD.

3.2.3. Bioassay of Plant Growth Promoting Substances

The *Bradyrhizobium* sp. (vigna) isolated from root nodules and *A. chroococcum* recovered from chilli rhizosphere were assessed for their ability to synthesize plant growth promoting substances (Table 3). Of these, only *Bradyrhizobium* sp. (vigna) produced 211 µmol α ketobutyrate/mg protein/h while *A. chroococcum* did not produce ACC deaminase. Even though, both *Bradyrhizobium* sp. (vigna) and *A. chroococcum* could solubilize insoluble P but the P-solubilization by *A.chroococcum* was 45.2% greater than those observed for *Bradyrhizobium* sp. (vigna). Also, A.chroococcumshowed 30% increase in IAA over *Bradyrhizobium* sp. (vigna) grown in Luria Bertani broth treated with 100 µg/ml tryptophan. Interestingly, both *Bradyrhizobium* sp. (vigna) and *A.chroococcum* secreted siderophores, EPS, HCN and ammonia, but no significant difference was observed among growth regulators.

Table 3. Quantitative assay of active biomolecules secreted by *Bradyhizobium* sp. (vigna) and *Azotobacterchroococcum.*

Bacterial strain	ACC deaminase activity (µmol α-ketobutyrate/mg protein/h)	P-solubilized (µg/ml)	IAA (µg/ml)	Siderophore			EPS (µg/ml)	HCN	Ammonia
				Zone on CAS agar (mm)	DHBA (µg/ml)	SA (µg/ml)			
Bradyhizobium sp. (vigna)	211±15	148±24	74±8	12±1.5	14±1.6	25±2.1	15±3	+	+
A. chroococcum	ND	215±11	96±9	12±1.1	15±1.6	28±2.4	19±2	+	+

Abbreviationsof plant growth promoting substances: CAS: Chrome Azurol S agar, DHBA: 2,3 Dihydroxy benzoic acid, SA: Salicylic acid, EPS: Exo-polysaccharides, ND: not detected, +: positive qualitative reaction, Values indicate mean ± S.D. of three independent replicates.

4. Discussion

In high input agricultural practices the use of biofertilizers especially those prepared from PGPR involving both phosphate solubilizers and N_2 fixers for optimum crop production has received greater attention due to low cost, easy production and lack of hazardous impact. Considering these properties, the hunt for soil microbiota capable of expressing variable plant growth activity has increased alarmingly. Realizing the importance of free living non symbiotic and phosphate solubilizing PGPR and symbiotic rhizobia in sustainable production of legumes, the present study was aimed at identifying and testing the influence of selected PGPR on overall performance of greengram under field soils fertilized with or without synthetic fertilizers. In the present study, strain AZ19was identified as Azotobacter by morphological, cultural and biochemical analysis and through melanin production while strain RB6 was identified as *Bradyrhizobium* by standard microbiological and plant infection test. There are four important species of *Azotobacter* viz., *A. chroococcum*, A. agilis, A. paspali and *A. vinelandii* of which *A. chroococcum* is most commonly found in Indian soils. A unique differentiating character of *Azotobacter* is its ability to form pigments. The pigment forming ability and types of pigment produced by each Azotobacter species is therefore, used as a marker for species differentiation. In the present study, aged cultures of *Azotobacter* formed an insoluble brown-black pigment commonly attributed to the presence of melanin. This melanin is formed as a result of oxidation of tyrosinase, a copper containing enzyme. Since Azotobacter produced melanin on Ashby medium, it was identified as *A. chroococcum* and hence, *Azotobacter* is considered *A. chroococcum* throughout the manuscript. In other studies, melanin excretion by nitrogen-fixing non-virulent bacterium *Azotobacter* was used as a marker for isolation and identification of *A. chroococcum* from soil samples (Aquilianti *et al.,* 2004; Tejera *et al.,* 2005; Banerjee *et al.,* 2014). However, other *Azotobacter* species forms fluorescent pigment (Jensen, 1954; Johnstone, 1955). Numerous authors have also isolated Bradyrhizobium from greengram nodules (Wani *et al.,* 2007a;Ahemad and Khan, 2011a).

The symbiotic nitrogen fixer *Bradyrhizobium* sp. (vigna) and asymbiotic nitrogen fixer *A. chroococcum* applied singly or as co-culture in the presence or absence of fertilizers favourably stimulated growth, nodulation, plant nutrition (N and P) and yield of greengram plants under field conditions. Generally, the combined application of *Bradyrhizobium* sp. (vigna) and *Azotobacter* showed a significant increase in overall performance of greengram plants compared to other single (fertilizers) treatments or simultaneous application of bacterial cultures. The composite culture of Bradyrhizobium sp. (vigna) and *A. chroococcum* among all treatments demonstrated highest increase in dry biomass of greengram plants at harvest (Figure 1). Similarly, the dual culture of *Bradyrhizobium* sp. (vigna) and *Azotobacter* resulted in maximum increase in nodule numbers (65/plant), nodule dry biomass (224 mg/plant) and leghaemoglobin [0.21 mM (g f m)-1], 50 days after sowing greengram plants compared to other treatments including the mixed application of urea and DAP. Additionally, since N and P are the two major elements affecting critically the growth and development of plants, the N and P contents in roots and shoots of inoculated and uninoculated plants were measured at harvest. In general, the N and P contents were at the maximum in shoots than in roots of field grown greengram plants inoculated with or without bioinoculants. Interestingly, the co-culture of *Azotobacter* and *Bradyrhizobium* sp. (vigna) producedlargest contents of N in roots(54 mg/g) and shoots (70 mg/g) while P contents in roots (0.46 mg/g) and shoots (0.54 mg/g) were found maximum following application of mixture of urea (25 kg/ha) and DAP (85 kg/ha) compared to other bacterial or fertilizer treatments. Combining an improved plant nutrient supply with N (*Bradyrhizobium*) and P (both by *Azotobacter* and *Bradyrhizobium*) together with other growth promoting substances with plant-growth promotion appears to have additive and possibly even multiplicative effects on greengram plants. Unlike other measured parameters, seed yield and seed protein were marginally better with combined application of urea and DAP over all treatments including co-culture of *Bradyrhizobium* sp. (vigna) and *Azotobacter*. However, the composite culture of *Azotobacter* and *Bradyrhizobium* sp. (vigna) significantly enhanced the seed yield and seed protein by 106% and 6%, respectively relative to control. The quantity and quality of greengram seeds were however, marginally better in *Bradyrhizobium* inoculated plants compared to *Azotobacter* inoculated plants. Similar increase due to inoculation with *Rhizobium* and *Azotobacter* in growth, symbiotic properties, nutrient uptake and seed attributes of legumes for example chickpea (Siddiqui *et al.,* 2014) and greengram following dual application of *Bradyrhizobium* and *Azotobacter* inoculants have been reported (Perveen *et al.,* 2002; Ara *et al.,* 2009). Additionally, the siderophore-producing rhizobial strains have been found more effective in nodulating legumes for instance chickpeas than other rhizobia (Duhan *et al.,* 1998). Similarly, the increased availability of P nutrition by phosphate solubilizing rhizobia (Kumar *et al.,* 2014) is reported to promote the rhizobial activity and concomitantly the biological N2 fixation (Giller, 2001) leading eventually to a substantial increase in the overall performance of legumes.

The beneficial impact of free living *Azotobacter* and nodule forming *Bradyrhizobium* sp. (vigna) on the biological and chemical characteristics of greengram plants has prompted us to identify factors involved in plant growth promotion. In order to find reasons forhow single or joint inoculation of *Azotobacter* and *Bradyrhizobium* sp. (vigna) facilitated the growth of greengram plants, the plant growth promoting activities of both bacterial cultures were assessed. Interestingly, both *A. chroococcum* and *Bradyrhizobium* sp. (vigna) used here produced considerable amounts of plant growth–promoting substances for example, both bacterial cultures solubilized insoluble P, secreted IAA, produced siderophores and EPS and showed a positive reaction to HCN and ammonia. However, ACC deaminase was

secreted only by *Bradyrhizobium* sp. (vigna) under in vitro conditions. Among plant growth regulators, ACC deaminase is an important plant growth regulator that induces metabolic changes and hence, increases the growth of plants indirectly by inhibiting/reducing ethylene synthesis (Glick *et al.,* 2007; Bal *et al.,* 2013; Magnucka and Pietr, 2015). Similar ACC deaminase production by rhizobia is reported (Ma et al. 2003; Bhattacharjee *et al.,* 2012). Solubilization of inorganic P is yet another important trait by which PGPR supplies soluble P and consequently enhance the growth of plants. In a P deficient soil, P is applied from external sources to fulfill P demands of crops. Interestingly, both *Azotobacter* and *Bradyrhizobium* used in this study solubilized insoluble P and following application, enhanced the yields of greengram. Therefore, both bacterial partners exhibited additional property of P solubilization in addition to their intrinsic nitrogen fixation ability. This finding could be of special interest for legume growers for soils deficient in both N and P sinceapplication of both *Bradyrhizobium* and *Azotobacter* together are likely to overcome the N and P deficiency. Furthermore, A. chroococcum showed 30% increase in IAA production over *Bradyrhizobium* sp. (vigna). Secretion of IAA by both *Azotobacter* and *Bradyrhizobium* sp. (vigna) is yet another microbiological trait that greatly influences the development of plants. IAA secreted as a secondary metabolite due to rich supply of substrates by PGPR is reported to control cell elongation and division, phototropism and apical dominance in plants (Remnas *et al.,* 2008; Ali *et al.,* 2009). Also, IAA aid in the production of longer roots with increased number of root hairs and lateral roots which are involved in nutrient uptake (Datta and Basu, 2000). Indole acetic acid also inhibits or delay abscission of leaves, induces flowering and fruiting (Zhao, 2010) and secretion of IAA by PGPR is reported (Shahab and Nasreen, 2009) Synthesis of siderophores may indirectly affect the growth of plants including legumes (Datta and Chakrabartty, 2014). Siderophores released by PGPR forms a complex with iron (Fe3+) in the rhizosphere and limits its availability to the phytopathogens and concomitantly prevent phytopathogens from causing damage to plants. In the present investigation, both *Bradyrhizobium* and *Azotobacter* showed siderophore activity as indicated by the development of orange color zone on CAS agar plates. The size of colored zone produced both by *Azotobacter* and *Bradyrhizobium* on CAS agar plates were nearly identical. However, *A. chroococcum* demonstrated 12% increase in salicylate compared to *Bradyrhizobium*. This result showed that both *Bradyrhizobium* and *Azotobacter* sp. can be beneficial for their antagonistic activity towards fungal pathogens. Similar siderophore production by *Azotobacter* (Muthuselvan and Balagurunathan, 2013) and rhizobia (Datta and Chakrabartty, 2014) is reported. The secretion of exopolysaccharides by *Bradyrhizobium* sp. (vigna) and *A. chroococcum* is another indirect plant growth promoting activity which is reported to favorably affect root colonization and consequently root ramification (Hirsch 1999) besides providing protection to bacterial cells against desiccation. Even though, both *Azotobacter* and *Bradyrhizobium* secreted considerable amounts of EPS, *Azotobacter* produced 27% more EPS than *Bradyrhizobium*. *Bradyrhizobium* and *Azotobacter* showed positive reaction to both HCN and ammonia also. Similar synthesis of plant growth promoting substances as observed in this study has also been reported to increase the growth and development of plants including legumes (Wani *et al.,* 2007; Ghosh *et al.,* 2015).The production of multiple plant growth regulators by *Bradyrhizobium* and *Azotobacter* suggest that the PGPR tested in this study could be exploited to develop co-culture and consequently to enhance the growth of legumes including greengram. However, the observed benefits following dual inoculations clearly indicate that the cumulative effect of these organisms which provided N (nitrogen fixers) and available P (*Bradyrhizobium* and *Azotobacter*) and improved nutrient absorption in addition to growth promoting substances resulted in overall improvement in greengram plants under field conditions.

5. Conclusion

The symbiotic nitrogen fixer *Bradyrhizobium* sp. (vigna) and asymbiotic nitrogen fixer *A. chroococcum* were isolated and identified from the greengram nodules and chilli respectively. The combined inoculation of *A. chroococcum* and *Bradyrhizobium* sp. (vigna) in the presence or absence of fertilizers was found superior than other treatments and enhanced growth, symbiosis, nutrient uptake and grain yields considerably. Moreover, the effects of mixed cultures of *A. chroococcum* and *Bradyrhizobium* were relatively greater than the sum of the individual inoculation effects, suggesting synergisms beyond simple additive effects. In addition, the maximum accumulation of N and P in inoculated plants suggested that both asymbiotic and symbiotic bacteria can live favourably in a microhabitat. The present finding therefore, strongly suggests that mixture of compatible pairing of asymbiotic bacterium (*A. chroococcum*) and symbiotic *Rhizobium* (*Bradyrhizobium*) endowed with multiple plant growth promoting activities can be used safely and sustainably to promote growth, symbiosis, nutrient uptake and yield of field-grown greengram while saving the chemical fertilizers.

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Author's Contributions

Designing and planning of the experiments were done by Prof. Mohd. Saghir Khan, and experiments were conducted and executed by Dr. Ees Ahmad. The overall editing, statistical analysis and preparation of the manuscript, according to the style of journal, were done by Dr. Almas Zaidi.

References

Ahmad E, Khan MS and Zaidi A. 2013. ACC deaminase producing *Pseudomonas putida* strain PSE3 and *Rhizobium*

leguminosarum strain RP2 in synergism improves growth, nodulation and yield of pea grown in alluvial soils. *Symbiosis*, **61**: 93-104.

Ahemad M and Khan MS. 2011a. Effect of pesticides on plant growth promoting traits of greengram symbiont, *Bradyrhizobium* sp. strain MRM6. *Bull Environ ContamToxicol*, **86**:384-388.

Ahmad F, Iqbal A andKhan MS.2005.Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan.*Turk J Biol*, **29**: 29-34.

Akhgar AR, Arzanlou M, Bakker PAHM andHamidpour M. 2014.Characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing *Pseudomonas* spp. in the rhizosphere of salt-stressed canola.*Pedosphere*, **24**: 461–468.

Alexander DB, Zuberer DA .1991.Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils 12: 39-45

Ali B, Sabri AN, Ljung K and Hasnain S. 2009. Quantification of indole-3-acetic acid from plant associated Bacillus spp. and their phytostimulatory effect on *Vigna radiata* (L.). *World J Microbiol Biotechnol*, **25**:519–526.

Ara N,Rokonuzzaman M and Hasan MN. 2009. Effect of *Bradyrhizobium* and *Azotobacter* on growth and yield of mungbean varieties. *J Bang AgricUniv*, **7**:7–13.

Aquilianti L, Favilli F and Clementi F. 2004.Comparison of different strategies for isolation and preliminary identification of *Azotobacter* from soil samples.*Soil BiolBiochem*, **36**: 1475-1483.

Bakker AW and Schipper B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. *Soil BiolBiochem*, **19**: 451–457.

Bal HB, Das S, Dangar TK andAdhya TK. 2013. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. *J Basic Microbiol*,**53**: 972–984.

Bhattacharjee RB, Philippe J, Clémence C, Bernard D,Aqbal S and Satya NM.2012.Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. trifolii SN10 promote rice growth, and in the process undergo colonization and chemotaxis. *BiolFertil Soils***48**: 173-182.

Bhattacharya PN and Jha DK. 2012. Plant growth promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol*,**28**: 1327-1350.

Banerjee A, Supakar S and Banerjee R. 2014. Melanin from the nitrogen-fixing bacterium *Azotobacter chroococcum*: A spectroscopic characterization. *PLoS ONE*, **9**: e84574. doi:10.1371/journal.pone.0084574.

Brick JM, Bostock RM and Silversone SE. 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane.*Appl Env Microbiol*, **57**: 535-538.

Buck JD and Cleverdon RC.1960. The spread plate as a method for the enumeration of marine bacteria. *Limnol Oceanogr*, **5**:78-80.

Datta C and Basu P. 2000. lndole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub *Cajanuscajan*. *Microbiol Res*, **155**: 123-127.

Datta B and Chakrabartty PK. 2014. Siderophore biosynthesis genes of *Rhizobium* sp. isolated from *Cicer arietinum* L.*3 Biotech*,**4**: 391–401.

Dworkin M and Foster J. 1958. Experiment with some microorganisms which utilize ethane and hydrogen. *J Bacteriol*, **75**:92–601.

Duhan JS, Dudeja SS and Khurana AL. 1998.Siderophore production in relation to N_2 fixation and iron uptake in Pigeon pea- *Rhizobium* symbiosis.*Folia Microbiologia*,**43**: 421–426.

Dye DW. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *NewZealand J Sci*, **5**: 393-416.

Frauque A, Haraguchi T, Hirota Oand Rahman MA. 2000. Growth analysis, yield, and canopy structure in maize, mungbean intercropping. *Bulletin of the Institute of Tropical Agriculture, Kyushu University Fukuoka, Japan*, **23**:61-69.

Giller K.2001.**Nitrogen Fixation in Tropical Cropping Systems**.CABI Publishing, Wallingford, UK.

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J andMcConkey B. 2007.Promotion of plant growth by bacterial ACC deaminase.*Critical Rev Plant Sci*,**26**: 227-242.

Ghosh PK, De TK, and Maiti TK. 2015. Production and metabolism of indole acetic acid in root nodules and symbiont (*Rhizobium undicola*) isolated from root nodule of aquatic medicinal legume *Neptuniaoleracea* Lour. *J Botany*, Article ID: 575067.

HassanW, David J and Bashir F.2014.ACC-deaminase and/or nitrogen-fixing rhizobacteria and growth response of tomato (*Lycopersicon pimpinellfolium* Mill.).*J Plant Interactions*,9: 869-882.

Hirsch AM. 1999. Role of lectins (and rhizobial exopolysaccharides) in legume nodulation. *Curr Opin Plant Biol*,**2**:320–326.

Holt JG, Krieg NR, Sneath PHA, Staley JT andWillams ST. 1994.**BergeysManual of Determinative Bacteriology**. Williams and Wilkins, Baltimore.

Iswaran V and Marwah TS. 1980. A modified rapid Kjeldahl method for determination of total nitrogen in agricultural and biological materials. *Geobios*,**7:** 281–282.

Jackson ML. 1967.**Soil Chemical Analysis**.Prentice-Hall of India, New Delhi, India.

*Jensen HL. 1954. The Azotobacteriaceae.Bacteriol Rev, **18**: 195-214.*

Johnstone DB. 1955. *Azotobacter fluorescence*. *J Bacteriol*, **69**: 481-482.

Khan MS, Ahmad E, Zaidi A andOves M. 2013. Functional aspect of phosphate-solubilizing bacteria: Importance in crop production. *In*: Maheshwari DK, Saraf M, Aeron A, (ed.),**Bacteria in agrobiology: crop productivity**. Springer-Verlag, Berlin Heidelberg, pp. 237-265.

King JE. 1932. The colorimetric determination of phosphorus. *Biochem J*, **26**: 292-297.

Kloepper JW and Schroth MN. 1978. Plant growth-promoting rhizobacteria on radishes. *Proc. 4th Intern.Conf. Plant Pathogen Bacteria.* Angers, France: Station de PathologieVégétaleetPhytobactériologie, INRA,**2**: 879–882.

Kumar, Kranthi G and Ram MR. 2014. Phosphate solubilizing rhizobia isolated from *Vigna trilobata*. *Amer J Microbiol Res*,**23**: 105-109.

Lemanski K and Scheu S. 2014. Incorporation of ^{13}C labelled glucose into soil microorganisms of grassland: Effects of fertilizer addition and plant functional group composition. *Soil Biol Biochem*,**69**: 38-45.

Lenin L and M. Jayanthi 2012 Indole acetic acid, gibberellic acid and siderophore production by PGPR isolates from rhizospheric soils of *Catharanthusroseus*. *Intern J PharmaceuBiol Arch*,**3**:933-938.

Lutenberg B and Kamilova F. 2009.Plant growth promoting rhizobacteria.*Ann Rev Microbiol***, 63**: 541-556.

Lowery OH, Rosebrough NJ, Farr AL and Randal RJ. 1951. Protein measurements with the Folin, Phenol reagent. *J BiolChem*, **193**: 265-275.

Ma W, Guinel FC and Glick BR. 2003. *Rhizobium leguminosarum*biovarviciae 1-aminocyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. *Appl Environ Microbiol*,**69**: 4396–4402.

Magnucka EG and Pietr SJ. 2015. Various effects of fluorescent bacteria of the genus *Pseudomonas* containing ACC deaminase on wheat seedling growth. *Microbiol Res*,**181**: 112–119

Mohite B. 2013.Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth.*J Soil Sci Plant Nutr*,**13**: 638-649.

Muthuselvan I and Balagurunathan R. 2013.Siderophore production from *Azotobacter* sp. and its application as biocontrol agent.*Intern J Curr Res Rev (IJCRR)*,**5**: 23-35.

Noreen S, Ali B and Hasnain S. 2012.Growth promotion of *Vigna mungo* (L.) by *Pseudomonas* spp. exhibiting auxin production and ACCdeaminase activity.*Ann Microbiol***, 62**:411–417.

Nosrati R, Parviz O, Horieh S, Iraj R and Malboobi MA. 2014.Phosphate solubilization characteristics of efficient nitrogen fixing soil*Azotobacter* strains. *Iran J Microbiol*, **6**: 285-295.

Narula N and Gupta KG. 1986. Ammonia excretion by *Azotobacter chroococcum* in liquid culture and soil in the presence of manganese and clay minerals. *Plant Soil*, **93**: 205-209.

Oskar AP, Bashan Y and de-Bashan LE. 2014. Proven and potential involvement of vitamins in interactions of plants with plant growth-promoting bacteria-an overview. *Biol Fertil Soils*,**50**:415–432.

Peix A, Ramirez-Bahena MH, Velazquez E. and Bedmar EJ. 2015. Bacterial association with legumes. *Crit Rev Plant Sci*, **34**:17-42.

Penrose DM and Glick BR. 2003.Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria.*Physiologia Plant*, **118**: 10-15.

Parveen S, Khan MS and Zaidi A. 2002. Effect of rhizophericmicroorganisms on growth and yield of greengram (*Phaseolus radiatus*). *Ind JAgric Sci*, **72**: 421-423.

Pikovskaya RI. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya*, **17**: 362–370.

Reeves MW, Pine L, Neilands JB and Balows A. 1983. Absence of siderophore activity in *Legionella* species grown in iron-deficient media.*J Bacteriol*, **154**: 324-329.

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J and Vanderleyden J. 2008. Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant Soil*, **302**: 149–161.

Sadasivam S and Manickam A. 1992. **Biochemical Methods for Agricultural Sciences**, Wiley Eastern Limited, New Delhi, India.

Saini P. 2012.Preliminary screening for plant disease suppression by plant growth promoting rhizobacteria.*Sci Res Rep*, **2**:246-250.

Shahab NA and Nasreen SK. 2009. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs Sadaf. *Afr J Agric Res*, 4: 1312-1316.

Sharma SK. 2000. Greengram. In: Rathore PS (Ed.), **Techniques and Management of Field Crop Production**, Agrobios (India), Jodhpur, Rajasthan, pp. 284-294.

Siddiqui A, Shivle R, Magodiya Nand Tiwari K. 2014.Mixed effect of *Rhizobium* and *Azotobacter* as biofertilizer on nodulation and production of chickpea, *Cicer arietinum*.*Biosci Biotech Res Comm*,**7**: 46-49.

Singh R, Arora NK, Gautam P and Lal S. 2013.Enhancement of plant growth of *Trigonella foenum-graecum* by coinculation of fluorescent *Pseudomonas* and *Rhizobium* for the sustainability of agriculture.*Asian J Plant SciRes*,**3**:74-79.

Somasegaran P and Hoben HJ. 1985.**Handbook for rhizobia: methods in legume *Rhizobium* technology**. Springer, New York.

Tejera N, Lluch C, Martìnez-Toledo MV and Gonzàlez-López J. 2005.Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant Soil*, **270**: 223-232.

Vincent JM. 1970.**A manual for the practical study of root-nodule bacteria**. IBP Handbook No. 15, Blackwell Scientific, Oxford.

Viruel E, Erazzú LE, Calsina LM, Ferrero MA, Lucca ME andSiñeriz F. 2014.Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. *J Soil Sci Plant Nutri*, **14**: 819-831.

Wani PA, Khan MS and Zaidi A. 2007. Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea.*J Plant Nutr Soil Sci*, **170**: 283–287.

Wani PA, Khan MS and Zaidi A. 2007a.Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants.*Chemosphere*, **70**: 36-45.

Zaidi A, Khan MS, Ahemad M and Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiol Immunol Hung*, **56**: 263-284.

Zhao Y. 2010. Auxin biosynthesis and its role in plant development.*Ann Rev Plant Biol*, **61**: 49-64.

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Analysis of Natural Food Composition of Fishes in Shatt Al-Arab River, Southern Iraq

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Abstract

A total of 1469 fishes of 39 species were caught between July 2010 and March 2011, using coastal seine net from three stations in Shatt Al-Arab River (Al-Daeir, Hamdan and Al-Fao). Environmental factors in the river were measured (water temperature (21.85±4.71°C), pH (7.96±0.25), salinity (3.26±2.25 ppt), D.O.(9.15 ±1.77 mg/L) transparency (37.29±16.08cm).The dominant species in Shatt Al-Arab River was *Carassius auratus* which comprises 20.76% and 42.90% from total numbers and weight, respectively. The feeding intensity (8.79 - 12.37 point/fish) for all fed fishes indicates that most fishes fed with half fullness stomach at all seasons. The food contents of 593 individuals belonging to 15 species were analyzed for reliable results. Sixteen food items used to calculate standardized diet compositions and trophic levels of fishes. Plant origin food items comprise 48.54% of preponderance index, while it was 39.43 and 12.03 % for animal and organic matter food items, respectively. Shatt Al-Arab feeding index showed two peaks at spring and autumn (61.87, 59.74, respectively).While the mean value of trophic level for vegetarian fish species was 2.06 which differ significantly (F=12.14,145.01 P= 0.013,0.00) from both species which depend on detritus (1.36) and on animal (3.38) food items, respectively, which were also significantly different (F=350.04,P=0.00) from each other.

Keywords: Shatt Al-Arab, Preponderance Index, Trophic Level, Feeding Intensity.

1. Introduction

The quality and quantity of the available natural food resources influence aspects of the life history of fishes including inter alia, growth rate, longevity, reproductive investment, sexual maturity and fecundity (King, 1994). The study of the food and feeding habits of fish species is a subject of continuous research because it constitutes the basis for the development of a successful fisheries management programmed on fish capture and culture and because the aquatic ecosystem is dynamic. The gut content is a reflection of the water quality, all other factors being constant (Oronsaye and Nakpodia, 2005; Ekpo *et al.,* 2014). Nature offers a great diversity of organisms that are used as food by fish, and these differ in size and the taxonomy group (Olojo *et al.,* 2003), most studies which are aimed at obtaining such information on food, feeding ecology are based on the analysis of gut content of organism caught from their natural habitats (George *et al.,* 2013; Job and Udo, 2002; Odum, 1971). The diet composition of fish within an assemblage is determined not only by the food availability but also, to some extent, by the factors related to the interspecific competition for food (Casaux and Barrera-Oro, 2013).

Southern Iraq is part of the Mesopotamian lowland bounded to the west by the Arabian plateau, to the east by the Zagros mountain range of Iran, and to the south by the Arab Gulf and Kuwait. The area is characterized by its saline soil washed by the waters of Tigris-Euphrates River system (Al-Daham and Yousif, 1990). During the past years, the Mesopotamian rivers were suffering from various problems, like the new hydrological projects, several large dams in Turkey, Syria, Iran, and Iraq have diverted water from the Tigris and Euphrates and their tributaries for irrigation, flood control, and hydroelectric power (Mohamed *et al.,* 2012).Thus, because of the distinguished environment besides the fluctuations during the last years, it was expected that Shatt Al-Arab river fish species, especially its feeding status, may reflect these alterations.

The present study describes the natural food composition, feeding intensity and feeding habits of the most abundant species caught from Shatt Al-Arab river, evaluating the possible effects of the environmental changes on stomach content; such important information is needed for the continuous successful management of this vital river.

2. Materials and Methods

2.1. Study Area

The Shatt Al-Arab River represents the most northwestern end of the Gulf (Fig. 1). It is formed by the confluence of the Tigris and Euphrates rivers at Qurna,

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flows southeastern direction to open in the Arabian Gulf south of Al-Fao city. The total length of Shatt Al-Arab River is about 204 km, the width varies from 400 to 1500m, and hundreds outlets in the form of small rivers and canals are found on both sides of Shatt Al-Arab River (Al-Lami, 2009; Mohamed *et al*.,2012).

Sampling on the river was conducted once a season from June 2010 to March 2011. Three sampling stations were selected: Al-Daeir (north), Hamdan (middle) and Al-Fao (south).

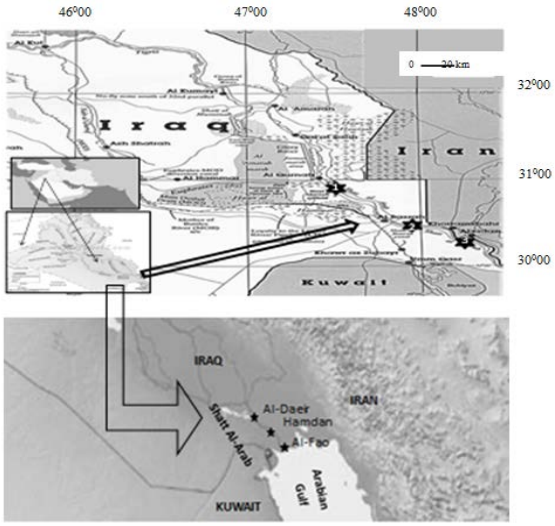

Figure 1.Map of the Shatt Al-Arab River showing the sampling area.

2.2. Fish Collection

Fishes were collected seasonally from Shatt Al-Arab River using coastal seine net with 100 meters length, 8 meters height and a mesh size 10×10 mm. The fish were preserved in cold ice box until reaching the laboratory where they were transferred into a freezer. Total lengths and weights of fishes were measured and the digestive tracts were removed and given a degree of fullness and emptied into a Petri dish to count different food items. Frequency of occurrence and point methods were used to analyze different food items (Hynes, 1950). The food contents of 593 individuals analyzed belonged to 15 species, which occurred in sufficient numbers for reliable results. For further details, see Mohamed *et al.* (2012) and Al-Lamy *et al.* (2015).

2.3. Data Analysis

The feeding data of fish species were analyzed using different parameters as follow:

- 1- Feeding intensity (Hyslop,1980)
  Feeding intensity = Σ points/ Σ feeding fish
- 2- Feeding index (Sarkar and Deepak, 2009)
  Feeding Index = P x 100/ X x N

where, P= total point of the gut that were examined, N= No. of guts examined, X= total points allotted to the full gut.

- 3- Index of Preponderance (Natarajan and Jhingran, 1961)
  I = ViOi/ Σ ViOi

where, I = Index of Preponderance of the food item; Vi = Percentage of volume (points) index of the food item; Oi = Percentage of occurrence index of the food item.

- 4- Feeding selectivity (Lawlor, 1980)
  PXi = Xi/ Σ i
- where Xi = quantity of item i in stomach of specie (i)and Σ i= sum of item (i)in all stomachs of all species present in station.
- 5- Trophic level (Corte´s,1999)
  TrL=1+ [Σ Pj.TLj]

where,TLj is the trophic level of each prey category j and Pj is the proportion of prey species j in the diet of species i Trophic level (TrL) of prey categories was taken from several published accounts (Table1).

Table 1. Prey categories used to calculate standardized diet compositions and trophic levels of fishes in Shatt Al-Arab River.

Code	Group	Trophic level*
Fish, Molluscs, Crustaceans, Zooplankton, Eggs and Worm	Animal	2.515
Plant, Ph.plank, Fil. Algae, Green algae, Spirogera and Diatom	Plant	1

*Calculated from Corte´s (1999).

2.4. Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) version 17.0 (2008) was used. Data were expressed as Mean ± SD and analyzed by ANOVA followed by LSD test for multi-group comparisons. A probability level of $P<0.05$ was considered statistically significant.

3. Results

3.1. Ecological Factors

The ecological factors showed seasonal changes in the studied stations of Shatt Al-Arab River (Table 2). All factors, except transparency and salinity, revealed obvious variations among different stations, especially between Al-Fao and the other two stations.

Table 2. Mean values of the environmental factors at the three studied Stations in Shatt Al-Arab river during 2010-2011

Environmental Factors	Station											
	Al-Daeir				Hamdan				Al-Fao			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Temperature(°C)	28.3	23.0	15.2	21.0	26.7	22.0	15.4	23.0	27.0	25.0	14.6	21.0
pH	8.3	8.2	8.0	7.8	7.7	7.9	8.1	7.6	8.0	7.8	7.9	8.2
Salinity (PSU)	1.2	0.8	1.8	1.9	1.4	2.3	3.8	2.0	5.8	6.2	7.6	4.3
O_2 (mg/L)	8.5	9.5	12.5	10	7.5	8.6	12.5	8.5	7.2	7.5	8.2	9.3
Transparency (cm)	47	56	55	43	47	42.5	48	36	12	8	20	33

3.2. Fish Species

A total of 39 fish species were caught from Shatt Al-Arab River during the four seasons belonging to fresh and marine environments. The total number of individuals were 1469 fish that ranged from 305 (*Carassius auratus*), which comprise about 20.76% and 42.90% from total numbers and weight, respectively, to only one individual for seven different species (Table 3). Only nine species (23%) occurred in all the four seasons while most species occurred in one season (44%) especially in summer (eight,21%).Also most species occurred in one station (46%) and two stations (41%) while only five species (13%)occurred at all three stations.

Table 3. Total numbers and total weights of fish species collected from the Shatt Al- Arab river, Southern Iraq (July 2010 to March 2011). Species are listed in order of abundance.

Rank	Species	Number		Weight		Appearance	
		Total	%	Total(gm)	%	station	season
1	*Carassius auratus*	305	20.76	30385.46	42.90	1,2,3	All
2	*Liza abu*	175	11.91	6569.64	9.27	1,2	All
3	*Tenualosa ilisha*	149	10.14	5108.41	7.21	1,2,3	All
4	*Chelon subviridis*	140	9.53	7468.23	10.54	1,2,3	All
5	*Johnius belangerii*	90	6.13	3224.08	4.55	3	S,W,Sp
6	*Thryssa hamiltonii*	89	6.06	1136.94	1.61	2,3	All
7	*Acanthopagrus arabicus*	66	4.49	2243.94	3.17	2,3	All
8	*Liza klunzingeri*	62	4.22	2658.85	3.75	2,3	A,S,Sp
9	*Poecilia latipinna*	57	3.88	265.07	0.37	1,3	S,W
10	*Thryssa whiteheadi*	57	3.88	748.32	1.06	1,2,3	All
11	*Johnius dussumieri*	56	3.81	2054.25	2.90	1,2	A,S
12	*Acanthobra mamarmid*	41	2.79	789.86	1.12	1,3	All
13	*Anodontostoma chacunda*	30	2.04	680.60	0.96	2	S
14	*Ilisha compressa*	28	1.91	513.98	0.73	3	All
15	*Sparidentex hasta*	24	1.63	2423.64	3.42	1,2,3	A,S,Sp
16	*Johnius sp.*	15	1.02	644.66	0.91	2,3	W,Sp
17	*Alburnus mossulensis*	9	0.61	291.02	0.41	3	A,S,W
18	*Boleophthalmus dussumieri*	7	0.48	40.78	0.06	1,2	A,S
19	*Scomberomorus commerson*	7	0.48	258.05	0.36	3	A
20	*Leuciscus vorax*	6	0.41	534.51	0.75	3	S
21	*Bathygobius fuscus*	6	0.41	106.03	0.15	2	A,S,W
22	*Cynoglossus bilineatus*	6	0.41	193.10	0.27	2,3	W
23	*Sillago sihama*	5	0.34	155.53	0.22	3	A,S,W
24	*Otolithes rubber*	5	0.34	155.63	0.22	2,3	A,S,W
25	*Carasobarbus luteus*	4	0.27	321.40	0.45	1,3	S
26	*Cyprinus carpio*	4	0.27	245.46	0.35	1,2	S,Sp
27	*Eleutheronema tetradactylum*	4	0.27	264.75	0.37	1,3	S
28	*Mastacemblus mastacemblus*	4	0.27	516.34	0.73	3	Sp
29	*Brachirus orientalis*	4	0.27	101.62	0.14	1,3	A,W
30	*Mystuspelusius*	3	0.20	203.30	0.29	1	S
31	*Arabibarbus grypus*	2	0.14	122.85	0.17	2	S
32	*Coptodon zilli*	2	0.14	67.60	0.10	2	A
33	*Amblygaster sirm*	1	0.07	22.80	0.03	2	A
34	*Ctenopharyngodon idella*	1	0.07	221.00	0.31	2	S
35	*Gerres oyena*	1	0.07	23.10	0.03	3	W
36	*Hemiculter leucisculus*	1	0.07	22.50	0.03	1	W
37	*Ilisha melastoma*	1	0.07	29.73	0.04	3	Sp
38	*Periophthalmus waltoni*	1	0.07	8.88	0.01	3	A
39	*Platycephalus indicus*	1	0.07	13.60	0.02	2,3	S
	Total	1469	100.00	70835.51	100.00		

3.3. Feeding Analysis

The feeding intensity for all the fed fishes in Shatt Al-Arab river during 2010-2011(Fig.2) demonstrates that all fishes exhibited a high feeding intensity in spring except in Al-Fao, where its fishes had the upper values in summer, no significant differences were noticed between stations (F= 0.035, P= 0.965),while in Shatt Al-Arab river, spring and autumn were higher than summer followed by winter, the overall values (8.79-12.37 point/fish) indicate that most fishes fed with half full stomach at all seasons.

1= Al-Daeir, 2 = Hamdan, 3 = Al-Fao - A=Autumn, W=Winter, S= Summer, Sp=Spring

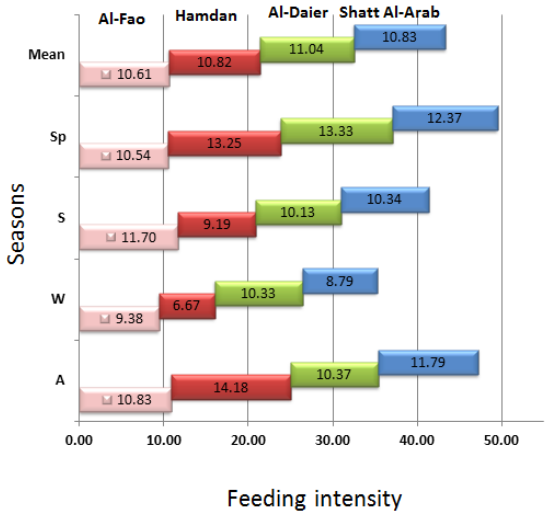

Figure 2. Seasonal variation in feeding intensity for all fish species in Shatt Al-Arab river.

From 39 species caught in Shatt Al-Arab River, only 15 species (Table 4) were included in the details of the feeding study because the number of the fed fishes was enough to be analyzed. Most fishes used in this analysis were caught from Hamdan and Al-Fao station (585, 512 individuals, respectively), while 272 of the analyzed fishes from Al-Daeir. Also *C. auratus* was the dominated species both in total numbers and fed fishes (86), while the other 14 species ranged between 15 to 82fed fish. Mean total length of fishes ranged between 6.37 to 16.46 cm and mean total weight between 4.12 to 57.41gm (*P. latipinna* and *C. subviridis*, respectively).

The main food items of the fifteen fish species were listed in Table 5 along with the identification code used in the following figures and tables; sixteen food categories were considered to calculate standardized diet compositions and trophic levels of fishes.

Preponderance index of fish species in Shatt Al-Arab River, as shown in Fig. 3, points out that there were two groups of feeding habits: The first contained eight species depending on vegetarian and detritus food items while the second contained the other seven species which depend on animal food items. This was confirmed in Fig.4 where plant origin food items comprise 48.54% of preponderance index, while it was 39.43 and 12.03 % for animal and organic matter food items, respectively; about 80.41% plant items comprise the food of six species, 73.97% organic matter of two species and 88.51% animal items of seven species (Fig.5).

No significant differences (F= 0.944, P= 0. 059) were noticed between stations in the values of feeding index (Fig. 6), the highest value found in Hamdan station at autumn (70.90) while the lowest value in the same station at winter. Shatt Al-Arab feeding index showed two peaks at spring and autumn (61.87, 59.74, respectively).

The mean value of the trophic level (Fig.7) for vegetarian fish species was 2.06 which differs significantly (F=12.14,145.01 P= 0.013,0.00) from both species which depend on detritus (1.36) and on animal (3.38) food items, respectively, which were also significantly different (F=350.04,P=0.00) from each other.

Table 4. Numbers, total length (cm)) and weight (gm) of abundant species from the Shatt Al- Arab river, Southern Iraq (July 2010 to March 2011).

Rank	Species	Numbers				No. fed	Mean	
		Al-Daeir	Hamdan	Al-Fao	Total		Length	Weight
1	*Carassius auratus**(F)	86	216	3	305	86	12.17	33.49
2	*Liza abu*(F)	100	75	0	175	63	13.83	32.76
3	*Tenualosa ilisha*(M)	16	51	82	149	82	15.48	34.01
4	*Chelon subviridis* (M)	13	87	40	140	71	16.46	57.41
5	*Johnius belangerii*(M)	0	0	90	90	40	14.02	33.87
6	*Thryssa hamiltonii*(M)	0	11	78	89	32	12.93	12.92
7	*Acanthopagrus arabicus*(M)	0	26	40	66	24	10.21	31.33
8	*Liza klunzingeri*(M)	0	45	17	62	15	12.22	24.62
9	*Poecilia latipinna**(F)	15	38	4	57	21	6.37	4.12
10	*Thryssa whiteheadi*(M)	1	3	53	57	50	12.14	12.15
11	*Johnius dussumieri*(M)	0	0	56	56	33	13.15	35.15
12	*Acanthobra mamarmid*(F)	39	2	0	41	26	12.63	19.95
13	*Anodontostoma chacunda*(M)	0	30	0	30	15	11.99	22.69
14	*Ilisha compressa*(M)	0	0	28	28	16	15.66	23.08
15	*Sparidentex hasta* (M)	2	1	21	24	19	13.96	44.91
Total		272	585	512	1369	593		

*Alien species, F= Freshwater fish, M= Marine fish

Table 5. Food items ingested by studied species. Identification codes are in parenthesis.

Animal	Vegetal	Detritus
Insect (IN)	Diatom (DI)	Organic detritus (OD)
Eggs (EG)	Filamentous Algae (FA)	Sediments (SD)
Zooplankton (ZP)	Spirogyra (SP)	
Crab (CR)	Phytoplankton (PP)	
Fish (FS)	Green algae (GA)	
Shrimp (SH)	Aquatic plant (AP)	
Mollusca (MO)		
Nematodes (NE)		

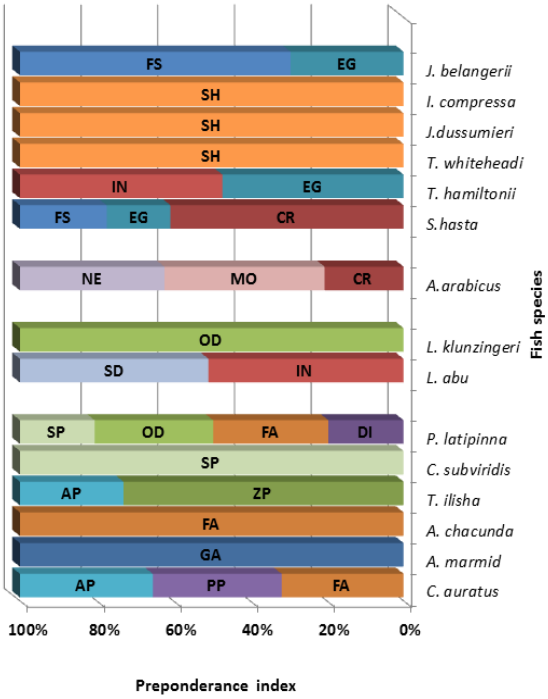

Figure 3. Preponderance index of primary food items of fish species in Shatt Al-Arab river. (Code of food items in Tab.4)

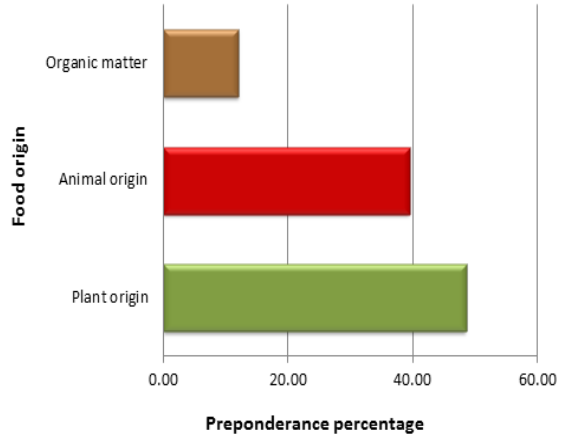

Figure 4. Preponderance index of food items origin of all studied fish species in Shatt Al- Arab river.

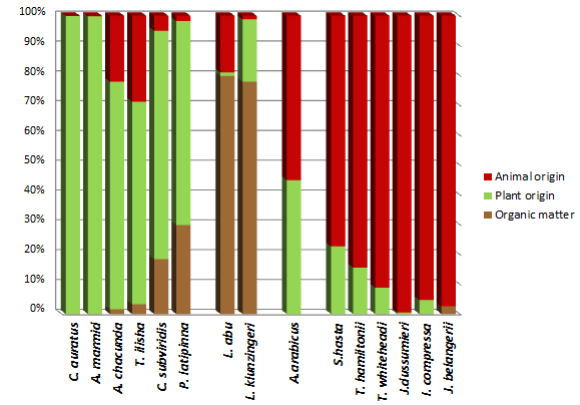

Figure 5. Feeding selectivity of fish species in Shatt Al-Arab river.

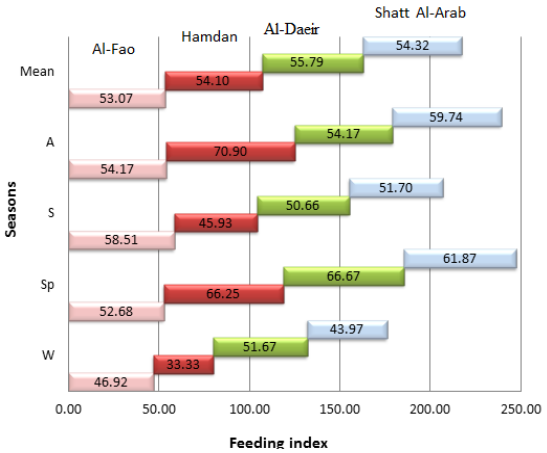

Figure 6. Feeding index of studied fish species in Shatt Al- Arab river.

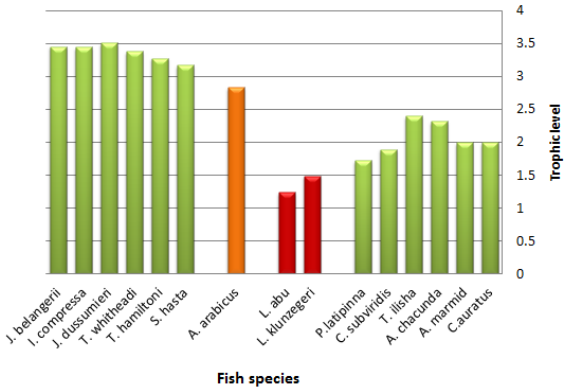

Figure 7. Trophic levels of studied fish species in Shatt Al- Arab river.

4. Discussion

The result of Mohamed *et al.*(2012), in the same period, confirmed that the physical properties, especially salinity, may provide some possible causes for the differences in the distribution, abundance and species composition along Shatt Al-Arab River, which refers to Al-Hassan *et al.* (1989) who stated that the marine species are limited to the middle and the lower regions of Shatt Al-Arab River and their number decreased toward the

upper reaches of the river, and freshwater fauna exhibited a reverse trend of distribution in the river. Mohamed *et al.,* (2015) reviewed the previous studies about the fish assemblage in Shatt Al-Arab river where the number of species was recorded in different papers, ranging from25 to 58 at different periods in the same region. Jasim *et al.* (2007) indicate that only 22 species were caught from Shatt Al-Arab river, mostly riverine species and 33 mostly marine species from Basrah canal during 2001-2002.

The study of Al-Dubakel (2011) showed that there has been an increase in the workers number in the marine fishing sector in the recent years offset decrease in catches, thereby reducing the revenues of fishermen and increasing the fishing effort to obtain sufficient quantities of fish, which in turn affects fish stocks, as it was found by Jabir (1995) that the rate of exploitation of the Shad was 0.8, which indicates that this fish was exposed to overfishing in the Shatt al-Arab River.

The limited number of feeding fish (15 from 39 species) in the present study reflects the dramatic environmental alterations including the "climate change", where Shatt Al-Arab river discharge has declined about – 34.7 % in recent years (Palmer *et al.,* 2008);this will affect the primary production and will also impact the fish (Jennings *et al.,* 2008).The highest primary production was encountered during spring 92.0 mg C/m3 / day (Al-Essa *et al.,* 2007).The annual production of fish in Shatt Al-Arab river is considers low compared to other water bodies (Jasim *et al.,* 2007).

The feeding intensity of the fishes in Shatt Al-Arab river was higher in spring and autumn which indicates appropriate water temperature (21- 25 °C) and resource availability, the majority of the studied fish (species and individuals) belong to marine environment, where more than 73% of fishes were caught from downstream where salinity ranged between 1.4 to 7.6 ppt in two stations (Hamdan and Al-Fao). Breine *et al.* (2011) stated that the contribution of marine migrants and estuarine species is higher in the mesohaline zone.

The composition of the diet and the prey items of most fish sampled at the Shatt Al-Arab River showed common agreement with those indicated for the same species in prior studies, with some minor differences in the main food items; moreover, the feeding habits did not show remarkable differences from the original grouping of the studied species. Delariva *et al.* (2007) indicate that most tropical fishes have variable diets and may exhibit high flexibility, ingesting all food items available in an aquatic environment. They also exhibit an acute ability to shift food quickly as a response to environment variations or food shortage. Therefore, changes in the diet of fish over time and space may be a function of food resources availability (Hahn *et al.,* 2004). This suggests that the diet composition of fish within an assemblage is determined not only by the food availability but also, to some extent, by factors related to the interspecific competition for food (Casaux and Barrera-Oro, 2013), so fish species forage on alternative prey or on different amounts of the same prey (Klemetsen, 1993; La Mesa *et al.,* 1997; Høines Bergstad, 2002). The preference shown by a species to a diet component or group is a biological strategy which discouraged the competition for the available food resource within a species (Olojo *et al.,* 2003; Job and Nyong, 2005; George *et al*.2013).

Love (1980) recorded the dominance of carnivores (85%) out of 600 species of fish. Pandian and Vivekanandan (1985) concluded that the majority of fishes resort to carnivory as against herbivory, detritivory and omnivory due to their relative low energy cost to maintain body temperature, the ease with ammonia excretion and their capacity to effectively digest a protein diet; this conclusion opposes the conclusion of the present study, the dominance was for species that depend on plant origin items (53%), carnivores (30%) and detritivory (17%);this may reflect the wide variety of food items that occurred in all the fish species which show that they are non-selective in feeding and each species can utilize various food sources. Shep *et al.* (2013) and Ekpo *et al.* (2014) observed such feeding on a wide range of food organisms that makes them euryphagous feeding, while Sarkar and Deepak (2009) explain the high incidences of animal parts in the stomach, particularly bones and scales resist digestion and tend to be over represented in gut content analysis. Some species radically changed the quality of their food while others did not. The changes affecting the diets of invertivores, piscivores and omnivores are not fundamental in the sense that they probably do not involve a strong modification of their feeding behavior. Conversely some herbivores and detritivores seemed to deeply modify the way they feed (Mérona and Vigouroux, 2006).

Many considered 'trophic level' as an operational term as the feeding habits and trophic level of the majority of fish groups are subjected to change depending on age, seasons, and the availability of prey and the area of distribution (Vander Zanden and Rasmussen, 1996; Cortes, 1999; Figueiredo *et al.,* 2005). Higher trophic levels of carnivore fishes were recorded in many species which reached four (Corte´s, 1999) where Abdurahiman (2006) concluded that it is mainly due to the presence of carnivorous fishes in the diet; he observed very low trophic in detritivores in accordance with the result of the present study. Vivekanandan *et al.* (2009) divided fishes occurring along the Indian coast into six groups ranging from one to more than four for detritivores and top predators, respectively.

5. Conclusion

Estuaries known to be very productive in terms of biota especially planktons, have been described as spawning, nursery, and feeding grounds. From the present study, we concluded that fish species in the Shatt Al, Arab River estuary consume more than one component of food and depended mainly on autochthonous food items. Many species had a significant commercial importance, so the stock of fish in the river should be evaluated accurately along with monitoring the magnitude of environmental instabilities and pollution in the estuary; this leads to qualify suitable management approaches.

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References

Abdurahiman, K. P. 2006. Studies on food and feeding of marine demersal finfishes with special reference to trophic interactions. Ph.D Thesis, CMFRI Kochi, India.

Al-Daham, N. K. &Yousif, A. Y. 1990.Composition, seasonality and abundance of fishes in the Shatt Al- Basrah Canal, an estuary in Southern Iraq. *ESTUAR COAST SHELF S*,**31**:411- 421.

Al-Dubakel A .2011.Commercial fishing and marketing of Hilsa Shad *Tenualosa ilisha* (Hamilton-Buchanon, 1822) in Basrah – Southern IRAQ.*Emirates J Food Agric***,23**:110-120.

Al-Essa, S.A.K., Abdullah, A.A.M. and Jassem, A.K. 2007.Primary production of phytoplankton in northern part of Shatt Al-Arab River-IRAQ.*J.Basra Agri. Sci*, **20**: 273- 287 (in Arabic).

Al-Hassan, L. A. J., N. A. Hussain and K. D. Soud. 1989. A preliminary annotated check-list of the fishes of Shatt Al-Arab River River, Basrah, Iraq. *Pol. Arch. Hydrobiol. J*, **36**: 283-288.

Al-Lami, O. R. T. 2009. Some Marine Properties for Arabian Gulf on North Part's Hydrology of Shatt Al Arab Riverbed. MSc thesis.Basrah University, Basrah, Iraq (in Arabic).

Al-Lamy, J. H.,Al-Dubakel, A. Y. andTaher, M. M. , 2015.Trophic breadth and dietary overlapfor ten fish species caught from Shatt Al-Arab River, Fao, southern Iraq. (In press).

Breine, J.,Maes, J., Ollevier, F.andStvens,M.2011. Fish assemblages across a salinitygradient in the Zeeschelde estuary (Belgium).*Belgian J. of Zool*, **141**:21-44.

Casaux, R. and Barrera-Oro, E.(2013). Dietary overlap in inshore notothenioid fish from theDanco Coast, western Antarctic Peninsula Polar Research. 32, 21319, http://dx.doi.org/10.3402/polar.v32i0.21319.(Dec.7,2015)

Corte´s E.1999. Standardized diet compositions and trophic levels of sharks.*CES Journal of MarineScience*, **56**: 707–717.

Delariva, R. L.,Hahn, N. S. and Gomes, L. C.2007. Diet of a Catfish before and after Damming of the Salto Caxias Reservoir, Iguaçu River.*Brazil. Arch. of Biol. and Tech*, **50**:767-775.

Ekpo, I. E., Essien-Ibok, M. A. and Nkwoji, J. N. 2014. Food and feeding habits and condition factor of fish species in Qua Iboe River estuary, AkwaIbom State southeastern Nigeria.Inter. *J. of Fish.andAqu. Stud*, **2**:38-46.

FigueiredoM, Morato T, Barreiros JP, Afonso P, Santos R. 2005. Feeding ecology of the white seabream, *Diplodussargus*, and the ballan wrasse, *Labrusbergylta*, in the Azores. *Fish Res*, **75**:107-119.

George, U.U.,Idung, J.U.,Andem, A.B. ,Okorafor, K.A. and Mowang, D. 2013. Diet composition and condition factor of *Ethmalosafimbriata* in the Cross River estuary.*Greener J. Biol Sci.*, **3**:244-252.

Hahn, N.S., Fugi, R. and Andrian, I.F. 2004.Trophic ecology of the fish assemblages. In: Thomaz, S. M. Agostinho, A. A. and Hahn, N. S. (Eds.) **The Upper Paraná River and its Floodplain: Physical aspects, Ecology and Conservation**. Backhuys Publishers Leiden, The Netherlands. pp. 247-269.

Høines, A. S. and Bergstad, O.A. 2002.Food partitioning by flatfishes on a herring spawning ground.*Sarsia* **,87**:19-34.

Hynes, H.B. N. 1950. The food of the freshwater sticklebacks (*Gastrosteusaculeatus*) and *Pygosteuspungitius*) with a review of methods used in studies of the food of fishes. *J. Anim. Ecol*, 19: 36-58.

Hyslop, D.J.(1980). Stomach analysis – A review of methods and their application.*J. Fish Biol*, **17**:411-429.

Jabir, M.K.1995. Preliminary assessment of the Sbour stock *Tenualosa ilisha* (Hamilton) in the Shatt Al-Arab River. Basrah, Iraq. *BasrahJ.Agric .Sci*, **8**: 49-64.

Jasim, A. A., Al-Dubaikel, A. Y.and Ahmed, S. M.2007.Fish production in BasrahCanal and Shatt Al-Arab.*Mesopotamia J. of Agriculture*, **35**: 52-58.(in Arabic).

Jennings, S., Mélin, F., Blanchard, J.L., Forster, R., Dulvy, N.K. and Wilson, R. 2008.Global-scale predictions of community and ecosystem properties from simpleecological theory. *Proc. Roy. Soc. Lond. B*, **275**: 1375-1383.

Job, B. E. and Nyong, E. A. 2005.Diet composition and condition factor of the African River Prawn (*Macrobrachiumvollenhovenii*) (Herklots, 1857) (Crustacea, Decapoda, Palaemonidae) of the Cross River Estuary, Nigeria.*Afr. J. Fish. Aquacult*, **4**:9-17.

Job, B.E. and Udo, P.J.2002. Food feeding and condition factor of estuarine catfish *Chrysichthysnigrodigitatus* (Lacepede) of the Cross River Estuary, Nigeria. *Afr. J. Fish Aquacult*, **3**:43-45.

King, R. P. 1994.Seasonal dynamics in the trophic status of *Papyrocranusafer* (Günther1868) (Notopteridae) in a Nigerian rainforest stream.*Rev. Hydrobiol. Trop*, **27**: 143-155.

Klemetsen, A. (1993). The food of the long-rough dab (*Hippoglossoidesplatessoideslimandoides* Bloch) in Balsfjorden, north Norway.*Sarsia*, **78**:17–24.

La Mesa,.M., Vacchi, M., Castelli, A. and Diviacco, G.1997 .Feeding ecology of two nototheniid fishes, *Trematomushansoni* and Trematomusloennbergii, from Terra Nova Bay, Ross Sea.*Polar Biol***,17**:62–68.

Lawlor, L. R. 1980. Overlap, similarity, and competition coefficients.*Ecolo* ,**61**:245- 251.

Love, R.M. 1980.**The chemical biology of fishes**.AcademicPress,London.

Mérona, B. de and Vigouroux, R.2006. Diet changes in fish species from a large reservoir in South America and their impact on the trophic structure of fish assemblages (Petit SautDam,French Guiana). *Ann. Limnol. Int. J. Lim*,**42**: 53-61.

Mohamed, A. R.,Hussein, S. A. and Lazem, L. F. 2015.Spatiotemporal variability of fishassemblage in the Shatt Al-Arab River, Iraq. *J. of Coastal Life Medicine*, **3**: 27-34.

Mohamed, A.R.,Resen, A. K and Taher, M.M. 2012..Longitudinal patterns of fish community structure in the Shatt Al-Arab River, Iraq. *Basrah J. of Sci*, **30**: 65-86.

Natarajan, A. V. and Jhingran, A. G.1961.Index of preponderance - a method of grading the food elements in the stomach analysis of fish, *Indian J. Fish*, **8**: 54-59.

Odum, E.P. 1971.**Fundamentals of ecology**. WB Saunders Company, Philadelphia.

Olojo, E.A.A.,Olurin, K.B. and Osiikoya, O.J. 2003. Food and feeding habit of *Synodontisnigrita* from the Osun River, SW Nigeria.*NAGA. World Fish Centre Quarterly***,26**:421- 424.

Oronsaye, C.G.; Nakpodia, F.A.2005. A comparative study of the food and feeding habits of *Chrysichthysnigrodigitatus* and *Brycinus nurse* in a tropical river. *Pak J SciInd Res*, **48**: 118- 121.

Palmer, M. A.,ReidyLiermann, C. A., Nilsson, C.,Flo¨rke, M.andAlcamo,J.2008 .Climate change and the world's river basins: anticipating management options. *Front. Ecol. Environ*, **6**, doi:10.1890/060148.

Pandian, T.J. and E. Vivekanandan.1985. Energetics of feeding and digestion. In:Tytler P and P. Calow (Eds), **Fish energetics**.pp.99-124 Croom Helm, London.

Sarkar, U. K. and Deepak, P. K. 2009. The diet of clown knife fish *Chitalachitala* (Hamilton - Buchanan) an endangered notopterid from different wiled population INDIA. *Electronic Journal of Ichthyology*, **1**: 11-20.

Shep, H. K., Ouattara, K.M.,Ouattara, M. A. and Gourene. G. 2013. Comparative analysis of diet of two sympatric species of Tilapia in Ayame man-made lake.*Livestock Res RurDevt***, 25**: 305-312.

Vander Zanden, M. J., and Rasmussen ,J. B..1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecological Monographs***, 66**:451–477.

Vivekanandan, E.,Gomathy, S.,Thirumilu, P.,Meiyappan M. M. and Balakumar, S. K. 2009 . Trophic level of fishes occurring along the Indian coast.*J. Mar. Biol. Ass. India*, **51**: 44 – 51.

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Vegetation-Soil Relationships in Wadi El-Rayan Protected Area, Western Desert, Egypt

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Abstract

The present study provides an analysis of the soil and vegetation composition at 10 sites in Wadi El Rayan Protected Area and concentrates on the environmental factors that affect plant species distribution. A total of 17 vascular plant species belonging to 13 botanical families was recorded. Poaceae, Chenopodiaceae, and Zygophyllaceae were the largest families identified. Chorological analysis revealed that 47% of the studied species are Pluri-regional, 41% are Bi-regional and 12% are Mono- regional. The recorded species extend their distribution all over the Saharo-Arabian (33%) followed by Irano-Turanian (24%), Mediterranean (22%), Palaeotropical (8%), Sudano-Zambezian (5%), Neotropical (5%), and Euro-Siberian (3%). The life-form spectrum revealed that the phanerophytes (35%) and geophytes helophytes (23%) are the most frequent, followed by chamaephytes (18%), therophytes (12%), hemicryptophytes (6%), and helophytes (6%). The dominant species were *Phragmites australis*, *Tamarix nilotica* and *Zygophyllum album*; while the co-dominant species were *Juncus rigidus*, *Nitraria retusa*, *Alhagi graecorum*, *Typha domingensis*, *Zygophyllum coccineum* and *Eucalyptus camaldulensis*. Variation in species diversity among different locations were evident, the Northeast of the Lower Lake(9 species), followed by the Southwest of the Lower Lake and the Northeast of the Upper Lake(6 species each) showed highest species richness, while the Southeast of the Lower Lake showed the lowest recorded species richness (one species). Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) Ordination techniques were used to examine the relationship between the vegetation and soil parameters; pH, electric conductivity, $CaCO_3$, organic matter and relative concentrations of cations.CCA analysis showed positive correlations of species and sites along the most important ecological gradients. Both ordination techniques clearly indicated the importance of these ecological factors on the distribution of the vegetation pattern in the area.

Keywords: Vegetation, Plant distribution, Wadi El Rayan, Soil, Desert, Egypt, Protected Area.

1. Introduction

Wadi El Rayan is located 140 km southwest of Cairo in the Fayoum Governorate on the Western Desert of Egypt. Its total area is 1759 Km² and it is classified by the Egyptian Environmental Affairs Agency (EEAA) as a managed Protected Area for the conservation of wild species and the sustainable utilization of natural resources (Nels, 1995). Although the Western Desert is characterized, in general, by poor plant diversity and cover (Boulos, 1975), Wadi El Rayan is rich with fauna and flora diversity (Osborn and Helmy, 1980; Saleh, 1987; Saleh *et al*., 1988a) and it also has unique geological and geomorphological features (Said, 1962). In 2011, Wadi El Rayan was recognized by the International Union for Conservation of Nature (IUCN) as one of the 20 Important Plant Areas (IPAs) in Egypt (Radford *et al*., 2011) and it was nominated by Egypt in 2012 as a wetland area of the Ramsar Convention (EEAA, 2012). The area is hyper-arid with low precipitation (mean annual precipitation of 10.1 mm of irregular rainfall) and hot summer (temperature is as low as 1.2°C in winter and as high as 48.4°C in summer) (Ayyad and Ghabbour, 1986; Saleh *et al*., 1988b). Wadi El Rayan depression has been used as a water reservoir for storing excess agricultural drainage water above the capacity of Lake Qarun. Two man-made lakes joined by a connecting channel, were constructed at two different levels (Zahran, 1970). Throughout time, Wadi El Rayan lakes have created a variety of habitats surrounding it, although the adverse consequences of their creation on the ecology of the area cannot be ignored (Saleh, 1987; Saleh *et al*., 1988a, b). The area became increasingly inhabited by people from adjacent villages and consequently a rapid economic development was established (IUCN, 1998). By February 2000, around 4840 feddans of reclaimed land were established. Irrigation water of these fields is

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pumped from the upper lake through pipeline running towards the west, entering the project area from the northwest and drains into the lower lake from southwest following the natural landscape gradient (Abdou, 2006).

Throughout the recent century, many researchers attempted to determine the factors controlling the plant species distribution (Glenn *et al*., 2002; He *et al*., 2007). The spatial distribution of plant species and communities in a small area of desert ecosystems is related to heterogeneous topography and landform pattern (Parker, 1991). The physiographic and edaphic factors play a paramount role in the distribution of plant communities in the Western Desert of Egypt (Ayyad, 1976). The distribution, pattern and abundance of plant species and communities in desert environments have been most often related to three groups of factors: rainfall (Kadmon and Danin, 1999), soil chemistry (Abd El-Ghani, 1998; Abd El-Ghani and Amer, 2003) and human impact including overgrazing and plant overcollection (Kassas, 1962). The distribution of species in saline and marshy habitat in many arid regions has been a subject of study by numerous authors (Kassas, 1957; Ungar, 1968; Maryam *et al*., 1995).The objective of the present study is to study the vegetation composition of Wadi El Rayan and to portray the relationship between the vegetation and the prevailing soil factors at the coastal zones of the lakes located in the study area.

2. Materials and Methods

2.1. Vegetation Sampling

Quantitative surveys of the vegetation in the study area were carried out between March and September 2013. Ten stands in the surrounding area of the lakes, including the upper and lower lakes and the connecting channel, were carefully identified ensuring a reasonable degree of physiographic and physiognomic homogeneity of habitat and vegetation. Also, care was taken to ensure a reasonable degree of physiographic and physiognomic homogeneity of both habitat and vegetation (Figure 1). At each stand, a total of five random quadrates were sampled (each of 10m × 10m; 50 quadrates in total), and a floristic list and count were recorded to determine flora density. Species richness was also determined according to Barbour *et al*. (1987).

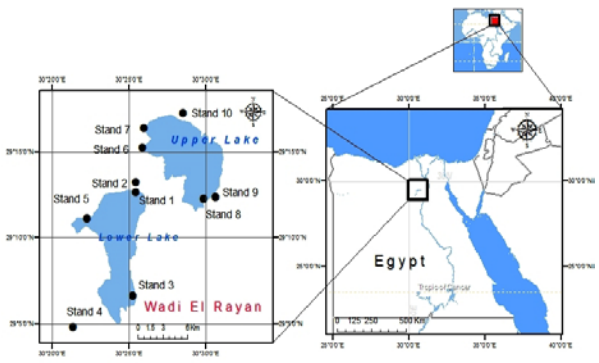


Figure 1. Egypt map focusing on Wadi El-Rayan and study stands

2.2. Soil Analysis

Surface soil samples (at depth of 25 cm, excluding the surface crust) were randomly collected at different stands to determine their physical and chemical characteristics.

Physical analysis: the soil texture was determined using a series of sieves: Soil samples were air-dried and passed manually through a 2-mm sieve to evaluate the gravel percentage. Particle size analysis was accomplished according to Piper (1950)to calculate the percentages of sand, silt, and clay and the classification of the soil texture type was accomplished according to the USDA soil texture triangle (USDA, 1993).

Chemical analysis: Calcium carbonate was measured by titration against 1.0 N HCl following Allen *et al*. (1976). Oxidizable organic carbon (as an indication of the total organic matter content) was measured according to Black (1965). Soil reaction (pH value), electrical conductivity, sulphates and chlorides were measured according to Jackson (1967). Bicarbonate was determined by titration using 0.1 N HCl (Allen *et al*., 1976). Extractable cations (Ca++ and Mg++) were determined (meq/L) by titration following Richard (1954),while,sodium and potassium ions (meq/L) were measured from air-dried soil using ammonium acetate solution at pH=7 (Allen *et al*., 1976).

2.3. Data Analysis

A floristic data; presence/absence data matrix of 10 stands and 17 species was applied for the classification by cluster analysis with the Community Analysis Package version (1.2) (Henderson and Seaby, 1999) using a Squared Euclidean Distance Dissimilarity Matrix with minimum variance (Ward's method) as the agglomeration criterion.

For vegetation classification, TWINSPAN (Two-Way Indicator Species Analysis; Hill, 1979) was applied using the Community Analysis Package software and the analysis was based on the Importance Values (IV) of the species. The diversity indices (Alpha diversity) were calculated at each site (Harper, 1999).

The vegetation and environmental factors were analyzed using ordination techniques. Classification (Siebert *et al*., 2002) and ordination (Pavlůet *et al*., 2003) are two possible ways to obtain results from multivariate data analysis.

All ordinations were performed using the CANOCO program (version 4.5) (TerBraak and Šmilauer, 2002; Hejcmanovā-Neźerková and Hejcman, 2006). Detrended Correspondence Analysis (DCA) was performed to detect the length of the environmental gradient. After DCA, Canonical Correspondence Analysis (CCA) was performed because the dataset was relatively heterogeneous and, therefore, the length of ordination axes in DCA was relatively long (Lepš and Šmilauer, 2003).

3. Results

3.1. Floristic Relations

In total, 17 plant species representing 13 families were identified throughout the surveys. The largest represented families were Poaceae (with three species), followed by Chenopodiaceae, and Zygophyllaceae (with two species for each) (Table 1).The life-form spectrum was dominated by phanerophytes (35%) and geophytes helophytes (23%), followed by chamaephytes (18%), therophytes (12%), hemicryptophytes (6%), and helophytes (6%) (Table 1).

Results of the chorological analysis (Table 2) show that the majority of the recorded species belong to the Saharo-Arabian chorotype (12 species; 33 %), followed by Irano-Turanian region (9 species / 24 %), Mediterranean region (8 species; 22 %), Palaeotropical (3 species; 8 %), Sudano-Zambezian (2 species; 5%), Neotropical (2 species; 5 %), and Euro-Siberian (1 species; 3 %). The relation between the frequencies of the recorded species and the number of the global phytogeographical regions show that the recorded species can be classified into 8 pluri-regional, 7 bi-regional and 2 mono-regional. Dominant species were *P. australis*, *T. nilotica* and *Z. album*, whereas the co-dominant species were *J. rigidus*, *N. retusa*, *A. graecorum*, *T. domingensis*, *Z. coccineum* and *E. camaldulensis*.

Variation in species richness was observed at different sites: Highest at Northeast of the Lower Lake (9 species), followed by the Southwest of the Lower Lake and the Northeast of the Upper Lake (6 species each); while the Southeast of the Lower Lake showed the lowest species richness (1species) (Table 3).

Examination of more detailed diversity indices enhanced the exploration of diversity in Wadi El Rayan. Table 3 shows different diversity indices along different stands. Stand 3 has the highest dominance value (=1) followed by stand 7 (=0.999). In contrary, the lowest Simpson's index value was recorded at stand 3 (=0) and highest at stand 4 (=0.574). Similarly, the highest Shannon's diversity index value was at stand 1 (=1.135) and lowest at stand 3(=0). The highest Buzas and Gibson's evenness index value was at stand 3(=1) and the lowest at stand 9 (=0.208). The highest Brillouin's index value was at stand 1 (=1.120) and the lowest at stand 3(=0). The highest Menhinick's richness index value was recorded at stand 4 (=0.570) and the lowest at stand 10 (0.012). Meanwhile, the highest Margalef's richness index value was recorded in stand 1 (=1.118) and the lowest at stand 3 (=0). Also, the highest equitability value was recorded at stand 5 (evenness index = 0.845) and the lowest value at stand 3 (=0). However, the highest Fisher's alpha index value was recorded at stand 4 (=1.359) and the lowest at stand 3 (=0.164). Berger-Parker dominance was the highest at stand 3 (=1), and the highest value of Chao1 index was recorded at stand 1 (=9).

The spatial distribution of some species showed wide ecological ranges; for example:

- *T. nilotica* and *P. australis* were commonly found around the two lakes.
- *J. rigidus* was distributed around the two lakes, with less representation around the Lower Lake (not recorded on its southern edge).
- *A. graecorum* was distributed at the edges of the Connecting Channel, northeast of the Lower Lake; southwest and northeast of the Upper Lake.
- *P. dactylifera* was sparse distributed around the two lakes.
- *I. cylindrica* and *P.dioscoridis* were distributed at the edges of the Connecting Channel and at the northeastern part of the Lower Lake.

Table 1.Recorded species, their families and life form at Wadi El Rayan.

No.	Species	Family	Life form
1	*Arthrocnemum macrostachyum (Moric.)k. Koch*	Chenopodiaceae	Chamaephytes
2	*Chenopodium murale* L.	Chenopodiaceae	Therophytes
3	*Pluchea dioscoridis* (L.)DC.	Compositae (Asteraceae)	Phanerophytes
4	*Cyperus laevigatus* L.	Cyperaceae	Geophytes Helophytes
5	*Imperata cylindrica* (L.) Raeusch.	Gramineae (Poaceae)	Geophytes Helophytes
6	*Phragmites australis* (Cav.)Trin. ex Steud.	Gramineae (Poaceae)	Geophytes Helophytes
7	*Polypogon monspeliensis* (L.) Desf.	Gramineae (Poaceae)	Therophytes
8	*Juncus rigidus* Desf.	Juncaceae	Geophytes Helophytes
9	*Alhagi graecorum* Boiss	Leguminosae (Fabaceae)	Hemicryptophytes
10	*Eucalyptus camaldulensis* Dehnh.	Myrtaceae	Phanerophytes
11	*Nitraria retusa* (Forssk) Asch	Nitrariaceae	Phanerophytes
12	*Phoenix dactylifera* L.,	Palmae (Arecaceae)	Phanerophytes
13	*Calligonum polygonoides* subsp. comosum *(L' Hér.)Soskov*	Polygonaceae	Phanerophytes
14	*Tamarix nilotica* (Ehrenb.) Bunge	Tamaricaceae	Phanerophytes
15	*Typha domingensis (Pers.) Poir. ex Steud.*	Typhaceae	Helophytes
16	*Zygophyllum album* L.f.	Zygophyllaceae	Chamaephytes
17	*Zygophyllum coccineum* L.	Zygophyllaceae	Chamaephytes

Table 2. The floristic regions of the flora around the lakes of Wadi El Rayan

Plant species	Floristic regions*
Alhagi graecorum	IR-TR + SA- AR + ME
Arthrocnemum macrostachyum	ME +SA-AR
Calligonum polygonoides sub. comosum	SA-AR + IR-TR
Chenopodium marale	Cosmopolitan
Cyperus laevigatus	Cosmopolitan
Eucalyptus camaldulensis	IR-TR
Imperata cylindrica	ME + IR-TR + SA-AR+PAL+NEO
Juncus rigidus	ER-SR + ME + IR-TR
Nitraria retusa	SA-AR + SU-ZA
Phoenix dactylifera	SA-AR + SU-AZ
Phragmites australis	ME + IR-TR + SA-AR + PAL + NEO
Pluchea dioscoridis	SU-ZA + SA-AR
Polypogon monospliensis	ME + IR-TR+ SA-AR
Tamarix nilotica	SA-AR + IR-TR
Typha domingensis	ME + IR-TR + PAL
Zygophyllum album	SA-AR + ME
Zygophyllum coccineum	SA-AR

*The floristic regions are abbreviated as follows: ME: Mediterranean, IR-TR: Irano-Turanian, SA-AR: Saharo-Arabian, ER-SR: Euro-Siberian, SU-ZA: Sudano-Zambezian, NEO: Neotropical, and PAL: Palaeotropical.

Table 3. Diversity indices along the study stands in Wadi El Rayan

	Stand 1	Stand 2	Stand 3	Stand 4	Stand 5	Stand 6	Stand 7	Stand 8	Stand 9	Stand 10
Taxa_S (species richness)	9	5	1	6	2	5	4	3	6	4
Dominance_D	0.487	0.843	1.000	0.426	0.603	0.915	0.999	0.995	0.920	0.978
Simpson_1-D	0.513	0.157	0.000	0.574	0.397	0.085	0.001	0.005	0.080	0.022
Shannon_H	1.135	0.335	0.000	1.119	0.586	0.203	0.006	0.018	0.223	0.062
Evenness_e^H/S	0.346	0.280	1.000	0.510	0.898	0.245	0.252	0.339	0.208	0.266
Brillouin	1.120	0.332	0.000	1.045	0.529	0.202	0.006	0.018	0.219	0.062
Menhinick	0.251	0.088	0.118	0.570	0.348	0.043	0.024	0.015	0.116	0.012
Margalef	1.118	0.495	0.000	1.062	0.286	0.422	0.293	0.190	0.633	0.259
Equitability_J	0.517	0.208	0.000	0.625	0.845	0.126	0.005	0.017	0.124	0.045
Fisher_alpha	1.306	0.580	0.164	1.359	0.469	0.490	0.354	0.252	0.731	0.313
Berger-Parker	0.679	0.915	1.000	0.604	0.727	0.956	0.999	0.998	0.959	0.989
Chao-1	9	5	1	7	2	5	4	3	6	4

3.2. Multivariate Analysis

3.2.1. Classification

Cluster analysis of species composition for each of the 10 studied stands is shown in Figure 2. Based on the results of the TWINSPAN analysis (using importance values 'IV' of the recorded species), the vegetation can be categorized into four vegetation groups (Figure 3), named after their leading dominant species: (A) *P. australis–T. nilotica*, (B) *J. rigidus*, (C) *A. graecorum*, and (D) *Z. album–N. retusa*. Different species of these groups can be easily linked to a habitat type: wetlands, Sabkha, sand sheets, sand dunes, desert areas edges of wetlands.

Statistical analysis of the physical properties of the soil showed a variation in soil texture: the most frequent type was sandy (70%) while the loamy sand was detected only at three stands (30%) (Table 4). The lowest recorded elevation was at El Modwara (-19m b.s.l) while the highest was at the northeastern part of the Upper Lake (16m a.s.l). The analyses of the chemical properties of the soil showed that it was slightly alkaline (with pH values ranging from 7.89 to 8.5); while the electrical conductivity values of soil extract showed a great variation ranging from 5.88 to 63.0 ds/m. The soil organic matter was low, ranging from 0.08 to1.2 % with the most frequent value of 0.61 %. However, significant differences in the soil chemical and physical characters were found (Table 5). The analyses of the soil chemical properties relationship with vegetation (Table 6) showed that the mean soil pH value for *P. australis* and *T. nilotica* was 8.14; 8.17 for *A. graecorum*; 8.19 for *J. rigidus*; and 8.5 for each of *P. monospeliensis*, *C. laevigatus and C. murale*. However, soil analyses showed that the soil was slightly alkaline, with pH values ranging from 7.89 at

Stand 10 (species: *J. rigidus, P. australis, T. nilotica and T. domingensis*) to 8.5 at Stand 1 (species: *A. graecorum, C. murale, C. laevigatus, I. cylindrica, J. rigidus, P. australis, P. dioscoridis, P. monspeliensis and T. nilotica)*. However, the most frequent value of soil pH in the study area was 8.11.

There was a great variation in Total Dissolved Solids (T.D.S) of different plant species. The mean T.D.S for *P. australis* was 11135.29 ppm; *T. nilotica*: 11212.16; *A. graecorum*: 6636.80; *Z. coccineum and A. macrostachyum:* 40320. However, high T.D.S variation across stands was measured ranging from 3763.2 ppm at Stand 9 (species: *A. graecorum, E. camaldulensis, J. rigidus, P. dactylifera, P. australis* and *T. nilotica*) to 40320 ppm at Stand 7 (species: *A. macrostachyum, P. australis, T. nilotica and Z. coccineum*).

Results showed that the study area is poor inorganic matter content, ranging from 0.08 % at Stand 4 (species: *C. polygonoides, N. retusa, P. dactylifera, P. australis, T. nilotica* and *Z. album)* to 1.2% at Stand 8, with the most frequent value of 0.61%. The study area showed a great variation of soil carbonate content, ranging from 3.81% at Stand 6 (species: *A. graecorum, J. rigidus, P. dactylifera, P. australis and T. nilotica*) to 37.9 % at Stand 1 (species: *A. graecorum, C. murale, C. laevigatus, I. cylindrica, J. rigidus, P. australis, P. dioscoridis, P. monspeliensis and T. nilotica)*, with the most frequent value of 7.16 %.

The soil calcium content ranged from19.3 meq/L at Stand 5 (species: *P. australis*and *T. nilotica*) to 115 meq/L at Stand 7 (species: *P. australis, T. nilotica, Z. coccineum and A. macrostachyum*). While, the soil magnesium content ranged from 3.6 meq/L at Stand 9 (species: *A. graecorum, E. camaldulensis, J. rigidus, P. dactylifera, P. australis and T. nilotica*) to 107 meq/L at Stand 7 (species: *P. australis, T. nilotica, Z. coccineum and A. macrostachyum*).

The soil sodium content ranged from 22.2 meq/L at Stand 4 (species: *C. polygonoides, N. retusa, P. dactylifera, P. australis, T. nilotica* and *Z. album*) to 590 meq/L at Stand 7 (species: *P. australis, T. nilotica, Z. coccineum* and *A. macrostachyum*). The soil potassium content ranged from 2.8 meq/L at Stand 10 (species: *J. rigidus, P. australis, T. nilotica* and *T. domingensis*) to 8.1 meq/L at Stand 3 (*T. nilotica*), with the most frequent value of 3.2 meq/L. The data indicated that Stand 7 has the highest value of bicarbonate content (4.01 meq/L - species: *P. australis, T. nilotica, Z. coccineum* and *A. macrostachyum*), while the lowest value was recorded at Stand 5 (1 meq/L - species: *P. australis* and *T. nilotica*), with the most frequent value of 2.11 meq/L. Soil collected at Stand 7 (species: *P. australis, T. nilotica, Z. coccineum* and *A. macrostachyum*) showed the highest chloride content value (700 meq/L),while the lowest value was recorded at Stand 5 (33.1 meq/L - species: *P. australis* and *T. nilotica*). Furthermore, soil sulphate contents were highest at Stand 7 (114.8 meq/L – species: *P. australis, T. nilotica, Z. coccineum* and *A. macrostachyum*) and lowest at Stand 9 (6.79 meq/L – species: *A. graecorum, E. camaldulensis, J. rigidus, P. dactylifera, P. australis* and *T. nilotica*).

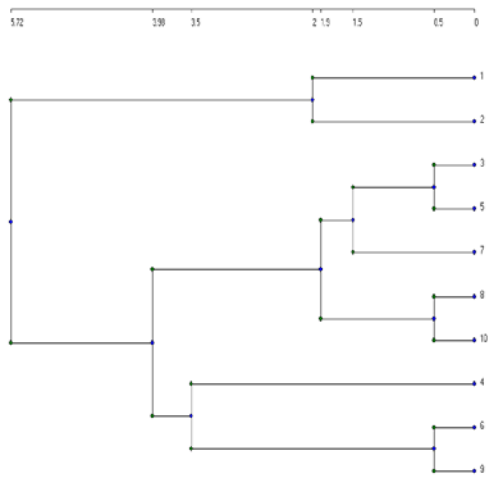
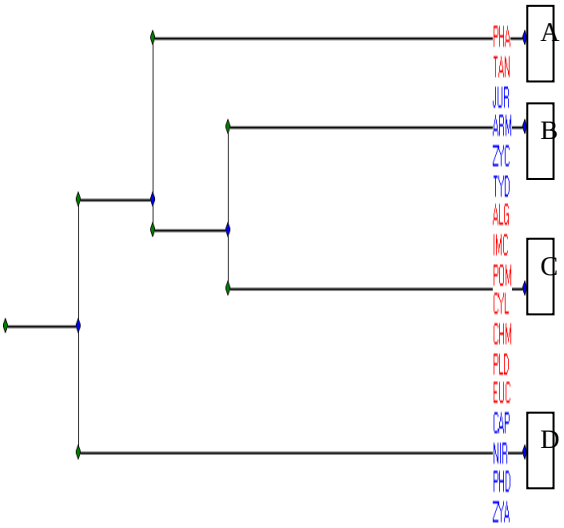


Figure2.Dendrograms showing cluster analysis of the studied stands.



Abbreviations:

PHA	*Phragmites australis*	*CAP*	*Calligonum polygonoides sub. comosum*
TAN	*Tamarix nilotica*	*ARM*	*Arthrocnemum macrostachyum*
JUR	*Juncus rigidus*	*NIR*	*Nitraria retusa*
ALG	*Alhagi graecorum*	*PHD*	*Phoenix dactylifera*
IMC	*Imperata cylindrica*	*ZYA*	*Zygophyllum album*
POM	*Polypogon monospeliensis*	*ZYC*	*Zygophyllum coccineum*
CYL	*Cyperus laevigatus*	*TYD*	*Typha domingensis*
CHM	*Chenopodium murale*	*EUC*	*Eucalyptus camaldulensis*
PLD	*Pluchea dioscoridis*		

Figure 3. TWINSPAN dendrogram of the 10 studied stands of the study area and 17 species based on their importance values. A-D are the four separated vegetation groups.

Table 4. The physical properties of soil samples collected within the study stands in Wadi El Rayan.

Location	Stand No.	Alt (m)	The saturation percentage (SP)	Texture (%)				Texture
				Coarse Sand	Fine Sand	Silt	Clay	
North East of the Lower Lake	1	-18	0.61	35.6	42.7	14.2	7.5	Loamy Sand
The Connecting channel	2	-2	0.43	39.6	46.4	9.5	4.5	Sandy
Southeast of the Lower Lake	3	-14	0.81	39.4	46.1	9.8	4.7	Sandy
Southwest of the Lower Lake	4	-3	0.08	38.8	44.5	10.4	6.3	Sandy
El Modwara (Northwest of Lower Lake)	5	-19	0.61	41.7	43.7	9.6	5.0	Sandy
Southwest of the Upper Lake	6	13	0.68	42.5	43.2	10.3	4.0	Sandy
Northwest of the Upper Lake	7	9	1.1	32.8	40.3	19.5	7.4	Loamy Sand
Southeast of the Upper Lake	8	9	1.2	35.0	42.5	16.2	6.3	Loamy Sand
Northeast of the Upper Lake	9	16	0.96	35.4	49.3	10.5	4.8	Sandy
North of the Upper Lake	10	13	20.1	44.7	47.5	5.3	2.5	Sandy

Table 5.Chemical properties of soil samples that collected within the study stands in Wadi El Rayan.

Location	Stand No.	pH	T.D. S ppm	EC ds/m	%	%	%	Cations (meq/l)				Anions(meq/l)		
					Organic matter	Organic carbon	$CaCO_3$	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
Northeast of the Lower Lake	1	8.50	4928	7.70	0.61	0.35	37.9	22.2	14.4	32.1	3.31	1.2	45.2	25.61
The Connecting Channel	2	8.11	9664	15.10	0.43	0.24	6.7	37.1	53.3	99.1	5.5	2.01	122.0	70.99
Southeast of the lower lake	3	8.15	11904	18.60	0.81	0.46	7.16	50.1	35.8	140.0	8.1	2.11	205.0	26.89
Southwest of the lower lake	4	8.12	3974.4	6.21	0.08	0.046	6.60	26.1	8.5	22.2	3.20	1.33	42.1	16.57
El Modwara (Northwest of Lower Lake)	5	8.11	3840	6.0	0.61	0.35	5.05	19.3	9.7	25.1	2.9	1.0	33.1	22.9
Southwest of the Upper Lake	6	8.05	8192	12.8	0.68	0.39	3.81	35.2	38.4	78.2	4.2	3.0	100.0	53.0
Northwest of the Upper Lake	7	8.0	40320	63.0	1.1	0.63	7.16	115.0	107.0	590.0	6.8	4.01	700.0	114.8
Southeast of the Upper Lake	8	8.49	10496	16.4	1.2	0.69	28.6	55.0	37.8	110.0	5.2	3.02	180.0	24.98
Northeast of the Upper Lake	9	8.02	3763.2	5.88	0.96	0.55	22.7	20.0	3.6	25.2	3.2	2.11	43.1	6.79
North of the Upper Lake	10	7.89	15040	23.5	0.2	0.11	8.6	57.8	25.1	199.2	2.8	2.9	244.2	37.8

Table 6. Mean values of chemical analysis of soil for different plant species within the study stands in Wadi El Rayan.

Species Name	pH	T.D.S ppm	EC ds/m	% Organic matter	% Organic carbon	% $CaCO_3$	Cations (meq/l) Ca++	Mg++	Na+	K+	Anions (meq/l) HCO_3-	Cl-	SO_4--
Phragmites australis	8.14	11135.29	17.40	0.65	0.37	14.12	43.08	33.09	131.23	4.12	2.29	167.74	41.49
Tamarix nilotica	8.14	11212.16	17.52	0.67	0.38	13.43	43.78	33.36	132.11	4.52	2.27	171.47	40.03
Juncus rigidus	8.19	8483.84	13.26	0.73	0.42	20.32	38.04	23.86	88.94	3.74	2.45	122.50	29.64
Alhagi graecorum	8.17	6636.80	10.37	0.67	0.38	17.78	28.63	27.43	58.65	4.05	2.08	77.58	39.10
Imperata cylindrica	8.31	7296.00	11.40	0.52	0.30	22.30	29.65	33.85	65.60	4.41	1.61	83.60	48.30
Polypogon monospeliensis	8.5	4928	7.7	0.61	0.35	37.9	22.2	14.4	32.1	3.31	1.2	45.2	25.61
Cyperus laevigatus	8.5	4928	7.7	0.61	0.35	37.9	22.2	14.4	32.1	3.31	1.2	45.2	25.61
Chenopodium murale	8.5	4928	7.7	0.61	0.35	37.9	22.2	14.4	32.1	3.31	1.2	45.2	25.61
Pluchea dioscoridis	8.31	7296.00	11.40	0.52	0.30	22.30	29.65	33.85	65.60	4.41	1.61	83.60	48.30
Calligonum polygonoides	8.12	3974.4	6.21	0.08	0.046	6.6	26.1	8.5	22.2	3.2	1.33	42.1	16.57
Arthrocnemum macrostachyum	8	40320	63	1.1	0.63	7.16	115	107	590	6.8	4.01	700	114.8
Nitrari aretusa	8.12	3974.4	6.21	0.08	0.046	6.6	26.1	8.5	22.2	3.2	1.33	42.1	16.57
Phoenix dactylifera	8.06	5309.87	8.30	0.57	0.33	11.04	27.10	16.83	41.87	3.53	2.15	61.73	25.45
Zygophyllum album	8.12	3974.4	6.21	0.08	0.046	6.6	26.1	8.5	22.2	3.2	1.33	42.1	16.57
Zygophyllum coccineum	8	40320	63	1.1	0.63	7.16	115	107	590	6.8	4.01	700	114.8
Typha domingensis	7.89	15040	23.5	0.2	0.11	8.6	57.8	25.1	199.2	2.8	2.9	244.2	37.8
Eucalyptus camaldulensis	8.02	3763.2	5.88	0.96	0.55	22.7	20	3.6	25.2	3.2	2.11	43.1	6.79

3.2.2. Ordination

There was a successive decrease in the Eigenvalues of the first three CCA axes. These Eigenvalues were somewhat higher for the DCA axes (Tables 7 and 8), which indicates that the important explanatory stand variables were not measured and included in the analysis or some of the variations were not explained by environmental variables (Franklin & Merlin, 1992; McDonald *et al.*, 1996).

However, The DCA analysis revealed information about the range of variation among the stands at the two lakes (Table 7 and Figure 4). Results showed that the first gradient is the longest, explaining about 36.8 % of the total species variability, whereas the second and higher axes explain much lower. Also, the first axis was very well correlated with the environmental data (r=1), and the correlation for the other axis was considerably lower. This suggests that the whole data set was governed by a single dominant gradient. The number of axis scores calculated for a species–environmental variable bi-plot was restricted in a DCA, by one or two defaults.

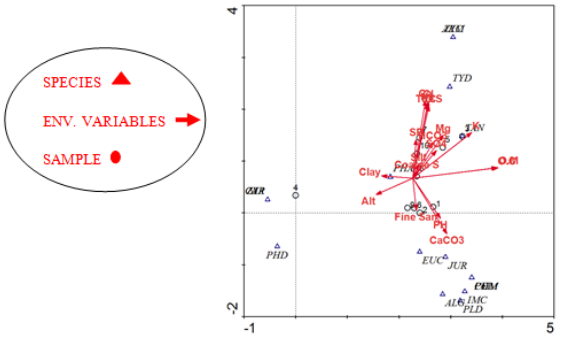
The first axis was negatively correlated with the altitude (Alt) and soil contents (fine sand and clay), with the increasing concentration of (K+, Mg++ and organic matter), positively correlated with (Na+, Cl^- , total dissolved salts with electrical conductivity), and with the increasing concentration of (SO_4^{--}, Ca++, and HCO_3^-), with the increasing concentration of $CaCO_3$ and pH gradient.

The positions of arrows for environmental variables suggest that there was a group of variables that were mutually highly positive correlated (Alt, $CaCO_3$, pH, clay and fine soil) and negative correlated with (Cl^-, K^+, Ca^{++} and organic matter) with the total dissolved salts and electrical conductivity. A closer inspection of the correlation matrix in the CANOCO Log View showed that the variables were indeed correlated, but in some cases the correlation is not very strong. The correlation matrix also confirmed that the correlation of all measured variables with the second axis was rather weak (Figure 4).

This pattern also appeared in the summary (Table 8), where the first axis explained more than the second, third and fourth axes do together. Comparing this summary with that from the DCA, it is noticed that the percentage variance explained by the first axis was the same as that explained by the first axis in the unconstrained DCA (36.8), where the species environment correlation was only slightly higher. The first axis of CCA was very similar (both for the species and for the sample scores) to the first axis of DCA (Figures 4and 5). However, the second axis was different: The CCA showed a remarkable arch effect – the quadratic dependence of the second axis on the first one.

Table7. Environmental parameters used in the DCA and their Eigenvalues.

Axes	1	2	3	4
Eigenvalues	0.756	0.25	0.099	0.02
Lengths of gradient	3.224	1.478	1.402	0.841
Species-environment correlations	1	1	1	1
of species data	36.8	49	53.8	54.8
of species-environment relation	28.7	43.5	0	0
The saturation percentage (SP)	0.0633	0.291	-0.1671	0.3675
Coarse S	0.0333	0.0572	-0.3723	-0.3823
Fine San	-0.0168	-0.2297	-0.2635	0.1383
Silt	0.0482	0.1123	0.4083	0.1891
Clay	-0.1756	-0.0325	0.3047	0.2017
PH	0.1121	-0.2511	-0.1829	-0.2658
EC	0.1754	0.5593	0.4979	0.3654
O.M	0.499	0.2137	0.4848	0.3654
$CaCO_3$	0.1318	-0.3548	-0.211	0.089
Ca	0.161	0.5932	0.4958	0.0906
Mg	0.2234	0.3657	0.3026	0.2062
Na	0.1689	0.5712	0.5148	0.3373
K	0.3882	0.4296	0.4114	0.0226
HCO_3	0.1553	0.2945	0.2447	0.3962
Cl	0.1775	0.5926	0.5381	-0.2252
SO_4	0.1667	0.2415	0.1335	0.1818
Altitude	-0.2286	-0.1794	-0.0585	0.3902



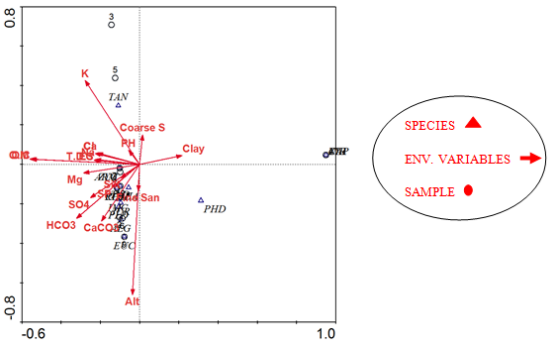
Abbreviations:

PHA	*Phragmitesaustralis*	*CAP*	*Calligonumpolygonoidessub .comosum*
TAN	*Tamarixnilotica*	*ARM*	*Arthrocnemummacrostachyum*
JUR	*Juncusrigidus*	*NIR*	*Nitrariaretusa*
ALG	*Alhagigraecorum*	*PHD*	*Phoenix dactylifera*
IMC	*Imperatacylindrica*	*ZYA*	*Zygophyllum album*
POM	*Polypogonmonospeliensis*	*ZYC*	*Zygophyllumcoccineum*
CYL	*Cyperuslaevigatus*	*TYD*	*Typhadomingensis*
CHM	*Chenopodiummurale*	*EUC*	*Eucalyptus camaldulensis*
PLD	*Plucheadioscoridis*		

Figure 4. The samples, species and environmental (circles, triangle and arrow, respectively) triplot of the DCA of the whole data set.

Table8. Environmental parameters used in the CCA and their Eigen values.

Axes	1	2	3	4
Eigenvalues	0.756	0.421	0.251	0.213
Species-environment correlations	1	1	1	1
of species-environment relation	36.8	57.3	69.5	79.9
The saturation percentage (SP)	-0.1404	-0.117	0.4948	0.8326
Coarse S	0.016	0.1498	-0.0919	0.7283
Fine San	-0.0061	-0.1349	-0.2887	0.5317
Silt	-0.0951	-0.0685	0.2729	-0.7654
Clay	0.2161	0.0451	0.0172	-0.7096
PH	-0.0557	0.0719	-0.4951	-0.1779
EC	-0.2312	0.0214	0.8418	-0.3918
O.M	-0.5586	0.0272	0.0966	-0.5516
$CaCO_3$	-0.1941	-0.2875	-0.3408	-0.0102
Ca	-0.2148	0.0581	0.848	-0.3053
Mg	-0.2848	-0.0435	0.6292	-0.5363
Na	-0.2242	0.0255	0.8548	-0.3825
K	-0.2772	0.4249	0.1586	-0.484
HCO_3	-0.3214	-0.2736	0.6972	-0.1547
Cl	-0.2264	0.0559	0.8486	-0.378
SO_4	-0.2508	-0.1699	0.6065	-0.4855
Altitude	-0.0358	-0.6612	0.438	0.1003



Abbreviations:

PHA	*Phragmitesaustralis*	*CAP*	*Calligonumpolygonoidessub .comosum*
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IMC	*Imperatacylindrica*	*ZYA*	*Zygophyllum album*
POM	*Polypogonmonospeliensis*	*ZYC*	*Zygophyllumcoccineum*
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CHM	*Chenopodiummurale*	*EUC*	*Eucalyptus camaldulensis*
PLD	*Plucheadioscoridis*		

Figure 5.The samples, species and environmental (circles, triangle and arrow, respectively) triplot of the CCA of the whole data set.

4. Discussion

The present study examined the environmental correlates of species distribution at different geomorphologic locations of Wadi El Rayan Protected Area along the coast of the two lakes. Both DCA and CCA assessed the soil-vegetation relationships: CCA analysis showed the relative positions of species and sites along the most important ecological gradients. Both ordination techniques clearly indicated that soil pH, electric conductivity (salinity), $CaCO_3$, organic matter, and relative concentrations of cations (Ca++, Mg++, Na+ and K+) were the most important parameters for the distribution of the vegetation pattern in the area.

The organic matter, considered as a key factor affecting the soil fertility of some desert ecosystems in Egypt (Sharaf El Din and Shaltout, 1985; Abd El-Ghani, 1998, 2000) and soils of arid lands have a low level of organic matter, are slightly acid to alkaline in reaction (pH) at the surface (Dregne, 1976). The soil chemistry affects the plant species composition through salinity levels (Sharma and Shankar, 1991; Kumar, 1996; Abbadi and El Sheikh, 2002), pH, calcium and organic carbon (Abd El-Ghani, 1998).The present study showed that Wadi El Rayan is characterized by a variety of soil types (based on their physical and chemical attributes), which is in accordance to its varying nutrient status (Amin, 1998). Literature reported, on average, a higher species richness in the area of Wadi El Rayan than the the one recorded in the present study (60 species: Abdou, 2002; 38 species: EEAA, 2003; 27 species: Azzazi, 2009; 13 species: Saleh *et al*., 1984). The lower number of the recorded species here is due to our confined scope towards wetlands and desert ecosystems; while others have also included species from the springs' oases and the cultivated lands. Furthermore, the human pressures may play a role in the distribution of the species (e.g., overgrazing, land encroachment, reduction in water levels due to decreased water collected in the lakes and tourism).Conservation of the vegetation in Wadi El Rayan requires stopping the severe human impacts that lead to the elimination of certain plant populations and, hence, the modification of the complex plant communities into simple fragile once. However, water supply is of great importance as it determines the Wadi El Rayan's future and its vegetation cover.

The ecosystems investigated in the present study are relatively simple, in which the survived species with stand stress environmental conditions, as Wadi El Rayan is located at the Sahara eco-region of the Palearctic eco-zone, the world's largest hot desert. However, the desert ecosystem of Wadi El Rayan consists of arid vegetation (xerophytic and halophytic plants) with a sparse plant cover except in wetlands ecosystem at the shoreline of the lakes which are characterized by some hydrophytic and halophytic plants.

Our results are in accordance with those of Amin (1998), who identified three major ecosystems in Wadi El-Rayan area: desert, lake and spring ecosystems. However, the spring ecosystem was not recognized in the area of the current study. Each of these ecosystems has its own habitat features that support the growth of variant plant communities, mainly hydrophytes, reed swamps, halophytes, and xerophytes. In the present study, the wetlands are represented by species *P. australis*, *T. nilotica*, *J. rigidus*, *T. domingensis*, *C. laevigatus*, *C. marale*, *P. monspeliensis*, *P. dioscoridis*, *E. camaldulensis* and *I. cylindrica*; while the desert is represented by *T. nilotica*, *A. graecorum*, *C. polygonoides sub. comosum*, *N. retusa*, *Z. album*, *Z. coccineum*, *A. macrostachyum* and *P. dactylifera*. The study area is dominated by two species: *P. australis* and *T. nilotica*. The latter is the most widely distributed plant in Wadi El Rayan and can be considered as the most successful species in the study area as it grows at a variety of ecosystems and habitats (e.g., desert areas, edges of wetlands and salt marshes). Other 15 species were recorded in a very limited abundance (see Table 1), amongst them are *T. domingensis* (which has a very limited distributional range in the study area to the northern edge of the Upper Lake) and *E. camaldulensis* an exotic (non-native to the area) species represented with very limited distribution (northeast edges of the Upper Lake) and typically grows along river banks and in alluvial valleys) (El-Hadidi and Boulos, 1989). Amin (1998) demonstrates the adaptation of different plant species in Wadi El Rayan to environmental stresses (extreme drought and salinity conditions), via (a) having a shedded green cortex (*Z. album* and *Z. coccineum*); (b) defoliation (*N. retusa*); (c) salt removal by secretion (*T. nilotica*); and (d) the ability to phytogenic mounds controlled mainly by the life-form of the species (*C. polygonoides*, *N. retusa*, *Z. album*, *Z. coccineum* and *T. nilotica*). Simpson (1932) reported that *T. domingensis* is more sensitive to salt than *P. australis* as the latter grows well in the Lake Mariut area, while the former is present only where the Lake receives fresh water from the Mahmudiya Canal. *A. graecorum* was a widely distributed species (Kassas, 1952) and was considered as a groundwater-indicating plant (Girgis, 1972). However, the dominant species *P. australis* was the most adaptive and suitable plant species for the quality of the water in Wadi El Rayan (EEAA, 2003).

A high variability of the recorded plants' growth forms was noticed, including shrubs with photosynthetic stems (e.g. *C. polygonoides*), succulent xerohalophytic semi-shrubs (e.g. *Z. album*), virgate and thorny trees and shrubs (e.g. *N. retusa*). The results of the present study are in accordance with the results of Beeftink (1977) and Zahran (1982) that the life-form chamaephytes and geophytes are able to withstand water logging, high salinity levels and a wide range of temperature variability. Also a high percentage of hemicryptophytes and therophytes in sandy dune habitats could be related to their ability to resist drought, sand accumulation and grazing (Danin and Orshan, 1990; Danin, 1996).

Chorological analysis of the floristic data showed that the Saharo-Arabian chorotype (33 %), Irano-Turanian (24 %) and Mediterranean (22 %) formed the major components of the floristic structure along the study area. Abd El-Ghani and Amer (2003) reported that the plants of the Saharo-Arabian species are good indicators for the quality of desert environmental conditions, while the Mediterranean species are more related to the mesic

environment. In arid and semi-arid regions, seasonal variations may lead to differential physiological responses in the plants inhabiting such environments, including adaptations to high temperature, drought, and salt stresses (Joyce *et al*., 1984; Murakeozy *et al*., 2003; Kusaka *et al*., 2005).Wickens (1977) and Boulos (1997) reported that the Saharo-Arabian region is characterized by few endemic species and genera, and the absence of endemic families.

Zahran and Willis (2009) reported that, in the extreme environmental conditions of arid lands, the interactions between its different components are of high significance, so that small changes in one component of the ecosystem can lead to substantial variations in others, creating distinct micro-habitats. In arid lands, the inter-relationships between soils, vegetation and atmosphere are so interconnected that they can hardly be considered, ecologically, as separate entities. However, extreme arid conditions, notably high salinity and high aridity, act as filters to species that are able to adapt in the hyper-arid environment.

5. Conclusion

In Wadi El Rayan, as hyper-arid environment, the wild plants have adapted to survive extreme climate conditions and soil factors, where both ordination techniques clearly indicated that soil pH, electric conductivity (salinity), $CaCO_3$, organic matter, and relative concentrations of cations were the most important parameters for the distribution of the vegetation pattern in the area. Considerations of the exploitation and conservation of wild plants must take ecological principles into account. Sustainable management of the floral biodiversity in Wadi El Rayan requires the termination of the severe human impacts that lead to eliminating certain plant populations and, hence, the modification of the complex plant communities into simple fragile once.

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References

Abbadi GA and El Sheikh MA. 2002. Vegetation analysis of Failaka Island (Kuwait). *Arid Environ J*, **50**: 153–165.

Abd El-Ghani M and Amer WM. 2003.Soil–vegetation relationships in a coastal desert plain of southern Sinai, Egypt. *Arid Environ J*, **55**: 607–628.

Abd El-Ghani MM. 1998. Environmental correlates of species distribution in arid desert ecosystems of eastern Egypt. *Arid Environ J*, **38**: 297–313.

Abd El-Ghani MM. 2000. Floristics and environmental relations in two extreme desert zones of western Egypt. *GLOBAL ECOL BIOGEOGR J*, **9**: 499–516.

Abdou WA. 2002. Environmental study on Wadi El Rayan, El Fayoum Governorate. MSc Thesis, Mansoura University, Mansoura

Abdou WA. 2006. Biodiversity study in Wadi El-Rayan, Nature Conservation Sector, Egyptian Environmental Affairs Agency, Egypt, pp. 32.

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C and Roberts JD. 1976. Chemical Analysis of Ecological Materials. Edited by S.B. Chapman. Blackwell Sci. Publ., Oxford, Chapter 8:166-411.

Amin SA. 1998. Ecological Study on the Plant Life in Wadi El-Rayan Area, El-Fayium, Egypt. PhD Thesis, Faculty of Science, Cairo University, Egypt.

Ayyad M. 1976. The vegetation and environment of the western Mediterranean coastal land of Egypt. IV. The habitat of non-saline depressions. *Ecol J*, **64**: 713–722.

Ayyad MA and Ghabbour SI. 1986. Hot deserts of Egypt and the Sudan. In: Evennari, M., Noy Meir, I. & Goodall. D (Eds.), Ecosystems of the world.ElSevier, Amesterdam, pp. 149-202

Azzazi MF. 2009. Present and Ancient Vegetation of Wadi El Raiyan, Egypt.The desert institute bulletin, Egypt. 69, No. 8 (2009): 463-475.

Barbour MG, Burk JH and Pitts WD. 1987.Terrestrial Plant Ecology, second ed. Benjamin/Cummings, Menlo Park, California.

Beeftink WG. 1977. The coastal salt marshes of western and northern Europe. In: Chapman V.J (Eds.), Ecosystems of the World 1. Elsevier, Amsterdam, pp. 109–155.

Black CA. 1965. Methods of Soil Analysis.American Society of Agronomy. Series 9, part 2: 771-1572.

Boulos L. 1975. The Mediterranean element in the flora of Egypt and Libya. – ColloquesInternat.CNRS 235. La flore du BassinMéditerranéan: essai de systématiquesynthétique: 119-124.

Boulos L. 1997. Endemicflora of the Middle East and North Africa. In: Barakat, H.N., Hegazy, A.K (Eds.), Reviews in Ecology: Desert Conservation and Development Metropole, Cairo, pp. 331

Danin A and Orshan G. 1990. Distribution of Raunkiaer life forms in Israel. *Vege Sci J*, **1**: 41–48.

Danin A. 1996. Plants of desert dunes. In: Cloudsley-Thompson J.L (Eds.), Adaptation of Organisms to the Desert. Springer Verlag, Heidelberg, pp. 156.

Dregne HE. 1976. Soils of Arid Regions. Elsevier, Amsterdam, pp.237.

EEAA [Egyptian Environmental Affairs Agency]. 2003. Monitoring Report on the third year of monitoring program. Wadi El-Rayan Protected Area, Nature Conservation Sector, Egyptian Environmental Affairs Agency.Egypt, pp.65.

EEAA [Egyptian Environmental Affairs Agency]. 2012. Information Sheet on Ramsar Wetlands to designate of Wadi El Rayan and Qaroun lakes as new Ramsar sites, submitted to Convention on Wetlands of International Importance (Ramsar Convention). Designation date: 04/06/12, Ramsar Site no. 2041.

El-Hadidi MN and Boulos L. 1989.The street trees of Egypt.The Amercan University in Cairo.ISBN.977-424-173-8.

Franklin J and Merlin M. 1992. Species–environment patterns of forest vegetation on the uplifted reef limestone of Atiu, Managia, Ma'uke and Miti'aro, Cook Islands. *Veg Sci J*, **3**: 3–14.

Girgis WA. 1972. Plant indicators in the Egyptian deserts. Bulletin of the Desert Institute, Arab Republic of Egypt, 21:511–515.

Glenn M, Robert E, Brian H, David RF, Jonathan H and Dana M. 2002. Vegetation variation across Cape Cod, Massachusetts:

environmental and historical determinants. *Biogeogr J*, **29**: 1439–1454.

Harper DAT. 1999.Numerical Palaeobiology.John Wiley & Sons.Chichester, 1999. ISBN 0 471 97405 6: pp. 468.

He MZ, Zheng JG, Lia XR and Qian YL. 2007. Environmental factors affecting vegetation composition in the Alxa Plateau, China. *Arid Enviro J*, **69**: 473–489.

Hejcmanovā-Neżerková P and Hejcman M. 2006.A canonical correspondence analysis (CCA) of the vegetation–environment relationships in Sudanese savannah, Senegal. *S. Afr. J. Bot*, **72**: 256 – 262.

Henderson PA and Seaby RMH. 1999. Community Analysis Package (CAP) Version 1.2. Lymington, UK: Pisces Conservation Ltd, IRC House, Pennington, Lymington, S041 8GN, UK.

Hill MO. 1979. TWINSPAN-A FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Ithaca, NY: Ecology and Systematics, Cornell University.

IUCN 1998.Wadi el Rayan Protected Area Project.Guidelines for overall Work Plan (1998 – 2001).Report 1.Prepared by G.H. MattraversMessana.

Jackson ML. 1967.Soil Chemical Analysis-Advanced Course.Washington Department of Soil Sciences.

Joyce PS, Paleg G and Aspinall D. 1984.The requirement for low-intensity light in the accumulation of proline as a response to water deficit. Journal of Experimental Botany, **35**: 209–218.

Kadmon R and Danin A. 1999.Distribution of plant species in Israel in relation to spatial variation in rainfall. *Veg Sci J*, **10**: 421–432.

Kassas MM. 1952. On the distribution of *Alhagimaurorum* in Egypt.Egyptian Academic of Science. Proc. **3**, 140–151.

Kassas MM. 1957. On the ecology of the Red Sea coastal land. *Ecol J*, **45**: 187-203.

Kassas MM. 1962. On the phytosociology of the desert vegetation of Egypt. Annals of Arid Zone, **I**: 54-83.

Kumar S. 1996. Trends in structural compositional attributes of dune-interdune vegetation and their edaphic relations in the Indian desert. *Veg*, **124**: 73–93.

Kusaka M, Ohta M and Fujimura T. 2005.Contribution of inorganic components to osmotic adjustment and leaf folding for drought tolerance in pearl millet. *Physiol Plant*, **125** (**4**): 474–489.

Lepš J and Šmilauer P. 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge, UK, pp. 282.

Maryam H, Ismail S, Alaa F and Ahmed R. 1995.Studies on growth and salt regulation in some halophytes as influenced by edaphic and climatic conditions. *Pak J Bot*, **27**: 151-163.

McDonald DJ, Cowling RM and Boucher C. 1996. Vegetation–environment relationships on a speciesrich coastal mountain range in the fynobs biome (South Africa). *Veg*, **123**: 165–182.

Murakeozy EP, Nagy Z, Duhaze C, Boucherean A and Tuba Z. 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of island saline vegetation in Hungary. *Plant Physiol J*, **160**: 395–401.

Nels CJ. 1995. Biodiversity in the balance Approaches to setting Geographic Conservation Priorities, Corpotate Press. Inc., Landover, Mary land, pp. 125.

Osborn DJ and Helmy I. 1980.The contemporary land mammals of Egypt including Sinai. *Fieldiana Zool* (New Series), **5**: 455-459.

Parker K. 1991.Topography, substrate, and vegetation patterns in the northern Sonoran Desert. *J. Biogeogr*, **18**: 151–163.

Pavlůet V, Hejcman M, Pavlůet L and Gaisler J. 2003. Effect of rotational and continuous grazing on vegetation of an upland grassland in the Jizerske´ Hory Mts., Czech Republic. *Folia Geobot J*, **38**: 21– 34.

Piper CS. 1950.Soil and Plant Analysis, University of Adelaide press. Australia, pp. 368.

Radford EA, Catullo G and MontmollinBde. 2011. Important Plant Areas of the south and east Mediterranean region: priority sites for conservation. IUCN, Gland, Switzerland and Malaga, Spain. Gland, Switzerland and Malaga, Spain: IUCN, VIII, pp.108.

Richard LA. 1954. Diagnosis and Improvement of Saline and Alkaline, Soils. U. S. Department Agr. Handbook, pp. 66.

Said, R. 1962.The Geology of Egypt. Elsevier, Amsterdam-New York, pp.1-377p

Saleh MA, Fouda M, Saleh MA, Abdel-Latif M and Wilson B. 1988a.Inorganic pollution of the Wadi El Raiyan lakes in Egypt and its impact on their fish and wildlife. *Arch Environ Contam Toxicol J*. **17**: 391-403.

Saleh MA, Saber SA and Saleh MA. 1988b. The structure of sand dune ecosystem of Wadi El Raiyan, Egypt, *Arid Enviro J*, **15**: 283-296.

Saleh MA. 1984. Investigations of Inorganic Pollutants in El Faiyum Aquatic Environment. FRCU Report, American. Egyptian Universities linkage program, AID, USA.

Saleh MA. 1987. The decline of gazelles in Egypt. *BiologicalConservation*. 39, 83-95.

Sharaf El Din A and Shaltout KH. 1985. On the phytosociology of Wadi Araba in the Eastern Desert of Egypt. Proceedings of the Egyptian Botanical Society, **4**: 1311–1325.

Sharma SK and Shankar V. 1991.Classification and ordination of vegetation of the Kailana catchments in the Indian Thar Desert ii.Woody Vegetation. *Trop Ecol J*, **32**: 269–286.

Siebert SJ, Van Wyk AE and Bredenkamp GJ. 2002. Vegetation ecology of Sekhukhuneland, South Africa: Combretumhereroense–Grewiavernicosa Open Mountain Bushveld. *S. Afr. J. Bot*, **68**: 475– 496.

Simpson ND. 1932. A Report on the Weed Flora of the Irrigation Channels in Egypt. Ministry of Works, Government Pess, Cairo.

TerBraak CJ and Šmilauer P. 2002. CANOCO Reference manual and CanoDraw for Windows user's guide: Software for Canonical. Microcomputer power, Itaca, 500 p

Ungar IA. 1968. Species-soil-relationships on the great salt plains of northern Oklahoma. *AM MIDL NAT J*, **80**: 392-406.

USDA [U.S. Department of Agriculture]. 1993. Soil Survey Manual. Handbook No. 18. Washington D.C, pp. 503

Wickens GE. 1977. Some of the phytogeographical problems associated with Egypt. Publications Cairo University Herbarium 7&8: 223–230.

Zahran MA and Willis AJ. 2009. The Vegetation of Egypt. Springer, 2nd edition, 437pp.

Zahran MA. 1970. Wadi El-Raiyan: A natural water reservoir, Western Desert, Egypt. Bull. Soc. Geogr. Egypte, XLIII–XLIV, 83–98.

Zahran MA. 1982. Ecology of the halophytic vegetation of Egypt. In: Sen D.N. and Rajpurohit K.S (Eds.), Tasks for Vegetation Science. Dr W. Junk Publisher, The Hague, pp. 3–20.

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Evaluation of Enset Clones for Their Reaction to Bacterial Wilt of Enset (*Xanthomonas campestris* pv. *musacearum*) in Gurage Zone, Southern Ethiopia

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Abstract

Bacterial wilt of enset is one of the major threats for the enset production in Gurage Zone, Southern Ethiopia. Even though the use of resistant clones has been an effective management strategy for the disease, such clones are not well identified. Hence, twenty five enset clones, collected from Gurage zone, were evaluated for their reaction to *Xanthomonas campestris* pv. *musacearum* (Xcm) in potted experiment under greenhouse conditions. Twelve suckers were grown for all the 25 clones and ten were inoculated with Xcm pathogen at a concentration of 10^8 cfu/ml and the other suckers were kept untreated as a control. All the 25 enset clones showed wilt symptom but at varying levels of the disease severity. Only one clone (Gezwet) was resistant, 7 clones moderately resistance, 6 clones susceptible and the other 11 clones were highly susceptible to the disease based on wilt incidence, incubation time and Area Under the Disease Progress Curve (AUDPC). The mean incubation period ranges from 16.2 (on Yeregye clone) to 42.2 days (on Gezwet clone). The complete wilting period was also longer for the resistant clone and shorter for susceptible clones. Therefore, Gezwet clone and the other moderately resistant clones were recommended for farmers to incorporate in their farming.

Keywords: *Xanthomonas campestris*, Bacterial wilt, Enset clones, Evaluation, Incidence.

1. Introduction

About 20% of the human population in Ethiopia depends on enset as a food source (Brandt *et al*., 1997). Enset (*Ensete ventricosum*, Ethiopian banana) is a multipurpose crop used as a source food for humans and animals, as medicine (Africa RISING, 2014), and in construction as well as in many cultural practices. This shows that cultivation of enset can significantly improve food security at household and at a national level. Enset is a staple food crop for over 20 million people in the southern part of Ethiopia (Dereje, 2012). The plant has a high nutritive value and is highly productive (Mohammed *et al*., 2013).

However, Bacterial Wilt of Enset (BWE), caused by *Xanthomonas campestris* pv. *musacearum* (Xcm), is the major enset production constraint for all enset producing regions. Bacterial wilt attacks enset at any developmental stage of the plant, including full maturity (Brandt *et al*., 1997). Fikre *et al*. (2012) reported cultural practices and sanitation (e.g., removal of infected plant and plant parts) control measures are the most principal control measures for BWE. On the other hand, curative mechanisms, the use of disease free sucker for planting material, crop rotation, and the use of resistant clones can serve as viable management options for BWE. The identification of infected plants and their early removal are seen as a key part of the control system.

Bacterial wilt of enset disease usually destroys enset plants resulting in total yield loss and threatening the livelihood of millions of people who depend on enset as a staple food source (Brandt *et al*., 1997). The disease attacks almost all varieties of commonly grown banana cultivars (Tripathi *et al*., 2007) and enset clones (Gizachew *et al*., 2008), but to varying extents. There is a high genetic diversity in cultivated enset populations in Ethiopia (Almaz *et al*., 2002; Birmeta, 2004; Yemane and Fassil, 2006; Bizuayehu, 2008). Farmers often cultivate various numbers of enset clones in mixture in their farms. They give vernacular names for each clone. There are more than 66 enset clones in Gurage Zone (Haile, 2009), which is one of the potential enset producing areas in Ethiopia.

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The presence of resistance/tolerance in enset clones to Xcm has been reported despite the fact that no clone was found to be completely resistant (Dereje, 1985; Gizachew *et al*., 2008, Tariku *et al*., 2015). However, further research is needed to consider the vast wealth of enset genetic resources in different enset-growing regions. Continuous and intense evaluation of enset clones for disease resistance is one of the basic requirements for effective and sustained implementation of integrated disease management programs.

Variable levels of clonal response to the Xcm disease were observed under farmers' field conditions and while using artificial inoculation (Anita *et al*., 1996). A number of studies have been conducted for the evaluation of some enset clones for Xcm pathogen (Dereje, 1985; Gizachew *et al*., 2008; Tariku *et al*., 2015); however, compared to the rich source of enset land races and the variability of the pathogen (Fikre and Gizachew, 2007; Befekadu *et al*., 2014), a continuous evaluation of the clones is important and recommended. Enset farmers commonly grow combinations of clones in their enset fields and each clone is basically grown for its specific use. Hence, this study was proposed to evaluate the enset clones for their reaction to Xcm at Gurage Zone, Southern Ethiopia.

2. Materials and Methods

Hypersensitivity Test

In order to separate the pathogenic and non-pathogenic bacterial isolates, the hypersensitivity test was conducted on tobacco (*Nicotiana tabacum* L.) plants. The inoculum was prepared by suspending bacterial cells from 48-hrs-old cultures into Sterilized Distilled Water (SDW) at a density of 10^8 cfu/ml. Then two milliliters of the bacterial cell suspension was injected into the leaves of two-month-old tobacco seedlings using hypodermic syringe and needle. The control plants were inoculated with distilled water. Isolates, showing complete collapse of tissues around the injection point, were considered as positive for the test and identified as pathogenic isolates (Quimio, 1992).

Pathogenicity Test

Bacterial isolates that induced hypersensitivity on the tobacco plants were subjected to pathogenicity test on susceptible enset clone, particularly on Astara clone (Gizachew *et al*., 2008). The enset suckers were grown in a greenhouse condition for two months. Bacterial colonies were grown on yeast extract dextrose calcium carbonate agar (YDC) medium for two days and suspended into SDW. One-year-old (two months after transplant) enset suckers were inoculated with 3 ml of the bacterial cell suspension of 460 nm (10^7-10^8 cfu/ml bacterial cell concentrations) by using a spectrophotometer. After development of the symptom, re-isolation of the pathogen was undertaken from infected leaf petiole at the point of inoculation and re-cultured and used for further work.

Enset Clones

A total of 25 enset clones (Table 1) was evaluated for reaction to Xcm pathogen at Plant Protection Site - Hawasa University, Hawasa, Ethiopia. Twelve one-year-old suckers of each of the 25 clones were collected from enset growing areas of Gurage Zone and grown in pots at Hawasa University in a greenhouse condition. The average temperature and relative humidity of the greenhouse during the experimental period was 22^{o}C and 70%, respectively. About 9 kg of well-mixed clay soil was placed into each pot. The enset clones were collected from areas with the same environmental conditions from farmer fields. The suckers were developed from a single corm for each clone. The clones were evaluated for their reaction to the pathogen under artificial inoculation. Lemat and Nechewe clones were included as tolerant/resistant checks, while Astra was used as a susceptible check (Gizachew *et al*., 2008).

Inoculum Preparation and Inoculation

Bacterial ooze was collected from the inoculated plants used in the pathogenicity test. The exudates were aseptically collected at the cut end of petioles and leaf sheaths with the help of tooth pick and suspended in SDW. A loopful of the suspension was streaked on YDC plate for multiplication of inoculum. The plates were incubated at 28^{o}C for 24 hrs. Pure bacterial colonies, showing light yellow mucoid growth typical of Xcm from the plate, were re-cultured on YDC agar and incubated at 28^{o}C for two days to produce enough bacterial culture for inoculation.

Two months after transplantation (at 4-7 leaf stages), the enset clones were inoculated by using hypodermic syringe and needle with 3 ml of 2-day-old bacterial suspension at the base of young leaf petiole. The concentration of bacteria was adjusted to 10^8 cfu/ml using spectrophotometer. Similarly, the control plants were inoculated with the same amount bacteria free of SDW. Ten suckers as replicates were inoculated with the pathogen and two suckers were inoculated with SDW as a control for each clone. Re-isolation of the pathogen from the inoculated plant was done at the end of the experiment which lasted for two months after inoculatin.

Disease Assessment

Disease data were taken 10 days after inoculation, then at a 7-day-interval for one month. The number of suckers showing wilt symptom, the time of the initial symptom (incubation period) and the complete wilting date were recorded. The percentage of the wilted plants (wilt incidence) at each assessment period was calculated according to the following formula:

$$\text{Incidence} = (NW/NT) \times 100$$

where, NT = the number of total tested plants and NW = the number of wilted plants.

The reaction of each clone was categorized into four resistance levels based on average wilt incidences at 35 DAI (days after inoculation) (Tripathi *et al*., 2007) as follows: Highly Susceptible (HS): 70-100% plants wilted, Susceptible (S): 40-69% plants wilted, Moderately Resistant (MR): less than 40% plants wilted, and Resistant (R): none of the plants wilted. Furthermore, the date of complete wilting, incubation time and average wilt incidence were used for the evaluation of clones. Similarly, percentage of wilted plants at each assessment period was used to calculate the Area Under Disease

Progress Curve (AUDPC) using the following formula (Shaner and Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n}[(Di_{i+1} + Di_i)/2] \times [t_{i+1} - t_i]$$

where Di_i = percentage of wilted plants at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

Data Analysis

Statistical software, SAS version 9.2 (SAS, 2002), was used with two-way ANOVA for analysis. Data on percent of disease incidence were arcsine transformed before the analysis. Significant difference among treatment means was tested using Duncan's Multiple Range Test (DMRT) at 5% probability level for significance. The experiment was arranged using Completely Randomized Design (CRD).

3. Results

Out of the 25 enset clones inoculated with Xcm pathogen, all of the clones showed symptoms of BWE at different assessment periods, while all the control plants inoculated with water did not show any wilt symptoms in all clones and at all assessment periods. Also, none of the evaluated enset clones was immune to the pathogen.

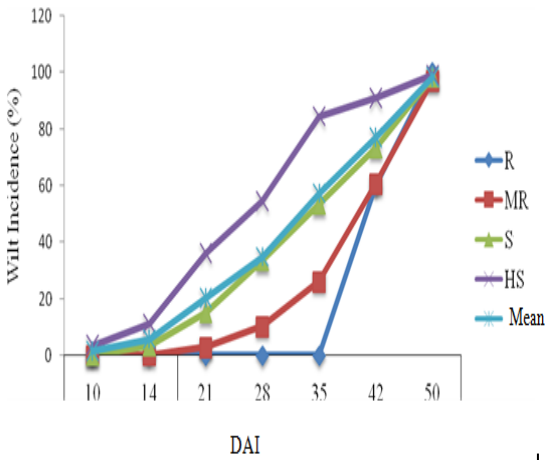
All inoculated clones' symptoms (yellowing and chlorosis) development started in the inoculated leaves. Symptom development after the artificial inoculation was similar to those observed in young plants following natural infection in the field. Significant differences ($p \leq 0.0001$) were observed in the incubation period, wilt incidence at the 35th day, complete wilting period, average incidence and AUDPC among the 25 enset clones evaluated for their resistance to Xcm pathogen. Symptom development started at the 10th day after inoculation and the mean incubation period of the clones varied from 16.2 (Yeregye) to 42.2 (Gezwet) days.

In this experiment the tested enset clones were categorized into four disease-rating groups based on their wilt incidence at 35 DAI. Accordingly, lower wilt incidence, longer incubation period, longer complete wilting period, lower mean incidence, lower AUDPC value and slow disease progression rate were associated with resistant clones, while the reverse held true for the susceptible clones (Figure 1).

The various enset clones showed significant differences in susceptibility to Xcm. The wilt incidence at the 35th DAI ranged from 0 to 100% for the evaluated enset clones. Gezwet was the only resistant clone to Xcm with no wilt incidence at 35 DAI (Figures 1 and 2), and with mean incubation period of 42.2 days and complete wilting of 71 days. Seven enset clones, namely Gimbwe, Terye, Agade, Yeshrakinke, Kechere, Badedat and Ferezye, were moderately resistant to Xcm. These clones showed wilt incidence of less than 40% at 35 DAI and an incubation period of 37.9-40.9 days. On the other hand, a complete wilting for these clones ranged from 63-70 DAI and there were no significant differences among them at 5% probability level for incubation period, complete wilting, mean incidence and AUDPC value (Tables 1 and 2).

Six enset clones, namely Kibinar, Yegendeye, Astara, Ewane, Wenadeye and Zober, were susceptible to the pathogen with an incidence at 35 DAI of 40-69%, incubation period of 31.2-38.5 days and a complete wilting from 56-70 DAI. These clones did not vary significantly from each other in disease parameters. However, Yegendiye clone performed well, with the exception of the incidence at 35 DAI all the parameters categorized it with resistant clones. The other eleven enset clones were found to be highly susceptible to Xcm pathogen with wilt incidence of 70-100% at 35 DAI, incubation period of 16.2-35.7 days and complete wilting period of 47.8-64.0 days (Table 1).

The average wilt incidence over the assessment periods ranged from 20.11 to 70.30% (Table 2). The maximum average wilt incidence was recorded on Yeregye (HS, 70.3%) and Lemat (HS, 64.29%). On the other hand, the average BWE incidence was the lowest on Gezwet (R, 20.11%) and Gimbwe (MR, 22.09%).



Disease progress was rapid on highly susceptible and susceptible clones, whereas a relatively slow progress was recorded on resistant and moderately resistant enset clones (Figure 1). Similarly, the disease progress curve was steeper initially for resistant and moderately resistant clones, while it increased faster for the susceptible and highly susceptible enset clones. The AUDPC value varied significantly ($p < 0.0001$) between the clones. The highest AUDPC (86.19%-day) was recorded on the clone Yeregye (HS), but the resistant clone Gezwet had the lowest AUDPC (21.25%-day) but not significantly different from the clones that are grouped moderately resistant, clones of numbers 2-8 (Table 2) at ($p \leq 0.05$).

Figure 1. Mean disease progress curve for resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS) clones as compared to the average (Mean) progress curve.

Table 1. Mean incubation period, complete wilting, wilt incidence at 35 DAI and disease rating for the 25 enset clones

No.	Clone name	Number of clone	Incidence at 35 DAI	Mean incubation	Complete wilting	Clone reaction rating*
1.	Gezwet	10	00.0 ^{h}	42.2 ^{a}	71.0 ^{a}	R
2.	Gimbwe	10	10.0 ^{hg}	40.9 ^{ba}	66.0 ^{bac}	MR
3.	Terye	10	20.0 ^{fhg}	38.5 ^{bac}	67.0 ^{bac}	MR
4.	Agade	10	30.0 ^{fheg}	39.5 ^{bac}	63.0 ^{bdac}	MR
5.	Yeshrakinke	10	30.0 ^{fheg}	39.8 ^{bac}	70.0 ^{ba}	MR
6.	Kechere	10	30.0 ^{egdf}	37.9 ^{ebdac}	64.0 ^{bdac}	MR
7.	Badedat	10	30.0 ^{egdf}	38.6 ^{bac}	68.0 ^{bac}	MR
8.	Ferezye	09	33.3 ^{feg}	38.0 ^{bdac}	67.8 ^{bac}	MR
9.	Kibinar	10	40.0 ^{fdeg}	32.6 ^{ebdgcf}	63.0 ^{bdac}	S
10	Yegendeye	10	50.0 ^{fdec}	38.5 ^{bac}	70.0 ^{ba}	S
11.	Zober	10	50.0 ^{fdec}	34.8 ^{ebdagcf}	62.2 ^{bdec}	S
12.	Ewane	10	60.0 ^{bdec}	34.3 ^{ebdagcf}	56.0 ^{fdeg}	S
13.	Wenadeye	10	60.0 ^{bdec}	31.6 ^{edhgcf}	60.0 ^{fdec}	S
14.	Astara	10	60.0 ^{bdec}	31.2 ^{eidhgcf}	66.0 ^{bac}	S
15.	Beresye	10	70.0 ^{bdac}	29.5 ^{eidhgf}	53.6 ^{fhg}	HS
16.	Shebrat	10	70.0 ^{bdac}	29.5 ^{eidhgf}	52.4 ^{fhg}	HS
17.	Teguaner	10	70.0 ^{bdac}	35.7 ^{ebdacf}	53.2 ^{fhg}	HS
18.	Demolejat	10	70.0 ^{bdac}	29.2 ^{eihgf}	62.0 ^{bdec}	HS
19.	Nechwe	10	80.0 ^{bac}	22.8 ^{ij}	51.4 ^{hg}	HS
20.	Kanchwe	10	80.0 ^{bac}	28.1 $^{ihg\ f}$	55.0 ^{fheg}	HS
21.	Yekeswe	10	90.0 ^{ba}	27. 8 ^{ihgf}	54.0 ^{fhg}	HS
22.	Bushrat	10	100.0 ^{a}	26.7 ^{ihg}	63.0 ^{bdac}	HS
23.	Oret	10	100.0 ^{a}	23.8 ^{ihj}	64.0 ^{bdac}	HS
24.	Lemat	10	100.0 ^{a}	18.4 ^{j}	57.4 ^{fdeg}	HS
25.	Yeregye	09	100.0 ^{a}	16.2 ^{j}	47.8 ^{h}	HS
CV (%)			12.38	15.70	12.50	
R^2value			0.91	0.63	0.64	

*This rating is based on average wilt incidences at 35 DAI (days after inoculation): Highly Susceptible (HS): 70-100% plants wilted, Susceptible (S): 40-69% plants wilted, Moderately Resistant (MR): less than 40% plants wilted and Resistant (R): none of the plants completely wilted. Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

Table 2. Arcsine transformed wilt incidence of the 25 enset clones at different disease assessment periods and their standardized AUDPC (%-day) values

		Wilt Incidence (%)								
		DAI[a]								
No.	**Clone**	**10**	**14**	**21**	**28**	**35**	**42**	**50**	**Mean**	**S AUDPC** (%-day)[b]
1.	Gezwet	0	0	0	0	0	50.77	90	20.11^{i}	21.25^{l}
2.	Gimbewe	0	0	0	0	13.29	51.33	90	22.09^{i}	23.00^{kl}
3.	Terye	0	0	0	13.29	26.57	57.1	76.72	24.81^{hi}	27.38^{kjl}
4.	Agade	0	0	13.29	13.29	32.9	50.77	90	28.61^{ghefi}	30.00^{kijl}
5.	Yeshrakinke	0	0	0	26.57	32.9	57.1	76.72	27.62^{ghfi}	30.88 ^{kijl}
6.	Kechere	0	0	0	13.29	32.9	51.33	90	26.79^{ghi}	28.25^{kjl}
7.	Badedat	0	0	0	0	32.9	50.77	90	24.81^{hi}	26.50^{kjl}
8.	Ferezye	0	0	15	30	34.62	42.12	90	30.25^{ghefi}	30.25^{kijl}
9.	Kibinar	0	26.57	26.57	32.9	39.23	57.1	90	38.91^{gcefd}	41.63^{gifh}
10.	Yegendye	0	0	13.29	26.57	45	50.77	76.72	30.34^{ghefi}	34.25^{kij}

11.	Zober	0	0	26.57	26.57	45	63.43	90	35.94^{ghefd}	40.75^{gih}
12.	Ewane	0	0	13.29	32.9	50.77	57.1	90	34.87^{ghefd}	40.63^{gih}
13.	Wenadye	0	0	26.57	51.33	51.33	57.1	90	39.48^{cefd}	47.63^{gefh}
14.	Astara	0	0	13.29	38.67	50.77	76.72	90	38.49^{gcefd}	46.13^{gefh}
15.	Beresye	0	0	32.9	39.23	57.1	63.43	90	40.38^{cebd}	49.50^{gefd}
16.	Shebrat	0	13.29	26.57	25.69	57.1	63.43	90	39.44^{cefd}	52.63^{cefd}
17.	Teguaner	0	0	0	13.29	57.1	57.1	90	31.07^{ghefi}	37.13^{ijh}
18.	Demolejat	0	0	32.9	39.23	57.1	76.72	76.72	40.38^{cebd}	50.38^{gefd}
19.	Nechwe	13.3	26.57	45	57.1	70.39	45.45	90	49.69^{cb}	66.06^{b}
20.	Kanchwe	0	0	32.9	45	63.43	76.72	90	44.01^{cbd}	54.88^{cebd}
21.	Yekeswe	0	0	19.62	45	76.72	90	90	45.91^{cbd}	56.75^{cebd}
22.	Bushrat	0	0	39.23	39.23	90	90	90	49.78^{cb}	60.25^{cbd}
23.	Oret	0	13.29	32.9	50.77	90	90	90	52.42^{b}	63.38^{cb}
24.	Lemat	26.6	39.23	50.77	63.43	90	90	90	64.29^{a}	77.25^{a}
25.	Yeregye	13.3	42.12	76.72	90	90	90	90	70.30^{a}	86.19^{a}
CV(%)									13.64	11.09
R^2									0.92	0.96

[a] days after inoculation; [b] Standard AUDPC (Area Under the Disease Progress Curve). Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

4. Discussion

In the present study, 25 enset clones from Gurage zone were evaluated for their reaction to Xcm pathogen under artificial inoculation and produced varying reactions. Some of the varieties were more tolerant to the disease while the others were susceptible. Generally, all the inoculated enset clones developed bacterial wilt symptoms to various intensities. A study by Tariku *et al*. (2015) and Gizachew *et al*. (2008) revealed that all the inoculated clones developed disease symptoms. Based on the evaluation of their reaction, none of the enset clones had a complete resistance to Xcm pathogen. Many reports indicate that there was no completely resistant enset clone to Xcm pathogen (Dereje, 1985; Gizachew *et al*., 2008), except for Mezya, which had a high resistance to the pathogen (Fikre and Gizachew, 2007). Similarly, no banana cultivar was found to be completely resistant to Xcm (Ssekiwoko *et al*., 2006; Biruma *et al*., 2007; Tripathi *et al*., 2007; Smith *et al*., 2008). None of the inoculated enset clones were recovered from Xcm infection.

This result partially agrees with the previous findings of Gizachew *et al.* (2008), who reported that Gezwet clone was susceptible, while in the current experiment it was found to be resistant but Astara was found to be a susceptible clone in both cases. Lemat and Nechwe showed a relative tolerance to Xcm (Gizachew *et al*., 2008). Conversely, both clones were found to be susceptible to the pathogen in this finding. Dereje (1985) reported that Agade was more susceptible to Xcm than the other clones tested, but here it was a moderately resistant clone. Similar findings were reported from Tariku *et al*. (2015) for Badedat clone which was found to be high resistant for BWE disease. In this experiment, Yeshrakinke was found to be a moderately resistant clone, which is in agreement with Anita *et al.* (1996), who reported that it was a tolerant clone to Xcm pathogen. Farmers in the study area also considered this clone as more tolerant to the disease.

In contrast, Gizachew *et al.* (2008) reported that Yeshrakinke was a susceptible clone. This variation might be due to the variations of isolates of Xcm pathogen (Fikre and Gizachew, 2007; Befekadu *et al*., 2014) though, this experiment was conducted in only one pathogenic isolate or it might be related to the genetic variations within the clones (a single clone may contain several sub clones). Fikre and Gizachew (2007) reported enset clones are not consistent for their resistance/tolerance across locations and time.

Both the susceptible (Astara) and the tolerant (Lemat and Nechewe) checks, used in the present study, were all found to be susceptible to the pathogen. Tariku *et al*. (2015) also reported that Astara was a susceptible clone. Although Daniel and Getaneh (2015) reported that some botanicals are effective to Xcm pathogen, no chemical is recommended to the pathogen. Hence, the use of resistant enset clones should be the most effective management approach. In the present experiment, eight tolerant clones were identified and most of them are preferred by the farmers for their agronomic trait.

5. Conclusion and Recommendations

In the present study, 25 enset clones were evaluated for their reaction to Xcm pathogen from Gurage zone in artificial inoculation. All the enset clones showed symptoms of chlorosis and/or necrosis on leaves of the inoculated plants in varying periods, whereas the control plants (inoculated with water only) did not show any kind of symptoms. However, the clones varied in their reaction to the pathogen, including incubation period, wilt incidence, complete wilting day and AUDPC value.

Among the 25 enset clones evaluated from Gurage zone, only Gezwet was the resistant clone, even clones, namely Gimbwe, Terye, Agade, Yeshrakinke, Kechere, Badedat and Ferezye, were moderately resistant, while six enset clones, namely Astara, Yegendeye, Zober, Ewane, Wenadeye and Kibinar, were categorized as susceptible enset clones. The other 11 enset clones were found to be highly susceptible to Xcm pathogen.

Considering the rich diversity of enset plants, it was anticipated that screening and evaluation of enset clones might provide a good source for effective management strategies of the disease.

The present study identified one resistant and seven moderately tolerant enset clones to the pathogen. Therefore, farmers should be encouraged to incorporate these clones in combination with other effective control measures into their farming systems. On the other hand, this study considered only 25 enset clones from Gurage zone. However, enset plant is genetically diverse in different locations and zones. Therefore, it is recommended that all enset clones be collected and evaluated for their reaction to the pathogen at the farm level of the country. These clones should also be further evaluated against a large number of Xcm isolates after being well-characterized into races or biotypes. The tolerant clones should also be further evaluated for their agronomic performances.

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References

Africa RISING (The Africa Research In Sustainable Intensification for the Next Generation). 2014. The Africa RISING enset research initiative in Ethiopia: Enhancing the productivity of farming systems. Transforming African agriculture through sustainable intensification. pp: 2.

Almaz N, Admasu T, van Treuren R and Visser B. 2004. AFLP analysis of enset clonal diversity in south and southwestrn Ethiopia for conservation. *Crop Sci*, **42**:1105-1111.

Anita S, Clifton H, Endale T and Gizachew WM. 1996. Enset need assessment project Phase 1 Report. Awassa, Ethiopia.

Befekadu H, Girma A and Fikre H. 2014. Physiological characterstics and and pathognicity of *Xanthomonas campestris* pv. *Musacearum* strains collected from enset and banana in Southwest Ethiopia. *African J. Biotechnol*, **13**: 2425-2434.

Biruma M, Pillay M, Tripathi L, Blomme G, Abele S, Mwangi M, Bandyopadhyay R, Muchunguzi P, Kassim S, Nyine M, Turyagyenda FL and Eden-Green S. 2007. Banana *Xanthomonas* wilt: a review of the disease, management strategies and future research directions. *African J. Biotechnol*, **6**: 953-962.

Birmeta G, Nybom H and Bekele E. 2004. Distinction between wild and cultivated enset (*Ensete ventricosum*) gene pools in Ethiopia using RAPD markers. *Hereditas*, **140**: 139-148.

Bizuayehu T. 2008. The enset (*Ensete ventricosum*) gardens of Sidama: composition, structure and dynamics of a traditional poly-variety system. *Genet. Resour. Crop Evol*, **10**:10722-10728.

Brandt SA, Spring A, Hiebsch C, McCabe ST, Tabogie E, Mulugeta D, Gizachew WM Yntiso G, Shigeta M and Tesfaye S. 1997. *The 'Tree Against Hunger'. Enset-based Agricultural Systems in Ethiopia.* American Association for the Advancement of Science. pp: 66.

Daniel K and Getaneh W. 2015. Evaluation of Different Botanical Plant Extracts and Other Material against Enset Bacterial Wilt (*Xanthomonas campestris* pv. *musacearum*) Disease in Oromia Regional State, Ethiopia. *ARPN J. of Science and Technology*, **5**: 68-73.

Dereje A. 1985. Studies on the bacterial wilt of enset *(Ensete ventricosum*) and prospects for its control. *Ethiopian J. Agric. Sci*, **7**: 1-14.

Dereje G. 2012. The Essence of Domestic Quarantine against Enset Bacterial Wilt during Technology Dissemination in Ethiopia. *In*: Mohammed B. and Y. Tariku H (eds.), Enset Research and Development Experiences in Ethiopia. *Proceedings of Enset National Workshop, 19-20 August 2010, Wolkite, Ethiopia*, pp. 97-106.

Fikre H and Gizachew WM. 2007. Evaluation of enset clone meziya against enset bacterial wilt. Proceeding of Africa Crop Science Conference. 8. 887-890. El-Minia, Egypt. African Crop Science Society.

Fikre H, Tariku H and Endale H. 2012. Research Achievements, Experiences and Future Directions on Bacterial Wilt of Enset. pp: 64-96. *In*: Mohammed Y. and Tariku H. (eds.). Enset Research and Development Experiences in Ethiopia. *Proceedings of Enset National Workshop*, 19-20 August 2010, Wolkite, Ethiopia.

Gizachew WM, Kidist B, Blomme G, Addis T A, Mengesha T and Mekonnen S. 2008. Evaluation of enset clones against enset bacterial wilt. *African Crop Sci. J*, **16**: 89-95.

Haile S. 2009. A Study on enset as a means of existence, social organization, and ethnical identification for the Gurage people. Master of Philosophy, University of Tromso.

Mohammed B, Gabel M and Karlsson LM. 2013. Nutritive values of the drought tolerant food and fodder crop enset. *African J. Agric. Res*, **8**: 2326-2333.

Quimio JA. 1992. Enset team support project Sidamo Gamo Gofa. Annual report of the plant pathologist: July 17,1991 to July 16,1992. Peasants Agricultural Development Program-PADEPIII. Awasa Research Center (IAR). Awasa, Ethiopia.

SAS 2002. SAS /STAT user's guide, version 9.0. SAS Institute Inc., Cary, NC.

Shaner G and RE Finney. 1977. The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. *Phytopathol*, **70**: 1183-1186.

Smith JJ, Jones DR, Karamura E, Blomme G and Turyagyenda FL. 2008. An analysis of the risk from *Xanthomonas campestris pv. musaceurum* to banana cultivation in Eastern, Central and Southern Africa. Biodiversity International, Montpellier, France. 1-29

Ssekiwoko F, Turyagyenda LF, Mukasa H, Eden-Green S and Blomme G. 2006. Systemicity of *Xanthomonas campestris* pv. *musacearum* (*Xcm*) in flower-infected banana plants. In: Saddler G, Elphinstone J and Smith J (eds), Programme and Abstract Book of the 4th International Bacterial Wilt Symposium, 17-20th July 2006. The Lakeland Conference Centre, Central Science Laboratory, York, UK, pp. 61.

Tariku H, Kassahun S, Endale H and Mengistu O. 2015. Evaluation of enset clones resistance against enset bacterial wilt disease (*Xanthomonas campestris* pv. *musacearum*). *J. Vet. Sci. Technol* , **6**: doi:10.4172/2157-7579.1000232

Tripathi L, Odipio J, Tripathi JN and Tusiime G. 2007. A rapid technique for screening banana cultivars for resistance to *Xanthomonas* wilt. *European J. Plant Pathol* , **121**: 9-19.

Yemane T and Fassil K. 2006. Diversity and cultural use of Enset (*Enset ventricosum* (Welw.) Cheesman) in Bonga in situ Conservation Site, Ethiopia. *A J. Plants, Peoples, and Applied Research* Ethnobotany Research & Applications, **4**: 147-157.

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Molecular-Based Identification of *Polystoma integerrimum* by 28S rDNA, Phylogenetic and Secondary Structure Analysis

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Abstract

The present study was carried out to molecular identification, phylogenetic evolutionary and secondary structure prediction of the monogenean, *Polystoma integerrimum* which was isolated from amphibians *Bufo viridis* and *B. regularis*, collected from various regions in Erbil City, Iraq. After the morphological examination of the parasite, molecular identification and phylogenetic relationships were carried out using 28S ribosomal DNA (rDNA) sequence marker. The result indicated that the query sequence (sample sequence) was 100% identical to this species of the parasite and the phylogenetic tree showed 97-99% relationships in the sequence of *P. integerrimum* and 28S rDNA regions for other species of *Polystoma*. In addition, the present finding was confirmed by the molecular morphometrics, according to the secondary structure of 28S rDNA region. The topology analysis produced the same data as the acquired tree. In conclusion, the primary sequence analysis showed the validity of *P. integerrimum*. Phylogenetic tree and secondary structure analysis can be considered as a valuable method for separating species of *Polystoma*.

Keywords: *Polystoma integerrimum*, 28S rDNA marker, Molecular identification, Phylogeny, Secondary structure.

1. Introduction

Polystoma integerrimum (Froelich, 1791) belongs to Phylum: Platyhelminthes, Class: Monogenea, Order: Polyopisthocotylea. Mainly infected amphibian hosts (a parasite of the excretory bladder of frog) freshwater terrapins. It has been also recorded in the Australian lungfish and the African hippopotamus (Du Preez, 2015). The parasite has a direct life cycle, typically; eggs are laid and hatch into water and become a free-swimming stage, then infect host (Koprivnikar *et al*., 2012).

Polymerase Chain Reaction- (PCR-)based techniques utilizing the 28S rDNA regions have proven to be a reliable tool for identifying the platyhelminthes species including *Polystoma* spp. and their phylogenetic relationships (Mollaret *et al*., 2000).

Phylogenetic relationships among members of Polystomatidae are controversial (Mollaret *et al.*, 2000). Resolving the interrelationships (phylogeny) of *P. integerrimum* and other members of Polystomatidae are especially significant issues in development, since the basal resolution critically affects our knowledge of primitive platyhelminthes. Study of the parasite morphology did not resolve a terminal polytomy (Du Preez, 2010) and it has been rejected in molecular analysis by Mollaret (1997).

Secondary structures of RNA are specially beneficial in the classification of organisms because they consist of certain properties that are not present in the primary region sequence, which represents morphological information; and the source of dependable secondary structure framework for 28S rDNA sequence would exhibit a basic step that directs a detailed knowledge of their biological functions, which gives a strength method for determining biologically relevant looping patterns in RNA structure (Chandni *et al*., 2012).

The first record of *P. integerrimum* in Iraq was done by Dauood (1974) from the green toads, *B. viridis*. Most studies on the monogenic members, especially *Polystoma*, were based on the morphological descriptions and diagnostic keys, in which the family Polystomatidae is

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distinguished by a well-evolved opisthaptor with six cup-like suckers.

In the present study, the first objective is to confirm the presence of *P. integerrimum* in amphibians collected in Erbil City (Iraq) using 28S rDNA sequence, then to establish the evolutionary relationships with other species of the *Polystoma* and provide a secondary structure modeling as a support of the primary molecular finding.

2. Materials and Methods

2.1. Description of the Study Area

Erbil City is the capital of Kurdistan Region, in the north of Iraq. The latitude of Erbil is 36.20, and the longitude is 44.01 (Figure 1). The Sami Abdul-Rahman Park lies near the center of the city. It is covered with different kinds of trees and grasses as the main green zone park in the city.

Various vertebrate animals live at the park, like pigeons, ducks, some reptiles, like turtles, and some amphibians, as frogs, in addition to different kinds of invertebrates, such as snails, crustaceans and aquatic insects.

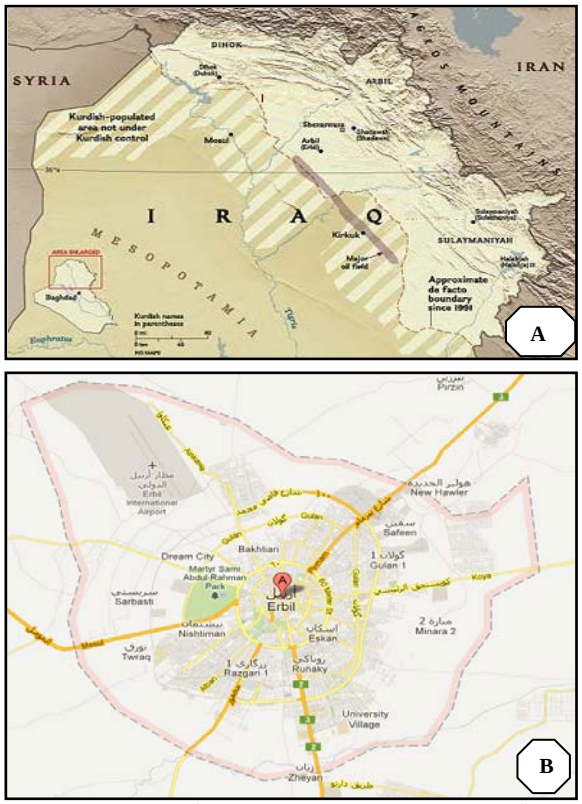


Figure 1: A- Map of Iraq showing northern part.B- Map of Erbil City showing studied area.

Shows the point of sample collection (red concentric circles).

2.2. Parasite Materials

A total of 33 frogs (21 of *Bufo viridis* and 12 of *B. regularis*) were collected by hand from different regions in Erbil City during the period of November 2014 to the end of April 2015. The frogs were anesthetized intra peritoneally with 2 ml of ketamine hydrochloride (50 mg/kg), and then the frogs were dissected as soon as possible. All viscera were removed and each of them was placed in a suitable Petri dish containing normal physiological saline 0.9% (Maulood, 2005). The following morphological descriptions and diagnostic keys were consulted for identification aid in the analysis of the distinctive features and finally the most determination of parasitic forms encountered (Yamaguti, 1963; Kudo, 1971; Smyth and Smyth, 1980).

2.3. DNA Extraction

Genomic DNA from *Polystoma* samples were obtained by employing kit of extraction (BIONEER, KOREA) according to the manufacture's instruction with few modifications (incubation time of tissue lyses step was extended into 3 hours and utilized absolute ethanol instead of isopropanol for DNA precipitation). The samples were macerated in mortar and pestle, and the contents were transferred into sterile tube containing 200 µl tissue lysis buffer and kept in incubator for 3 hrs. Qualification and quantification of DNA concentration were performed by using NanoDrop (ND- 1000, USA). Samples of DNA genomic with (A260–A320) / (A280–A320) ratio more than 1.7 and outputs more than 0.5µg were obtained.

2.4. DNA Amplification and Sequencing

A region of 28S rDNA was amplified by Polymerase Chain Reaction (PCR). The primers were universal, forward primer C1 (ACCCGCTGAATTTAAGCAT at position 25), and reverse primer C3 (CTCTTCAGAGTACTTTTCAAC at position 390), they were designed and selected by Mollaret *et al*. (2000) and expected to be specific to Platyhelminthes. PCR reaction and condition were performed using MJ Research, Applied Biosystem (AB) thermal cycler. Fifty µl reaction mixture was prepared in PCR tubes containing 2 µl DNA template, 25 µl OnePCR^{TM} master mix (GENEDIREX, KOREA), 1 µl for each primer and 21 µl double demonized water (ddH_2O). The cycling conditions comprised of initial denaturation at 94˚C for 5 min, 35 cycles of denaturation at 94˚C for 45 sec, annealing temperatures at 51˚C for 45 sec and extension at 72˚C for 45 sec, and final extension at 72˚C for 5 min. Agarose gel electrophoresis was employed to check the efficiency of PCR reactions. The samples were prepared and run in 2% gel of agarose then stained with ethidium bromide that makes the DNA visible under UV light, with the expected size of the PCR product was 365 bps.

In the present study, ABI 3130X nucleotide sequence analyzer (SINGAPORE) was used to find the nucleotides order of 28S rDNA from *P. integerrimum*. The PCR fragments of the *P. integerrimum* were excised from the agarose gel and used as a source of DNA template for sequence specific PCR amplification.

2.5. Phylogenetic and Secondary Structure Analysis

The regions of sequences were aligned employing muscle multiple alignment with the neglectful gap and expansion penalties employed by this program. The results of 28S rDNA regions were entered in the MEGA 6.0 (Barry, 2013) for constructing the evolutionary developmental trees. The phylogenetic trees of the *P.*

integerrimum were constructed using character state method (maximum likelihood). Branch support was given employing 1000 bootstrap replicates. Secondary structures of 28S rDNA regions of *P. integerrimum* were expected by the online MFold package (version 3.5) (Chandni and Hridaya, 2012). MFold is the broad employed algorithms for secondary structure of RNA expectation that are dependent on a search for the minimal free energy state.

3. Results

3.1. Molecular Based Identification

The sequence from DNA of *P. integerrimum* was 28S rDNA of 315bp (amplified fragment was 365bp, while after sequencing 50 miss-nucleotides were excluded, related to quality of sequencing analysis) put to BLAST then compared with other stored species of *Polystoma* sequences from GenBank. The BLAST results indicated that the query sequence was 100% identical to *P. integerrimum (*Figure 2).

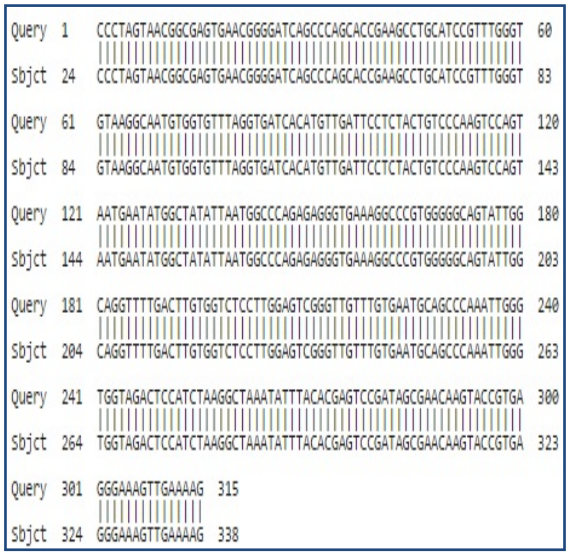
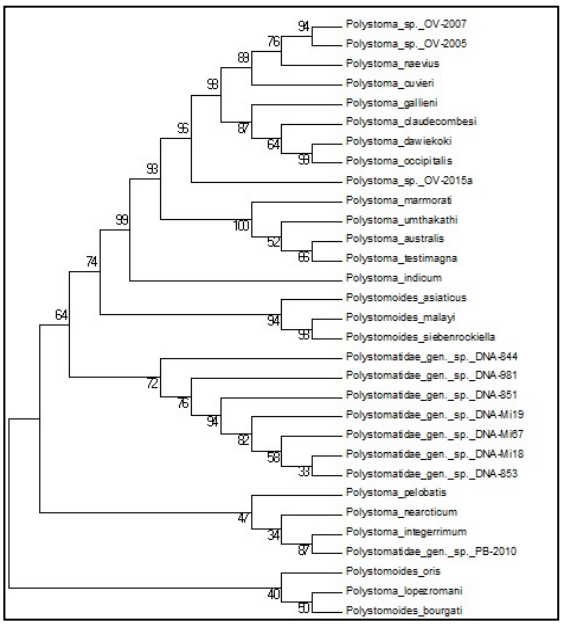


Figure 2. Pairwise alignment of 28S rDNA sequence of *P. integerrimum*. Query is the study or sample sequence and Sbjct is the GenBank sequence.

3.2. Phylogenetic Tree Analysis

Phylogenetic tree showed 97-99% relationships in comparing the sequence of *P. integerrimum* and stored 28S rDNA regions for the other species of *Polystoma*. Phylogenetic analysis employing the diverse method, maximum likelihood method as mentioned in Figure 3. It was revealed and indicated that the topology was the same among acquired trees with significant bootstrap support for the clades. For the bootstrap analysis, the values of 70% and above represented the accuracy of evolutionary development and showed reliable grouping among various species of *Polystoma*.

Figure 3. Phylogenetic positioning of P. integerrimum according to sequences of 28S rDNA employing maximum likelihood method, (Tamura 3-parameter model with invariant sites).



3.3. Secondary Structure Analysis

Predicted 28S rDNA secondary structural properties with the highest negative free energy ΔG= -113.00 Kcal/mol of *P. integerrimum* to provide the principal information for the evolutionary developmental analysis. The secondary sequence properties of 28S rDNA structure as represented in Figure 4 were analyzed according to loops and conserved stems. In the structure of *P. integerrimum*, the loops arrangements exhibit according to their numbers and classified into: hairpin loop, interior loop, multi loop, bulge loop and exterior loop (Figure 5). The topology was only based on the expected RNA secondary structure of 28S rDNA sequence, which determined most associations among the species researched.

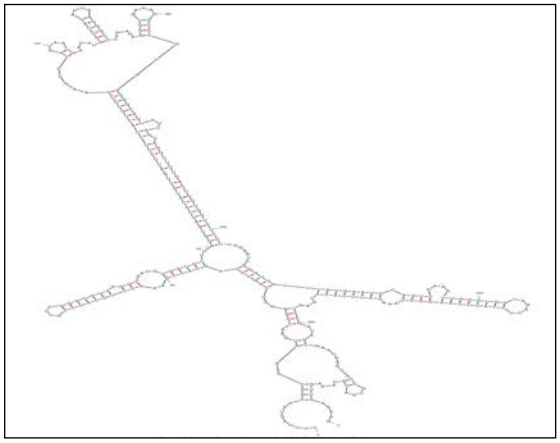


Figure 4: Schematic representation of the 28S rRNA expected secondary sequence of *P. integerrimum.*

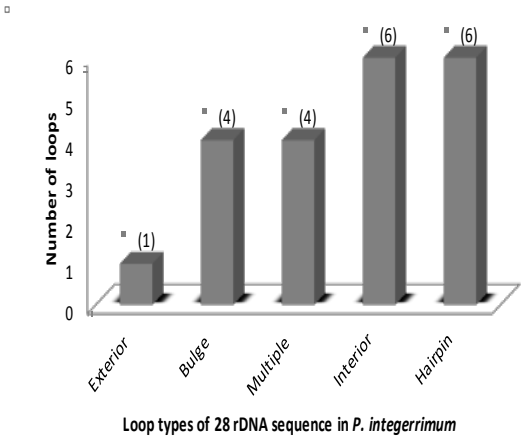


Figure 5: Arrangement of various loops of 28S rDNA sequence region of *P. integerrimum.*

4. Discussion

Polystoma is a cosmopolitan and diverse genus (Raharivololoniaina *et al*., 2011). Its-diversification might be related to host-specificity and host diversification by parallel evolution (Bentz *et al*., 2001). The present study includes several steps based on molecular characterization; phylogeny and secondary structure analysis of *P. integerrimum*.

P. inegerrimum species genetically well distinguished from the other available species of *polystoma* previously identified with the same rDNA sequence fragment marker, available at the National Center for Biotechnology Information (NCBI). The primary sequence analysis, using universal primers of study sample, revealed that the parasite belongs to species *P. inegerrimum* (Figure 2).

If the bootstrap value for a given interior branch of a phylogenetic tree is 70% or higher, then the topology at that branch is considered reliable according to the phylogenetic analysis rule (Chandni *et al*., 2012). The current data revealed that the bootstrap value was more than 70% for the acquired tree. The tree topologies according to phylogenetic analysis showed *P. integerrimum* as molecularly closely related with *P. pelobatis* and *P. nearcticum* species (Figure 3).

In addition, phylogenetic findings were confirmed using secondary structure analysis as a tool of bioinformatics, RNA loops were employed for rectification the alignment. Molecular morphometrics is intended to analyze phylogenetic relationships based on similarities among some structural characteristics of folded nucleotide molecules (Bernard *et al*., 2000). Molecular morphometrics has been reported to be the strongest tool in comparison to classical primary structure analysis, due to the only consideration of the size variations for homologous structural segments, whereas molecular morphometrics represents the folding pattern of RNA molecule (Bernard *et al.*, 2000). The topology of the present study, based on the expected RNA secondary sequence of the 28S rDNA structure, showed and determined most relationships among the species studied; for instance the structure of *P. integerrimum*, total number loops preference were 21 loops, types of the loops according to their numbers were exterior loop (1), bulge loop (4), multi loop (4), interior loop (6) and hairpin loop (6), respectively, as presented in Figure 5. RNA loops and conserved stems appeared to be complementary to classical primary sequence analysis in phylogenetic studies. Incorporation of secondary structure information allows improved estimates of phylogeny among several *Polystoma* species.

PCR-based techniques utilizing the 28S rDNA sequences have proven to be a reliable tool to identify the helminth species and their phylogenetic (Chandni *et al*., 2012). In platyhelminth systematics, rDNA genes, in general, have been used successfully and 28S rDNA, in particular, to estimate the relationships existing among the platyhelminthes (Morand *et al*., 2015). Therefore, many previous studies on phylogeny of the Polystamatidae members have been achieved using 28S rDNA and 18S rDNA. Mollaret *et al*. (2000) reported the phylogeny of Monopisthocotylea and Polyopisthocotylea (platyhelminthes) that were obtained from using 28S rDNA sequence analysis. The phylogenetic relationships of the families Polystomatidae and Sphyranuridae (subclass Polystomatoinea) within tetrapod monogenean parasites were investigated by using partial 18S rDNA sequences (Sinnappah *et al*., 2001). *Polystoma floridana* was described by Du Preez *et al*. (2007) as a new species of the Polystomatidae parasitic in the urinary bladder of *Hyla cinerea* by using 28S rDNA (analysis).

5. Conclusions

The results of the present study were concluded that the genus *polystoma* is the most diverse genus. Therefore, it is difficult to separate the species and sub-species from any phases in their life cycle only by relying on the morphology. For this reason, a dozen presumably distinct species remain unnamed. Primary sequence analysis revealed the validity of *P. integerrimum*. Phylogenetic tree and secondary sequence analysis could be a valuable method for separating species of *Polystoma*. The phylogenetic position of *P. integerrimum* within subfamily Polystomatinae renews interest in the facultative alternation of generations observed in anuran polystomatids.

References

Barry GH. 2013. Building phylogenetic tree from molecular data with mega. *Mol Biol Evolu*, **30**:1229-35.

Bernard B, Marie-Anne G, Monique M and Jean SD. 2000. Cirripede Phylogeny Using a Novel Approach: Molecular Morphometrics. *Mol Biol Evol*, **17**:1435-1445.

Bentz S, Leroy S, Du Preez LH, Mariaux J, Vaucher C and Verneau O. 2001. Origin and evolution of African Polystoma (Monogenea: Polystomatidae) assessed by molecular methods. *Int J Parasitol*, **31**: 697-705.

Chandni V, Anshu C and Hridaya SS. 2012. PCR-based molecular characterization, phylogenetic analysis and secondary structure of the 28S rDNA of Thaparocleidus wallagonius (Monogenea: Dactylogyridae) – the most primitive species of this genus from India. *Bioinformation*, **8**: 816-819.

Charlotte KO and Paul FL. 2004. Genes and DNA. Columbia Univ. Press. 217 pp.

Dauood KS. 1974. Studies on the Protozoan and Trematode Parasites of Some Amphibian From Nienava District, North Iraq. MSc Thesis, Mosul University, Mosul, Iraq.

Du Preez LH, Verneau O and Gross T. 2007. *Polystoma floridana* n. sp. (Monogenea: Polystomatidae) a parasite in the green tree frog, *Hyla cinerea* (Schneider), of North America. *J Zoota*, **1663**: 33-45.

Du Preez LH. 2010. *Polystoma vernoni* n. sp. (Monogenea: Polystomatidae) from the sharp nosed grass frog Ptychatidae Oxyrhynchus (Smith, 1849) in South of Africa. *J Helminthol*. **84**:295-294.

Du Preez LH. 2015. *Eupolystoma namibiensis* n. sp. (Monogenea: Polystomatidae) parasitic in *Poyntonophrynus hoeschi* (Ahl, 1934) of Namibia. *Afr. J Zool*, **88** :1-5.

Koprivnikar J, David JM, Jason RR, Sarah AO, Thomas RR and Pieter TJ. 2012. Macroparasite Infections What Can They Tell Us. *Int Associ Ecolo H*, **9**: 342–360.

Kudo RR. 1971. **Protozoology**, fifth ed, Charles C. Springfield.

Maulood IM. 2005. Effects of L-arginine and Melatonin on Plasma Endothelin-1 level in Rats with Induced Diabetes Mellitus or Pulmonary Fibrosis. Ph.D Thesis, Salahaddin University, Erbil, Iraq.

Mollaret I, Jamieson BG and Justine JL. 2000. Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *Int J Parasitol*, **30**: 171-185.

Mollaret I, Jamieson BG and Adlard RD. 1997. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *J Mol Bioch Parasitolo*, **90**:433-8.

Morand S, Krassnove BR and Littlewood TJ. 2015. Parasite diversity and diversification. Cambridge Univ.Press. 462pp.

Raharivololoniaina L, Verneau O., Berthier P, Vences M, Du Preez LH. 2011. First monogenean flatworm from a microhylid frog host: Kankana, a new polystome genus from Madagascar. *J Parasitolo Int* , **60**: 465–473.

Sinnappah ND, Lim LH, Rohde K, Tinsley R, Combes C and Verneau O. 2001. A paedomorphic parasite associated with a neotenic amphibian host: phylogenetic evidence suggests a revised systematic position for Sphyranuridae within anuran and turtle polystomatoineans. *J Mol Phylogenet Evol*, **18**: 189-201.

Smyth JD, Smyth MM. 1980. Frogs as host-parasite systems I. The Macmillan Press Ltd, London.112pp.

Yamaguti S. 1963. Systema helminthum, Vol. IV: Monogenea and Aspidocotylea. Intersci Publ, New York: 699pp.

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Growth, Water Relation and Physiological Responses of Three Eggplant Cultivars under Different Salinity Levels

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Abstract

Eggplant (*Solanum melongena* L.) is an important traditional crop that is cultivated worldwide. Salinity is one of the major abiotic stress factors that impact crops yield. Seeds of three eggplant cultivars (Blacky, Pearly F1, and Classic) were evaluated under salt stress. Seedlings were treated with salinity solutions induced by a 3:1 ratio of calcium chloride and sodium chloride to four concentration levels measured as electrical conductivity (EC) [1.2 dS/m (control), 2.0 dS/m, 4.0 dS/m, and 8.0 dS/m] for 65 days. Plants had a higher root dry weight when irrigated with 4.0 and 8.0 dS/m solutions. Eggplant cultivars varied in their response to salinity. At both control and 4.0 dS/m, Blacky cultivar had the highest plant height, stem diameter, RGR, NAR, leaf area, stem, leaf, root, and total plant dry weights. However, Blacky had the lowest in all these parameters when subjected to 8.0 dS/m. Results indicate that high salinity levels may alter the pattern of dry matter distribution that preferred investment in roots than in the other plant parts. Under 4.0 dS/m, Blacky seedlings were triggered to develop adaptive mechanisms that could better tolerate saline conditions than when irrigated with 2.0 dS/m water.

Keywords: Eggplant, salt stress, water relations, gas exchange, relative growth rate, net assimilation rate.

1. Introduction

Eggplant (*Solanum melongena* L.) is a traditional crop, cultivated mainly in Asia, Southern Europe and the Mediterranean countries. In 2008, about 1.96 million ha were devoted for eggplant cultivation worldwide (FAO, 2010). In the 21stcentury, some major problems concerning water resources and the increase in soil and water salinization appeared (Shrivastava and Kumar, 2015).

Salinity is one of the major abiotic stress factors that threaten crops yield (Yamaguchi and Blumwald, 2005; Yasar *et al*., 2006; and Shahbaz and Ashraf, 2013), mainly in countries where supplemental irrigation is needed for the crops (Flowers, 2004). When evaporation is greater than precipitation and salts are present in high amounts in the soil, a white layer of dry salt on the soil surface is formed in a process called salinization (Unlukara *et al*., 2010). In 2014, Shurivastava and Kumar reported that high salinity adversely impacted20% of cultivated lands, and 33% of the irrigated agricultural lands. Moreover, there is an annual increase in salinized areas at a rate of 10% due to low precipitation, irrigation with saline water and poor cultural practices (Shurivastava and Kumar, 2014). More than 50% of the arable land is expected to reach high levels of salinity by the year 2050 (Jamil *et al*., 2011).

It is well known that plant metabolism is adversely affected by water stress. Salinity reduces plant growth (Parida and Das, 2005; Paul, 2012) either through osmotic inhibition of water uptake by roots or specific ion effects, which affects cell division, cell expansion, and stomatal conductance (Munns, 2002; Abed El-Azeem *et al*., 2012). The rate and the amount of water that plant roots can absorb are reduced with high soil salinity. This reduction is due to high osmotic pressure of the soil solution leads to physiological drought due to low water availability (Kozlowski, 1987). Around 5% of the productive land all around the world showed reduction in growth, yield and

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development at physiological and biochemical activity levels due to high salt concentrations (Ghassemi and Jakeman, 1995; Munns and Tester, 2008).

Irrigation with a low water quality is a common source of salts; salts accumulate as water is used by the crop or evaporates directly from the soil (Unlukara *et al*., 2010). In the root zone, soil is considered saline when the Electrical Conductivity (EC) of the saturation extract exceeds 4 dS/m (approximately 40 mM NaCl) at 25 ^{o}C (Shrivastava and Kumar, 2015).Most crops do not grow well under saline conditions. Only salt tolerant plants (halophytes) can grow properly in soils with accumulated salts (Glenn and Brown, 1999).

Eggplant is classified as a very sensitive (Fu *et al*., 2013) to moderate sensitive vegetable crop (Akinci *et al*., 2004; Yasar *et al*., 2006), so more attention on salinity adverse effects is required to improve crop performance, increase productivity and profitability (Akinci *et al*., 2004). Knowing the salinity levels threshold of different eggplant varieties and the impact on the crop yield in response to increasing soil salinity is crucial (Heuer *et al*., 1986). Determining salt tolerance of different eggplant varieties and cultivars helps in minimizing the injury of salinity impact (Akinci *et al*., 2004). Plant tolerance to salinity stress can be determined by identifying the plant responses to different physiological parameters (Chartzoulakis and Loupassaki, 1997).

The objective of this work is to study the physiological response of the three most cultivated eggplant cultivars (Blacky, Pearly and Classic) in Jordan to increasing salinity.

2. Materials and Methods

2.1. Study Location

This study was conducted in a greenhouse at The Hashemite University, Zarqa, 32°05’ N Latitude and 36°06 E Longitudes. Greenhouse day temperatures were in the range of 20-35°C, and mean midday photosynthetic photon flux density (PPFD) was 365 µmol.s-1.m-2measured by a quantum sensor (LI_250A; LICOR.)

2.2. Plant Material and Experimental Design

Seeds of three eggplant (*S. melogena* L.) cultivars (Blacky, Hi-Tech, Denmark; Pearly F1, Hi-Tech, Denmark; and Classic, Harris moran, China) were used for this experiment. Seeds were germinated in trays containing peatmoss (KEKKILA, European Union).

After 30 days, seedlings were transplanted into 5 L pots containing autoclaved mixture media of fumigated peatmoss: perlite: soil (1:1:1v/v). Cloth screens were placed in the bottom of the pots to prevent soil loss. Transplanted seedlings were kept well irrigated in the greenhouse for a month. Plants were fertilized using (Nutri-Leaf 60, USA) (20N-20P- 20K fertilizer) at a rate of 5g/L water one week after transplantation for one time.

Uniform plants from each cultivar were assigned randomly to one of four irrigation treatments (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m) for 65 days. The experimental design was completely randomized block design. There were five experimental blocks, each containing a total of 12 plants (3 cultivars x 4 salinity levels). Extra 24 plants (8 from each cultivar) were used to determine the initial growth characteristics before applying the salinity treatment.

2.3. Initial Seedling Traits

On the day the irrigation treatments were initiated,8 plants, from each cultivar, were harvested to determine the initial plant growth traits. The harvested plants were separated into leaves, stem and roots. Data recorded at that time included leaf area, leaf dry weight, stem dry weight and root dry weights. Leaf area (cm2) was measured using a portable leaf area meter (LI-3000A; LI-COR; Lincoln, Nebr. USA). Roots were washed by tab water to remove soil mixture. Oven dry weights of leaves, stems, and roots were determined after drying to a constant weight at 65oC (data not shown).

2.4. Salinity Treatments

A 3:1 ratio of calcium chloride and sodium chloride were respectively diluted in water to prepare the stock solution. Treatment solutions were made by adding stock solution to tap water until the desired salinity levels were achieved. All readings were recorded using an EC meter (Milwaukee SPEM500). On the same day when the initial data were recorded the remaining five blocks were then watered with salinity treatment (EC 2.0 dS/m), to prevent salt shock, except for the control that was watered with tap water (1.2 dS/m). The EC of the irrigated water were continuously and gradually increased until each desired salinity level achieved (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m). Once all the experimental plants were receiving their designated salinity level, all treatments were irrigated manually every two days to the field capacity for the total duration of 65 days.

2.5. Physiological Traits

Chlorophyll Concentration Index (CCI) was determined biweekly by averaging two midday readings of each plant. Two youngest fully-expanded mature healthy leaves were selected and measured using plant chlorophyll concentration meter (LI-250A OPTIC-SCIENCES CCM-200). Transpiration and stomatal conductance (gs) were measured biweekly using a steady-state porometer (LI-1600; LICOR; Lincoln, Nebr.). Plant height was measured biweekly from soil surface to the top of the plant for each plant.

2.6. Final Harvest

At the end of the experiment (65 days), all plants were harvested. Harvested plants were washed, air dried on filter paper, separated into leaves, stems and roots. Leaf area (cm2) was determined using a portable leaf area meter (LI-3000A; L-ICOR; Lincoln, Nebr. USA). Stem diameters were measured using an electronic 0-150 mm digital caliper (Swiss). Leaves, stems and roots oven dry weights were determined after drying plant parts to constant weight at 65oC.

Leaf discs from two youngest fully expanded mature leaves of all plants were used to determine Relative Water Content (RWC). RWC was calculated using the equation (FW-DW/SW-DW)(100). Where FW is the fresh weight and DW represents fresh weight sample oven dried at 68 °C and SW represents saturated weight of sample, which

was immersed overnight in distilled water (Bsoul *et al.*, 2006).

Relative growth rates were calculated using the equation of Gutschick and Kay (1995): RGR = (ln W2 - ln W1)/ (T2 - T1), where W2 was the final dry weight at day 121 (T2), and W1 was the initial DW determined from initial data harvest on day 1 (T1). Net assimilation rates (NAR) were calculated as: NAR = M2 - M1/T2 - T1 X log L2 - log L1/L2 - L1, where M2 was the final dry weight at day 65 (T2), and M1 was the initial DW determined from the initial recorded on day one of the experiment (T1). Leaf area ratio (cm2.g-1) was calculated as SLA= leaf area/leaf dry weight. Specific stem length (cm.g-1) was calculated as SSL= stem height/ stem dry weight.

2.7. Statistical Analysis

Statistical analysis was performed using SAS 9.1 software for Windows (2003). Significant differences between values of all parameters were determined at P≤ 0.05 using ProcGlm, PDIFF, ANOVA and Duncan's Multiple Range Tests.

3. Results

Regardless of the treatment, there were no significant differences among cultivars in terms of stem, leaf, shoot, root, and total plant dry weights. In addition, root/shoot ratio, plant height and stem diameter were not significantly different among the three cultivars (Table 1). Regardless of the cultivars, there were no significant differences among the treatments for all these parameters either, except for the root dry weight. Both treatments 4.0 ds/m and 8.0 ds/m accumulated the highest root dry weights (0.63g and 0.57g, respectively)(P= 0.028) (Table 1).However, there were obvious significant cultivar treatments interaction effects for stem, leaf, shoot, root, total plant dry weights, plant height, specific stem length and stem diameter (Table 1).

Blacky cultivar had the highest stem (0.7 g) leaf (2.2 g) root(0.5 g) and total plant dry weights (3.5 g) under control treatment(Figure 1 A-D). On the other hand, when irrigated with 2.0dS/m treatment, Blacky cultivar was among the lowest in terms of stem, leaf, root, and total plant dry weights, while Pearly cultivar maintained the highest. But, under 4.0 dS/m Blacky cultivar restored the maximum stem, leaf, root and total plant dry weights as compared with the other cultivars (Pearly and Classic) (Figure 1 A-D). When irrigated with 8.0 dS/m, Blacky stem, leaf, and total plant dry weight were affected the most. Blacky had stem dry weight about half less, leaf area more than 3 fold less, and more than two fold less total plant dry weight than that when irrigated with 4.0 dS/m water. In addition, Blacky had the lowest stem, leaf and total plant dry weights when compared with Pearly and Classic cultivars at 8.0 dS/m(Figure 1 A, B and D).

Under 2.0 dS/m, Blacky had the shortest plant height, while at 4.0 dS/m, Blacky cultivar height recovered and had the tallest plant height (16.6 cm) at 4.0 dS/m. At 8.0 dS/m Blacky height had no significant difference than the other cultivars (Figure 1 E). No significant differences were found among cultivars in the stem diameter under all treatments, except for Blacky that had the lowest stem diameter (3.83 mm) when irrigated with water with an EC of 8.0dS/m (Figure 1 F).

Table 1. Plant biomass dry weights, root to shoot ratio, plant height, Stem diameter (SD), relative water content (RWC),and specific stem length (SSL) of three eggplant cultivars (Blacky, Pearly, and Classic) subjected to four salinity treatments (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m) for 65 days.

Cultivar	Stem DW (g)	Leaf DW (g)	Shoot DW (g)	Root DW (g)	Plant DW (g)	Root/ Shoot (g)	Plant height (cm)	SD (mm)	RWC (%)	SSL ($cm \cdot g^{-1}$)
BLACKY	0.65[z]a	1.38a	2.03a	0.48a	2.50a	0.26a	15.3a	4.2a	0.51[z]b	24.33a
PEARLY	0.64a	1.14a	1.78a	0.51a	2.29a	0.28a	14.7a	4.3a	0.56a	25.37a
CLASSIC	0.66a	1.43a	2.08a	0.53a	2.61a	0.26a	14.5a	4.4a	0.57a	23.28a
Treatment										
Control	0.61a	1.28a	1.89a	0.41b	2.29a	0.23a	14.8a	4.2a	0.54a	25.78a
2.0dS/m	0.64a	1.06a	1.69a	0.42b	2.11a	0.24a	14.8a	4.2a	0.54a	25.37a
4.0 dS/m	0.67a	1.72a	2.39a	0.63a	3.01a	0.28a	15.1a	4.5a	0.55a	23.10a
8.0 dS/m	0.68a	1.21a	1.88a	0.57a	2.45a	0.31a	14.5a	4.3a	0.55a	23.05a
P-value										
Cultivar	0.93	0.37	0.44	0.78	0.58	0.84	0.27	0.29	0.017	0.388
Treatment	0.59	0.06	0.11	0.028	0.08	0.13	0.81	0.17	0.957	0.253
[zz]CxT	0.0009	0.001	0.0007	0.024	0.001	0.21	0.023	0.01	0.178	0.0004

[z]Means (n = 5) within columns followed by the same letter were not statistically different. Means were assessed at $P \leq 0.05$ using ProcGlm, PDIFF option of SAS.

[zz]CxT: interaction between cultivar and treatment.

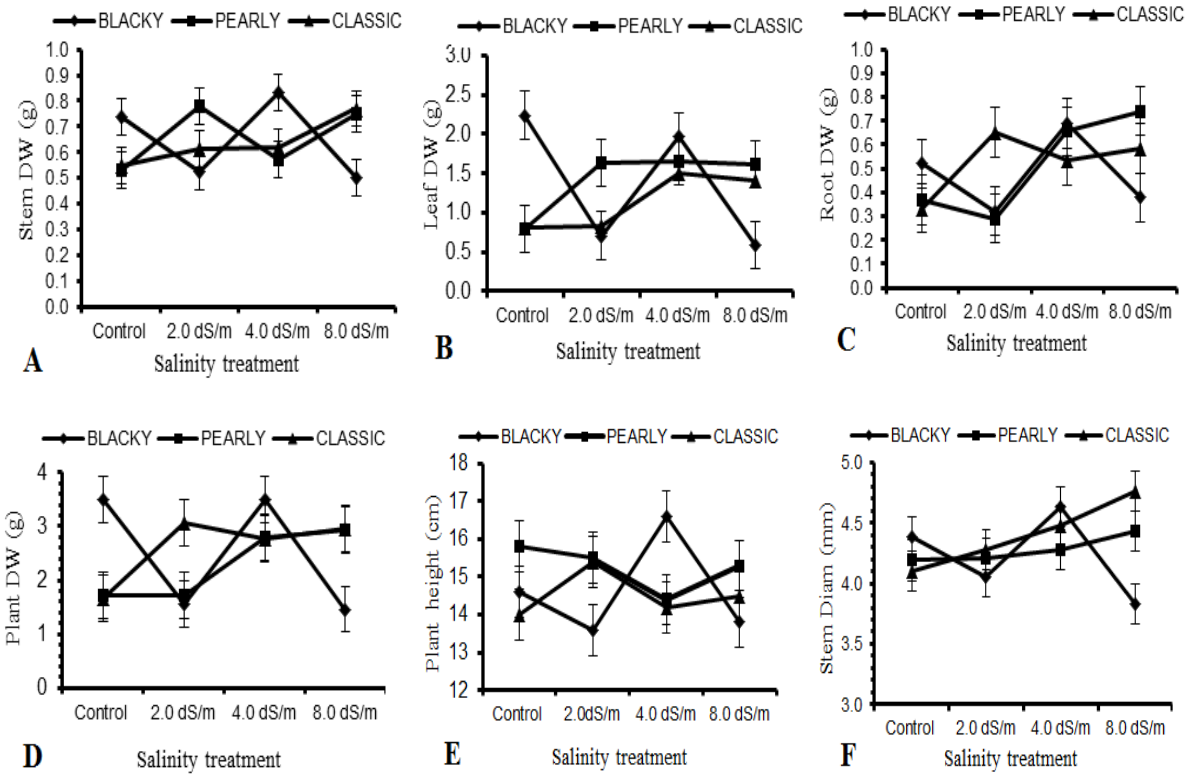

Figure 1. Plant stem dry weight (A), leaf dry weight (B)dry weight (C), total plant dry weight (D) plant height (E), and Stem diameter (F), of three eggplant cultivars(BLACKY, PEARLY, and CLASSIC) subjected to four salinity treatments (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m) for 65days.Each point represents a mean ± SE (n = 5).

Significant differences were recorded among cultivars at the end of the experiment in their RWC (P= 0.017) (Table 1), NAR (P= 0.002), and RGR (P=0.0301) (Table 2). Blacky cultivar had the lowest RWC (0.51 %), and RGR (0.016 g•g-1•d-1) while Pearly and Classic had the maximum and similar RWC and RGR. Classic cultivar had the highest NAR (0.044 mg•cm-2•d-1)and was significantly higher than that of Pearly and Blacky (Table 2).

Regardless of cultivars RGR had significant differences among treatments (*P*=0.0358), plants irrigated with 2.0 dS/m had the lowest RGR (0.016 $g•g^{-1}•d^{-1}$)with no significant differences among the other treatments (Table 2).On the other hand, there were no significant differences among treatments for RWC (*P*= 0.957) (Table 1), NAR (*P*= 0.065), SSL (*P*= 0.253), and LA (*P*= 0.11) (Table 2). However, cultivar treatment interaction effects were highly significant for NAR (*P*= 0.0005), RGR (*P*= 0.0003), SSL (*P*= 0.0004), and LA (*P*= 0.003), while there was no significant treatment cultivar interaction for the RWC (*P*= 0.178) (Table 1).Chlorophyll content index, stomatal conductance and transpiration had no significant differences among cultivars (*P*-values = 0.44, 0.32, and 0.15, respectively)treatments (*P*-values = 0.84, 0.80, and 0.76 respectively) and cultivar*treatment interaction (*P*-values = 0.68, 0.39, and 0.58, respectively) (Table 2).

Blacky cultivar had the highest RGR (*P*= 0.0003) and NAR (*P*= 0.0005) under control treatment, but it had the lowest RGR and NAR under 2.0 dS/m (Figure 2 A and B).However no significant differences were recorded among cultivars under 4.0 dS/m. under the highest salinity treatment 8.0 dS/m, Blacky cultivar had about half RGR and four-folds less NAR than both Pearly and Classic cultivars. In addition, if we compare the NAR and RGR for the three cultivars, we will notice that the NAR and RGR followed the same trend under all treatments (Figure 2 A and B).

Despite that the Classic cultivar had the highest SSL (30.4 $cm•g^{-1}$) under control treatment (*P*= 0.0004), its SSL continued to decrease as the EC increase and had the lowest SSL (19.8 $cm•g^{-1}$) under 8.0 dS/m (Figure 2 D). On the other hand, Blacky cultivar had the lowest SSL under control treatment (20.1 $cm•g^{-1}$), but under 8.0 dS/m Blacky had the highest SSL (28.2 $cm•g^{-1}$)(Figure 2 D).

When irrigated with tap water, Blacky cultivar had the highest LA (498.7 cm^2) (*P*= 0.003) and about four folds more than Classic (142.1 cm^2), but its LA reduced to about four folds (142.1 cm^2) under 2.0 dS/m. However, Blacky had among the highest LA under 4.0 dS/m (377.7 cm^2). Under 8.0 dS/m Blacky LA had the lowest LA (140.1 cm^2)(Figure 2 E).

Plants chlorophyll content index was maximum (38.1) (*P*<0.0001) after 28 days from the time when plants were irrigated with salinity treatments. However, plants CCI reached (29.6) after 42 days and continued to decrease to the lowest (9.3) after 65 days and lost about four folds (Figure 3 A). After 28 days plants had the lowest transpiration rate (21.7$mmol•m^{-2}•s^{-1}$) (*P*<0.0001), but had the highest (63.2$mmol•m^{-2}•s^{-1}$) after 65 days (Figure 3 A).Plants stomatal conductance was the lowest (345.6 $mmol•m^{-2}•s^{-1}$) (*P*<0.0001) after 28 days then continued to increase to the highest (2092.0 $mmol•m^{-2}•s^{-1}$) at the end of the experiment (Figure 3 B).

Table 2. Plant net assimilation rate (NAR), relative growth rate (RGR), leaf area (LA), Chlorophyll content index (CCI), stomatal conductance g_s, and transpiration (Trans.) of three eggplant cultivars(BLACKY, PEARLY, and CLASSIC) subjected to four irrigation treatments (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m) for 65days.

Cultivar	NAR ($mg•cm^{-2}•d^{-1}$)	RGR ($g•g^{-1}•d^{-1}$)	LA (cm^2)	CCI	g_s ($mmol•m^{-2}•s^{-1}$)	Trans. ($mmol•m^{-2}•s^{-1}$)
BLACKY	0.024b	0.016b	290.7a	10.4a	2102.9a	63.56a
PEARLY	0.032b	0.018a	234.2a	8.3a	2175.2a	63.77a
CLASSIC	0.044a	0.020a	283.3a	9.1a	1972.4a	62.31a
Treatment						
Control	0.028a	0.017a	278.1a	9.9a	2099.9a	63.7a
2.0dS/m	0.028a	0.016b	214.7a	8.3a	2064.1a	62.8a
4.0 dS/m	0.028a	0.021a	342.9a	9.7a	2036.5a	62.8a
8.0 dS/m	0.033a	0.018a	241.9a	9.2a	2133.5a	63.5a
P-value						
Cultivar	0.002	0.0301	0.43	0.44	0.32	0.15
Treatment	0.0651	0.0358	0.11	0.84	0.80	0.76
[zz]CxT	0.0005	0.0003	0.003	0.68	0.39	0.58

[z]Means (n = 5) within columns followed by the same letter were not statistically different. Means were assessed at $P \leq 0.05$ using ProcGlm, PDIFF option of SAS.

[zz]CxT: interaction between cultivar and treatment.

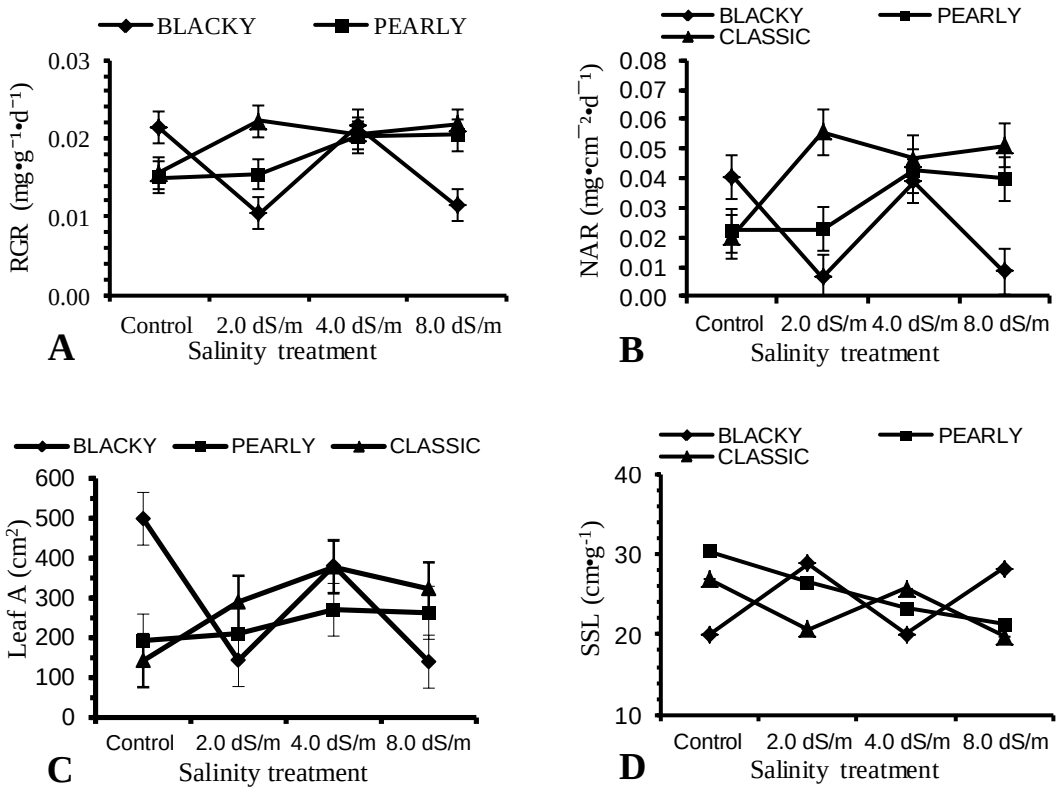

Figure 2.Relative growth rate (A),Plant net assimilation rate (B), leaf area (C), and specific stem length (D) of three eggplant cultivars (BLACKY, PEARLY, and CLASSIC) subjected to four irrigation treatments (1.2 dS/m (control), 2.0dS/m, 4.0 dS/m, and 8.0 dS/m) for 65 days. Each point represents a mean ± SE (n = 5).

4. Discussion

Developmental traits associated with water deficit responses are important to quantifying plant adaptation mechanisms to water shortage (Blum, 1996). The scant differences of stem, leaf, shoot and total dry weights among cultivars and treatments at the end of the experiment is devoted to varied and altered plant growth and physiological responses of the cultivars to the salinity levels during the experiment. Fu *et al*. (2013) reported that eggplant capability to survive and grow during stress periods were enhanced according to their morphological and anatomical responses .

Cano *et al.* (1998) suggested that root growth is the most indicative parameter for salt tolerance. Because the roots are more sensitive to salt stress than the other plant parts, the only effect of treatment was higher root dry weight in plants irrigated with4.0 dS/m and 8.0 dS/m water. Savvas and Lenz (2000) had the same results and reported that the only effect of salinity treatments was on the root dry weight that was greater in eggplants exposed to NaCl-salinity. Results indicate that the high salinity levels may actually alter the partitioning pattern of dry matter preferring investment in the roots. Similar results were also recorded in kiwifruit (Chartzoulakis *et al*., 1995) and in beans (Seemann and Critchley, 1985).Maintenance of root growth during physiological drought is an obvious advantage to maintain an adequate water supply, and varied with plant genetics (O'Toole and Bland, 1987).

Blacky cultivar had the highest stem, leaf, and root and total plant dry weights under control treatment. But, all of these parameters had extreme reduction as salinity treatment increased slightly to 2.0 dS/m (Figure 1 A-D). However, plant parts dry weights recovered and increased sharply to the highest at 4.0 dS/m. On the other hand, Blacky failed to withstand high salt concentration and its dry weights reduced to the lowest at 8.0 dS/m (Figure 1 A-D). According to the results of many studies, plants may differ in their salinity response to vegetative growth and root development. The vegetative dry weight of eggplant decreased with increasing soil salinity. But it is not unusual to observe an increase in the yield with an initial increase in salinity. The positive effect of low salinity on shoots of several plants has been reported by many other authors. The cause is not known but could be related to mineral nutrition (Unlukara *et al*., 2010; Andriolo *et al*., 2005).Plants lose most of the water through leaves. Thus, Blacky cultivar escaped low available water through the restriction of leaves growth to about 70% less than the control (Figure 2 E). Reducing leaf size was considered as first symptom of water deficit (Mohd *et al*. 2004).Torrecillas *et al*. (1995) found that tomato had less leaf area under water deficit compared to control plants (Saei *et al*.,2006).

Increasing the stem diameter of Pearly and Classic cultivars at 8.0 dS/m has advantages over Blacky cultivar even, though; the cultivars had no significant differences at low salinity treatments. Wide stem diameter could provide easier path for water to supply the upper plant parts (Bsoul *et al*., 2016; Lis *et al*., 1989).

The RWC of Blacky averaged 51% suggesting that the foliar of Blacky cultivar endure low RWC yet maintain adequate photosynthesis. Chaves (1991) reported that photosynthetic activity is reduced when RWC ranges from 40% to 70%. RGR represents to which extent a plant invests its photosynthesis in current growth and enhances its capacity for future photosynthesis (Fitter and Hay, 2002). Efficiency of eggplants to accumulate dry matter under salt stress (NAR) is cultivar-dependent. Blacky cultivar was more efficient in dry matter accumulation at 4.0 dS/m but that efficiency (NAR) dropped to the minimum when irrigated 8.0 dS/m water. RGR and NAR of Blacky followed similar trend (Figure 2 B). RGR and NAR data suggest that rapid growth is not advantageous under salinity conditions to conserve resources.

Because SSL indicates length of stem allocated to each unit of biomass, this ratio could be used to determine stem's mechanical strength (Bsoul *et al*., 2006). Black cultivar maintained lowest SSL at 4.0 dS/m (Figure 2 D), which indicates that Blacky had the strongest stem and better water conduction rout to the above plant parts. In addition, the strong stem maintains plant erection habit and prevents logging, which would be more suitable trait under that salinity level.

At the end of the experiment, cultivars and treatments had no effect on stomatal conductance, transpiration, and Chlorophyll content index. Eggplant cultivars begin to lose their chlorophyll content when treated with saline water (Figure 3 A). At increasing levels of salinity, chlorophyll degradation occurs (Malibari *et al*., 1993; Salama *et al*., 1994). Excess salt in chloroplasts causes shrinkage of thykaloids and stacking of adjacent membranes of grana. Reduction of chloroplasts occurred also as a result of ionic imbalances (Blumwald *et al*., 2000). Reduction of stomatal conductance and transpiration rate are considered as adaptations to protect plants from dehydration. It is known that both stomatal conductance and transpiration rate decrease with a higher vapor pressure deficit that is a consequence of elevated temperature (Bunce, 2000; Lloyd and Farquhar, 2008). The increasing in g_s and transpiration rate were affected by the day temperature when data were recorded (33.8 ^{o}C ± 5.6) more than salinity treatments or cultivars. Similar results were reported with tomato plants under water deficit (Bsoul *et al*., 2016).

5. Conclusion

Results indicated that high salinity levels may alter the pattern of dry matter distribution that preferred investment in roots for more water resources. Eggplants could maintain acceptable growth with a RWC around 51 %.Under saline conditions stomatal conductance and transpiration rate were affected and lessened by the high day temperature more than the increased salt concentration. Chlorophyll content of eggplants was adversely affected by salt stress. Under moderate salinity levels around 4.0 dS/m some eggplant cultivars like Blacky were triggered to develop adaptive mechanisms that could tolerate saline conditions better than when irrigated with 2.0 dS/m water. Our results are nominating Pearly and Classic eggplant cultivars for cultivation under high salinity levels as they gained better adaptive characteristics than Blacky at 8.0 dS/m., while Blacky is suitable when irrigated with 4.0 dS/m water.

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References

Abd El-Azeem, S Elwan, M Sung J and Ok Y. 2012.Alleviation of Salt Stress in Eggplant (*SolanumMelongena* L.) by Plant-Growth-Promoting Rhizobacteria. *Commun Soil Sci Plant Anal*, **43**:1303–1315.

Akinci IE, Akınci S, Yilmaz K and Dikici H .2004. Response of eggplant varieties (*Solanummelongena*) to salinity in germination and seedling stages. *New Zealand J. Crop and Hortic. Sci*, **32**:1993-2000.

Andriolo JL, Luz GL, Witter MH, Godoi RS, Barros GTandBortolotto OC. 2005.Growth and yield of lettuce plants under salinity. *Hort. Bras*, **23**: 931-934.

Blum A. 1996. Crop responses to drought and interpretation of adaptation. *Plant Growth Regul*, **20**:135-146.

Blumwald, E, AharonGS and ApseP. 2000. Sodium transport in plant cells. *Biochim.Biophys.Acta*, **1465**: 140-151.

Bsoul EY, Al-Afaeshat A and Qaryouti M. 2016. Growth, water relation and physiological responses of drought stressed Irhaba tomato landrace as compared with Amani and GS-12 cultivars. *J FOOD AGRIC ENVIRON*, **14**: 78-84.

Bsoul E, St. Hilaire R, and VanLeeuwen D. 2006.Bigtooth maples exposed to asynchronous cyclic irrigation show provenance differences in drought adaptation mechanisms. *J. Amer. Soc. Hort. Sci*, **131**:459-468.

Bunce JA. 2000. Responses of stomatal conductance to light, humidity and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field. *Glob Chang Biol*, **6**: 371-382.

Cano EA, Perez-Alfocea F, Moreno V, Caro M and Bolarin MC. 1998.Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. *Plant Cell Tiss Org Cult*, **53**:19-26.

Chartzoulakis K. and Loupassaki MH. 1997. Effects of NaCl salinity on germination, growth, gas exchange and yield of greenhouse eggplant. *Agric. Water Manage*, **32**: 214-225.

Chartzoulakis KI, Therios NM and Noitsakis B. 1995.Growth, ion content and photosynthetic performance of salt-stressed kiwifruit plants. *Irrigation Sci*, **16**: 23-28.

Chaves MM.1991. Effect of water deficit on carbon assimilation. *J. Expt. Bot*, **42**:234-239.

FAO. (2010). http://faostat.fao.org/site/567/default.aspx#ancor.

Fitter AH and Hay RKM. 2002. Environmental physiology of plants, third ed. Academic. Calif.

Flowers TJ. 2004. Improving crop salt tolerance. *J. Exp. Bot*, **55**:307-319.

Fu QS, Yang RC, Wang HS, Zhao B, Zhou CL, Ren SX, and Guo YD. 2013. Leaf morphological and ultrastructural performance of eggplant(*Solanummelongena*L.) in response to water stress. *Photosynthetica*, **51**: 109-1.

Ghassemi F and Jakeman AJ. 1995. Salinization of land and water resources. First ed. CABI, UK.

Glenn EP, Brown JJ and Blumwald E. 1999.Salt tolerance and crop potential of halophytes.*Crit Rev Plant Sci*, **18**:227-255.

Gutschick, V.P. and LE.Kay. 1995. Nutrient-limited growth rates: quantitative benefits of stress responses and some aspects of regulation. *J. Exp. Bot*, **46**: 995-1009.

Heuer B, Meiri A and Shalevet J. 1986.Salt tolerance of eggplant. *Plant and Soil*, **95**:9-13.

Jamil A Riaz S Ashraf M and Foolad MR. 2011. Gene expression profiling of plants under salt stress Crit. *Rev. Plant Sci*, **30**:435-458.

Jones RW, Pike LM, Yourman L.F. Salinity influences cucumber growth and yield. *J. Amer. Soc. Hort. Sci*, **114**: 547-551, 1989.

Kozlowski TT. 1987. Soil moisture and absorption of water by tree roots. *Arboriculture J*, **13**:39-46.

Lis H, Huguet G, Schoch P G and P Oriando. 1989. Response of peach-tree growth and cropping to soil water deficit at various phenological stages of fruit development. *J. Hortic. Sci*, **64**:541-52.

Lloyd J and Farquhar GD. 2008. Effects of rising temperatures and [CO_2] on the physiology of tropical forest trees. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 1811-1817.

Malibari AA, Zidan MA, Heikal MM, El-Shamary S. 1993.Effect of salinity on germination and growth of alfalfa, sunflower and sorghum. *Pak J Bot*, **25**:156-60.

Mohd R, Ismail M, Yusoff K and Mahmood M. 2004. Growth, water relations, stomata conductance and proline concentration in water stressed banana (*Musa* spp.) plants. *Asian J. Plant Sci*, **3**:709-713.

Munns R and Tester M. 2008.Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol*, **59**: 651-681.

Munns R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*, **25**: 239 250.

O'Toole JC and Bland WL.1987. Genotypic variation in crop plant root systems. *Adv Agron*, **41**:91-145.

Parida AK and Das AB. 2005.Salt tolerance and salinity effects on plants. *Ecotoxicol. Environ. Saf*, **60**:324-349.

Paul D. 2012. Osmotic stress adaptations in rhizobacteria. *J. Basic Microbiol*, **52**:1-10.

Saei A, ZamaniZ, TalaieA, and Fatahi R. 2006. Influence of drought stress periods on olive (Olea europaea L. cv. Zard) leaves stomata. *Int J Agric Biol*, **4**: 430–433.

Salama S, Trivedi S, Busheva M, Arafa AA, Garaband Erdei L. (1994). Effects of NaCl salinity on growth, cation accumulation, chloroplast structure and function in wheat cultivars differing in salt tolerance. *J. Plant Physiol*, **144**: 241-247

Savvas D. and Lenz F. 2000. Effects of NaCl or nutrient-induced salinity on growth, yield, and composition of eggplants grown in rockwool . *Sci Hort*,**84**: 37-47.

Seemann J and Critchley C. 1985. Effects of salt stress on the growth, ion content, stomatalbehaviour and photosynthetic capacity of a saltsensitive species, *Phaseolus vulgaris* L. *Planta*, **164**:151-162.

Shahbaz M and Ashraf M. 2013.Improving salinity tolerance in cereals Crit. *Rev. Plant Sci*, **32**: 237-249.

Shrivastava P and Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J Biol Sci*, **22**: 123-1312.

Torrecillas A, Guillaume C, Alarcón JJ and Ruizsánchez M C. 1995. Water relations of two tomato species under water-stress and recovery.Plant Sci, **105**: 169-176.

Unlukara A,Kurung A,Kesmez G,Yurtsevene E, and Suarez D. 2010. Effects of salinity on eggplant (*SolanumMelogena* L.) growth and evapotranspiration. *Irrig.and Drain*, **59**: 203-214.

Yamaguchi T and Blumwald E. 2005.Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci*, **10**:615-620.

Yasar F Ellialtioglu S and Kusvuran S. 2006. Ion and lipid peroxide content in sensitive and tolerant eggplant callus cultured under salt stress. *Eur. J. Hort. Sci*, **71**:169-172.

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Germination and Emergence Characteristics of Annual Ground Cherry (*Physalis divaricata*)

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Abstract

Recently, annual ground cherry *Physalis divaricata* (*P. divaricata*) has become a very serious damaging weed in a wide range of summer annual crops and some minor crops in western Iran. However, data regarding the seed germination and seedling emergence behavior of this weed are limited. Therefore, laboratory experiments were conducted in Razi University, Kermanshah, Iran during 2013 to evaluate the effect of temperature, KNO_3, GA_3, light, burial depth, pH, osmotic and salt stresses on seed germination and emergence of *P. divaricata* collected from Kermanshah, Islamabade-Gharb, Sarpole-Zahab. Results indicated that the seeds of *P. divaricata* germinated over a relatively wide range of temperatures. Though, the greatest germination occurred under the alternating temperature regime of (20/35°C 16 h low and 8 h high). Generally, light had a stimulatory effect on seed germination of *P. divaricata*. Emergence of *P. divaricata* decreased with the increase in burial depth. The highest emergence percentage was recorded for seeds buried at soil depth of 0 and 1 cm (75 and 55, respectively). The seeds failed to emerge when they were buried deeper than 4 cm. The salt concentration 46.09 mM NaCl resulted in 50% inhibition of seed germination. An osmotic potential of 0.43 MPa led to the inhibition of seed germination by 50%. Germination positively responded to increasing pH level. The maximum percentage of seed germination was shown at pH 10. Overall, germination increased when GA_3 and KNO_3 were added to the germination media. The results revealed that *P. divaricata* has the potential to become a noxious weed in further areas in Iran. Using cultivation, especially conducted during night, to deprive photoblastic seeds of *P. divaricata* from light would be beneficial in reducing its seed germination and removing newly established seedlings.

Keywords: burial depth, light, osmotic stress, salt stress, temperature.

1. Introduction

Annual ground cherry (*Physalis divaricata* L.) belongs to the family of solanaceae. It is an invasive erect weed with a length of 15-60 cm. Flowers are solitary with yellow color and cup-shaped, which appear from May to July. *P. divaricata* produces 4 to 70 seeds per berry depending on environmental conditions. The number of seeds produced can range from 126 to 16,300 per plant. This weed prefers nutrient-rich soils. According to the observations and preliminary tests of the present stufy (data not published), the freshly harvested seeds showed high levels of dormancy. Recently, *P. divaricata* has become a very serious damaging weed in a wide range of summer annual crops, such as sugar beet (*Beta vulgaris* L.), tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), maize (*Zea mays* L.), and some minor crops in western Iran. In addition to the substantial reduction in crop yield, it causes harvest problems and reduces crop quality due to sticky materials released from its berries. Seed germination is one of the most important stages in the life cycle of *P. divaricata*. Like other nightshade family weeds, *P. divaricata* seeds apparently have no special tools to disperse for short and long distances. Therefore, its survival highly depends on seed dormancy (Defelice, 2003; Zhou *et al.*, 2005; Taab & Andersson, 2009; Stanton *et al.*, 2012). Factors, such as seed burial depth (Penny & Neal, 2003; Wilson *et al.*, 2006), temperature (Fandrich & Mallory-Smith, 2005; Foley, 2008), light (Malik *et al.*, 2010; Huebner, 2011), osmotic potential (Boyd & Van Acker, 2004) and their interactions with internal conditions of seeds can highly affect seed germination and seedling emergence. Because *P. divaricata* is a very close relative to other Solanaceae crops, its chemical control is difficult, especially in the fields of these crops (Gorski & Wertz, 1987). A better understanding of the factors affecting seed germination and seedling emergence of *P. divaricata* could help to develop effective control measures, predict its invasion potential and make

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critical weed management decisions (Ghersa & Holt, 1995).

In spite of the fact that *P. divaricata* is a problematic and invasive weed for farming systems in western Iran, data describing the response of seed germination and seedling emergence of this weed to environmental factors is limited (Alam *et al.*, 2011; 2013). Hence, the aim of the present study is to determine the effect of some major environmental factors, including temperature, KNO3, GA3, light, burial depth, pH, osmotic and salt stresses on seed germination and emergence of *P. divaricata.*

2. Materials and Methods

2.1. Plant Material

The seeds used in the present study were collected from an infested maize field of Research Farm of Paradise of Agricultural and Natural Resources, Razi University, Kermanshah (34°18′N47°03′E; elevation 1519 m), Iran, during early September 2013. Seeds were also collected from an infested potato field located in Islamabade-gharb city (34°03′N46°40′E; elevation 1335 m), Iran, during late September 2013. In addition, seeds were collected from a tomato field belonged to Sarpole-Zahab city (34°35′N45°50′E; elevation 536 m), Iran, in July 2013. Meteorological conditions of these regions are given in Fig. 1. These different sites, which are heavily infested by P. divaricata, were chosen to test the relationship between seed germination of *P. divaricata* and environmental conditions. Seeds were stored at room temperature for10 days.

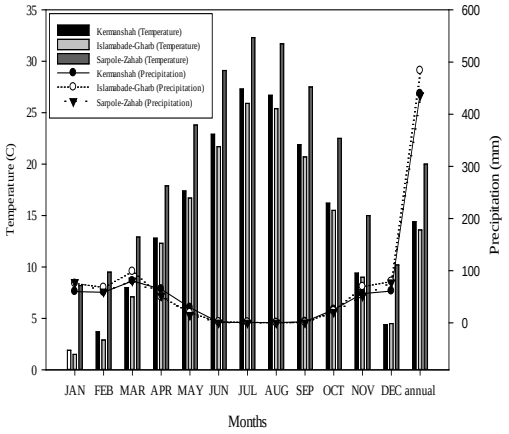


Figure 1. Long-term precipitation and temperature for Kermanshah, Sarpole-Zahab and Islamabad-Gharb during (1955-2014)

2.2. Germination Test

Four replications of 25 seeds of *P. divaricata* were placed in 9-cm Petri dishes lined with two layers of Whatman No. 1 filter paper, which were moistened with either 5 ml deionised water or other solutions when required. The Petri dishes were sealed with parafilm to prevent evaporation and then placed in a germination chamber in the physiology laboratory of the campus of Agricultural and Neutral Resources, Razi University, Kermanshah, Iran. Germination tests were conducted for 14 days at a light/dark temperature range of 20/35°C with the high temperature during the light cycle. Seeds were considered to have germinated when the radicle emerged. The number of germinated seeds was counted daily until day 14 of germination. The temperature and light conditions used here are according to the preliminary tests (data not shown).

2.3. Effect of Light and Temperature on Germination

Incubation temperatures consisted of constant (10, 20, 30 and 40°C) and fluctuating (5/10, 10/20, 15/25 and 20/35°C) (8 h low/16 h high) temperatures under light/dark cycle and continuous dark. For light treatments, dishes were sealed in transparent polyethylene bags while dark controls were wrapped in aluminum foil. All treatments were chosen according to the preliminary tests (data not shown).

Preliminary experiments showed no differences in germination among ecotypes collected from Islamabade-gharb, Sarpole-Zahab and Kermanshah in response to salt stress, pH, osmotic stress and burial depth treatments. Therefore, all experiments were carried out with seeds collected from Kermanshah.

2.4. Effect of Salt Stress on Seed Germination

Seeds were exposed to ten levels of salinity using solutions containing 0, 2, 4, 8, 16, 32, 64, 128, 256 and 512 mM NaCl. The experiment was carried out under the conditions described in the general procedure for germination test above.

2.5. Effect of pH on Seed Germination

Evaluated pH values were 4, 5, 6, 7, 8, 9 and 10. Buffered solution of different pH values were prepared based on Chen et al. (2009). The pH of 2 mM potassium hydrogen phthalate buffer was adjusted to 4 with 1 N HCl. The pH of 2 mM MES [2-(N-morpholino) ethanesulfonic acid was adjusted to 5 and 6 with 1 N NaOH. The pH of 2 mM solution HEPES [N -(2-hydroxymethyl) piperazine- N-(2-ethanesulfonic acid)] was adjusted to 7 and 8 with 1 N NaOH. Buffer solutions of pH 9 and 10 were prepared with 2 mM Mtricine [N -Tris (hydroxymethyl) methylglycine]. Petri dishes were incubated under light and temperature regimes as described for the germination test above.

2.6. Effect of Osmotic Stress on Seed Germination

The effect of osmotic stress on germination was assessed by incubating the seeds in polyethylene glycol solutions (PEG-6000) with the osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, - 0.8 and - 1.0 MPa. The solution was prepared based on the method described by Boyd and Hughes (2011). The seeds were incubated in the light and temperature regimes as mentioned for the germination test above.

2.7. Effect of Burial Depth on Seedling Emergence

The burial depths consisted of 0, 1, 2, 3, 4, 6, 8 and 10 cm. Thirty *P. divaricata* seeds were placed at the specified depths in a cup. Cups were watered daily and kept at room temperature (~25 °C). Emergence was counted weekly for 28 d or until emergence no longer occurred. Then, soil was sieved to find germinated seeds which could not reach the soil surface (suicidal germination). Seedlings were considered emerged when the two cotyledons could be seen at the soil surface.

2.8. Effect of GA3 and KNO3 on Seed Germination

Seeds were placed on filter paper moistened with 7 ml of distilled water containing GA3 (5 and 10 mM) and KNO3 (25 and 50 mM). In the control Petri dishes, only the distilled water was used. Petri dishes were then incubated according to the germination test above.

2.9. Statistical Analysis

All experiments were conducted as a completely randomized design with four replications and repeated twice. Unless otherwise noted, data were pooled across runs because of the lack of the significant run by-treatment interactions. Data were analyzed using PROC GLM in SAS and means were compared using LSD at the 0.05 level of probability. The following model was fitted to the data obtained from the salt and osmotic stress experiments using Sigma Plot software (version 12.0, SyStat Software, Inc., Point Richmond, CA, USA):

$$G\ (\%) = Gmax/[1+(X/X50)\ Grate] \quad (1)$$

where, G: Total germination (%) at NaCl concentration or osmotic potential x, Gmax: maximum germination (%), X50: the NaCl concentration or osmotic potential for the 50% reduction in the maximum germination (%) and Grate: germination rate or the slope of the curve (Chauhan *et al.*, 2006b)

The seedling emergence data resulted from the burial depth experiment were fitted to the following sigmoidal model (Norsworthy & Oliveira, 2006):

$$E(\%) = Emax/(exp(-(X-X50/Erate)) \quad (2)$$

where, E: seedling emergence percentage at burial depth x, Emax: maximum seedling emergence, X50: the soil depth at which emergence is reduced by 50% and Erate: emergence rate or the slope of the curve.

3. Results

Seed Germination under Different Conditions

Light and Temperature

Preliminary experiments (data not shown) demonstrated that the freshly harvested mature seeds of *P. divaricata* exhibited dormancy. Germination of seeds from three ecotypes of *P. divaricata* (Islamabade-Gharb, Sarpole-Zahab and Kermanshah) was studied over a series of constant and alternating temperatures under dark and light/ dark regimes. Seeds collected from Sarpole-Zahab with mean germination percentage of 15.77 showed the highest percentage of seed germination (Fig. 2).

In general, for all temperature regimes light had stimulatory effect on seed germination of *P. divaricata* and its effect was more obvious at the lower temperatures (Fig. 2).

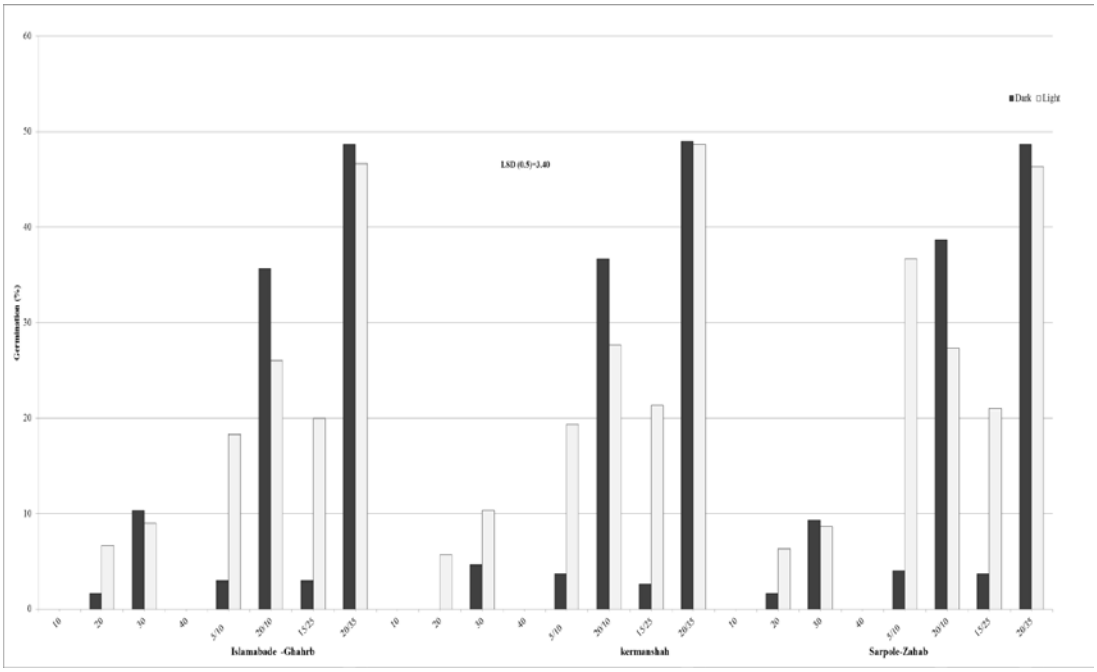


Figure 2 Seed germination of *P. divaricata* collected from different locations as influenced by different light and temperature regimes

Moreover, seeds exposure to a cycle of 8 h dark/ 16 h light enhanced germination percentage by 4.15 compared with the continuous darkness (Fig. 2). Different ecotypes responded to light regime identically so that under light/dark cycle germination percentage of all ecotypes was higher than that under complete darkness (3.04, 4.38 and 5.04%, respectively) (Fig. 2).

Burial Depth

Emergence of *P. divaricata* decreased with an increase in burial depth. At Day 14 After Burying (DAB), the higher emergence percentages (75 and 55) were recorded for the burial depth of 0 and 1 cm, respectively (Fig. 3).

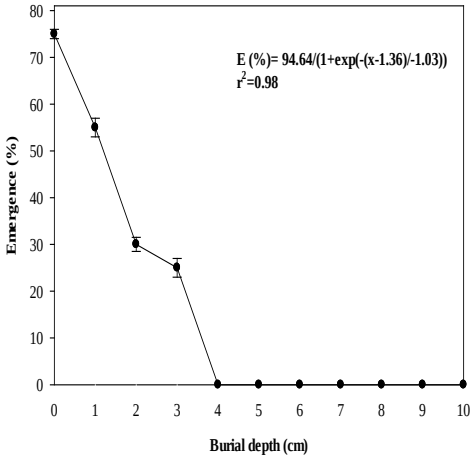


Figure 3 .Effect of different seed burial depths on seedling emergence of *P. divaricata.*

Salinity Stress

A three-parameter logistic model described well the relationship between germination of *P. divaricata* and salt concentration (Fig. 4). The germination occurred over a wide range of the salt concentration (from 0 to near 180 mM NaCl). A significant decrease in seed germination was shown with the increase in salt concentration (seed germination of 76% at the 0 mM to 0% at the 180 mM) (Fig. 4).

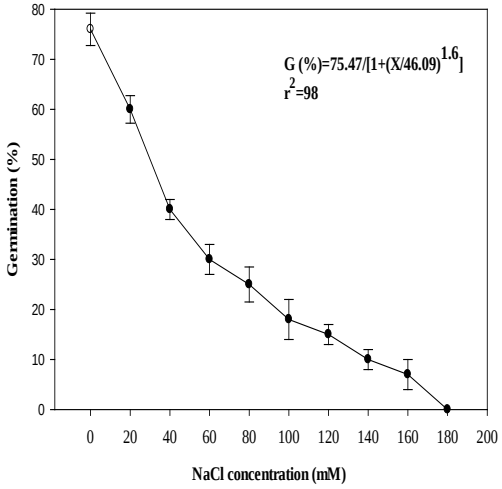


Figure 4. Seed germination of *P. divaricata* as influenced by different NaCl concentrations.Vertical bars represent standard error of the mean.

At the lowest concentration of NaCl (10 mM), seed germination of *P. divaricata* was reduced by 8.1% compared with the control (the 0% of salt concentration). However, *P. divaricata* seed germination was more than 40% at NaCl concentrations up to 40 mM. Even at 100 mM NaCl, seed germination was 18% and it reached 10% at140 mM (Fig. 4).

Osmotic Stress

A functional three-parameter logistic model was fitted to the germination data (%) obtained under different osmotic levels (Fig. 5). Germination decreased as osmotic stress level was increased (Fig. 5). Significantly, the greater number of seeds (80%) germinated in the control treatment (distilled water) when compared with other osmotic treatments (Fig. 5). Germination rapidly decreased when osmotic potential reached -0.5 MPa. Very limited number of seeds could germinate at osmotic potentials greater than -0.8 MPa and above -1.0 MPa, no germination occurred.

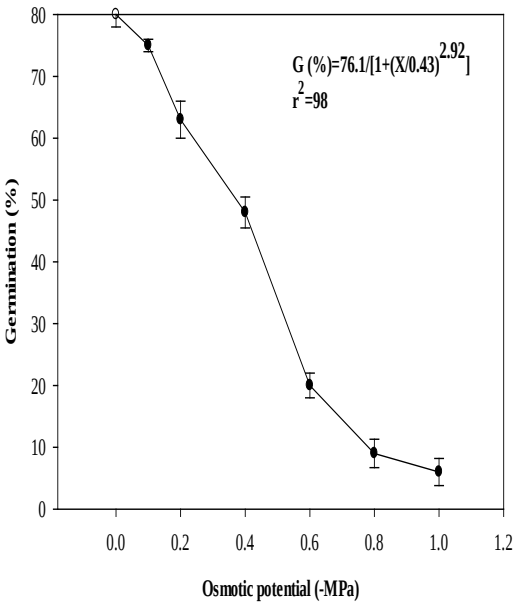


Figure 5 Seed germination of *P. divaricata* as influenced by different osmotic stress levels

pH

P. divaricata seeds showed a germination value more than 44% over a pH range of 4 to 10 (Fig. 5). Maximum and minimum germination percentages were recorded at pH values of 4 and 10 (44.7 and 86 %), respectively. Seed germination increased linearly in response to increasing pH (y=5.86X+25.29, R2=95). In general, *P. divaricata* seeds notably performed better in alkaline pHs, as the higher germination percentage were recorded at the pH levels above 7 with a maximum value at pH 10 (Fig. 6). Acidic condition (pH values lower than 7) significantly decreased *P. divaricata* germination.

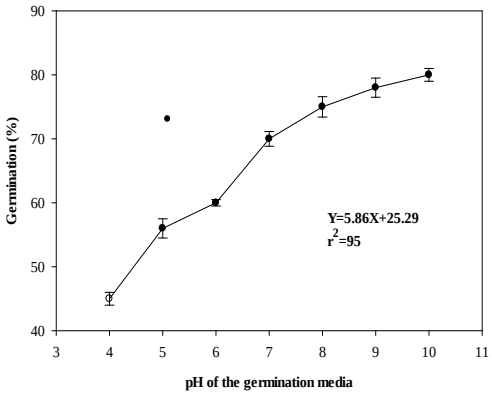
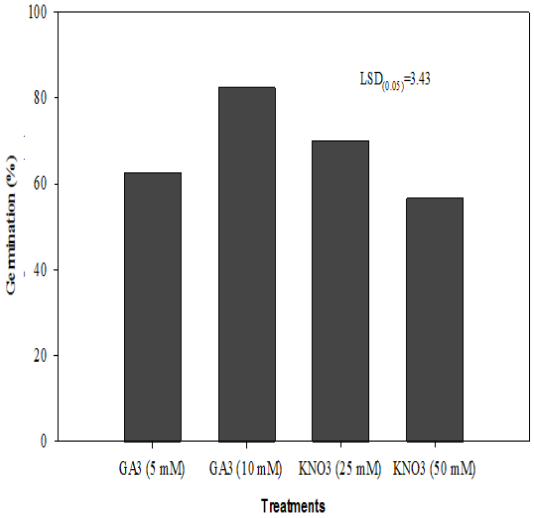


Figure 6 . Seed germination of *P. divaricata* as influenced by different pH levels.Vertical bars represent standard error of the mean

KNO3 and GA3

KNO3 at concentration of 25 and 50 mM stimulated germination to a maximum level of 70.22 and 56.70 %, respectively (Fig. 7). In general, germination declined with concentrations of KNO3 greater than 50 mM (data not shown). Overall, germination increased when GA3was added to the germination solution (Fig. 7). Addition of 10 mM of GA3 increased seed germination by 82.43% relative to the control.



Figuer 7. Effect of GA_3 and KNO_3 incubation on Germination percentage of *P. divaricata.*

4. Discussion

There was statistically substantial difference between seed germination (%) of ecotypes collected from Kermanshah and Islamabade-Gharb ecotypes with that of Sarpole-Zahab. In opposition to our results, an earlier work conducted on seed dormancy and germination of 44 ecotypes of Johnson grass [*Sorghum halepense* (L.) Pers.] by Taylorson and McWhorter (1969) indicated a negligible difference among the different ecotypes. Similar to our results, several works have demonstrated that seed germination varies greatly among ecotypes of different species (Meyer & Monsen, 1991; Galloway, 2002; Pita Villamil *et al.*, 2002). As a general rule, the conditions under which seeds mature on the mother plant can determine the level of seed dormancy (Meyer & Allen, 1999; Baskin & Baskin, 2014). The most important factor in this regard is temperature (Sharif-Zadeh & Murdoch, 2000). Consistent with our results, seeds produced by plants grown at higher temperatures have a lower dormancy level than those produced at lower temperatures. It is probably due to the fact that the environmental conditions can affect seed germination by affecting their chemical composition and seed provisioning (Galloway, 2002). Sarpole-Zahab is located in a warmer climatic condition relative to Kermanshah and Islamabad and *P. divaricata* seeds collected from this region had lower dormancy level which could be attributed to its warmer weather conditions.

Generally, light had a stimulatory effect on the seed germination of *P. divaricata*. Similar results obtained by De Cauwer et al. (2014) who demonstrated that Galinsoga spp. seeds required light for germination as well as light dependency varied among populations. Likewise, Malik et al. (2010) reported that wild radish (*Raphanus raphanistrum* L.) requires light for optimum seed germination. However, the study conducted by Del Monte and Dorado (2011) revealed the negative effect of light on seed germination in great brome (Bromus diandrus Roth.). Stimulatory and inhibitory effects of light on seed germination have also been reported for licorice weed (Scopa riadulcis L.) (Jain & Singh, 1989) and wild oat (Avena fatua L.), respectively (Sharma & Born, 1978). Previous studies demonstrated that seed germination of nightshade species, which have close botanical relationships with *P. divaricata*, was not sensitive to light (Taab & Andersson, 2009; Stanton *et al.*, 2012). This is in contrast to seed germination of P. divaricata, which was significantly enhanced by light. The light, either by the phytochrome system or by altering the balance of germination promoters and inhibitors in the embryo, may affect germination of P. divaricata. Both mechanisms could guarantee that germination occurs far away from other plants and close to the soil surface (Schutte *et al.*, 2014). The seeds of *P. divaricata* germinated over a relatively wide range of temperatures. The greatest germination occurred under the alternating temperature of 20/35°C, irrespective of lightness regime. Among the constant temperature treatment, the seeds of *P. divaricata* failed to germinate at the 10 and 40°C. It is well documented in several very old and recent studies that fluctuating temperatures greatly improve seed germination of different weed species (Morinaga, 1926; Ghersa *et al.*, 1992; Lee *et al.*, 2011; Wu *et al.*, 2015). The higher seed germination of *P. divaricata* at temperature regime of 20/35°C can be a major component of the mechanism that enables its seeds to detect soil depth. Usually, in a soil profile the most temperature fluctuation occurs in the upper layers of soil and it diminishes in response to increasing soil depth. Therefore, the *P.*

divaricata seeds located in the upper layers have more chance for germination and seedling formation. This can be explained by the relatively small size of *P. divaricata* seeds. Commander et al. (2008) also reported that alternating temperatures improved the germination of a range of Solanum species which their seed size is almost similar to that of P. divaricata.

The emergence of *P. divaricata* decreased sharply when seeds were buried deeper than 3-cm. This might be due to the lack of the seed germination stimulants such as light and fluctuating temperatures at the deeper layers of a soil profile. Diminishing seedling emergence with increasing burial depth has also been reported in other weed species (Prostko *et al.,* 1998, Sabila *et al.,* 2012; Schutte *et al.,* 2014). It can be concluded that *P. divaricata* germinates predominately near the soil surface, with a few seedlings emerging from the seeds buried beyond 3 cm. This confirms that *P. divaricata* seeds can germinate better at the presence of light and fluctuating temperatures as shown in the present study. These conditions usually exist at the surface layers of soil. *P. divaricata* emergence drastically reduced at the soil depth more than 3 cm and reached zero at the 4 cm. A practical finding is that burying seeds through tillage may effectively reduce the *P. divaricata* infestation in crop fields. This can explain the reason why this weed species is more dominant in less disturbed habitats such as gardens, roadside and edge of tilled fields in western Iran (personal observation).

The salt concentration required to inhibit the germination of *P. divaricata* was 250 mM. This suggests that *P. divaricata* can tolerate some levels of salt stress and even at the salinity levels up to 180 mM a portion of its seeds may still germinate. It is concluded that this weed species can pose a serious invasion threat for habitats of salty soil. This conclusion is strongly supported by the fact that the farms, located in the western Iran having a more or less saline soil, are heavily infested by *P. divaricata*. Further studies are needed to dissect the mechanisms involved in salt tolerance of P. divaricata. The seeds of other weed species, such as rigid rye grass (Lolium rigidum Gaudin) and common reed (Phragmites australis (Cav.) Trin ex Steudel), have shown some level of tolerance to salinity as they germinated by 50% at the 40 mM of NaCl (Chauhan *et al.,* 2006c). Chauhan et al. (2006a) also reported a germination percentage of 7% for the annual sow thistle (Sonchus oleraceus L.) (a common weed species in western Iran) at salt concentration of 160 mM.

Very limited number of seeds could germinate at osmotic potentials more than -0.8 MPa. This shows that *P. divaricata* can tolerate low water potentials and germinate under very dry and intense water stress conditions. Other weed species have shown varying sensitivity to water stress. Reddy and Singh (1992) reported that the germination of hairy beggar ticks (*Bidens pilosa* L.) decreased linearly with increasing osmotic stress. In another study, hemp dogbane (*Apocynum cannabinum* L.) germination rapidly decreased at osmotic potentials below -0.25 MPa (Webster & Cardina, 1999). However, tolerance to severe water stress conditions has been reported in some weed species, such as turnip weed (*Rapistrum rugosum* (L.) All.) and members of the Brassicaceae family (Ray *et al.,* 2005). It is worth noting that turnip weed and Brassicaceae family are dominant weed species in western parts of Iran, especially in Kermanshah province, which is also the habitat for P. divaricata. Therefore, under low soil water potentials and not dryland farming, *P. divaricata* is expected to grow well and compete vigorously with the irrigated summer crops.

Acidic conditions (pH values lower than 7) significantly decreased seed germination of P. divaricata. Oliveira and Norsworthy (2006) reported that optimal germination of other weedy members of solanaceae family was at pH 6 to 8. Our findings are compatible with those obtained by Ahmadi (1999), based on the fact that most of the soils infested by *P. divaricata* are alkaline. This compatibility can intensify the invasion potential of *P. divaricata* in western regions of Iran, the regions with soil pH higher than 7. However, the results of the present study cannot be fully conclusive and further research needs to be conducted to assess the impact of pH on seed germination of this weed under soil conditions.

Generally, germination increased when GA3 and KNO3 were added to the germination media. GA3 is known to reduce the adverse effects of germination inhibitors (Zhou *et al.,* 2005). The increase of seed germination by GA3 has been demonstrated in other weed species. This is because GA has an important role in the mobilization of food reserves in the seeds to nourish the growing embryo (Toyomasu, 1993). Nevertheless, results revealed that light was a stimulatory factor of seed germination of *P. divaricata*. Therefore, this response is likely to be due to the breakage of physiological dormancy in the seeds of this species. It is well documented that KNO3 and GA3 can overcome seed dormancy (Fawcett & Slife, 1978; Foley & Chao, 2008). From the results of this experiment, it could be argued that the application of N-containing fertilizers may stimulate the germination of *P. divaricata* seeds under field conditions. This agronomic operation is common across infested areas with P. divaricata. Bouwmeester and Karssen (1993) also reported stimulation in seed germination of hedge mustard (*Sisymbrium officinale* L.) by the addition of nitrate.

5. Conclusion

The simple and most important result of the present study is that seeds of *P. divaricata* have innate dormancy and this dormancy could be alleviated by some seed dormancy breaking factors. This is contrary to the results reported by Mousavi

and Ahmadi (2008) who suggested that the reason of seed dormancy of *P. divaricata* was the mucilage content of the berries, which inhibits seed germination. Therefore, washing out mucilage by normal irrigation and by rain will result in a high germination percentage. In addition, the berries are decomposed during autumn and winter seasons, which eventually lead to a dormancy breakage and a seed germination in the following season.

As it is well demonstrated, the survival of weed seeds in the soil is important, since potential weed problems exist as long as weed seeds remain alive in the soil (Schutte *et al.,* 2014). The survival of weed seeds during log time surely could not depend only on mucilage content of its fruits, because they cannot be eradicated from the field easily by irrigation or by destroying the emerging seedlings. Seed dormancy ensures that the seeds do not germinate under closed canopies and in very deep depths of soil profile by detecting light quality and temperature fluctuations (Ghersa *et al.,* 1992).

In conclusion, *P. divaricata* is an emerging problem in summer crops in the western parts of Iran and this problem is exacerbated by seed dormancy and the absence of an efficient herbicide-based management system. Further research is required to elucidate the details of *P. divaricata* seed germination, especially those related to the effect of real soil conditions. However, the present study provides preliminary information on the effect of some factors on seed dormancy germination. Long-term studies are needed to determine the impact of management and climatic factors on the persistence of *P. divaricata* seed banks. This important information is required for the development of management strategies for this weed species.

References

AHMADI H. (1999). *Applied Geomorphology (Wind erosion)*. University of Tehran Press.

ALAM JN, ALIZADE H AND HR MASHHADI. 2011. FREQUENCY, DAMAGE AND COMPARATIVE PHONOLOGY OF ANNUAL GROUND CHERRY (PHYSALIS DIVARICATA L.) WEED IN SUGAR BEET FIELDS .NOT SCI BIOL, **3(2)**:129-134

ALAM J N,B NASIRI, AMIRI H AND MITRA KHOSRAVI SIMA JORABI. 2013. BIOLOGY OF ANNUAL GROUND CHERRY (PHYSALIS DIVARICATA L.) WEED IN LORESTAN, IRAN. INTL J FARM & ALLI SCI, **6**:2322-4134

BASKIN CC AND BASKIN JM .2014. *Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination*. In: BOUWMEESTER HJ & KARSSEN CM (Eds.)Elsevier Science.

Annual Changes in Dormancy and Germination in Seeds of *Sisymbrium officinale* (L.) Scop. *New Phytologist* **124**, pp. 179-191.

BOYD N AND VAN ACKER R. 2004. Seed germination of common weed species as affected by oxygen concentration, light, and osmotic potential. *Weed Sci,* **52**: 589-596.

BOYD NS AND HUGHES A .2011. Germination and Emergence Characteristics of Spreading Dogbane (*Apocynum androsaemifolium*). *Weed Sci,* **59**: 533-537.

CHAUHAN BS, GILL G AND PRESTON C .2006. Factors affecting seed germination of annual sowthistle (*Sonchus oleraceus*) in southern Australia. *Weed Sci,* **54**: 854-860.

CHAUHAN BS, GILL G AND PRESTON C .2006. Factors affecting turnipweed (*Rapistrum rugosum*) seed germination in southern Australia. *Weed Sci,* **54**:1032-1036.

CHAUHAN BS, GILL G AND PRESTON C .2006. Seedling recruitment pattern and depth of recruitment of 10 weed species in minimum tillage and no-till seeding systems. *Weed Sci***,54**:658-668.

CHEN GQ, GUO SL AND HUANG QS .2009. Invasiveness evaluation of fireweed (*Crassocephalum crepidioides*) based on its seed germination features. *Weed Biol. Manag,* **9**: 123-128.

COMMANDER LE, MERRITT DJ, ROKICH DP, FLEMATTI GR AND DIXON KW .2008. Seed germination of Solanum spp. (Solanaceae) for use in rehabilitation and commercial industries. *Aust J Bot,* **56**: 333-341.

DE CAUWER B, DEVOS R, CLAERHOUT S, BULCKE R AND REHEUL D.2014. Seed dormancy, germination, emergence and seed longevity in *Galinsoga parviflora* and *G.quadriradiata*. *Weed Res,* **54**: 38-47.

DEFELICE MS .2003.The Black Nightshades, *Solanum nigrum* L. et al.—Poison, Poultice, and Pie. *Weed Technol,* **17**: 421-427.

DEL MONTE JP AND DORADO J .2011. Effects of light conditions and after-ripening time on seed dormancy loss of Bromus diandrus Roth. *Weed Res***, 51**: 581-590.

FANDRICH L AND MALLORY-SMITH C .2005.Temperature Effects on Jointed Goatgrass (*Aegilops cylindrica*) Seed Germination. *Weed Sci***, 53**: 594-599.

FAWCETT RS AND SLIFE FW .1978. Effects of Field Applications of Nitrate on Weed Seed Germination and Dormancy. *Weed Sci,* **26**: 594-596.

FOLEY ME .2008. Temperature and Moisture Status Affect Afterripening of Leafy Spurge (*Euphorbia Esula*) Seeds. *Weed Sci,* **56**: 237-243.

FOLEY ME AND CHAO WS .2008. Growth Regulators and Chemicals Stimulate Germination of Leafy Spurge (*Euphorbia esula*) Seeds. *Weed Sci***,56**: 516-522.

GALLOWAY LF .2002. The effect of maternal phenology on offspring characters in the herbaceous plant Campanula americana. *Ecol,* **90**: 851-858.

GHERSA CM, ARNOLD RLB AND MARTINEZ-GHERSA MA .1992. The Role of Fluctuating Temperatures in Germination and Establishment of Sorghum halepense. Regulation of Germination at Increasing Depths. *Funct Ecol,* **6**: 460-468.

GHERSA CM AND HOLT JS .1995. Using phenology prediction in weed management: a review. *Weed Res,* **35**: 461-470.

GORSKI SF AND WERTZ MK .1987. Tomato (*Lycopersicon esculentum*) and Eastern Black Nightshade (*Solanum ptycanthum*) Tolerance to Aciflurofen. *Weed Technol***, 1**: 278-281.

HUEBNER CD.2011. Seed Mass, Viability, and Germination of Japanese Stiltgrass (*Microstegium*

vimineum) under Variable Light and Moisture Conditions. *Invasive Plant Sci Manag* **,4**: 274-283.

JAIN R AND SINGH M.1989. Factors Affecting Goatweed (*Scoparia dulcis*) Seed Germination. *Weed Sci***, 37**: 766-770.

LEE J, CHAUHAN BS AND JOHNSON DE .2011.Germination of Fresh Horse Purslane (*Trianthema portulacastrum*) Seeds in Response to Different Environmental Factors. *Weed Sci*, **59**: 495-499.

MALIK MS, NORSWORTHY JK, RILEY MB AND BRIDGES W .2010. Temperature and Light Requirements for Wild Radish (*Raphanus raphanistrum*) Germination over a 12-Month Period following Maturation. *Weed Sci*, **58**: 136-140.

MEYER SE AND ALLEN PS .1999. Ecological Genetics of Seed Germination Regulation in Bromus tectorum L.. II. Reaction Norms in Response to a Water Stress Gradient Imposed during Seed Maturation. *Oecol***, 120**: 35-43.

MEYER SE AND MONSEN SB.1991.Habitat-Correlated Variation in Mountain Big Sagebrush (*Artemisia Tridentata* SSP. Vaseyana) Seed Germination Patterns. *Ecol***, 72**:739-742.

MORINAGA T .1926. Effect of Alternating Temperatures Upon the Germination of Seeds. *Am J Bot***, 13**: 141-158.

MOUSAVI S AND AHMADI A .2008. Environment factor effect on germination of seeds of ground cherry weed (in Persain with English abstract). *Plant path*,**76**: 10 – 30.

NORSWORTHY JK AND OLIVEIRA MJ .2006. Sicklepod (*Senna obtusifolia*) germination and emergence as affected by environmental factors and seeding depth. *Weed Sci*, **54**:903-909.

OLIVEIRA MJ AND NORSWORTHY JK .2006. Pitted morningglory (*Ipomoea lacunosa*) germination and emergence as affected by environmental factors and seeding depth. *Weed Sci*, **54**: 910-916.

PENNY GM AND NEAL JC .2003. Light, Temperature, Seed Burial, and Mulch Effects on Mulberry Weed (*Fatoua villosa*) Seed Germination. *Weed Technol*, **17**: 213-218.

PITA VILLAMIL JM, PÉREZ-GARCÍA F AND MARTÍNEZ-LABORDE JB .2002. Time of seed collection and germination in rocket, Erucavesicaria (L.) Cav. (Brassicaceae). *Genet. Resour. Crop Evol***, 49**: 47-51.

PROSTKO EP, WU H-I AND CHANDLER JM .1998. Modeling Seedling Johnsongrass (*Sorghum halepense*) Emergence as Influenced by Temperature and Burial Depth. *Weed Sci*, **46**: 549-554.

RAY J, CREAMER R, SCHROEDER J & MURRAY L .2005. Moisture and temperature requirements for London rocket (*Sisymbrium irio*) emergence. *Weed Sci*, **53**: 187-192.

REDDY KN & SINGH M .1992. Germination and Emergence of Hairy Beggarticks (*Bidens pilosa*). *Weed Sci*, **40**: 195-199.

SABILA MH, GREY TL, WEBSTER TM, VENCILL WK & SHILLING DG .2012. Evaluation of Factors That Influence Benghal Dayflower (*Commelina benghalensis*) Seed Germination and Emergence. *Weed Sci*, **60**: 75-80.

SCHUTTE BJ, TOMASEK BJ, DAVIS AS et al. 2014. An investigation to enhance understanding of the stimulation of weed seedling emergence by soil disturbance. *Weed Res*, **54**: 1-12.

SHARIF-ZADEH F AND MURDOCH AJ .2000. The effects of different maturation conditions on seed dormancy and germination of Cenchrus ciliaris. *Seed Sci Res*, **10**:447-457.

SHARMA MP AND BORN WHV .1978. The Biology of Canadian Weeds.: 27. AVENA FATUA L. *CAN J PLANT SCI*, **58**: 141-157.

STANTON R, WU H AND LEMERLE D .2012. Factors Affecting Silverleaf Nightshade (*Solanum elaeagnifolium*) Germination. *Weed Sci*, **60**: 42-47.

TAAB A AND ANDERSSON L .2009. Primary Dormancy and Seedling Emergence of Black Nightshade (Solanum nigrum) and Hairy Nightshade (*Solanum physalifolium*). *Weed Sci*, **57**:526-532.

TAYLORSON RB AND MCWHORTER CG .1969. Seed Dormancy and Germination in Ecotypes of Johnsongrass. *Weed Sci*, **17**: 359-361.

Toyomasu, T, Tsuji, H, Yamane, H, Nakayama, M, Yamaguchi, I, Murofushi, N, Takahashi, N and Inoue, Y .1993. Light effects on endogenous levels of gibberellins in photoblastic lettuce seeds. *Plant Growth Regul*, **12**: 85-90**.**

WEBSTER TM AND CARDINA J .1999. *Apocynum cannabinum* Seed Germination and Vegetative Shoot Emergence. *Weed Sci*, **47**: 524-528.

WILSON DG, BURTON MG, SPEARS JF AND YORK AC .2006. Doveweed (*Murdannia nudiflora*) germination and emergence as affected by temperature and seed burial depth. *Weed Sci*, **54**: 1000-1003.

WU X, LI J, XU H AND DONG L .2015. Factors Affecting Seed Germination and Seedling Emergence of Asia Minor Bluegrass (*Polypogon fugax*). *Weed Sci***, 63**: 440-447.

ZHOU J, DECKARD EL AND MESSERSMITH CG .2005. Factors Affecting Eastern Black Nightshade (*Solanum ptycanthum*) Seed Germination. *Weed Sci*, **53**: 651-656.

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