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Full Length Research Paper

Performances of elite amaranth genotypes in grain and leaf yields in Northern Tanzania

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Amaranth is one of the most commonly produced and consumed indigenous vegetables on the African continent. In Tanzania amaranth constitutes about 5.3% of total vegetable hectarage planted annually. Most cultivated varieties of amaranth are landraces with relatively poor leaf and grain yield. This study was conducted to identify genotypes with potential for dual purpose (leaf and grain) use for promotion or further cultivar development. An experiment was carried out in two seasons at AVRDC - The World Vegetable Center in Arusha, Tanzania from Feb to May and June to Sep 2012. Fourteen genotypes were used in a randomized complete block design. Results indicated that leaf yield differed significantly among the genotypes in both trial 1 ($p \leq 0.01$) and 2 ($p \leq 0.05$). The highest leaf yields were obtained in genotypes RVI00117 (32.8 t/ha) and RVI00002 (14 t/ha) in trial 1 and 2, respectively. The lowest leaf yields were obtained from genotypes RVI00121 and RV00090 (4 and 6.3 t/ha) in trials 1 and 2, respectively. There were significant differences ($p \leq 0.001$) among genotypes for grain yield obtained after leaf harvesting. Genotype RVI00022 had the highest seed yield (1971.3 kg/ha) over the two seasons. Where leaf was not harvested, genotype RVI00121 had the highest seed yield (2920 kg/ha) over the two seasons. From this study, we recommend genotypes RVI00121 and RVI00001 for grain production. For dual purpose use, we recommend RVI00007 during warm and wet conditions and RVI00022 during cool and dry condition.

Key words: Amaranth, leaf yield, seed yield, genotype performance,

INTRODUCTION

Amaranth (*Amaranthus* spp.), a C_4 plant, is extensively grown as a green leafy vegetable and for its grain in many tropical countries in Africa, Central and Southern America, Mexico and parts of Asia (DAFF, 2010). It is one of the oldest food crops in the world; evidence of its cultivation is dating back 6700 BC (Itúrbide and Gispert,

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1994; DAFF, 2010). The crop is one of few plant species whose leaves are eaten as a vegetable and can also be grown for their seeds. This is the case of some introduced varieties of American origin (Wu et al., 2000). Grain amaranth is not commonly cultivated in Africa (Grubben and Denton, 2004). However recently, a few farmers have taken the growing of grain amaranth more seriously and are supplying millers and supermarkets in Zimbabwe, Kenya, Uganda and Ethiopia (Achigan-Dako et al., 2014). The genus *Amaranthus* consists over 60 species, several of which are cultivated as leaf vegetables, grains, or ornamental plants, while others are considered weeds (Maboko, 1999; DAFF, 2010). However, the majority of the species grown for vegetables are represented by *Amaranthus dubius, A. lividus,* and *A. hybridus* (Mlakar et al., 2010). Three principal species most considered for grain include, *A. hypochondriacus, A. cruentus* and *A. caudatus* (Teutonico and Knorr, 1985; Muyonga et al., 2008; Mlakar et al., 2010).

Amaranth is one of the most commonly produced and consumed indigenous vegetables on the African continent (Grubben and Denton, 2004). It is extensively grown as a green leaf vegetable in many tropical countries in Africa like Tanzania, Benin, Togo, Sierra Leone, DR Congo and Kenya. It is also common in tropical areas outside Africa like in India, Bangladesh, Sri Lanka and Caribbean (Grubben and Denton, 2004). Of the more than 78,000 ha of vegetables planted annually in Tanzania, amaranth constitutes about 5.3% (National Bureau of Statistics, 2012). A study by Keller (2004) indicates that amaranth is an important traditional leafy vegetable in northeast Tanzania, listed first in the top five vegetables grown in the region.

The combination of its anatomical features and its C_4 metabolism might have contributed to its wide geographical adaptation under diverse environmental conditions (Stallknecht and Schulz-Schaeffer, 1993; Kaul et al., 1996). Amaranth is an annual crop that grows rapidly and is harvested within 3 to 4 weeks after sowing for leaves, while the grain can be harvested 60 to 90 days. The crop is tolerant to common vegetable insect pest and less labour-demanding (Maundu et al., 2009). There is no distinct separation between the vegetable and grain types, except black grains are not preferred by most farmers and consumers. Leaves of young plants grown for grain are used not only for human consumption but also used as animal feed, in South America, Africa, Asia and Eastern Europe (Kaul et al., 1996; Muyonga et al., 2008). Amaranth leaf can be used as greens in salads, boiled or fried in oil and mixed with meat or fish. Cooked greens can be used as side dish in soups or as an ingredient in sauce and baby food (Mlakar et al., 2010). The grain of amaranth can also be used in numerous recipes ranging from popped amaranth snack, porridge, stiff porridge, chapatti (flat bread), bread, creamy soup, pancakes, cakes, scones, pizza, etc.

Amaranth leaves are rich in vitamins A (2917 IU) and vitamin C (43.5 mg), while both leaves and grains contain, iron (2.32 mg; 2.1 mg), calcium (215 mg; 47 mg), potassium (611 mg; 135 mg), phosphorus (148 mg; 50 mg) and protein (2.46 g; 3.8 g), respectively. All of these are essential nutrients lacking in most people's diets.

Despite its positive agronomic and nutritional characteristics, the majority of cultivated genotypes of amaranth in Africa including Tanzania are low yielding relative to their potential of up to 40 tons and 600 kg per ha for leaf and grain, respectively (Svirskis, 2003; Moinester, 2007). Only a few improved varieties are available as a result of which the majority of farmers grow their local cultivars. Studies for both leaf and grain yield and its contributing quantitative and qualitative traits are scarce (Shukla et al., 2006). However, there are a number of germplasm collections available in AVRDC genebank for evaluation and direct release and/or use in breeding programs. Harvest of leaves and grain from the same plant (dual-purpose) allows smallholder farmers to exploit the full nutritional benefits of amaranth. Therefore, the current study was conducted to identify dual purpose (leaf and grain) amaranth genotype for possible release as new varieties or further cultivar enhancement.

MATERIALS AND METHODS

Genetic materials and experimental design

A total of 14 amaranth lines were evaluated on-station at AVRDC - The World Vegetable Center, Regional Center for Africa (AVRDC-RCA), Arusha, Tanzania (Table 1). Materials selected were based on suitability for using in grain and leaf such as grain colour (brown or cream). The materials were evaluated for leaf and grain yields in two trials. In trial 1, plants were evaluated for both leaf and grain yields. Side leaves were continuously harvested/picked weekly allowing the plant to flower and give grain. In trial 2, the genotypes were grown for grain yield evaluation without leaf harvesting. The experiments were conducted in 2012 in two seasons, first season (Feb - May) and second season (May - Sep). The trials were laid out in randomized complete block design (RCBD) with three replications in a plot size of two rows at 60 cm spacing between rows and 25 cm between plants; there were 24 plants per row.

Experimental location

The trial site, AVRDC-RCA's research station, is located in Arusha, Tanzania at 1290 m a.sl, and 4.8° N latitude and 37° E longitude. The site has clay loam soil with a pH ranging 6.0 to 6.7. The average temperature during the first season (Feb - May) was 25.1°C with a mean daily maximum of 28.5°C and daily minimum of 20.5°C, while the average during the second season (May - Sep) was 24.3°C with 26.1 and 21°C mean daily maximum and minimum, respectively. The location has bimodal rainfall with the main rainfall occurring from Feb to Jun and the short rain from Sep to Dec. The total amount of rainfall received during the first and the second season was 322.1 and 32.7 mm, respectively. Average relative humidity in the first and second season was 86.3 and 80.8%, respectively.

Table 1. Entry, genotype and origins of amaranth genotypes used in experiments at AVRDC-RCA Arusha, Tanzania February to May and May to September 2012.

Entry	Genotype code	Genebank collection name	Origin
1	RVI00007	AH-TL	Tanzania
2	RVI00130	HTT	Kenya
3	RVI00089	MELANGE	Madagascar
4	RVI00138	BRESIL	Madagascar
5	RVI00090	PARIS (A)	Madagascar
6	RVI00116	DB 2006306	USA
7	RVI00002	IP-5	Zambia
8	RVI00001	AM-25	Uganda
9	RVI00117	SIMON FARM	Sudan
10	RVI00022	TZSMN 102	Tanzania
11	INCA	INCA	-
12	RVI00086	RED INFLORESCENCE	Sudan
13	RVI00121	AH-NL	Tanzania
14	RVI00021	TZSMN 82	Tanzania

General agronomic practices

Land was ploughed and harrowed by tractor, and ridges were made manually by hand hoe. Seeds were sown directly at the rate of 1 kg per ha by drilling after mixing with sand in 1:4 seed to sand ratio. Seed was sown on the 7th Feb in 1st season for both trial 1 and 2, and 29th May 2012 in the 2nd season for trial 1 and 2. Thinning was carried out twice at 14 and 22 days after sowing (DAS) leaving a spacing of 25 cm between plants and a total of 24 plants per row. Fertilizer was applied at the rate of 200 kg/ha Diammonium phosphate (DAP) 18:46:0 as a basal application at sowing, and at 120 kg/ha urea (46:0:0) as side-dressing in two split applications, 60 kg/ha each, at two and six weeks after sowing. Selecron® (a.i. profenofos 720 g/l EC) was used to control cutworm and whiteflies at the rate of 1 ml/l of water while Actellic® (a.i. pirimiphos-methyl, 1.5 ml/l) was used to control, aphids and caterpillars twice at 14 and 42 DAS. Folicur (a.i. Tebuconazole 430 g/l) at the rate of 1 ml/l and Ridomil (a.i. Metalaxyl-M) at 3 g/l of water were used to control dumping off once at 7 DAS. Weed was controlled by hand-hoeing at 2-weeks interval starting 14 days after germination, but the frequency reduced as the plants grew forming canopy. Furrow irrigation was used to supplement rainfall.

Data collection

Data collected in trial-1 (experiment with leaf harvesting) included leaf yield, number of leaf harvested per plant, leaf length and width, number of branches per plant, days to 50% flowering, plant height and grain yield. Grain yield was measured in trial-2 (the experiment without leaf harvesting) to see the potential of the genotypes in grain yield when grown without leaf harvested. The first leaf harvesting per plot was started 6 weeks after sowing and continued at bi-weekly interval until a total of 4 harvests in the first season and 3 harvests in the second season. The leaf harvesting was done by plucking off tender leaves without topping. Fresh leaf weight was measured immediately using a kitchen balance (model Globe Brand; Globe Food Equipment Company Dayton, Ohio, USA). At each harvest, number of leaves harvested per plot was counted. Leaf length and width (cm), number of branches per plant, and plant height (cm) at flowering stage were measured on 10 plants randomly selected per plot. In the experiment without leaf harvesting, the materials were allowed to flower and give grain without any disturbance. Grain yield harvesting in both experiments was conducted when inflorescence colour had turned yellow. Plants were cut and threshed and clean grains were put in net bags and dried on seed drier (locally made with air blowing by fan under neath) to 6.5% moisture content before weighing using an electronic balance.

Data analysis

Data collected were subjected to both individual and combined analyses of variances (ANOVA) using CoStat version 6.204 (CoHort Software, CA, USA). Correlation analysis was performed to see the association among the various parameters.

RESULTS

Genotype (G) by Season (S) interactions were significant for leaf yield per plant, leaf yield per ha, number of leaves per plant, number of branches per plant, days to 50% flowering and plant height.

Leaf yield

The best leaf yielding genotypes in season-1 were not the best in season-2 and vice versa (Table 2). The highest

Table 2. Mean of fresh leaf yields and number of leaves harvested in 14 amaranth genotypes evaluated in leaf harvested experiment for two seasons, Feb-May and May-Sep 2012, AVRDC-RCA, Arusha, Tanzania.

Genotype code	Leaf yield (g plant^{-1})		Leaf yield (t ha^{-1})		No. of leaves harvested per plant	
	S1	S2	S1	S2	S1	S2
RVI00007	299.8^{b}	178.3^{abc}	19.9^{b}	11.9^{abc}	86.6^{bc}	89.1^{bc}
RVI00130	253.4^{b}	120.8^{cd}	16.9^{b}	8.1^{cd}	82.8^{bcd}	69.3^{cd}
RVI00089	251.7^{b}	100.9^{cd}	16.8^{b}	6.7^{cd}	74.2^{bcd}	67.8^{cd}
RVI00138	272.2^{b}	97.7^{d}	18.2^{b}	6.5^{d}	48.8^{cd}	47.1^{de}
RVI00090	273.1^{b}	93.8^{d}	18.2^{b}	6.3^{d}	46.1^{d}	30.3^{e}
RVI00116	273.3^{b}	124.8^{cd}	18.2^{b}	8.3^{cd}	81.4^{bcd}	50.2 ^{de}
RVI00002	314.3^{b}	210.3^{a}	20.9^{b}	14^{a}	91.3^{ab}	154.2^{a}
RVI00001	266.3^{b}	205.1^{ab}	17.8^{b}	13.7^{ab}	90.8^{ab}	117.6^{b}
RVI00117	492.3^{a}	130.1^{bcd}	32.8^{a}	8.7^{bcd}	127.9^{a}	77.6^{cd}
RVI00022	248.2^{b}	168.2^{abcd}	16.5^{b}	11.2^{abcd}	67.9^{bcd}	95.5^{bc}
INCA	273.5^{b}	132.4^{bcd}	18.2^{b}	8.8^{bcd}	90.7^{ab}	89.5^{bc}
RVI00086	305.3^{b}	128.1^{bcd}	20.4^{b}	8.5^{bcd}	83.6^{bcd}	76.5^{cd}
RVI00121	211.1^{b}	165.9^{abcd}	14.1^{b}	11.1^{abcd}	56.4^{bcd}	80.3^{cd}
RVI00021	308.9^{b}	131.5^{bcd}	20.6^{b}	8.8^{bcd}	94.1^{ab}	67.9^{cd}
F-test	**	*	**	*	**	***
Lsd $_{(0.05)}$	95.6	68.4	6.4	4.6	33.9	30.4
CV (%)	19.7	28.7	19.7	28.7	25.2	22.8

ns non-significant; * significant ($p<0.05$); ** highly significant ($p<0.01$); *** highly significant ($p<0.001$). Means within the same column followed by the same letter(s) are not significantly different at 5% probability level based on DMRT.

fresh leaf yield in season-1 was obtained in genotype RVI00117 (32.8 t/ha) followed by genotypes RVI00002 (20.9 t/ha) and RVI00021 (20.6 t/ha). The lowest leaf yield was obtained in genotype RVI00121 (14.1 t/ha). The highest mean leaf yield in season-2 was obtained in genotypes RVI00002 (14 t/ha) and RVI00001 (13.7 t/ha), while the lowest yield was in genotype RVI00090 (6.3 t/ha).

Number of leaf harvested per plant

The differences among the genotypes were significant at $p\leq0.01$ in season-1 and at $p\leq0.001$ in season-2. Genotype RVI00117 had the highest mean leaf number harvested per plant in season-1, while RVI00002 gave the highest in season-2 (Table 2). The lowest mean leaf number harvested per plant in both seasons was in genotype RVI00090.

Number of branches per plant and plant height

The genotypes significantly differed in number of branches per plant at $p\leq0.001$ in season-1 and at $p\leq0.05$ in season-2. Genotype RVI00002 had many number of branches per plant in both seasons (Table 3). On the other hand a few numbers of branches per plant were observed in genotypes RVI00022 in season-1 and in genotype RVI00021 in season-2. Some of the tallest genotypes in season-1 were not the tallest in season-2. RVI00002 and RVI00090 were the tallest genotypes in season-1 while RVI00130 was the tallest in season-2 followed by RVI00001 and RVI00002 (Table 3).

Days to 50% flowering

RVI00007 and RVI00001 were the earliest genotypes in season-1, whereas genotype RVI00130 was the earliest in season-2 (Table 3). The longest number of days to attain 50% flowering in season-1 was recorded in genotypes RVI00090, RVI00116 and RVI00002, while in season-2 the longest number of days was observed in RVI00002.

Grain yield, leaf length and leaf width

Grain yield in both harvested and non-harvested experiments, leaf length and leaf width were three traits for which G×S interactions were non-significant in this

Table 3. Mean days to flowering, plant height and number of branches per plant in 14 amaranth genotypes evaluated in leaf harvested experiment for two seasons, Feb-May and May-Sep 2012, AVRDC-RCA, Arusha, Tanzania

Genotype code	Days to 50% flowering		Plant height (cm)		No. of branches per plant	
	S1	S2	S1	S2	S1	S2
RVI00007	37.3^{b}	48^{cd}	182.9^{abc}	76.8^{abc}	20.9^{bcd}	11.4 ^{b}
RVI00130	40.7^{ab}	42^{e}	150.2^{de}f	85.2^{a}	17.3^{cde}	11.4 ^{b}
RVI00089	42^{ab}	51.7^{c}	171.3^{bcd}	74.8^{abc}	25.1^{ab}	10.7^{b}
RVI00138	43.3^{ab}	59.7^{b}	191.1^{ab}	69.6^{bcd}	22.4^{bc}	11.1^{b}
RVI00090	47.7 ^{a}	61.7^{b}	210^{a}	73.5^{abc}	20.9^{bcd}	11.1^{b}
RVI00116	47.7^{a}	57.7^{b}	142.8^{de}f	50.5^{e}	23.8^{ab}	11.6 ^{b}
RVI00002	46^{a}	76^{a}	211.1^{a}	83^{ab}	29.2^{a}	16.2^{a}
RVI00001	37^{b}	43^{e}	140^{e}f	83.9^{ab}	16de	11.7^{b}
RVI00117	42^{ab}	59.7^{b}	151.9^{de}f	56.9^{de}	20.3^{bcd}	11.1^{b}
RVI00022	40.7^{ab}	45de	126.5f	77.7^{abc}	13.4^{e}	11.6 ^{b}
INCA	37.7^{b}	44de	160^{cde}	79.8^{ab}	16.8^{cde}	12^{b}
RVI00086	45.7^{a}	59.7^{b}	148.7^{de}f	64.9^{cd}	22.2^{bc}	11.8^{b}
RVI00121	35.7^{b}	48^{cd}	181^{bc}	76.8^{abc}	17.3^{cde}	11.6 ^{b}
RVI00021	41^{ab}	43^{e}	140.2^{e}f	70.7^{bc}	15.9^{de}	9.7^{b}
F-test	*	***	***	***	***	*
Lsd $_{(0.05)}$	6.9	4.3	26.5	12.5	5.4	2.6
CV (%)	9.9	4.9	9.6	10.2	16.1	13.3

ns non-significant; * significant (p<0.05); ** highly significant (p<0.01); *** highly significant (p<0.001). Means within the same column followed by the same letter(s) are not significantly different at 5% probability level based on DMRT.

study. Combined analysis of variance indicated that there was a significant difference (p≤0.001) among genotypes in grain yield (Table 4). The highest mean grain yield in non-leaf-harvested experiment was observed in genotype RVI00121 (2921 kg/ha) followed by RVI00022 (1961 kg/ha), whereas the lowest yield was observed in genotype RVI00002 (1085 kg/ha). On the other hand in trial-1, where leaves were harvested, the highest grain yield was recorded in genotype RVI00022 (1971 kg/ha) and RVI00021 (1929 kg/ha). The differences among genotypes in leaf length and width were significant in both seasons. Genotype RVI00086 had the longest leaf, while the shortest leaf was recorded in genotype RVI00116 (Table 4). The broadest leaf was recorded in genotype RVI00138 and the narrowest in genotype RVI00089.

Correlation of yield parameters

Correlation analysis conducted among traits on the average of data of the two seasons indicated that leaf yield per plant had strong positive correlation with number of leaf per plant, while it was not correlated with other traits (Table 5). Grain yield per plant indicated negative correlation with days to 50% flowering and branch number per plant, implying that genotypes with late flowering and few number of branches per plant had low seed yield and vice versa. There was no correlation between grain yield and leaf yield.

DISCUSSION

Leaf and grain yield

The variations in leaf and seed yield between the two seasons might be due to the influence of the growing environment condition. The first season was characterized by warm (mean temp 25.1°C) and wet (322 mm rainfall), while the second season was cool (mean temp 24°C) and dry (32.7mm rainfall) (Figure 1). Warm and wet conditions seems to be optimum for amaranth production since it affects other traits like plant height and number of branches which might affect directly or indirectly leaf and grain yield.

It has been reported that fresh leaf yield of amaranth may vary from 10 to 70 t ha^{-1}, while seed yield ranges from 1 to 6 t ha^{-1} (Svirskis, 2003). Grain yield could go below 1 t/ha. Gupta et al. (1994) reported grain yields of 0.3 t and 0.7 t ha^{-1} under unfavorable and optimum growing conditions in Kenya, respectively. Leaf yield

Table 4. Combined ANOVA for mean grain yields, leaf length and width of 14 amaranth genotypes in leaves harvested and leaves not harvested across two seasons, Feb-May and May –Sep 2012, AVRDC-RCA, Arusha, Tanzania.

Genotype code	Grain yield in leaves not harvested trial		Grain yield in leaves harvested trial		Leaf length	Leaf width
	g/plant	Kg/ha	g/plant	Kg/ha	cm	cm
RVI00007	18.1^{c}	1204.7^{c}	23.9^{ab}	1591^{ab}	18.1^{bcd}	10.1^{abcd}
RVI00130	24.9^{bc}	1659.9^{bc}	24.1^{ab}	1609.5^{ab}	17.8^{cd}	9.1^{cde}
RVI00089	20.1^{bc}	1341.7^{bc}	16^{bcd}	1069.4^{bcd}	17.3^{cd}	7.3f
RVI00138	17.5^{c}	1164.5^{c}	15.2^{cde}	1012.5^{cde}	19.9^{ab}	11.2^{a}
RVI00090	22.8^{bc}	1519.6^{bc}	15.1^{cde}	1006.9^{cde}	19.4^{abc}	10.7^{ab}
RVI00116	17.6^{c}	1170.8^{c}	7.1^{e}f	472.5^{e}f	16.3^{d}	9.4^{cde}
RVI00002	16.3^{c}	1085.3^{c}	6.7f	449.4f	17.4^{cd}	8.5^{e}
RVI00001	29.8^{b}	1988.4^{b}	23.6^{ab}	1572.9^{ab}	18.8^{abc}	8.9^{de}
RVI00117	25.5^{bc}	1702.9^{bc}	15.9^{bcd}	1059.3^{bcd}	18.9^{abc}	9.6^{bcde}
RVI00022	29.4^{b}	1961.1^{b}	29.6^{a}	1971.3^{a}	18.6^{abc}	9.6^{bcde}
INCA	26.8^{bc}	1786.2^{bc}	23.4^{abc}	1557.1^{abc}	17.3^{cd}	8.5^{e}
RVI00086	24.1^{bc}	1606.9^{bc}	10.6^{de}f	707.2^{de}f	20.6^{a}	10.2^{abc}
RVI00121	43.8^{a}	2920.9^{a}	14.8^{de}f	986.6^{de}f	18.2^{bcd}	10^{abcd}
RVI00021	25.4^{bc}	1692.2^{bc}	28.9^{a}	1929^{a}	18.4^{bcd}	9.9^{bcd}
F-test	***	***	***	***	***	***
$Lsd_{(0.05)}$	9.4	623.7	7.4	496.2	1.88	1.1
Seasons						
1	26.5^{a}	1764.1^{a}	20.8^{a}	1387.6^{a}	19.3^{a}	9.6^{a}
2	22.4^{b}	1493.8^{b}	15.6^{b}	1040.3^{b}	17.4^{b}	9.4^{a}
F-test	*	*	***	***	**	ns
S * G	ns	ns	ns	ns	ns	ns
$Lsd_{(0.05)}$	3.5	235.7	2.8	187.5	0.7	0.4
CV (%)	33.1	33.1	35.3	35.3	8.8	9.9

ns non-significant; * significant (p<0.05); ** highly significant (p<0.01); *** highly significant (p<0.001). Means within the same column followed by the same letter(s) are not significantly different at 5% probability level based on DMRT.

Table 5. Pearson's correlation coefficients of selected parameters showing relationships among yield parameters at AVRDC-RCA, Arusha, Tanzania, 2012.

Yield parameter	LYGP	SYGP	LNP	BNP	LL	LW
SYGP	-0.02^{ns}					
LNP	0.76**	0.13^{ns}				
BNP	0.21^{ns}	-0.76**	0.23^{ns}			
LL	0.09^{ns}	-0.04^{ns}	-0.24^{ns}	-0.19^{ns}		
LW	-0.03^{ns}	-0.03^{ns}	-0.49^{ns}	-0.24^{ns}	0.68**	
D50F	0.22^{ns}	-0.85***	-0.03^{ns}	0.84***	0.13^{ns}	0.14^{ns}

Non-significant difference (ns) was considered when P>0.05, * when P≤0.05, ** when P≤0.01 and *** when P≤0.001. LYGP=Leaf yield g per plant, SYGP=Seed yield g per plant LNP=Leaf number per plant, BNP=Number of branch per plant, LL=Leaf length in cm, LW= Leaf width in cm and D50F=Days to 50% flowering.

reported in the present study were generally lower, but comparable to those reported earlier for Amaranthus

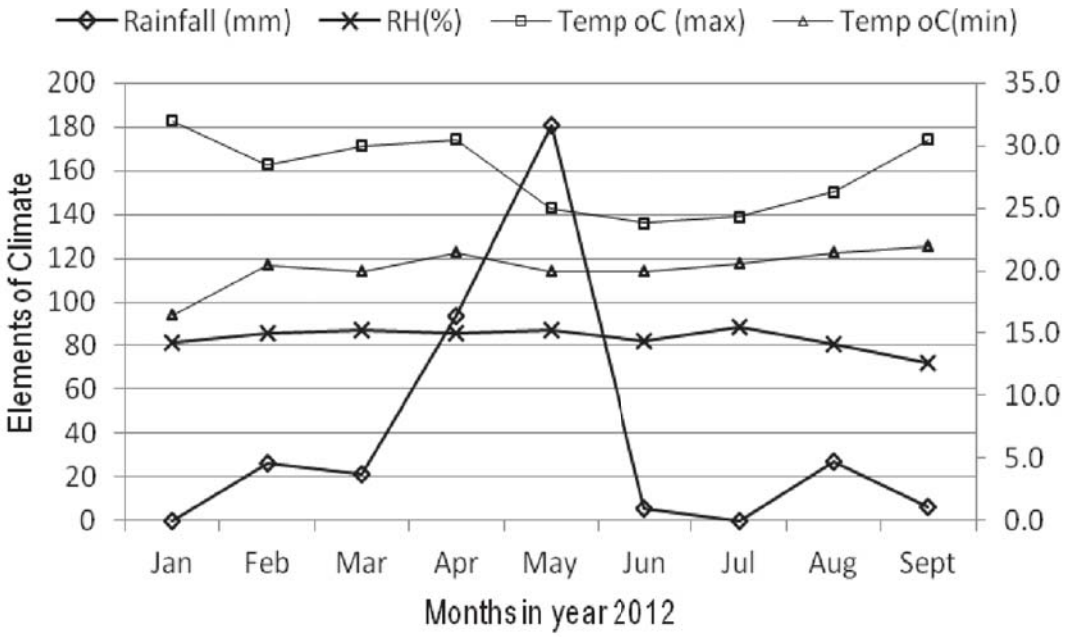

Figure 1. Maximum (max.) and minimum (min.) temperature (Temp), monthly rainfall (mm) and mean monthly relative humidity (RH) at AVRDC-RCA, Arusha. Source: Tengeru Met. Station.

species, *A. cruentus*, *A. hypochondriacus* and *A. dubius* (Oluoch et al., 2009) that varied between 17.8 t and 32 t/ha with different harvesting techniques. The higher values reported in the earlier study may be explained by differences in harvesting methods and genotypes evaluated. In the present study, differences among the genotypes in leaf and grain yields indicate their differences for dual purpose or grain amaranths.

In general, the grain yield reported in this study was within the yield ranges reported earlier (Svirskis, 2003). Variations among genotypes in grain yield in leaf harvested experiment and in leaf not harvested indicate that in many cases leaf defoliation reduces grain yield. Removal of specific green tissues inhibits photosynthesis and alters sink-source relationships. Leaf harvesting/defoliation limit the production of exportable sugars which are required as a resource for meristematic activity and for the growth of sink organs, mainly the grain in this case. Saidi et al. (2007) reported the highest grain loss in cowpea when leaf harvesting frequency was as per appearance. In the present study, however, some genotypes (RVI00007 and RVI00021) gave higher grain yields in leaf harvested experiment than under leaf not harvested experiment. We observed in these genotypes, where leaves were harvested there were few branches and light inflorescence that were not breaking/loading. However, in plots where leaves were not harvested both branching and inflorescences became heavy resulting in lodging, and breakage of inflorescences during windy and/or rainy days. This resulted in significant grain yield loss before harvesting.

Plant height and days to 50% flowering

Both plant height and days to 50% flowering were affected by season. Plant height ranged from 127 to 211 cm and 51 to 85 cm in the first and second season, respectively. The same trend was observed in days to 50% flowering where the entries took more days in the second season. These variations can be attributed to differences in genotypes response to the different seasons. In the first season the weather condition was warm and wet while the second season was cool and dry. Vegetable amaranth has been reported to achieve optimum growth at temperature ranges 25 to 30°C (Whitehead et al., 2002). The result of the current study is in agreement with the finding of Kauffmann and Weber (1990) who reported that some traits of amaranths such as plant height, days to maturity and plant architecture are affected by environmental conditions.

Number of leaves and branches per plant

Differences observed in number of leaves harvested and number of branches per plant in each season might be due to genotype and seasons differences. Highest leaf yield was harvested in Season-2.The genotype RVI00002 took longer time to flower in both seasons as compared

to other genotypes, and therefore its vegetative phase extended, which resulted in higher number of branches as well as leaves harvested. This observation is in line with findings by Okokoh and Bisong (2011) who observed the sharp decline of leaf productivity in *A. cruentus* after on-set of flowering.

Relationship among yield parameters

Weak negative correlations between leaf yield with seed yield and leaf width, suggest that high leaf yielding genotype had relatively low grain yield as well as thinner leaves. This was shown by the genotype RVI00002, which had relatively higher leaf yield in both seasons, but low in grain yield.

Conclusions

Amaranth is one of the vegetables that have potential for nutrition and food security, and income diversification. There is, therefore, a need of improving its productivity. It was indicated from this study that genotypes RVI00121 and RVI00001 were the best for grain production while RVI00007 and RVI00022 were recommended for dual purpose (leaf and grain) during warm wet and cool dry conditions, respectively; further study might be required to understand the effects of environment on yield and quality of both leafy and grain, and genotype by environment interaction. Generally, genotypic differences appear to strongly affect the choice of amaranth for leaf, grain or dual purpose production.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Stability analysis of components characters in cowpea (*Vigna unguiculata* (L.) Walp)

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Stability of yield and its attributes were assessed for nineteen genotypes over twelve environments (two seasons 2009 and 2010 × six planting dates), to determine the quantitative responses of cowpea genotypes. The interaction between genotypes and environments (G×E) were significant for all the characters studied characters except pod length, hundred seed weight and weight of pods per plant. The longest pods and heaviest hundred seeds weight were produced by genotype TVU 21, IT82C-116, providing the highest number of seeds per plant. Whereas, Sudany genotypes gave the highest number of pods per plant and heaviest seeds per plant, Blackeye Crowder genotypes had the heaviest pods per plant and total dry seed yield. The best season and planting date are fall season, third planting date (August, 15th) for most studied traits. The stable genotypes were Chinese Red, IT81D1064, IT85F2205 and Sudany for total dry seed yield.

Key words: Sowing dates, stability parameters, genotype × environment, selection, grain yield.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most ancient crops known to man. In Egypt, cowpea is a popular vegetable crop. The total area under cultivation of this crop was estimated at 9155 feddans (feddan= 4200 m^2) for dry seed in 2008 with a mean production of 980 kg/fed. Also, the area that produced green pods was 10064 feddans with a mean of 5.19 ton/fed (Department, Agriculture, Statistics, Ministry of Agriculture, Giza, Egypt). Stable performance of cowpea genotypes across contrasting environments is essential for the successful selection of stable and high yielding varieties (Dashiell et al., 1994; Ariyo, 2000; Ahmed et al., 2005; Yousaf and Sarwar, 2008). Combination of genotypes stability with high yield is an important criteria for selecting high yielding and stable genotypes. Therefore, a number of techniques that simultaneously coupled with high yield and stability of performance have been proposed. The regression technique (Eberhart and Russell, 1966) has been used. In this technique, the response of genotypes to a given environment is considered. G x E cannot be avoided, in fact, it is an important limiting factor for testing the efficiency of any breeding programme. The occurrence of large genotype × environment (G × E) interaction affects the recommendations of the breeders in selecting genotypes for specific environment. Genotype × environment analysis is used to provide unbiased estimates of yield and other agronomic characteristics and to determine yield stability or the ability to withstand both predictable and unpredictable environmental variation (Kamdi, 2001).

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Table 1. Source, seed color and growth habit of the tested cowpea genotypes.

Genotype	Seed color	Growth habit
1. Dokii 331	White with black eye	Determinate
2. Kaha 1	Yellowish-white	Determinate
3. Cream 7	Yellowish-white	Determinate
4. IT91K-118-20	Light Brown	Determinate
5. IT93K2045-20	Light Brown	Determinate
6. TVU-21	White with red eye	Indeterminate
7. IT82D-889	Light Brown	Determinate
8. Chinese Reds	Dark Brown	Indeterminate
9. IT81D1064	Dark Brown	Determinate
10. IT85F-2205	Light Brown	Determinate
11. IT90K-1020-6	Light brown	Determinate
12. Blackeye Crowder	White with black eye	Determinate
13. IT82C-16	Dark Brown	Determinate
14. IT82-812	Light Brown	Indeterminate
15. Sudany	Black	Indeterminate
16. Cream 12	Yellowish-white	Determinate
17. Monarch Blackeye	White with black eye	Determinate
18. Azmerly	White with black eye	Determinate
19. Black Crowder	Black	Indeterminate

The regression coefficient (b_i) and genotype mean yield were used together as measure of adaptation (Bilbro and Ray, 2000). Genotype with b = 1.0 was considered as adapted to al environments, genotype with b < 1.0 was considered adapted for low yielding environments and genotype with b > 1.0 was considered as better adapted for high yielding environments, depending upon the genotype mean yield. De Rocha et al. (2007a) found that TE97-321G-4, EVX-92-49E and EVX-63-10E cowpea lines were highly adaptable, but only the last one was highly predictable. The BRS Guariba cultivar as well as EVX-92-49E and TE97-321G-4 lines best expressed their genetic potential in environments of high yield. Taiwo (2007) reported that IT 98K-1111-1, IT 86D-1010, IT 86D-719, IT 93K-452 and IT 97K-503-1 were identified to be of a high fodder yield and stable genotypes performance across performance environment. Ajeigbe et al. (2008) found that IT98K-506-1, IT97K-1113-7, IT97K-1069-6, IT97K-1092-2, IT97K-1069-5, IT98K-131-2 and IT97K-568-18 produced higher grain and fodder yielders than the other varieties. The objective of this investigation were to assess the magnitude of G×E interaction as well as the relative performance and stability of 19 cowpea genotypes under abiotic (heat) stress of Upper Egypt environmental conditions, to identifying the most stable genotypes for this stress.

MATERIALS AND METHODS

Study sites and experimental design

The field experiments were conducted at Faculty of Agriculture Farm, South Valley University, Qena Governorate, Egypt, during the growing seasons of 2009 and 2010. The material used in this study and sources of the investigated genotypes are shown in Table 1. These nineteen genotypes were evaluated in summer and fall seasons of 2009 and 2010. In each season, the genotypes were arranged in a Randomized Complete Block Design (RCBD) with three sowing dates viz, March, 15th, 30th and April, 15th in the summer seasons of 2009 and 2010, and July, 15th, 30th and August, 15th in the fall seasons of 2009 and 2010. Each genotype was represented by single row and was repeated three times, the length of the row was 3 m, 60 cm apart and plants spaced 20 cm from each other. Then, different agricultural production practices that is, fertilization and pest management were applied as per the commercial cowpea production in Egypt.

Data collection

The measured traits included

(1) Pod length (cm): Ten normal and fully dry pods for each genotype from each plot were taken to determine dry pod length and the average were recorded.
(2) Number of pods/plant: Average pod number of ten plants for each genotype from each plot was estimated.
(3) Number of seed per pod: Recorded from 10 pods per plant at harvesting time and the average was estimated.
(4) Hundred seed weight (gram): Average weight of the ten samples for each genotype in each plot was determined.
(5) Average seed weight/plant (gram): Ten plants from each genotype were taken from each plot to determine the weight of seeds/plant (gram) and the average was recorded.
(6) Average pod weight (gram): Ten normal and fully dry pods for each genotype from each plot were taken to determine dry pod weight and the average were recorded.
(7) Total dry seed yield (ton/fed.): Estimated as the weight of the dry seed per plot.

Data from all plots were subjected to analysis of variance (Steel and Torrie, 1980). Stability parameters were worked out according to (Eberhart and Russell, 1966).

Table 2. Means of pod length of the nineteen genotypes under twelve environments.

Genotype	Pod length (cm)												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	13.73	12.50	14.8	13.33	12.43	14.40	14.10	14.70	14.80	13.43	14.97	15.47	14.06
Kaha 1	12.73	12.63	14.00	12.33	11.77	12.40	13.10	13.70	13.47	13.94	14.05	14.05	13.18
Cream 7	15.4	15.17	16.47	15.00	15.10	14.73	15.77	16.37	16.13	16.00	17.00	17.00	15.85
IT91K118-20	10.97	11.73	12.57	12.17	12.33	11.57	12.70	12.20	13.97	11.90	12.00	12.23	12.20
IT93K2045-20	14.00	13.86	15.00	14.14	14.07	14.80	14.80	15.00	15.77	15.20	15.90	15.57	14.84
TVU 21	18.40	17.90	19.40	18.00	17.97	18.20	17.10	17.50	18.47	18.83	19.00	19.80	18.38
IT81D-889	15.20	14.63	16.27	14.80	14.57	15.87	15.70	15.50	15.60	16.23	17.00	16.63	15.67
Chinese Red	12.13	11.60	13.20	11.73	11.53	12.80	13.47	13.10	13.87	13.13	13.33	13.87	12.81
IT81D1064	12.80	12.67	13.87	12.40	12.60	13.47	14.27	13.77	15.87	14.37	15.50	14.50	13.84
IT85F2205	13.63	13.13	14.70	13.23	13.07	14.30	14.85	14.98	15.30	14.50	15.80	15.37	14.41
IT90K1020-6	11.07	11.50	12.13	10.67	11.10	11.73	11.93	12.03	13.80	11.93	12.67	12.80	11.95
Blackeye Crowder	12.73	11.50	13.80	12.33	12.43	13.40	13.77	13.70	14.80	13.10	14.40	14.47	13.37
IT82C-16	17.73	16.50	18.80	17.33	16.77	18.40	18.00	17.10	18.17	18.10	19.60	19.47	18.00
IT82- 812	12.20	11.60	13.27	11.80	12.20	12.87	14.53	13.37	13.93	13.73	13.37	13.93	13.07
Sudany	11.00	9.53	10.00	9.67	9.93	9.17	10.43	10.73	10.80	11.43	12.23	12.00	10.58
Cream 12	15.73	14.50	16.8	15.33	14.43	16.40	15.63	16.00	16.30	16.03	16.63	17.17	15.91
Monarch Blackeye	10.90	10.17	11.97	10.50	10.10	11.57	11.93	11.87	12.63	12.27	12.60	12.63	11.60
Azmerly	15.73	15.50	16.8	15.33	15.43	16.40	14.90	15.70	16.07	16.13	16.57	17.17	15.98
Black Crowder	15.07	14.17	16.13	14.67	14.10	15.73	15.00	15.37	16.00	15.40	16.00	16.40	15.34
Environmental mean	13.74	13.20	14.74	13.41	13.26	14.12	14.31	14.35	15.04	14.51	15.19	15.29	14.26

E1 = Summer season 2009, First date E5 = Fall season 2009, Second date E9 = Summer season 2010, Third date, E2 = Summer season 2009, Second date, E6 = Fall season 2009, Third date, E10 = Fall season 2010, First date, E3 = Summer season 2009, Third date E7 = Summer season 2010, First date E11 = Fall Third 2010, Second date E4 = Fall season 2009, First date E8 = Summer season 2010, Second date E12 = Fall Third 2010, Third date.

RESULTS AND DISCUSSION

The combined analysis variance (Table 9) revealed that highly significant differences among genotypes (G), environments (E) as well as interaction between genotypes and environments (G×E) for most of the studied traits. These results indicated that cowpea genotypes responded differently to the diverse environmental conditions, differences were due to the genetic variations among genotypes and environmental factors and climatic conditions, among others. Similar results were obtained by Teixeira et al. (2007) and Akande and Balogun (2009). The mean response of each trait is outlined below.

Pod length (cm)

The mean performance of genotypes is presented in Table 2. The average pod length of the 19 genotypes over all environments ranged from 18.38 cm (TVU 21) to 10.58 cm (Sudany). The delay in planting date increased the fresh pod length in all seasons. These results agree with those reported by Ali et al. (2004) and Rashwan (2010) who found that the delay in plating date until Dec. 15 increased the fresh pod length. Partitioning the genotype × environment interaction mean square (Table 10) that (G×E) mean squares were estimated with insignificant value. The stability parameters ($\bar{x}$, bi and s^2d) of the individual genotypes are illustrated in Table 11.

Table 3. Means of number of seeds per pods of the nineteen genotypes under twelve environment.

Genotype	Number of seeds per pods												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	9.33	10.00	10.00	9.33	10.33	10.00	10.67	11.00	10.33	10.33	11.00	10.67	10.25
Kaha 1	7.67	8.67	8.33	7.67	8.67	8.33	9.67	9.33	8.33	8.33	10.33	8.33	8.64
Cream 7	11.00	12.00	12.33	11.00	12.00	12.33	12.67	10.00	11.00	11.33	10.67	11.00	11.44
IT91K118-20	8.00	10.33	11.00	10.33	11.00	11.33	12.00	11.33	11.33	11.33	10.33	11.33	10.81
IT93K2045-20	10.33	11.33	12.00	11.67	11.33	12.00	10.67	9.00	10.00	10.67	10.67	10.67	10.86
TVU 21	10.67	11.67	10.67	10.67	11.33	10.67	12.67	11.33	12.00	12.00	10.67	12.00	11.36
IT81D-889	9.33	10.33	9.00	9.33	10.67	9.00	11.00	11.00	11.00	11.00	10.00	11.00	10.22
Chinese Red	8.33	9.33	8.00	8.33	10.00	8.00	11.00	10.00	10.00	10.33	10.00	10.33	9.47
IT81D1064	8.33	9.33	8.67	8.33	9.33	8.67	9.33	8.33	9.00	9.67	9.00	9.33	8.94
IT85F2205	8.33	9.33	8.67	8.33	9.33	8.67	10.33	8.00	9.33	9.67	10.00	10.33	9.19
IT90K1020-6	7.67	8.33	9.00	7.67	9.33	9.00	11.00	10.33	11.00	9.33	9.33	8.67	9.22
Blackeye Crowder	10.33	9.33	10.67	10.33	10.67	10.67	12.67	12.67	13.33	11.00	9.67	11.33	11.06
IT82C-16	12.67	13.67	13.67	12.67	13.67	13.67	9.33	9.00	9.33	13.00	12.00	13.00	12.14
IT82- 812	8.33	9.33	9.00	8.33	9.33	9.00	10.33	9.33	10.00	10.33	9.00	9.67	9.33
Sudany	9.33	7.67	7.67	9.00	8.00	8.33	9.33	10.00	10.67	9.67	8.67	9.67	9.00
Cream 12	11.00	11.33	12.00	7.33	12.00	12.00	11.67	12.33	8.00	11.33	11.33	11.33	10.97
Monarch Blackeye	7.67	8.67	7.67	7.67	8.67	7.67	11.33	10.00	10.67	9.67	11.33	9.67	9.22
Azmerly	9.33	10.33	10.33	9.33	10.33	10.33	11.33	11.00	11.33	10.33	11.00	10.67	10.47
Black Crowder	10.33	11.33	11.00	10.33	11.33	11.33	11.33	11.00	11.33	11.33	12.00	11.67	11.19
Environmental mean	9.37	10.12	9.98	9.35	10.39	10.05	10.96	10.26	10.42	10.56	10.37	10.56	10.20

11. All genotypes except IT85F2205 and Monarch Blackeye exhibited non significant stability parameters from unity and zero for regression coefficient (bi) and deviation from regression (s^2d), respectively. The genotypes IT91K118-20, Sudany and Kaha 1 appeared to be stable and exhibited below average response to different environments, ($b_i < 1$) their genotypes were considered to perform relatively better in less favorable environments. The genotypes Cream 7, TVU 21, IT81D-889 and Azmerly could be considered good inserted gave a pod length more than the overall of average genotypes besides their stability. The genotypes IT93K2045-20, Black Crowder, Azmerly and IT81D-889 might be considered superior, because they should be the tallest pod length when compared with the average overall genotypes besides their stability. Similar results were reported by Akande and Balogun (2009), Teixeira et al. (2007) and Sarutayophat et al. (2007).

Number of seeds per pod

Results in Table 3 showed that average number of seeds per plant of genotypes overall environments ranged from 12.14 (IT82D-16) to 8.64 seeds per pods for genotype (Kaha 1), with an average of (10.20) seeds per pods for all genotypes. These are in accordance with the finding of Rajput (1994) who observed that sowing on 10th March recorded significantly more number of pods per plant (16.0), seeds per pod (13.2), seed yield (12.1 q ha-1), stover yield (24.7 q ha-1) and harvest index (34.5%) compared to sowing in 18th February and 30th March. The highest number of seeds per pod was that of genotype IT82D-16, in the third planting date (August 15th and April 15th) at two seasons (summer and fall), while, the lowest was for genotypes Kaha 1, in the first planting date in fall season (July, 15th).

Table 4. Means of number of pods per plant traits of the nineteen genotypes under twelve environments.

Genotype	Number of pods per plant												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	54.33	54.67	56.33	55.67	57.33	56.00	53.33	53.67	54.67	57.00	58.67	57.67	55.78
Kaha 1	34.00	34.67	36.33	34.67	37.00	35.67	31.67	32.00	33.00	35.33	38.67	38.67	35.14
Cream 7	53.67	55.00	55.33	54.67	56.67	55.00	52.00	52.33	53.33	55.67	58.00	57.67	54.94
IT91K118-20	48.33	49.33	50.33	49.33	51.33	50.00	46.33	46.67	47.67	50.00	53.00	52.67	49.58
IT93K2045-20	41.33	42.33	43.33	42.67	44.33	43.00	40.33	40.67	41.67	44.00	45.67	45.67	42.92
TVU 21	43.00	44.33	44.67	44.00	46.00	44.00	41.67	42.00	43.00	45.33	47.00	46.67	44.31
IT81D-889	37.00	37.33	39.33	38.00	40.33	38.33	36.33	36.67	37.67	40.00	41.33	41.00	38.61
Chinese Red	49.00	50.33	50.67	51.00	52.00	51.00	47.67	48.00	49.00	51.33	54.00	53.33	50.61
IT81D1064	35.67	35.67	38.00	37.67	38.67	38.33	35.00	35.33	36.33	38.67	40.67	39.33	37.44
IT85F2205	44.67	45.33	47.00	45.67	47.67	46.33	44.00	44.33	45.33	47.67	49.33	48.67	46.33
IT90K1020-6	34.67	35.00	38.00	37.00	38.67	38.00	36.33	37.33	37.00	35.33	40.00	39.00	37.19
Blackeye Crowder	62.67	63.33	64.67	64.00	65.33	65.00	61.00	61.67	62.00	63.67	68.00	65.00	63.86
IT82C-16	34.33	35.00	37.67	35.67	38.00	36.67	36.00	36.67	36.67	34.00	38.67	37.67	36.42
IT82- 812	38.33	38.67	41.00	40.33	41.00	41.00	39.00	39.33	40.00	37.67	43.33	41.00	40.06
Sudany	66.33	67.33	67.67	66.67	68.67	67.33	65.67	66.33	66.67	66.67	70.33	67.33	67.25
Cream 12	51.00	51.67	52.67	51.67	53.33	51.67	51.00	51.67	51.67	51.00	54.67	52.67	52.06
Monarch Blackeye	33.67	34.67	35.00	34.67	36.00	35.00	33.33	34.00	34.00	34.00	38.00	35.00	34.78
Azmerly	57.00	57.67	58.67	58.33	59.33	58.33	57.00	57.67	57.67	57.33	61.33	58.67	58.25
Black Crowder	55.67	56.00	57.33	57.00	57.67	57.33	55.67	56.33	56.33	55.33	60.00	57.33	56.83
Environmental mean	46.04	46.75	48.11	47.30	48.91	47.79	45.44	45.93	46.51	47.37	50.56	49.21	47.49

The differences among the tested genotypes (G) were highly significant; also, environmental (E) effect and the interactions between genotypes and environments (G×E) were highly significant as shown in Table 9. Most of this interaction was in a linear function with the environmental values as indicated by greater magnitude of the G×E (linear) mean squares (5.51) in comparison with the estimated value for E+ (G×E) mean squares (3.04), which appeared also highly significant. These results were presented in Table 10. These results appeared to be in harmony with those obtained by Torres et al. (2008) and Akande and Balogun (2009).

The stability parameters ($\bar{x}$, bi and s^2d) of the individual genotypes are illustrated in Table 11. The results indicated that all genotypes values were non-significant except IT93K2045-20, Chinese Red and Monarch Blackeye were significant, genotypes Dokii 331, IT93K2045-20 and Black Crowder were considered specially adapted to unfavorable environments because the regression coefficient of theses genotypes less than one ($b_i < 1$) while, genotypes IT91K118-20, IT82C-16 and Blackeye Crowder could consistently performed better under favorable environments because their regression coefficient (bi) were more than one. The genotypes IT93K2045-20, Dokii 331, Cram 7, and Black Crowder might be consider superior because they gave high mean values for number of seeds per pods above the grand mean, besides their stability. These results is in agreement with those obtained by Damarany (1994b), Ushakumari et al. (2002), Dahiya et al. (2007a, b, c) and Singh et al. (2007).

Number of pods/plant

Average number of pods per plant of genotypes overall environments ranged from (67.25) for

genotype Sudany to (34.78) pods per plant for genotype Monarch Blackeye, with an average of (47.49) pods per plant for all genotypes, data are presented in Table 4. The highest number of pods per plant was for genotype Sudany at fall season at second planting date (July, 30th), in both seasons, while, the lowest was for genotype Monarch Blackeye at summer season at first planting date (March, 15th), in both seasons. The significance of genotype by environment interaction in regional variety trials or in selection for wide adaptation has been reviewed by other workers (Becker and Leon, 1988; Crossa et al., 1990; Cooper and DeLacy, 1994). Other studies (Allen and Allen, 1981; Singh and Rachie, 1985; Damarany, 1994a; Ishiyaku et al., 2005) pointed out the existence of significant genotypic differences in cowpea for yield and agronomic traits. However, most of the studies were conducted under single location or controlled environments that might underestimate the environmental as well as genotype by environment interaction.

Results illustrated in Tables 9 and 10 showed that the differences among all genotypes (G) and environments (E) were highly significant. Also, the interactions between genotypes and environments (G×E) were highly significant. Also, highly significant effect of E (linear) was reported, indicating that the studied trait was highly influenced by the combination of environment. G×E (linear) item was highly significant, suggesting that cowpea genotypes were different in their response to environments. Similar results were reported by Teixeira et al. (2007) and Torres et al. (2008).

The estimated stability parameters ($\bar{x}$, bi and s^2d) of the studied genotypes for number of pods per plant indicated that Sudany, Cream 12, Azmerly and Monarch Blackeye genotypes were stable ($b_i < 1$) with high mean values, while, IT90K1020-6, IT82C-16 and IT82-812 genotypes were stable with the mean values lower than the grand mean. On the other hand, Dokii 331, Cream 7, IT91K118-20 and Chinese Red were unstable ($b_i > 1$) and could consistently do better in favorable environments. These results are presented in Table 11. Similar results were obtained by Ushakumari et al. (2002) and Dahiya et al. (2007b).

Hundred seed weight (gram)

Average hundred seed weight (gram) of genotypes overall environments ranged from 22.16 (gram) for genotype TVU 21 to 11.63 (gram) hundred seed weight (gram) for genotype Chinese Red, with an average of 14.89 (g) hundred seed weight (gram) for all genotypes. The data were presented in Table 5. These results are in agreement with that obtained by Damarany (1994b), Dahiya et al. (2007b, c), Peksen (2007) and De Rocha et al. (2007b). The highest hundred seed weight was that of genotype TVU 21, in the third planting date at fall season, while, the lowest was for genotypes Chinese Red, in the third planting date (April, 115th), in summer season. The stability parameters ($\bar{x}$, bi and s^2d) of the individual genotypes are illustrated in Table 11. The results indicated that all genotypes values were non-significant except IT81D-889 and IT82C-16 were highly significant, genotypes Azmerly, IT81D-889, Blackeye Crowder and Black Crowder were considered specially adapted to unfavorable environments because the regression coefficient of theses genotypes less than one ($b_i < 1$) while, genotypes Dokii 331, IT91K118-20, IT82C-16 and IT85F2205, Blackeye Crowder could consistently performed better under favorable environments because their regression coefficient (bi) were more than one. The genotypes IT82D-889 and Azmerly might be consider superior because they gave high mean values for hundred seeds weight above the grand mean, besides their stability. These results are in agreement with those obtained by De Rocha et al. (2007b and Akande and Balogun (2009).

Average seed weight/plant (gram)

The performance of tested genotypes is presented in Table 6. The results indicated that average weight of seeds per plant of the various genotypes ranged from 67.81 g for (Sudany) to 37.03 g for (Kaha 1), with an average of 48.05 g for all genotypes. The heaviest weight of seeds per plant 69.23 and 68.80 g was found for (Sudany) in summer season, at third plating date, in both seasons, respectively. While, the lightest of 35.80 g was found for (Kaha 1) genotype in fall season at first planting date. These results are in agreement with that obtained by Ushakumari et al. (2002), and Dahiya et al. (2007b, c).

The joint regression analysis of variance is presented in Table 9. The differences among the tested genotypes (G) were highly significant; also, environmental (E) effect and the interactions between genotypes and environments (G×E) were highly significant as shown in Table 10. Most of this interaction was in a linear function with the environmental values as indicated by greater magnitude of the G×E (linear) mean squares in comparison with the estimated value for E+ (G×E) mean squares, which appeared also highly significant. These results appeared to be in harmony with those obtained by Dahiya et al. (2007a, b).

The estimated stability parameters ($\bar{x}$, bi and s^2d) of the studied genotypes for average seed weight indicated that Cream 7, Azmerly, Blackeye Crowder, Dokii 331 and Black Crowder genotypes were stable ($b_i < 1$) with high mean values, while, Kaha 1, and IT85F2205 genotypes were stable with the mean values lower than the grand mean. On the other hand, Sudany, Monarch Blackeye, IT82-812 and IT82C-16 genotypes were unstable ($b_i > 1$) and could consistently do better in favorable environments (Table 11). Similar results were obtained by

Table 5. Means of hundred seeds weight per plant traits of the nineteen genotypes under twelve environments.

Genotype	Hundred seed weight(g)												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	18.40	18.40	18.40	20.60	19.82	20.09	19.10	18.73	18.47	19.70	20.37	20.00	19.34
Kaha 1	11.93	11.70	11.93	13.90	13.12	13.63	12.13	12.87	12.00	12.90	13.47	13.30	12.74
Cream 7	12.87	12.80	12.87	15.00	14.22	14.56	13.47	13.93	12.93	14.00	14.60	14.40	13.80
IT91K118-20	12.43	12.53	12.43	14.73	13.96	14.13	12.90	13.33	12.50	13.73	14.03	14.13	13.40
IT93K2045-20	16.03	15.70	16.03	17.90	17.12	17.73	15.73	15.53	16.10	17.07	16.87	17.30	16.59
TVU 21	21.70	21.33	21.70	23.53	22.76	23.39	21.27	20.67	21.77	22.53	22.30	22.93	22.16
IT81D-889	13.50	13.10	13.50	15.30	14.52	15.19	13.67	14.23	13.57	14.30	14.63	14.70	14.18
Chinese Red	10.80	10.60	10.80	12.80	12.02	12.49	11.00	11.77	10.87	12.10	12.13	12.20	11.63
IT81D1064	10.93	10.93	10.93	13.13	12.36	12.63	10.70	10.67	11.00	12.13	11.83	12.53	11.65
IT85F2205	15.77	15.43	15.77	17.63	16.86	17.46	15.13	14.47	15.83	16.63	16.27	17.03	16.19
IT90K1020-6	12.60	12.50	12.60	14.70	13.92	14.29	12.90	13.97	12.67	13.70	14.03	14.10	13.50
Blackeye Crowder	12.80	12.90	12.80	15.10	14.32	14.49	13.20	13.73	12.87	14.10	14.33	14.50	13.76
IT82C-16	15.47	15.67	15.47	17.87	17.09	17.16	15.90	15.60	15.53	16.87	17.03	17.27	16.41
IT82- 812	12.70	12.37	12.70	14.57	13.79	14.39	12.73	14.27	12.77	13.57	13.87	13.97	13.47
Sudany	11.73	11.40	11.73	13.60	12.80	13.43	11.23	11.10	11.80	12.60	12.37	13.00	12.23
Cream 12	11.37	11.30	11.37	13.50	12.70	13.06	11.47	11.67	11.43	12.50	12.60	12.90	12.16
Monarch Blackeye	16.43	16.37	16.43	18.57	17.77	18.13	17.17	16.97	16.50	17.57	18.30	17.97	17.35
Azmerly	17.90	17.43	17.90	19.63	18.83	19.59	17.27	17.50	17.97	18.63	18.40	19.03	18.34
Black Crowder	13.10	13.00	13.10	15.20	14.40	14.79	13.80	13.63	13.17	14.20	14.93	14.60	13.99
Environmental mean	14.13	13.97	14.13	16.17	15.39	15.82	14.25	14.45	14.20	15.20	15.39	15.57	14.89

Ushakumari et al. (2002) and Dahiya et al. (2007b).

Average pods weight/plant (gram)

The performance of tested genotypes is presented in Table 7. The results indicated that average weight of seeds per plant of the various genotypes ranged from 82.00 (g) for Blackeye Crowder to 50.36 (g) for Chinese Red, with an average of 64.45 (g) for all genotypes. The heaviest weight of pods per plant 85.67 (g) was found for Blackeye Crowder in fall season, at second plating date, while, the lightest of 45.33 (g) was found for Chinese Red in summer season at third planting date. These results are in agreement with that obtained by Hazra et al. (1999), and De Rocha et al. (2007b).

The joint regression analysis of variance is presented in Table 10. The differences among the tested genotypes (G) were highly significant, also, environmental (E) effect, while, the interactions between genotypes and environments (G×E) were insignificant, as shown in Table 9. Indicating the presence of genetic variability among these genotypes and the suitability of stability analysis. These results appeared to be in harmony with those obtained by Chattopadhyay et al. (2001) evaluated twenty cowpea genotypes for stability in yield and its components such as number of pods per plant, pod length, and pod weight and revealed that the significant genotype and environment interaction was observed for all characters except pod length.

The estimated stability parameters ($\bar{x}$, bi and s^2d) of the studied genotypes for average pods weight are presented in Table 11. All genotypes exhibited insignificant stability parameters from unity and zero for regression coefficient (bi) and deviation from regression (s^2d), the results indicated that Dokii 331, TVU 21, Blackeye Crowder, Black

Table 6. Means of weight of seeds per plant traits of the nineteen genotypes under twelve environments.

Genotype	Average weight of seeds per plant (cm)												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	52.53	56.57	56.17	54.73	56.23	56.20	54.73	56.23	55.87	55.27	56.77	56.03	55.61
Kaha 1	38.03	37.73	36.90	35.80	37.47	37.10	35.82	37.47	36.77	36.33	38.00	36.93	37.03
Cream 7	51.70	56.23	56.10	54.77	55.97	56.27	54.77	55.97	55.93	55.30	56.50	56.10	55.47
IT91K118-20	44.70	50.37	51.10	49.37	50.13	50.87	49.37	50.13	50.53	49.90	50.67	50.70	49.82
IT93K2045-20	46.40	44.43	43.13	41.47	44.00	43.13	41.47	44.00	42.80	42.00	44.53	42.97	43.36
TVU 21	52.67	47.10	44.80	43.53	46.77	45.17	43.53	46.77	44.83	44.07	47.30	45.00	45.96
IT81D-889	44.77	40.60	39.17	37.83	40.27	39.03	37.83	40.27	38.70	38.37	40.80	38.87	39.71
Chinese Red	46.03	51.13	50.97	50.07	50.63	51.20	50.07	50.63	50.87	50.60	51.17	51.03	50.37
IT81D1064	43.43	38.80	36.93	35.77	38.17	36.60	36.00	38.40	36.50	36.30	38.70	36.43	37.67
IT85F2205	47.50	47.17	45.77	44.57	46.30	45.70	44.90	46.63	45.70	45.17	46.90	45.57	45.99
IT90K1020-6	48.10	38.30	36.17	34.57	37.50	35.87	34.90	37.83	35.87	34.97	37.90	35.73	37.31
Blackeye Crowder	62.67	66.17	66.20	64.63	65.47	65.83	64.97	65.80	65.83	65.03	65.87	65.70	65.35
IT82C-16	47.73	38.03	36.00	34.27	37.37	35.73	34.60	37.70	35.73	34.67	37.77	35.60	37.10
IT82- 812	51.40	42.00	40.10	38.10	41.37	39.87	38.43	41.70	39.87	38.50	41.77	39.73	41.07
Sudany	68.30	67.20	69.23	67.20	66.53	68.80	67.53	66.87	68.80	67.60	66.93	68.67	67.81
Cream 12	50.27	53.20	53.20	51.37	52.60	52.97	51.70	52.93	52.97	51.77	53.00	52.83	52.40
Monarch Blackeye	54.10	38.53	35.93	34.47	37.97	35.73	34.80	38.30	35.73	34.87	38.37	35.60	37.87
Azmerly	56.93	59.07	59.00	57.40	58.50	58.63	57.73	58.83	58.63	57.80	58.90	58.50	58.33
Black Crowder	55.77	57.60	57.30	55.13	56.60	56.87	55.47	56.93	56.87	55.53	57.00	56.73	56.48
Environmental mean	49.63	48.96	48.11	46.58	48.41	47.98	46.77	48.60	47.83	47.05	48.89	47.83	48.05

Crowder and Azmerly genotypes were stable ($b_i < 1$) with high mean values, while, Kaha 1, Cream 7 and IT81D1064 genotypes were stable with the mean values lower than the grand mean. On the other hand, IT82C-16 and IT82-812 genotypes were unstable ($b_i > 1$) and could consistently do better in favorable environments. Similar results were obtained by Hazra et al. (1999) and De Rocha et al. (2007b).

Total dry seed yield (ton/fed.)

Results in Table 8 showed that total dry seed yield of genotypes overall environments ranged from 0.989 (ton/fed.) for (Blackeye Crowder) to 0.328 (ton/fed.) dry seed yield for (IT81D1064), with an average of 0.706 (ton/fed.) dry seed yield for all genotypes. These are in accordance with the finding of Kurubetta (2006), he found that time of sowing influenced significantly the seed yield per plant. June second fortnight sowing recorded significantly higher seed weight (14.40 g plant^{-1}) compared to July first fortnight (8.51 g plant^{-1}) and July second fortnight (6.12 g plant^{-1}) sowing. However, July first fortnight was significantly superior to July second fortnight sowing, Damarany (1994c), Torres et al. (2008), Yousaf and Sarwar (2008) and Akande and Balogun (2009). The highest dry seed yield 1.200 (ton/fed) was that for (Azmerly), in fall season at third planting date (August, 15th), while, the lowest 0.270 (ton/fed.) was for IT81D1064 in the summer season at third planting date (April, 15th).

The joint regression analysis of variance is presented in Table 10. The differences among the tested genotypes (G) were highly significant, also, environmental (E) effect, while, the interactions between genotypes and environments (G×E) were non-significant, as shown in Table 9. These results indicated that cowpea genotypes responded differently to different environmental conditions, suggestion the importance of assessment of

Table 7. Means of weight of pods per plant traits of the nineteen genotypes under twelve environments.

Genotype	Average weight of pods per plant (gram)												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	70.33	70.00	69.33	75.33	75.67	76.00	70.00	68.67	69.67	72.33	76.33	76.00	72.47
Kaha 1	59.00	59.00	58.00	64.00	64.33	64.67	59.00	57.67	58.00	61.33	65.33	64.67	61.25
Cream 7	59.33	59.00	58.33	64.33	64.67	65.00	59.00	57.67	58.67	61.33	65.33	65.33	61.50
IT91K118-20	57.67	57.67	56.67	62.67	63.00	63.33	57.67	56.33	56.67	60.00	64.00	65.33	60.08
IT93K2045-20	63.67	62.00	62.67	68.67	69.00	69.33	62.00	60.67	64.33	64.33	68.33	69.00	65.33
TVU 21	77.33	78.00	76.33	82.33	82.67	83.00	78.00	76.67	75.67	80.33	84.33	84.67	79.94
IT81D-889	61.33	61.00	60.33	66.33	66.67	67.00	61.00	59.67	60.67	63.33	67.33	69.33	63.67
Chinese Red	47.33	48.33	46.33	52.33	52.67	53.00	48.33	47.00	45.33	50.67	54.67	58.33	50.36
IT81D1064	56.00	54.00	55.00	61.00	61.33	61.67	54.00	52.67	57.00	56.33	60.33	60.67	57.50
IT85F2205	56.67	56.33	55.67	62.00	62.00	62.00	56.33	54.33	56.00	58.67	63.00	61.00	58.67
IT90K1020-6	69.00	68.33	68.00	74.33	74.33	74.33	68.33	66.33	68.67	70.67	75.00	74.33	70.97
Blackeye Crowder	80.33	78.67	79.33	85.67	85.67	85.67	78.67	77.67	81.00	81.00	85.33	85.00	82.00
IT82C-16	69.00	68.33	68.00	73.67	74.33	75.00	68.33	67.33	68.67	70.67	74.67	77.67	71.31
IT82- 812	68.33	66.00	67.33	73.00	73.67	74.33	66.00	65.00	69.67	68.33	72.33	74.00	69.83
Sudany	52.00	51.67	51.00	56.67	57.33	58.00	51.67	50.67	51.33	54.00	58.00	59.33	54.31
Cream 12	56.00	54.67	55.00	60.67	61.33	62.00	54.67	53.67	56.33	57.00	61.00	61.67	57.83
Monarch Blackeye	61.67	60.67	60.67	66.33	67.00	67.67	60.67	59.67	61.67	63.00	67.00	69.67	63.81
Azmerly	67.67	66.33	66.67	72.33	73.00	73.67	66.33	65.33	68.00	68.67	72.67	73.33	69.50
Black Crowder	71.00	69.67	70.00	75.67	76.33	77.00	69.67	68.67	71.33	72.00	76.00	75.67	72.75
Environmental mean	63.35	62.61	62.35	68.28	68.68	69.09	62.61	61.35	63.09	64.95	69.00	69.74	65.43

genotypes under different environments in order to identify the best genetic make up for particular environment. These results appeared to be in harmony with those obtained by Nwofia et al. (2007), Padi (2007), Peksen (2007), De Rocha et al. (2007 a,b), Sarutayophat et al. (2007) and Taiwo (2007).

The stability parameters ($\bar{x}$, bi and s^2d) of the individual genotypes are illustrated in Table 11. All genotypes except IT93K2045-20, Dokii 331, Chinese Red and Cream 12 exhibited highly significant stability parameters from unity and zero for regression coefficient (bi) and deviation from regression (s^2d), respectively. The genotypes Dokii 331, Cream 12, IT81D-889, Chinese Red, IT81D1064, IT85F2205 and Sudany appeared to be stable and exhibited below average response to different environments, ($b_i < 1$), their genotypes were considered to perform relatively better in less favorable environments. Genotypes Cream 7, IT91k118-20, TVU 21 and Blackeye Crowder were unstable because regression coefficient (bi) more than one. The genotypes Dokii 331 and Cream 12, could be considered good inserted gave dry seed yield more than the overall of average genotypes besides their stability. Similar results were reported by Patel et al. (2005), conducted the experiment in loamy sandy soil with cowpea which revealed that sowing in 2nd March recorded significantly higher seed and haulm yield compared to sowing in 15th February, 17th March and 2nd April, (Gurushara and Sharma, 2004; Jena, 2003; Singh and Singh, 2000; Obiadalla-Ali et al., 2007; Rashwan, 2010).

Conclusion

The study identified considerable degree of genotypic differences and average stability for yield in cowpea when tested under various environments. The best genotypes were Dokii 331 and Cream 12. These genotypes were most stable that would be suitable for growth parameters under the test

Table 8. Means of total dry seed yield traits of the nineteen genotypes under twelve environments.

Genotype	Total dry seed yield (ton/fed.)												
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Mean
Dokii 331	0.950	0.920	0.920	0.950	1.000	1.100	0.968	0.929	0.890	0.960	0.988	1.101	0.973
Kaha 1	0.610	0.598	0.592	0.750	0.800	0.852	0.628	0.599	0.569	0.735	0.787	0.858	0.698
Cream 7	0.730	0.660	0.610	0.800	0.850	0.900	0.720	0.680	0.594	0.796	0.836	0.886	0.755
IT91K118-20	0.600	0.600	0.550	0.800	0.820	0.902	0.680	0.550	0.520	0.744	0.793	0.950	0.709
IT93K2045-20	0.610	0.570	0.549	0.680	0.700	0.740	0.601	0.575	0.550	0.657	0.708	0.757	0.642
TVU 21	0.700	0.670	0.660	0.850	0.900	0.950	0.711	0.680	0.640	0.836	0.895	0.955	0.787
IT81D-889	0.340	0.330	0.300	0.322	0.380	0.400	0.358	0.310	0.300	0.328	0.380	0.400	0.346
Chinese Red	0.400	0.380	0.360	0.480	0.500	0.552	0.400	0.380	0.360	0.477	0.490	0.549	0.444
IT81D1064	0.320	0.300	0.280	0.330	0.350	0.390	0.328	0.299	0.270	0.326	0.343	0.401	0.328
IT85F2205	0.650	0.600	0.550	0.730	0.750	0.792	0.640	0.589	0.569	0.698	0.760	0.799	0.677
IT90K1020-6	0.360	0.300	0.300	0.345	0.375	0.402	0.342	0.300	0.331	0.328	0.385	0.400	0.347
Blackeye Crowder	0.910	0.880	0.860	1.050	1.100	1.150	0.930	0.887	0.830	1.040	1.080	1.161	0.990
IT82C-16	0.850	0.800	0.750	0.950	0.980	1.003	0.853	0.790	0.755	0.946	0.985	1.000	0.889
IT82- 812	0.810	0.780	0.760	0.260	0.270	0.290	0.850	0.810	0.689	0.245	0.277	0.298	0.528
Sudany	0.590	0.550	0.510	0.623	0.650	0.680	0.583	0.539	0.519	0.597	0.655	0.697	0.599
Cream 12	0.850	0.810	0.790	0.890	0.900	0.943	0.901	0.800	0.750	0.847	0.890	0.978	0.862
Monarch Blackeye	0.870	0.830	0.800	1.000	1.040	1.100	0.881	0.820	0.799	0.987	1.073	1.097	0.941
Azmerly	0.800	0.700	0.700	1.000	1.100	1.200	0.773	0.732	0.699	1.000	1.072	1.190	0.914
Black Crowder	0.880	0.860	0.810	1.070	1.100	1.152	0.910	0.851	0.810	1.064	1.112	1.147	0.980
Environmental mean	0.675	0.639	0.613	0.731	0.767	0.816	0.687	0.638	0.602	0.716	0.764	0.822	0.706

Table 9. Combined analysis of variance for studied traits of 19 genotypes under various environments.

SOV	d.f	pod length (cm)	No. of seeds per pods	No. of pods per plant	hundred seeds weight (g)	weight of seeds per plant (g)	weight of pods per plant (g)	total dry seed yield Kg/fed.
Environments (E)	11	36.41**	12.69**	130.58**	34.46**	48.53**	598.04**	738283.36 **
Replication/ E	24	24.65	2.13	10.56	20.82	34.68	47.56	1416.64
Genotypes (G)	18	157.15**	39.02**	3661.90**	295.29**	3319.79**	2548.05**	1820938.13 **
G × E	198	0.79NS	2.50**	1.74**	0.23NS	18.63**	1.57NS	30879.28 **
Error	432	1.14	0.78	1.48	0.45	2.25	2.87	1249.18

* Significant at P < 0.05; ** highly significant at P < 0.01.

Table 10. The joint regression analysis of variance for the studied traits.

SOV	d.f	pod length (cm)	No. of seeds per pods	No. of pods per plant	Hundred seeds weight (g)	weight of seeds per plant (g)	weight of pods per plant (g)	total dry seed yield Ton/fed.
Genotypes (G)	18	157.24**	39.02**	3661.90**	295.29**	3319.78**	2548.05**	1820898.68**
E + (G×E)	209	2.28**	3.04**	8.52**	2.03**	20.20**	32.96**	41725.26**
E (linear)	1	340.45**	139.64**	1436.38**	379.09**	533.83**	6578.29**	3591930.96**
G×E (linear)	18	1.03^{NS}	5.51**	4.56**	0.08^{NS}	90.06**	0.73^{NS}	222547.50**
Pooled deviation	190	0.62^{NS}	2.09**	1.38^{NS}	0.23^{NS}	10.88**	1.57^{NS}	5909.44**
Pooled error	432	1.14	0.78	1.48	0.45	2.25	2.87	1249.18

*Significant at P < 0.05; ** highly significant at P < 0.01.

Table 11. Genotype average performance over 12 environments, and stability parameters of 19 cowpea genotypes.

Genotype	Pod length (cm)			No. of seeds per pods			No. of pods per plant			hundred seeds weight (g)			weight of seeds per plant (g)			weight of pods per plant (g)			total dry seed yield Kg/fed.		
	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d	$\bar{X}$	Bi	s²d
Dokii 331	14.06	1.20^{NS}	0.59	10.25	0.96^{NS}	0.33	55.78	1.07^{NS}	0.72	19.34	1.05^{NS}	0.18	55.61	0.01*	4.52	72.47	0.96^{NS}	0.35	0.973	0.82^{NS}	2127.16
Kaha 1	13.18	0.94^{NS}	0.50	8.64	1.05^{NS}	1.22	35.14	1.45**	1.52	12.74	0.95^{NS}	0.13	37.03	0.81**	0.09	61.25	0.95^{NS}	0.87	0.698	1.41**	1786.88
Cream 7	15.85	0.91^{NS}	0.50	11.44	0.37^{NS}	2.06	54.94	1.20^{NS}	1.16	13.80	0.97^{NS}	0.20	55.47	-0.23*	5.56	61.50	0.97^{NS}	0.25	0.755	1.37**	975.67
IT91K118-20	12.20	0.41^{NS}	1.43	10.81	1.65^{NS}	1.42	49.58	1.35*	1.13	13.40	1.03^{NS}	0.11	49.82	-0.62*	8.48	60.08	1.02^{NS}	0.98	0.709	1.90**	2313.73
IT93K2045-20	14.84	0.92^{NS}	0.13	10.86	-0.51*	2.31	42.92	1.12^{NS}	0.97	16.59	1.02^{NS}	0.20	43.36	1.51**	0.26	65.33	1.00^{NS}	1.36	0.642	0.98^{NS}	322.70
TVU 21	18.38	0.64^{NS}	1.26	11.36	1.17^{NS}	0.62	44.31	1.07^{NS}	1.16	22.16	1.03^{NS}	0.51	45.96	2.40**	4.43	79.94	0.98^{NS}	2.67	0.787	1.57**	2099.50
IT81D-889	15.67	0.96^{NS}	0.36	10.22	1.37^{NS}	0.98	38.61	1.08^{NS}	1.15	14.18	0.89**	0.10	39.71	1.83*	2.42	63.67	1.05^{NS}	0.74	0.346	0.44**	791.74
Chinese red	12.81	1.10^{NS}	0.24	9.47	1.87*	0.98	50.61	1.24^{NS}	1.06	11.63	0.96^{NS}	0.12	50.37	-0.53**	5.86	50.36	1.11^{NS}	8.01	0.444	0.93^{NS}	573.68
IT81D1064	13.84	1.38^{NS}	0.76	8.94	0.76^{NS}	0.30	37.44	1.14^{NS}	1.09	11.65	1.09^{NS}	0.19	37.67	1.97*	3.85	57.50	0.98^{NS}	2.66	0.328	0.51**	272.77
IT85F2205	14.41	1.23*	0.21	9.19	1.31^{NS}	0.84	46.33	1.09^{NS}	0.90	16.19	1.07^{NS}	0.77	45.99	0.98**	0.10	58.67	0.94^{NS}	1.59	0.677	1.16*	657.26
IT90K1020-6	11.95	1.00^{NS}	0.63	9.22	1.82^{NS}	1.65	37.19	0.87^{NS}	3.35	13.50	0.94^{NS}	0.27	37.31	3.10*	16.55	70.97	0.99^{NS}	0.57	0.347	0.44**	940.41
Black eye Crowder	13.37	1.26^{NS}	0.37	11.06	1.27^{NS}	3.88	63.86	1.22^{NS}	0.90	13.76	1.03^{NS}	0.10	65.35	-0.12**	3.09	82.00	0.97^{NS}	1.64	0.990	1.55**	1296.05
IT82D-16	18.00	1.16^{NS}	0.78	12.14	-1.49^{NS}	9.47	36.42	0.67^{NS}	3.75	16.41	1.11**	0.11	37.10	3.11*	15.50	71.31	1.07^{NS}	1.26	0.889	1.27**	1890.71
IT82- 812	13.07	1.04^{NS}	0.95	9.33	1.28^{NS}	0.26	40.06	0.83^{NS}	2.73	13.47	0.87^{NS}	0.48	41.07	3.11*	13.79	69.83	1.02^{NS}	3.40	0.528	-2.88**	82418.73
Sudany	10.58	0.94^{NS}	1.53	9.00	0.47^{NS}	2.84	67.25	0.74*	0.95	12.23	1.02^{NS}	0.31	67.81	-3.42*	73.98	54.31	1.01^{NS}	0.48	0.599	0.82**	238.80
Cream 12	15.91	1.04^{NS}	0.44	10.97	1.28^{NS}	7.23	52.06	0.65**	0.82	12.16	1.03^{NS}	0.01	52.40	0.11*	2.76	57.83	0.99^{NS}	0.57	0.862	0.83^{NS}	1649.81
Monarch black eye	11.60	1.26**	0.14	9.22	2.36*	2.59	34.78	0.75*	0.87	17.35	1.00^{NS}	0.16	37.87	4.26*	42.68	63.81	1.06^{NS}	0.79	0.941	1.55**	1822.09
Azmerly	15.98	0.65^{NS}	0.72	10.47	1.20^{NS}	0.37	58.25	0.73*	0.78	18.34	0.97^{NS}	0.28	58.33	0.20**	1.47	69.50	0.99^{NS}	0.57	0.914	2.57**	6115.00
Black Crowder	15.34	0.96^{NS}	0.25	11.19	0.80^{NS}	0.29	56.83	0.73^{NS}	1.29	13.99	0.99^{NS}	0.15	56.48	0.50^{NS}	1.39	72.75	0.95^{NS}	1.04	0.980	1.76**	3986.68
Mean		14.26			10.20			47.49			14.89			48.05			65.43			0.706	
L.S.D of G. M		0.528			0.434			0.602			0.331			0.743			0.839			17.503	
S. E (bi)		0.240			0.866			0.246			0.062			1.790			0.046			1.089	

localities or other similar environments.

Conflict of Interest

The authors have not declared any conflict of interest.

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Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers

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Moringa peregrina **plants were grown under four levels of saline water (0, 3000, 6000, 9000 ppm), and treated with sprayed Hoagland solution containing different concentrations of ZnO and Fe_3O_4 Nano-Particles (NP) (30, 60 and 90 mg/L); the normal Hoagland solution was used as a control. Results show that salinity levels significantly reduced growth parameters (plant height, root length, number of leaves, number of branches, shoot and root fresh and dry weights). Also, chlorophyll, carotenoids and crude protein levels decreased meanwhile proline and total carbohydrate levels, antioxidant non-enzymes (vitamins A and C) and enzymes (POD and SOD) increased.** ***Moringa*** **plants sprayed with Hoagland-containing ZnO and Fe_3O NP showed an enhancement in growth parameters either under normal or saline conditions when compared to control. Also, spraying plants with Hoagland-containing ZnO and Fe_3O NP resulted in significant reduction in Na^+ and Cl^- and an increase in N, P, K^+, Mg^{2+}, Mn^{2+}, Fe, Zn; total chlorophyll, carotenoids, proline, carbohydrates, crude protein levels, antioxidant non-enzymes and enzymes when compared to control, normal Hoagland sprayed-plants. Generally, this enhancement of salt tolerance was considerable in plants sprayed with 60 mg/L ZnO and Fe_3O_4 NP and grown either in saline and non-saline conditions.**

Key words: *Moringa peregrine*, nanofertilizers, salt stress, growth parameters, chemical composition.

INTRODUCTION

Moringa peregrina (Forssk.) Fiori (Moringaceae) is a tree (4 to 15 m) (Boulos, 1999). Its seeds have different economic and medical importance. Due to its unique composition, the extracted oil is highly valued for preparing cosmetics, cooking, and lubricating purposes (Somali et al., 1984). *Moringa* plants are considered a valuable source for many useful components such as vitamins A, B and C, and provide humans with minerals,

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protein and amino acids (Price, 2000). As a result of uncontrolled and indiscriminate use of this plant in many activities, the tree has decreased in numbers and become rare in Egypt (Zaghloul et al., 2008). New lands are considered as promising areas to cultivate this crop. The notable problems facing the plants cultivation in the reclaimed lands are drought, salt and heat stresses (abiotic stresses) which adversely affect the growth and productivity of the plants. Salt stress is one of the most devastating problems that limits the crop's production worldwide by imposing its effect through osmotic stress, Na^+ and Cl^- toxicity and ions uptake imbalance leading to deficiency in N, P, K^+, Ca^{2+} and micronutrients (Munns, 2005). Salt stress was reported to decrease the growth and yield of the plants as it affects the organic, ion contents and metabolic activity in the stressed plants. Accumulation of organic solutes is another mechanism that enables the plant to tolerate salt stress. Osmoprotectants (sugars, glycine betaine, proline, mannitol, etc) generally found in cytosol, plays an important role in osmotic adjustment as well as protection of enzymes and proteins (Munns and Tester, 2008). It was suggested that this osmprotecants work as scavengers of ROS (reactive oxygen species) which are induced by salt stress and negatively affect the lipid membrane and enzyme activity. Attempts have been made to increase plants' tolerance against salt stress. These efforts include classical breeding, gene transfer, seed priming, foliar application of osmoprotectants and inorganic compounds (Chen et al., 2007).

Nanoparticles (nano-scale particles; NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002). Nanofertilizers have been developed and have provided a new efficient alternative to normal regular fertilizers. The properties of nano-particles (more surface area) may help in increasing the reactive points of these particles and hence increase the reactivity of these nanoparticles. This leads to changes in the physio-chemical properties of these nanoparticles which help in the absorption of fertilizers in plants (Anonymous, 2009). The promoting effect of nano-particles on seedling growth and development were reported by Zhu et al. (2008). Also, nano-iron oxide compared to other treatments such as organic materials and iron citrate facilitated photosynthesis and iron transfer in peanut leaves (Liu et al., 2005). Nanoparticles can be divided into groups; metal based materials such as nanogold, nanozinc, nanoaluminum; and nanoscale metal oxides like TiO_2, ZnO and Al_2O (Ruffini and Roberto, 2009).

Foliar application of macro and micronutrients has been reported as an effective method to increase salt tolerance in plants and have been suggested to ameliorate the adverse effect of salt stress (Hamayun et al., 2011). This promoting effect can be attributed to the increased and enhanced nutrient uptake of micronutrients through the leaf or root as a result of root improvement. Foliar application may also offer a solution to overcome root restriction caused by salt stress (El-Fouly et al., 2004). Fe is critical for chlorophyll formation and photosynthesis and is important in enzyme systems and plant respiration (Malakouti and Tehrani, 2005). For most plants, zinc is an essential component of enzymes and participates in the synthesis of chlorophyll and other proteins (Vallee and Auld, 1990). The effect of nano fertilizers on plant growth in general and specifically under salt stress by investigating the effect of Hoagland solution containing ZnO and Fe_3O_4 NPs on *M. peregrina* plants grown under different levels of salinity is therefore the aim of this study.

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and National Institute of Laser Enhanced Sciences (NILES) Cairo University, Giza, Egypt during the two seasons of 2013 and 2014.

One year old seedlings of *M. bergrina* were obtained at the in the first and second seasons, respectively, from Orman Botanical Garden, Cairo, Egypt. Then the seedlings were transplanted into 25 cm diameter-plastic bags filled with 6 kg sandy soil, and watered every 3 days with Hoagland's nutrient solution (Hoagland and Arnon, 1950) for plant maintenance.

Soil analysis

The soil texture was sandy having the following characteristics: 30.82% coarse sand, 62.61% fine sand, 1.22% silt, 5.35% clay, pH 7.75, EC 1.15 dS/m, organic matter 0.08%, available N 6.9 ppm, available P 6.2 ppm, available K 64 ppm, $CaCO_3$ 0.26%, and water holding capacity 14.5%.

Salinity treatments

Two weeks after transplanting (in both seasons), the salinity treatments were initiated after 10 days. Four levels of salinity (Control, 3000, 6000, and 9000 ppm) were used for testing salt stress. The different saline water concentrations were prepared using a mixture of synthetic seawater salt obtained from Sigma Company. At each irrigation, the plants were watered till 100% of soil field capacity (F.C.). To maintain the required soil medium salt levels, the soil EC was measured periodically by portable EC meter.

Nano treatments

***Synthesis of ZnO and Fe_3O_4 magnetic nanoparticles (NPs)*:** Zinc acetate [Zn (H_3COO) $2H_2O$.], NaOH and isopropyl alcohol (2-propanol) with 99.5% reagent grade were obtained from Sigma Aldrich (99.9%). 0.073 mmol Zn (OAc). $2H_2O$ was dissolved at 60°C in 50 ml 2-propanol under stirring. In a second flask, 1.5 mmol NaOH was dissolved under vigorous stirring in 25 ml^2 propanol at 60°C. NaOH solution was added drop wise under stirring to the acetate solution. The product was stirred for an hour at 60°C and then cooled to room temperature. The precipitate was washed twice

with 2-propanol and centrifuged at 4500 rpm for 30 min (Bardhan et al., 2007). In addition, the Fe_3O_4 magnetic nanoparticles were prepared by coprecipitation of Fe^{3+} and Fe^{2+} at a molar ratio of 2:1 with aqueous ammonia (0.3 mol/L) as precipitating agent (Laurent et al., 2008).

Characterization of ZnO and Fe3O4 NPs: The size and shape of ZnO and Fe_3O_4 nanoparticles were observed directly by transmission electron microscope (TEM) (FEI, Netherland) The TEM samples were prepared by placing a few drops of the solution on a carbon-coated copper grid (Okenshoji Co., Ltd.).

Seedlings were sprayed monthly with Hoagland solution which replaced Zn and Fe with mixed ZnO and Fe_3O_4 NPs (30 (T1), 60 (T2), and 90 (T3) mg/L) after 10 days of adding salinity. Also, the normal Hoagland solution was used as a control (T0). Spraying was carried out between 09:00 and 11:00 AM.

Experimental design

The experiment was based on a Randomized Complete Block Design (RCBD) with two factors, including 16 treatments and three replicates. The first factor was control (without NPs application) and 3 levels of mixed ZnO and Fe_3O_4 NPs applications; the second factor had four irrigation water salinity treatments with each block consisting of 80 plants (five plants/ treatment). The seedlings were harvested at 90 days (in the two seasons, respectively) in order to determine the growth parameters and carry out chemical analysis.

Growth parameters

Plant height (cm), root length (cm), number of branches/plant, numbers of leaves/plant, stem diameter (cm), fresh and dry weight of shoots (leaves and stems) and roots (g/ plant) were also recorded.

Chemical analysis

Leaf pigments and total carbohydrates: Total chlorophyll and carotenoid contents were extracted using the method described by Nornai (1982). Total carbohydrates (%) in the dried leaves were also determined as described by Dubois et al. (1956).

Determination of macro and micronutrients and crude protein: Dried leaves samples were digested and the extract analyzed to determine nitrogen (N%) using the modified micro-Kjeldahl method, phosphorus (%) by Jackson (1967); K and Na% using a flame spectrophotometer (Jameel and Kahayri, 2002); while Ca, Fe, and Zn were determined by atomic absorption (Allen et al., 1984). The proline content in fresh leaves was also determined according to Bates et al. (1973). Also, protein % was determined as described by James (1995).

Antioxidant non-enzymes and enzymes determination: Antioxidant non-enzymes (vitamins A and C) were measured according to AOAC (1999) using dried leaves. Meanwhile, enzymes extraction was carried out using fresh leaf tissues at 40°C in buffer solution (3: 1 buffer:fresh weight v/v) in a pastel. It was mortared with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were stored in separate aliquots at 8°C (Vitoria et al., 2001). Antioxidant enzymes were assayed as follows; peroxidase (POD) by spectrophotochemically according to Amako et al. (1994) and superoxide dismutase (SOD) by photochemical method as described by Giannopolitis and Ries (1977). Enzymes activities were expressed as units/min/mg protein.

Statistical analysis

The data were subjected to statistical analysis of variance and the means were compared using the least significant difference (LSD) test at the 5% level, as described by Little and Hills (1978).

RESULTS AND DISCUSSION

Characterization of photo-catalysts by TEM

The shape and diameter of the nanoparticles used were observed with TEM. TEM image emphasized that ZnO presents in spherical nanoparticle form, with a diameter range of about 10-15 nm (Figure 1a) while Fe_3O_4 nanoparticles diameter ranges from approximately 10 to 12 nm with an almost spherical shape.

Effect of salt stress on salt stressed-plants

Table 1 shows that growth parameters (plant height, root length, stem diameter, number of leaves, number of branches) decreased in response to different concentrations of salinity and this reduction was significant in plants treated with the two levels of salinity (6000 and 9000 ppm). Furthermore, the shoot and root fresh and dry mass of the *Moringa* plants decreased significantly under salinity conditions compared to those of control plant.

Soil salinity adversely affects plant growth through several physiological and biochemical means like ion toxicity, osmotic stress, nutritional imbalance, biochemical and physiological disorders (Kao et al., 2003). Salt stress resulted in the reduction in the number of leaves and branches and stunted shoot growth in *Acacia saligna* (Soliman et al., 2012). Moreover, Bello and Igbokwe (2013) reported that salt stress reduced height of both *Acacia senegal* and *Parkia biglobosa*. The first reduction in plant growth may be attributed to the initial sudden increase in osmotic pressure as stated by Hajibagheri et al., (1989) thus suggesting that high salinity might inhibit root and shoot elongation due to slowing down of water uptake by the plant. Over time, Na^+ and Cl^- will accumulate to toxic concentrations in the shoot resulting in premature leaf senescence and death due to the ionic component of salt (Munns and Tester, 2008; Hairmansis et al., 2014). The accumulated amounts of ions enter the plant through the transpiration stream thereby causing cells injury in the transpiring leaves which may cause further reductions in photosynthesis processes thereby

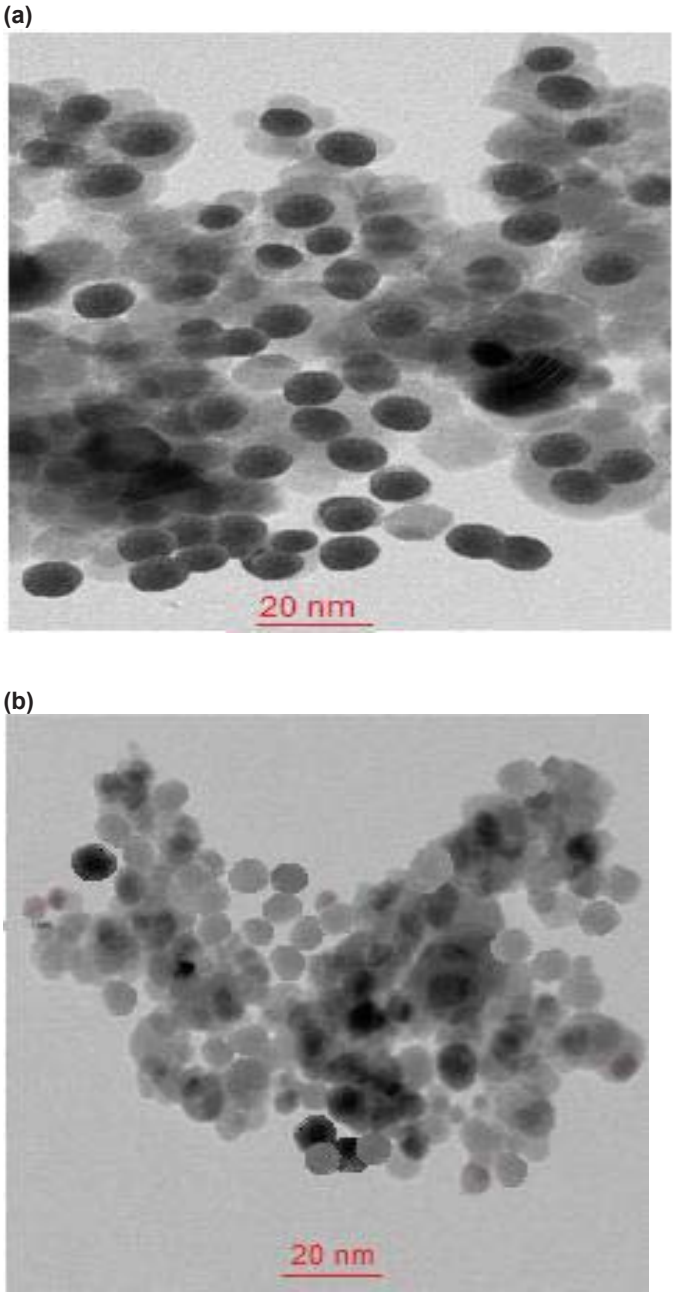

Figure 1. TEM image of the prepared nanoparticles. (a) ZnO, (b) Fe_3O_4.

leading to growth reduction (El-Fouly et al., 2002; Munns et al., 2006).

Total chlorophyll (Chl a and b) and carotenoides contents were significantly lower in plants grown under salt stress conditions than those recorded in control plants (Table, 2). A reduction of 48% in total chlorophyll contents of *Moringa* leaves was recorded at the third salinity concentration (9000 ppm). An inhibition in chlorophyll biosynthesis, activation in the chlorophyllase and/or destruction of chloroplast structure (Gunes et al., 1996) could have contributed to lowering the pigment content under saline conditions.

The results also showed an increase in proline and charbohydrate concentrations in leaves of *Moriga* plants in response to different levels of salt stress (Table 2). In this regard, increased free amino acids and proline in response to salt stress has been investigated by many researchers in many plants (Gunes et al., 1996; Sharma et al., 2010). One of the strategies that plants use to cope with salt stress is osmoprotectant synthesis of low molecular weight molecules such as sugars, proline and glycine betaine which play an important role in osmotic adjustments and protection of protein and lipids from (ROS). These further results in the protection of plasma membrane integrity and enzyme function. Also, it plays an important role as a scavenger for free radicals which protects cells from ROS actions. Proline serves as a storage sink for carbon and nitrogen and it is a free-radical. It also stabilizes subcellular structures (membranes and proteins), and buffers cellular redox potential. Hence, these organic osmolytes are known as osmoprotectants. These organic solutes may contribute to osmotic adjustment, protecting cell structure and function, and/or may serve as a metabolic or an energetic reserve (Chen and Murata, 2000).

Crude protein was found to decrease in response to salt stress. Protein synthesis has been considered as a possible primary target of salt toxicity because in vitro protein synthesis systems are dependent on physiological potassium and are inhibited by sodium and chloride (Morant-Avice et al., 1998). Considering the evidences on plant soluble protein response to salinity, there is a marked difference between the species and varieties. Thus, proteins may play a role in osmotic adjustment. According to Pareek et al. (1997), proteins may be synthesized *de novo* in response to salt stress, or may be present constitutively in low concentrations and increased when plants are exposed to salt stress.

Raising the salt concentration significantly increased antioxidant non-enzymes (vitamins A and C) and enzymes (POD, and SOD) in tissues of *Moringa* leaves (Figure 2) in both seasons. Accordingly, the lowest values of the non-enzymatic and enzymatic antioxidants were found in control plants irrigated with tap water, whereas the highest values were found in plants irrigated with water containing the highest salt concentration (9000 ppm). Such results are in harmony with Foyer and Noctor (2009), Cazzonelli and Pogson (2010) and Boguszewska and Zagdańska (2012). They found that many plants produce significant amount of a potential source of compounds such as non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD and SOD) to prevent toxidative stress caused by oxygen and photons. Piotr

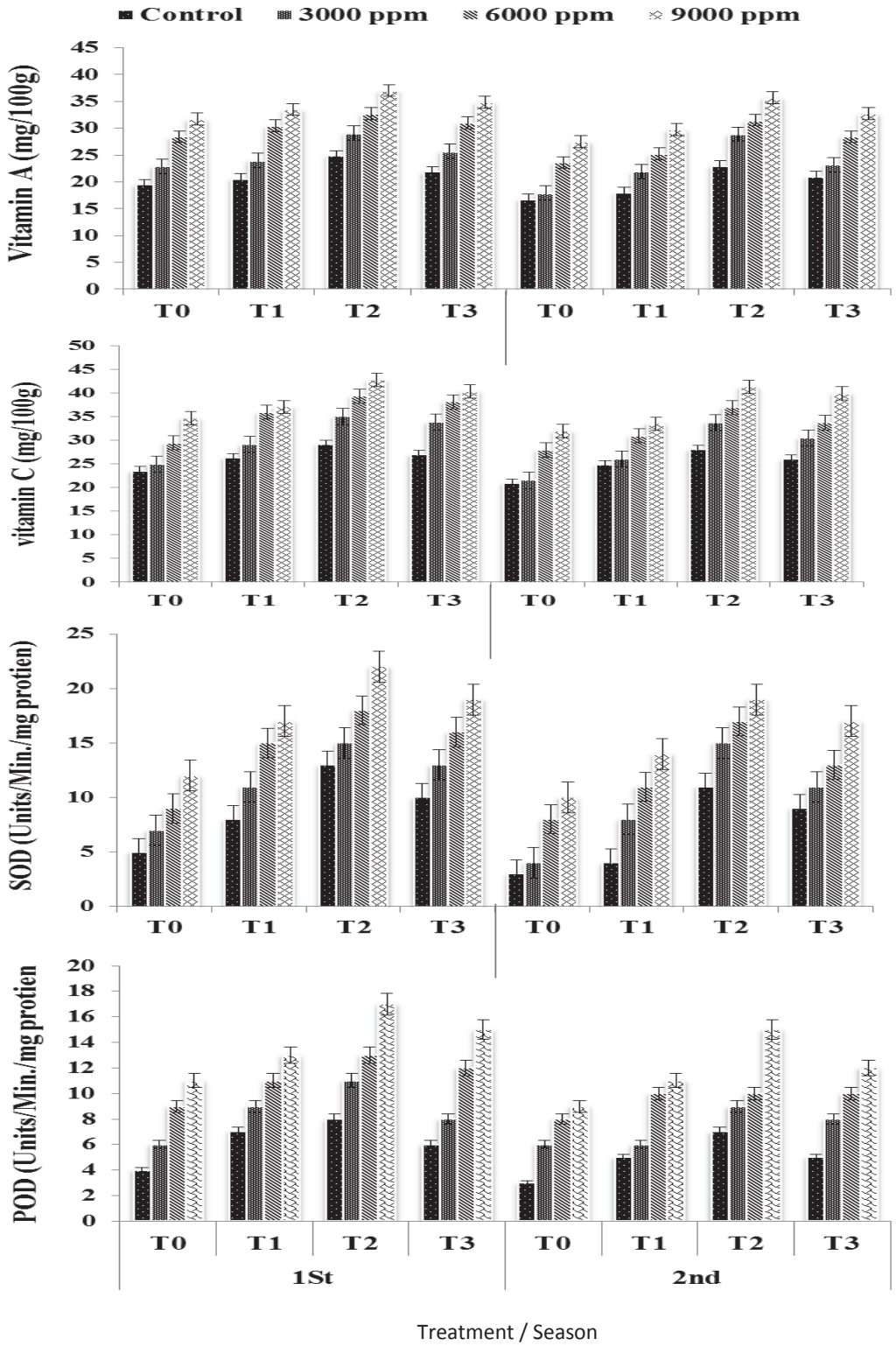

Figure 2. Effect of salt stress and nanofertilization on non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD, and SOD) in *M. pregrina* during 2013 and 2014.
T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and $Fe3O_4$ NPs, T2= 60 mg/L ZnO and $Fe3O_4$ NPs and T3= 90 mg/L ZnO and $Fe3O_4$ NPs.

Table 1. Effect of salt stress and nanofertilization on growth parameters of *M. pregrina* during 2013 and 2014.

Treatment			Plant height (cm)	Root length (cm)	Stem diameter (cm)	No. of leaves/ plants	No. of branches/ plant	Fresh weight of leaves and stems (g/ plant)	Dry weight of leaves and stems (g/ plant)	Fresh weight of roots (g/ plant)	Dry weight of roots (g/ plant)
Control	T0	1St	45.00	24.00	2.00	29.00	11.33	21.13	8.90	95.40	46.70
	T1		59.33	29.33	2.37	39.00	23.00	24.80	11.07	128.50	63.25
	T2		70.00	38.33	3.00	52.00	27.67	32.10	16.05	149.43	74.05
	T3		67.00	33.00	2.70	48.33	26.00	28.30	13.15	142.60	70.63
	T0	2nd	41.33	19.33	0.90	24.00	9.00	18.57	7.95	91.70	45.18
	T1		50.00	24.00	1.60	35.00	16.00	21.90	9.28	125.90	61.95
	T2		67.67	32.00	2.00	47.00	22.00	29.20	14.60	140.30	69.65
	T3		60.33	29.67	1.90	43.00	20.00	28.00	12.00	136.80	67.73
3000 ppm	T0	1St	39.33	22.00	1.80	25.00	9.00	18.80	8.73	92.70	45.35
	T1		46.33	24.00	2.10	34.67	18.00	23.43	10.41	121.90	60.28
	T2		66.67	35.00	2.80	49.67	24.00	30.70	14.68	142.07	70.68
	T3		59.67	29.33	2.50	43.00	22.00	26.63	12.32	134.80	66.73
	T0	2nd	34.00	17.33	0.70	21.00	8.00	17.20	7.72	87.90	42.95
	T1		40.00	21.00	1.20	30.00	12.00	19.50	8.75	118.50	58.25
	T2		61.67	30.00	1.90	43.00	19.00	26.67	11.67	136.80	67.73
	T3		53.00	27.00	1.70	41.00	16.33	21.90	9.62	130.40	64.53
6000 ppm	T0	1St	31.33	18.67	1.20	19.67	7.00	15.30	6.95	84.10	41.38
	T1		40.00	20.00	1.73	26.00	11.33	20.09	9.38	117.60	58.8
	T2		52.67	30.33	2.10	37.00	18.33	27.37	12.96	136.70	68.02
	T3		47.33	25.00	2.00	32.00	15.67	24.47	11.91	128.90	63.73
	T0	2nd	25.00	13.00	0.53	16.67	5.00	12.79	5.59	80.40	39.53
	T1		38.33	17.33	1.17	22.33	8.00	17.90	8.02	114.9	56.45
	T2		47.33	25.00	1.60	33.00	15.00	24.44	10.89	131.60	65.30
	T3		41.67	21.00	1.30	30.00	11.00	20.13	8.73	123.70	61.35
9000 ppm	T0	1St	28.33	13.33	0.90	15.00	4.67	13.44	6.31	71.10	35.12
	T1		31.33	18.00	1.30	20.00	8.33	15.97	7.56	113.50	56.08
	T2		43.33	28.67	1.60	30.00	13.00	21.57	10.35	128.90	64.08
	T3		38.33	21.33	1.45	24.00	10.33	18.85	8.94	120.40	59.50
	T0	2nd	21.00	11.00	0.43	12.33	3.33	11.80	4.70	67.40	33.03
	T1		27.67	14.33	0.80	16.33	5.33	13.65	6.42	109.80	54.23
	T2		40.00	20.00	1.17	28.67	11.00	18.87	8.83	123.23	61.12
	T3		32.00	18.33	1.00	22.33	9.00	15.90	7.18	118.70	58.85
LSD (0.05)											
S		1St	1.69	4.07	0.30	8.01	1.53	2.92	1.13	5.92	2.36
N			5.73	6.53	0.66	5.04	2.03	5.06	2.38	3.10	1.59
N× S			11.46	13.06	1.33	10.08	4.07	10.12	4.76	6.20	3.18
S		2nd	2.44	2.64	0.26	8.33	1.24	5.59	2.38	10.88	5.26
N			5.93	5.99	0.28	6.18	2.23	3.69	1.43	6.81	3.34
N× S			11.58	11.98	0.56	12.36	4.47	7.38	2.86	13.61	6.69

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and Fe3O4 NPs, T2= 60 mg/L ZnO and Fe3O4 NPs and T3= 90 mg/L ZnO and Fe3O4 NPs. S= salinity treatments, N= nano treatments. 1st = first season, 2nd = second season.

and Klobus (2005) and Wu et al., (2007) reported that ascorbic acid is an important antioxidant which reacts not only with H_2O_2 but also with O_2, OH and lipid hydroperoxidases. In addition, Shao et al. (2006) and Abogadallah (2010) indicated that ascorbic acid concentration significantly increases in turf grass during water deficiency. Mittler (2002) and Akram et al. (2012) reported that the enzymatic antioxidants SOD and POD

Table 2. Effect of salt stress and nanofertilization on chemical composition of *M. pregrina* during 2013 and 2014 seasons.

Treatment			Total chlorophylls content (mg/g fresh weight)	Carotenoids content (mg/g fresh weight)	Total carbohydrate (% of dry weight)	Proline content (µ moles/g fresh weight)	Crude Protein (%)
Control	T0	1St	1.66	0.75	19.00	13.00	18.94
	T1		1.70	0.84	23.00	15.00	20.88
	T2		2.29	1.14	29.33	19.333	22.63
	T3		1.96	0.92	27.67	18.00	22.13
	T0	2nd	1.47	0.68	15.67	17.33	18.06
	T1		1.53	0.72	19.33	18.67	19.94
	T2		2.15	1.01	25.67	23.67	21.50
	T3		1.69	0.843	23.33	21.33	20.63
3000 ppm	T0	1St	1.63	72.33	22.33	16.00	17.81
	T1		1.59	0.78	28.33	19.33	18.69
	T2		2.24	1.11	33.67	22.67	20.06
	T3		1.81	0.89	30.33	20.33	19.31
	T0	2nd	1.22	0.59	17.67	19.67	16.69
	T1		1.43	0.69	24.67	21.33	17.69
	T2		2.18	1.06	29.33	28.33	19.38
	T3		1.67	0.84	27.33	25.00	18.13
6000 ppm	T0	1St	1.09	0.55	26.00	20.33	12.81
	T1		1.32	0.68	33.00	25.00	14.31
	T2		2.18	1.08	39.33	29.33	17.19
	T3		1.59	0.81	37.33	27.33	15.25
	T0	2nd	0.99	0.43	23.33	23.00	12.31
	T1		1.25	0.58	29.67	30.33	13.50
	T2		2.02	0.98	35.33	33.00	15.13
	T3		1.51	0.74	31.33	30.33	14.313
9000 ppm	T0	1St	0.86	0.41	32.33	25.67	11.00
	T1		1.05	0.50	37.67	27.67	11.88
	T2		1.49	0.79	43.33	32.33	14.19
	T3		1.13	0.57	40.00	30.67	12.63
	T0	2nd	0.75	0.32	28.00	28.00	9.69
	T1		1.01	0.48	33.00	31.33	11.69
	T2		1.30	0.66	39.33	38.33	13.63
	T3		1.02	0.51	37.67	35.33	12.38
LSD (0.05)							
S		1St	0.03	0.03	0.94	1.76	0.09
N			0.08	0.05	3.89	4.02	0.47
N×S			0.15	0.09	7.78	8.04	0.94
S		2nd	0.06	0.06	1.03	1.54	0.33
N			0.07	0.05	3.05	3.27	0.50
N×S			0.13	0.11	6.10	6.53	1.00

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and $Fe3O_4$ NPs, T2= 60 mg/L ZnO and $Fe3O_4$ NPs and T3= 90 mg/L ZnO and $Fe3O_4$ NPs. S= salinity treatments, N= nano treatments.

are considered to be the first line of defense against ROS thus the simultaneous increase in the activity of these enzymes contributes to a decrease in the deleterious effects of H_2O_2 under stress. Also, POD activity increased in eggplant plants under saline conditions (Shaheen et al., 2013). Other studies also reported that salt stress-induced enhanced POD and SOD activities were observed in sunflower (Akram et al., 2012) and pistachio plants (Abbaspour, 2012). Thus, it becomes clearly evident that non-enzymatic and enzymatic antioxidant status of plants for ROS scavenging is an important salt tolerant trait.

Salinity stress significantly increased percentage Na, Cl and Ca and reduced percentage K, Mg and P in the leaves of *Moringa* plants (Table 3). Salinity may result in the disturbance of uptake and utilization of essential nutrients due to competition and interactions of soluble salts with mineral nutrients (Gouia et al., 1994). Ionic imbalance occurs in the cells due to over accumulation of Na^+ and Cl^- and reduced uptake of other mineral nutrients, such as K^+, Ca^{2+}, Mg^{+2} and No^- and Mn^{2+} thus leading to growth suppression (Karimi et al., 2005).

Effect of ZnO and Fe_3O_4 NPs- containing Hoagland solution on salt stressed-plants

The foliar application of nano-iron and -zinc containing-Hoagland solution caused a significant increase in previously mentioned growth parameters in comparison to control plants (Table 1). This promoting effect of these nano-applications was not only noticed in the growth of salt-stressed plants, but also did promote the growth in plants grown under normal conditions. The most interesting result is that under the highest salinity level 9000 ppm, the increment in shoots fresh weight, number of leaves and plant height reaches up to 60, 100 and 53 % respectively in plants treated with the T2 (6 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) over control plants (Hoagland-sprayed plants). This increment was recorded in the first and second seasons. It means that the T2 treatment has a strong promoting effect either in stress or non-stress conditions. It also noticed that T1 (3 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) and T3 (9 mg/l ZnO and Fe_3O_4 NPs-containing Hoagland solution) also has promoting effect on growth parameters of plants grown under both stress and non-stress conditions in comparison to Hoagland-sprayed plants but is however less than those found in the T2 treatment. The aforementioned data are in trustworthiness with Aslam et al. (1993) who mentioned that growth parameters have been used as an indicator of salt tolerance in plants e.g. shoot weight. Meanwhile, significant increase in biomass, with respect to length or diameter of stem, leaves and dry weight (DW) of plants was observed by spraying *Moringa* plants with the combination of zinc and iron nano fertilizers. This indicates that proper concentration of zinc is required for dry matter accumulation and plant growth (Dimkpa et al., 2013).

Improved salt tolerance by addition of nutrients has been reported in many plants (Zhu et al., 2004 on cucumber; Al-Aghabary et al., 2005 on tomato). In addition, application of micronutrients is reported to enhance photosynthetic activities which lead to an increase in cell division and elongation thereby increasing vegetative biomass. It was also found that foliar spray of zinc sulfate (Yildirim et al., 2008) and treatment of seedlings with zinc sulfate before transplanting (Tzortzakis, 2010) leads to relieve symptoms of salt stress.

Nano-technology can offer opportunities to enhance yield and counter environmental stress. By using nano-particles, we aim to delay releasing fertilizers. Nano-particles have high reactivity because of the larger specific surface area and increased reactivity of these areas on the particle surface. These features simplify the absorption of fertilizers and pesticides that are produced in nano scale (Anonymous, 2009). The application of nano-particles to plants can be beneficial (seedling growth and development) or non-beneficial (prevent root growth) (Zhu et al., 2008). These results are in agreement with the findings of Liu et al., (2005) who concluded that nano-iron oxide facilitated photosynthesis and iron transfer to the leaves of peanut when compared to organic materials and iron citrate. In addition, Sheykhbaglou et al. (2010) found that the nano-iron oxide had significant effects on the dry pod weight; leaf with dry pod, and yield of soybean compared to other treatments. In pumpkin, iron oxide NPs increased root elongation which was attributed to Fe dissolution (Wang et al., 2011). Thus, the positive effects of appropriate zinc and Fe concentrations on fresh and dry weight, plant height, number of leaves and branches under NaCl stress could be explained by the replacement of Fe and Zn with nano forms.

Foliar applications with nano-iron and nano-zinc containing-Hoagland solutions at different concentrations lead to increased total chlorophyll, carotenoids, proline content, total carbohydrates and crude protein percentage more than those recorded in Hoagland-sprayed plants either in non-stress or stress conditions (Table 2). At the highest level of salinity (9000 ppm), increased percentage values in chlorophyll content resulted from the application of the nano form of Fe and Zn Hoagland solution. This increase reached 73% in both seasons when treated with T2 and was noticed in increased leaf numbers. In addition, iron plays an important role in the photosynthetic reactions as it is a component of ferrodoxin, an electron transport protein associated with chloroplast (Hazra et al., 1987). Iron also activates several enzymes and contributes in RNA synthesis and

Table 3. Effect of salt stress and nanofertilization on macro and micro nutrients in *M. pregrina* during 2013 and 2014 seasons.

Treatment		N (%)	P (%)	K (%)	Na (%)	Cl (%)	Ca (%)	Mg (%)	Fe ppm	Zn ppm
T0		3.03	0.30	2.19	0.35	0.18	0.55	0.55	87.46	95.40
T1	1St	3.34	0.43	2.39	0.31	0.17	0.74	0.90	95.61	103.73
T2		3.62	0.59	2.62	0.23	0.11	1.02	1.32	121.25	140.50
T3		3.54	0.47	2.45	0.29	0.13	0.88	1.18	110.50	128.90
Control										
T0		2.89	0.26	1.89	0.41	0.23	0.47	0.48	82.72	88.50
T1	2nd	3.19	0.37	2.14	0.37	0.21	0.60	0.78	90.85	99.87
T2		3.44	0.48	2.33	0.29	0.16	0.93	1.19	117.54	123.80
T3		3.30	0.40	2.20	0.35	0.19	0.79	0.93	100.25	110.60
T0		2.85	0.27	1.77	0.30	0.27	0.76	0.41	80.26	86.77
T1	1St	2.99	0.32	2.00	0.35	0.25	1.10	0.82	87.92	95.63
T2		3.21	0.48	2.29	0.29	0.18	1.25	1.02	100.53	119.48
T3		3.09	0.37	2.07	0.31	0.23	1.16	0.96	93.51	105.56
3000 ppm										
T0		2.67	0.24	1.63	0.42	0.31	0.63	0.40	76.77	70.29
T1	2nd	2.83	0.28	1.83	0.44	0.28	0.99	0.68	80.39	89.80
T2		3.10	0.39	2.00	0.34	0.21	1.17	0.95	93.34	106.58
T3		2.90	0.32	1.90	0.41	0.25	1.10	0.79	88.36	97.28
T0		2.05	0.22	1.59	0.47	0.40	1.01	0.32	72.70	69.34
T1	1St	2.29	0.27	1.79	0.41	0.33	1.30	0.64	80.26	80.48
T2		2.75	0.36	1.93	0.33	0.26	1.46	0.95	98.42	102.80
T3		2.44	0.30	1.88	0.37	0.29	1.39	0.77	90.53	88.77
6000 ppm										
T0		1.97	0.19	1.44	0.54	0.47	0.85	0.28	64.36	57.14
T1	2nd	2.16	0.22	1.63	0.49	0.39	1.19	0.57	74.61	77.83
T2		2.42	0.31	1.77	0.38	0.29	1.35	0.90	95.43	93.32
T3		2.29	0.26	1.70	0.45	0.34	1.26	0.73	87.33	80.87
T0		1.76	0.17	1.37	0.64	0.45	1.15	0.24	39.3	44.81
T1	1St	1.90	0.23	1.45	0.48	0.40	1.54	0.48	55.21	76.92
T2		2.27	0.29	1.67	0.40	0.30	1.73	0.87	67.85	90.41
T3		2.02	0.25	1.58	0.45	0.36	1.60	0.59	60.44	87.33
9000 ppm										
T0		1.55	0.12	1.3	0.70	0.50	1.10	0.21	32.45	30.20
T1	2nd	1.87	0.16	1.37	0.55	0.46	1.30	0.35	49.47	69.47
T2		2.18	0.25	1.59	0.47	0.35	1.50	0.81	63.32	87.83
T3		1.98	0.21	1.46	0.51	0.41	1.39	0.53	57.48	80.30
LSD (0.05)										
S		0.001	0.12	0.22	0.001	0.05	0.08	0.20	3.98	5.57
N	1St	0.08	0.07	0.23	0.04	0.03	0.08	0.06	6.78	5.87
N× S		0.15	0.14	0.47	0.08	0.05	0.17	0.12	13.57	11.73
S		0.05	0.03	0.33	0.03	0.05	0.08	0.07	4.99	6.28
N	2nd	0.08	0.06	0.22	0.04	0.04	0.07	0.10	4.98	9.23
N×S		0.16	0.12	0.44	0.08	0.08	0.14	0.19	9.97	18.46

T0= normal Hoagland solution (control), T1 = 30 mg/L ZnO and $Fe3O_4$ NPs, T2= 60 mg/L ZnO and $Fe3O_4$ NPs and T3= 90 mg/L ZnO and $Fe3O_4$ NPs.
S= salinity treatments, N= nano treatments. 1st = first season, 2nd = second season.

improves the performance of photosystems (Malakouti and Tehrani, 2005). Moreover, iron oxide NPs have been reported as facilitators for iron and photosynthate transfer to the leaves of peanut (Liu et al., 2005). Meanwhile, Zn plays an important role in many biochemical reactions within the plants like chlorophyll and carbohydrate formation (Corredor et al., 2009), increased photochemical reduction rates (Kumar et al. 1988), chloroplast structure, photosynthetic electron transfer as well as photosynthesis (Romheld and Marschner, 1991); in enzyme structure involved in amino acid biosynthesis (Cakmak et al., 1989). These results agree with those of El-Kereti et al. (2013) and El-Feky et al. (2013). The results presented in Figure 2 also indicates that in both seasons, the foliar application of a combination of ZnO and Fe_3O_4 NPs in Hoagland solution significantly increased non-enzymatic (vitamins A, and C) and enzymatic antioxidants (POD and SOD) in *Moringa* seedlings in comparison with control plants. The elevated amount in non-enzymatic and enzymatic antioxidants may be attributed to the beneficial effects of Fe and Zn represented in the increasing liberation of more nutrients from the unavailable reserves through correcting iron and zinc deficiency thus resulting in photosynthesis efficiency, increasing amino acids and vitamins to be absorbed by plant roots. This may be attributed to the importance of iron as a cofactor for many enzymes that catalyze unique biochemical reactions that are essential plant development such as chlorophyll and thylakoid syntheses and chloroplast development (Miller et al., 1995). Meanwhile, zinc is an essential element for plants that act as a metal component of various enzymes or as a functional structure or regulatory cofactor for protein synthesis and photosynthesis (Marschner, 1995). Also, Chang and Sung (1998) concluded that priming with antioxidant compounds such as ascorbic acid could increase free radical scavenging enzymes such as superoxide dismutase (SOD), and peroxidase in seeds.

Salt-stressed *Moringa* plants accumulated lower amounts of Na^+, Cl^- and higher amount of N, K^+, P, Ca^{2+}, Mg^{2+}, Fe and Zn upon foliar application of ZnO and Fe_3O_4 NPs-containing Hoagland solution when compared to those of the salt-stressed plants that received only foliar application of Hoagland solution (Table 3). The accumulation of less Na^+ is an important indicator of salt tolerance in plants as those subjected to foliar applications with ZnO and Fe_3O_4 NPs-containing Hoagland solution showed less accumulation of Na^+ in their shoots either in stress or non-stress conditions. The reduction of Na^+ in shoots of *Moringa* plants grown under the highest salinity level and sprayed with T2 reached 37 and 32 % in first and second seasons, respectively, in comparison to plants that received only Hoagland solution and grown under the same salinity level (9000 pm). At highest salinity level, the increase of K^+ in T2- sprayed plants reached 21 and 22% in both the first and second seasons, respectively, over Hoagland-sprayed plants. The importance of determining percentage Na^+ and K^+ in the plants is because they reflect salt tolerance in plants (Tunçtürk et al., 2011). Foliar feeding with micronutrients could partially alleviate the adverse effect of NaCl on nutrients uptake through improving root growth and preventing nutritional disorders and consequently resulting in an increase in nutrients uptake by the roots (El-Fouly et al., 2002). Also, zinc may help nutrient translocation from aged cells to newborn cells (Rockenfeller and Madeo, 2008). Zinc may, therefore, play an important role in membrane permeability, phospholipids (P) accumulation, and free oxygen radical scavenging. These results correlate with the findings of Qu et al. (2009) who reported that zinc application could alleviate possible Na^+ and Cl^- injury in plants.

Our results reveal that salt toxicity in *Moringa* plants can be alleviated by foliar spray of nano- zinc and iron. The results are consistent with Cakmak and Marschner (1988) who reported that zinc could play an important role in the maintenance of the structural integrity of the plasma membrane and thus control Na and other toxic ions uptake. Similarly, Saleh and Maftoun (2008) observed that zinc application reduced Na^+ concentration in rice shoot. Cakmak and Marschner (1988) reported that under zinc application, the activity of membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase producing reactive oxygen species (ROS), decreased.

In this study, alleviation of salt stress can be attributed to two reasons: first, promoting effect of spraying nutrients in Hoagland solution on *Moringa* plants grown under salt stress conditions and control conditions; second, the properties of ZnO and Fe_3O_4 NPs (larger specific surface area and moew reactive areas) that help in enhanced enzyme activity related to salt tolerance. Thus the Fe_3O_4 NPs were found to induce oxidative stress and higher antioxidative enzyme activity than the bulk Fe_3O_4 particles.

Conclusion

In this study, we succeeded in showing that salt stress can be alleviated in *Moringa* plants using foliar applications of ZnO and Fe_3O_4 NPs-containing Hoagland solution in comparison to spraying only with normal solution. Growth parameters and chemical composition related to salt tolerance were enhanced when nano-forms of Fe and Zn were used in Hoagland solution (60 mg/L).

Conflict of Interest

The authors have not declared any conflict of interest.

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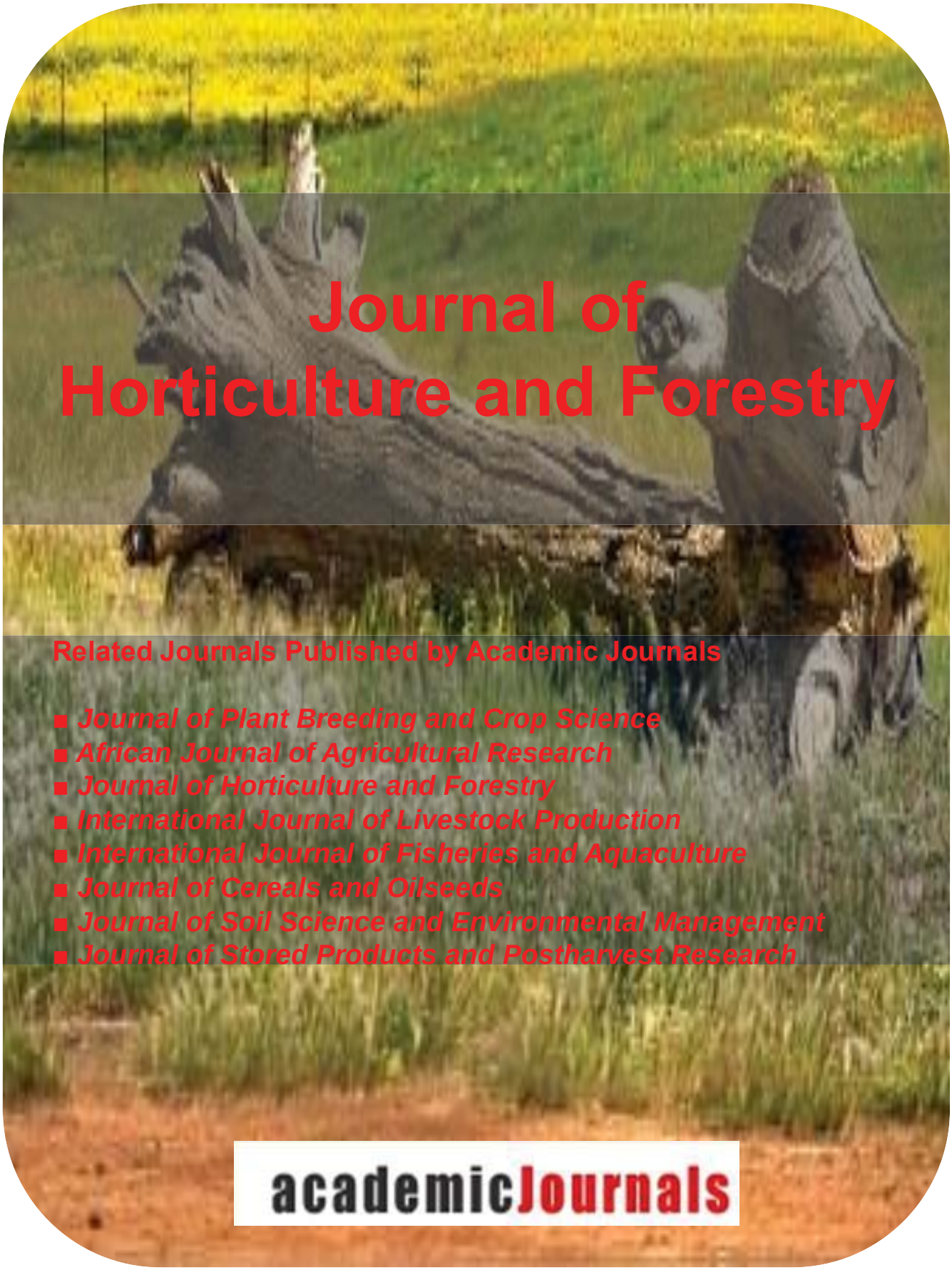

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