Asian J Agri & Biol. 2018;6(1):115-124.

AJAB

Original Research Article

Salicylic acid improves physiological traits of *Zea mays* L. seedlings under copper contamination

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Received:
September 09, 2017

Accepted:
February 12, 2018

Published:
March 27, 2018

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Abstract

A pot sand culture experiment was conducted to study the effect of copper (Cu) on maize plant growth with or without salicylic acid (SA). Nutrient medium (i.e. half strength Hoagland) along with sixteen different concentrations of Cu and SA were applied as rooting medium twice a week. The lower level of salicylic acid (0.1 mM) increased the biomass production, length of shoot and root, number and area of leaves. While higher level of salicylic acid (10.0 Mm) reduced all the growth parameters. The excess copper (10.0 mM) reduced the shoot and root length, biomass production, number and area of leaves. In both harvests copper accumulations in root had highest value by treating with 10 mM SA and 5 µM copper contamination and minimum value by applying the same concentration of SA but lowest concentration of copper 0.5 µM. While copper accumulation in shoot is not effected by addition of different levels of copper and salicylic acid at both harvest levels. Results indicate that SA application may be one approach to improve growth of this crop under copper contamination but high concentration of SA can decrease the crop growth and Cu accumulation in roots increased with increased Cu contamination.

Keywords: Plant hormone; Cu accumulation, Plant growth

Introduction

Salicylic acid (SA) is an endogenously produced phenolic compound, which is regarded as an important plant growth regulator (Shakirova et al., 2003) and a signaling molecule and plays vital role in regulating various physiological processes in plant systems(Hayat et al., 2010; Rivas-San Vicente and Plasencia, 2011; Wani et al., 2017). Under adverse environmental conditions, itis actively involved in conferring plants resistance against various biotic (Hussain et al., 2008) and abiotic stresses (Noreen and Ashraf, 2010) including the heavy metal stress (Liu et al., 2016; Shakirova et al., 2016; Wani et al., 2017). Heavy metal contaminated soil soriginating from natural depositions and anthropogenic activities have posed serious environmental, nutritional and human health implications (Zhao et al., 2013; Najam et al., 2015; Clemens and Ma, 2016; Shakoor et al., 2017). Expensive reclamation process has alternatively forced to go for phytoremediation technologies, which are considered to be innovative, cost effective and environment friendly solution for remediation of soils contaminated with heavy metals (Bennett et al., 2003; O'connor et al., 2003; Wang et al., 2003; Ehsan et al., 2016).Plants plays vital role in remediation of contaminated soils; however, they must have intrinsic tolerance against higher concentration of heavy metal contamination (Krämer, 2010). Certain plants have been reported to have ability to tolerate heavy metal toxicity including *Zea mays* L. (Lombi et al., 2001), *Brassica juncea* L. (Bennett et al., 2003) *Polygonum* *hydropiper* L. (Wang et al., 2003). Copper is one of the most destructive heavy metals and may induce growth abnormalities in maize (Brennan et al., 2014). High concentrations of copper are toxic for plants and have direct or indirect impacts on plant growth and development due to interference with various physiological processes (Liu et al., 2014; Adrees et al., 2015; Rizwan et al., 2017), restrict root development and ultimately results in inhibition of plant growth (Weckx and Clijsters, 1996; Madejonb et al., 2009; Kopliku and Mesi, 2012).Plenty of scientific literature is available to highlight copper toxicity in plants and its negative impacts on growth parameters, mineral composition and chlorophyll contents, and activities of several key enzymes (Mocquot et al., 1996; Chen et al., 2000; Brun et al., 2003; Ouzounidou and Ilias, 2005; Maksymiec and Krupa, 2006; Sağlam et al., 2016). Maize seedling exposed to copper solution lead to significant inhibition of root growth, although its impact on shoot growth remained non-significant (Bashmakov et al., 2005). Plant roots have strong affinity to absorb copper ions thus hindering the development of fine roots and uptake of trace element (particularly Fe) (Lexmond and Van der Vorm, 1981; Minnich et al., 1987). Macnicol and Beckett (1985) reported that in maize plants copper toxicity (21 mg/kg) in shoots can lead to significant yield reduction (10% yield reduction). Subsequent studies reconfirmed 20 mg/kg copper concentration in maize leaf or shoot as critical thresholds of Cu toxicity indicator (Mocquot et al., 1996; Borkert et al., 1998). SA improved antioxidant capacity and influences adaptability to the toxicity of Hg and Pb by preventing oxidative stress (Zhou et al., 2009; Wang et al., 2011). Further, the role of SA against Cu stress was investigated in tobacco and cucumber (Strobel and Kuc, 1995). This study was also aimed to investigate the potential effectiveness of salicylic acid in protecting maize (*Zea mays* L.) against copper toxicity.

Materials and Methods

Seeds of maize hybrid 30Y87 were sown in petri dishes under natural light condition (with average temperature and relative humidity ranges from 35°C to 60%, respectively) in a glasshouse at Bahauddin Zakariya University, Multan, Pakistan. The ceiling of glasshouse was covered with a green sheet. The seeds were kept moist with distilled water. After germination, five seedlings were transplanted in each of 128 plastic pots of 28 cm diameter, containing 3.5 kg of thoroughly washed dried sand, which were irrigated with 250ml water containing half strength of Hoagland Solution(as described in Table A).

Table – A: Composition of nutrient solution

Compound	Concentration of Stock Solution g/L	½ strength Hoagland soln. mL Stock Solution/1L
Macronutrients		
KNO_3	101.10	3
$Ca(NO_3)_2 \bullet 4H_2O$	236.16	2
$NH_4H_2PO_4$	115.08	1
$MgSO_4 \bullet 7H_2O$	246.48	0.5
Micronutrients		
H_3BO_3	0.773	1
$MnSO4 \bullet H_2O$	0.169	1
$ZnSO_4 \bullet 7H_2O$	0.288	1
$CuSO_4 \bullet 5H_2O$	0.062	1
H_2MoO_4	0.04	1
Fe-EDTA	22.74	3
KCl	1.864	1

Pots were arranged in 16 sets (treatments, including control), each containing eight pots. Total 128 pots were used for this experiment, 64 pots for 1st harvest and other 64 for 2nd harvest.

Plants were exposed to Copper (Cu, copper sulphate) through nutrient media. Salicylic acid (SA) was firstly dissolved in small amount of Sodium hydroxide (NaOH) solution and then added to the mineral medium. After three days of germination plants were exposed to treatments including T1 (0.0mM SA + 0.5 µM Cu),T2 (0.1mM SA + 0.5µM Cu), T3 (1.0mM SA + 0.5µMCu), T4 (10.0mM SA + 0.5µM Cu), T5 (0.0mM SA + 2.5µM Cu), T6 (0.1mM SA + 2.5µM Cu), T7 (1.0mM SA + 2.5µM Cu), T8 (10.0mM SA + 2.5µM Cu), T9 (0.0 mM SA + 5.0µM Cu), T10 (0.1mM SA + 5.0µM Cu), T11 (1.0mM SA + 5.0µM Cu), T12 (10.0mM SA + 5.0µM Cu), T13 (0.0mM SA + 10.0µM Cu), T14 (0.1 mM SA + 10.0µM Cu), T15 (1.0mM SA + 10.0µM Cu) and T16 (10.0mM SA + 10.0µM Cu).

Nutrient medium along with corresponding copper and salicylic acid treatments were changed twice a week. The pots were slightly agitated and watered with minimum amount of tap water when required. Maize seedlings were harvested twice i.e., 15 and 30 days after sowing (DAS) during course of experimentation. Harvested seedlings were cleaned by mild shaking and plant length (cm), fresh and dry weights (g) of both shoots and roots were measured using standard procedure. Moreover, number of leaves and leaf area (cm^2), copper accumulation in shoots and roots were also recorded. Shoot and root dry weights of sampled plants were recorded from weighted freshly harvested plants, after covering each component in separate paper envelopes and dried for 48 h at 80°C. Di-acid digestion method was used to determine copper concentrations in roots and shoots by using atomic absorption spectrophotometer (Spurgeon et al., 1994).

Statistical analysis

Collected data was statistically analyzed with MSTAT-C and treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955) at 5% level of probability.

Results

Shoot and Root Length

Maize plants harvest at 15 DAS showed that when treated with 0.lmM SA + 0.5 µM Cu, it produced maximum shoot and root length (Table 1). Whereas, minimum shoot length was produced when 10.0mM SA was applied in combination with5.0 µM Cu. Minimum root length was recorded in plants treated with 10.0 mM SA + 5.0 µM Cu. Maximum shoot length at 30 DAS was produced when maize plants were exposed to 0.l mM SA and 0.5 µM Cu, however it was decreased subsequently when treated with higher concentration of coppers (0.lmM SA + 2.5 µM Cu, 0.0 mM SA + 5.0 µM Cu and 10.0mM SA + 2.5 µM Cu, respectively) (Table 2). Maize plants produced the maximum root length when treated with 0.lmM SA and 2.5 µM Cu. While, it was gradually decreased by following order as 10.0mM SA + 10.0 µM Cu, 1.0 mM SA with 5.0 µM Cu and 1.0 mM SA with 10.0 µM Cu, respectively.

Biomass Production

Highest fresh weight of shoot and root was produced when plants were treated with 0.lmM SA + 0.5 µM Cu, while lowest fresh weights of shoot and root were observed under application of 10.0mM SA + 5.0 µM Cu (Table 3). Similarly, the maximum dry weights of shoots and roots were also produced under treatment with 0.lmM SA + 0.5 µM Cu; whereas lowest dry weights were observed when plants were treated with 10.0mM SA + 5.0 µM Cu (Table 3).Maximum fresh weights of both shoot and root at 30 DAS were recorded when plants were treated with 0.1 mM SA + 0.5 µM Cu (Table 4). However, shoot fresh weight was decreased at higher concentration of Cu as well as SA. The root fresh weight decreased as 1.0mM SA with 10.0 µM Cu, 1.0mM SA + 2.5 µM Cu, 0.0mM SA + 5.0 µM Cu and 10.0mM SA + 0.5 µM Cu respectively. Data recorded at 30 DAS indicated the maximum shoot and root dry weight at 0.1 mM SA + 0.5 µM Cu. Subsequent decline in shoot dry weight was observed in 0.1 mM SA + 5.0 µM Cu, 0.lmM SA + 2.5 µMCu, 10.0mM SA + 10.0 µMCu and 10.0mM SA + 0.5 µMCu, treatments respectively. The comparisons among treatment means indicate the maximum root dry weight being decreased by subsequent conditions as 0.1 mM SA + 5.0 µMCu, 0.0mM SA + 2.5 µMCu, 10.0 mM SA + 2.5 µM Cu and 10.0mM SA + 0.5 µMCu, respectively.

Number of leaves and leaf area

By comparing the means, treatment with 0.lmM SA + 0.5 µM Cu yielded maximum number of leaves and leaf area; while 10.0mM SA + 5.0 µM Cu produced minimum number of leaves and leaf area (Table 1). This is an outcome of 15days harvest. Plants harvest at 30 DAS indicates that the maximum numbers of leaves were recorded under 0.lmM SA + 0.5 µM Cu and minimum at I0.0mM SA + 2.5 µM. And comparisons among treatment means indicate the maximum leaf area at 0.lmM SA + 0.5 µM Cu and minimum at 10.0mM SA + 0.5 µM Cu (Table 2).

Copper Accumulation in shoots and roots

Plants exposed to different levels of copper and salicylic acid had no significant effect on copper accumulation in shoot at 15 DAS (Table 5).The accumulation of Cu in root observed at 0.0mM SA + 5.0 µM Cu was maximum.

When higher concentration of copper and salicylic acid (l0.0mM SA + 10.0 µM Cu) was applied, then no effect was recorded on copper accumulation in roots. But when higher concentration of salicylic acid and lower concentration of copper (10.0mM SA + 5.0 µM Cu) applied, the accumulation of copper in roots was increased. The data presented indicate that the addition of different levels of copper and salicylic acid has no effect on copper accumulation in shoot at 30 DAS. The accumulation of Copper in root observed at 0.0mM SA + 5.0 µM Cu is more as compared to control (0.0mM SA + 0.5 µM Cu). When higher concentration of copper and salicylic acid (l0.0mM SA + 10.0 µM Cu) applied then no effect was calculated on copper accumulation in roots. But when higher concentration of salicylic acid and lower concentration of copper (10.0mM SA + 0.5 µM Cu and l0.0mM SA + 5.0 µM Cu) was applied, the accumulation of copper in roots increased as compared to control (0.0mM SA + 0.5 µM Cu).

Table -1: Effect of different levels of salicylic acid and copper on number of leaves, leaf area and lengths of root and shoot of maize seedlings at 15 days after sowing.

Treatment	SA (mM)	Cu (µM)	Number of Leaves	Shoot Length (cm)	Root Length (cm)	Leaf Area (cm^2)
T_1	0.0	0.5	5.5^{bcde}±0.19	46.46^{bcde}±1.57	28.61^{ab}±2.00	131.88^{ab}±10.50
T_2	0.1	0.5	6.2^{a}±0.16	52.71^{a}±1.25	31.81^{a}±1.74	145.75^{a}±9.99
T_3	1.0	0.5	5.5^{bcde}±0.19	47.99^{abed}±1.21	28.61^{be}±2.54	102.81^{be}±15.10
T_4	10.0	0.5	5.12^{de}±0.12	28.41^{g}±1.80	19.35^{d}±1.87	55.55^{e}±4.28
T_5	0.0	2.5	5.6^{bcd}±0.18	46.18^{cde}±1.43	25.18^{ab}±1.02	117.77^{abc}±17.08
T_6	0.l	2.5	5.9^{ab}±0.22	50.35^{abc}±1.17	28.39^{ab}±1.00	124.58^{abc}± 11.25
T_7	1.0	2.5	5.4^{bcde}±0.18	49.02^{abed}±1.40	26.76^{abe}±0.90	123.61^{abc}±3.26
T_8	10.0	2.5	5.4^{bcde}±0.18	33.02^{f}±0.92	25.48^{be}±2.72	71.88^{de}±6.76
T_9	0.0	5.0	5.9^{ab}±0.12	45.19^{de}±0.70	22.95^{cd}±1.49	105.51^{be} ±12.96
T_{10}	0.1	5.0	5.8^{abc}±0.16	51.10^{ab}±1.03	27.14^{abe}±1.77	120.38^{abc}±5.69
T_{11}	1.0	5.0	5.4^{bcde}±0.18	48.55^{abed}±1.88	29.55^{ab}±1.36	130.25^{ab}±2.24
T_{12}	10.0	5.0	5.4^{bcde}±0.18	35.05^{f}±2.40	25.25^{be}±1.42	96.66^{cd}±7.03
T_{13}	0.0	10.0	5.0^{e}±0.19	35.66^{f}±0.95	18.28^{d}±0.97	67.39^{e}±3.63
T_{14}	0.1	10.0	5.25^{cde} ±0.16	46.50^{bcde}±1.64	27.15^{abe}±1.01	117.32^{abc}±9.59
T_{15}	1.0	10.0	5.9^{ab} ±0.22	45.06^{de}±l.51	27.91^{abe}±2.00	118.42^{abc}±8.15
T_{16}	10.0	10.0	5.7^{abc}±0.16	42.69^{e}±1.34	22.52^{cd}±1.09	97.53^{cd}±7.36

Each value represents mean of 8 replicates ± SEM.
Means sharing the different letters are significantly different from each other's at 0.05%.
SA, Salicylic acid; Cu, copper

Table – 2: Effect of different levels of salicylic acid and copper on number of leaves, leaf area and lengths of root and shoot of maize seedlings at 30 days after sowing.

Treatment	SA (mM)	Cu (µM)	Number of Leaves	Shoot Length (cm)	Root Length (cm)	Leaf Area (cm^2)
T_1	0.0	0.5	9.12^{de}±0.23	71.84^{cd}±1.53	36.59^{cd}±1.27	338.35^{be}±18.8
T_2	0.1	0.5	10.62^{a}±0.30	81.32^{a}±0.61	44.80^{a}±1.30	445.31^{a}±28.8
T_3	1.0	0.5	8.62^{ef}±0.32	66.82^{ef}±2.16	38.82^{c}±1.20	272.90^{de}±23.5
T_4	10.0	0.5	8.00^{fg}±0.00	56.16^{g}±2.15	30.40^{e}±1.45	133.66^{f}±14.6
T_5	0.0	2.5	9.25^{cde}±0.31	72.61^{cd}±0.96	36.42^{cd}±1.05	306.58^{cd}±23.7
T_6	0.l	2.5	9.62^{bcd}±0.07	77.50^{ab}±1.23	45.35^{a}±0.98	304.58^{cd}±33.2
T_7	1.0	2.5	9.12^{de}±0.23	68.04^{def}±1.69	44.35^{a}±0.91	239.44^{e}±10.4
T_8	10.0	2.5	7.88^{fg}±0.13	57.70^{g}±2.77	30.28^{e}±1.54	161.64^{f}±19.3
T_9	0.0	5.0	8.25^{fg}±0.16	71.34^{cde}±1.03	6.65^{cd}±2.59	262.62^{de}±11.0
T_{10}	0.1	5.0	9.75^{bed}±0.06	79.26^{ab}±0.94	43.15^{ab}±1.13	263.14^{de}±13.1
T_{11}	1.0	5.0	10.00^{abc}±0.19	74.86^{be}±2.11	36.41^{cd}±1.31	364.52^{b}±29.6
T_{12}	10.0	5.0	8.00^{fg}±0.00	57.29^{g}±1.02	33.44^{de}±1.33	300.56^{cd}±6.9
T_{13}	0.0	10.0	7.75^{g}±0.16	54.64^{g}±1.06	30.66^{e}±0.46	116.69^{cd}±4.61
T_{14}	0.1	10.0	9.75^{bed}±0.37	70.56^{cdef}±1.82	38.44^{c}±0.75	322.22^{bed}±3l.5
T_{15}	1.0	10.0	10.12^{ab}±0.18	78.68^{ab}±0.71	43.75^{a}±0.88	378.09^{b}±5.9
T_{16}	10.0	10.0	10.12^{ab}±0.30	66.34^{f}±1.47	39.76^{be}±0.93	337.89^{be}±6.5

Each value represents mean of 8 replicates ± SEM.
Means sharing the different letters are significantly different from each other's at 0.05%.
SA, Salicylic acid; Cu, copper

Table- 3: Effect of different levels of salicylic acid and copper on fresh and dry weights of maize seedlings at 15 days after sowing.

Treatment	SA (mM)	Cu (µM)	Fresh Weight(g)		Dry Weight(g)	
			Shoot	Root	Shoot	Root
T_1	0.0	0.5	3.34 ^{a}±0.21	1.21^{abc} ±0.16	0.30^{ab}±0.02	0.21^{ab} ±0.02
T_2	0.1	0.5	3.46^{a}±0.17	1.48^{a} ±0.19	0.31^{a} ±0.02	0.23^{a} ±0.02
T_3	1.0	0.5	2.08^{abc}±0.12	1.25^{abc} ±0.10	0.18^{def}±0.01	0.09^{fgh} ±0.01
T_4	10.0	0.5	1.08^{c}±0.13	0.65^{fg} ±0.06	0.11^{g} ±0.01	0.08^{gh} ±0.01
T_5	0.0	2.5	2.81^{ab}±0.18	0.94^{cdefg}±0.10	0.24^{bed}±0.02	0. I I^{fgh} ±0.02
T_6	0.l	2.5	2.87^{ab}±0.36	1.09^{bed} ±0.17	0.24^{bed}±0.03	0.17^{ede} ±0.02
T_7	1.0	2.5	2.83^{ab}±0.26	1.04^{bcde} ±0.16	0.24^{bed}±0.02	0.13^{efg} ±0.02
T_8	10.0	2.5	1.56^{be}±0.17	0.63^{fg} ±0.05	0.15^{egf}±0.01	0.09^{gh} ±0.01
T_9	0.0	5.0	2.24^{abe}±0.19	0.59^{g} ±0.04	0.21^{cd} ±0.02	0.10^{fgh} ±0.01
T_{10}	0.1	5.0	2.36^{abe}±0.16	0.88^{cdefg}±0.04	0.23^{cd} ±0.02	0. I 7^{cde} ±0.02
T_{11}	1.0	5.0	2.82^{ab}±0.26	0.93^{cdefg}±0.08	0.25^{bc} ±0.02	0.14^{def} ±0.01
T_{12}	10.0	5.0	2.32^{abc}±0.20	0.71^{efg} ±0.01	0.14^{fg} ±0.01	0. I I^{fgh} ±0.02
T_{13}	0.0	10.0	1.62^{be}±0.07	0.57^{g} ±0.07	0.11^{g} ±0.01	0.07^{h} ±0.02
T_{14}	0.1	10.0	2.43^{abe}±0.21	0.99^{cdef} ±0.10	0.22^{cd}±0.02	0.12^{efg} ±0.01
T_{15}	1.0	10.0	3.37^{a}±0.25	1.38^{ab} ±0.20	0.30^{ab}±0.02	0.20^{abe} ±0.02
T_{16}	10.0	10.0	2.74^{ab}±0.10	0.77^{defg} ±0.20	0.20^{cde}±0.02	0.I8^{bed} ±0.01

Each value represents mean of 8 replicates ± SEM.
Means sharing the different letters are significantly different from each other's at 0.05%.
SA, Salicylic acid; Cu, copper

Table – 4: Effect of different levels of salicylic acid and copper on fresh and dry weights of maize seedlings at 30 days after sowing.

Treatment	SA (mM)	Cu (µM)	Fresh Weight (g)		Dry Weight (g)	
			Shoot	Root	Shoot	Root
T_1	0.0	0.5	9.08^{bc}±0.49	4.78^{bcd}±0.13	0.94^{cd}±0.06	0.46^{be}±0.02
T_2	0.1	0.5	11.20^{a}±0.62	6.82^{a}±0.23	1.19^{a}±0.08	0.62^{a}±0.06
T_3	1.0	0.5	6.43^{e}±0.73	4.72^{bed}±0.17	0.76^{def}±0.08	0.41^{cd}±0.02
T_4	10.0	0.5	3.59^{f}±0.58	2.79^{f}±0.35	0.44^{h}±0.04	0.23^{e}±0.03
T_5	0.0	2.5	8.83^{c}±0.42	4.05^{de}±0.63	0.94^{cd}±0.06	0.41^{cd}±0.10
T_6	0.l	2.5	11.00^{a}±0.73	5.51^{b}±0.14	0.86^{cdef}±0.06	0.47^{be}±0.01
T_7	1.0	2.5	6.68^{de}±0.52	4.17^{ede}±0.20	0.73^{ef}±0.06	0.31^{de}±0.05
T_8	10.0	2.5	3.96^{f}±0.47	2.59^{f}±0.11	0.53^{gh}±0.06	0.24^{e}±0.02
T_9	0.0	5.0	8.54^{c}±0.58	3.71^{e}±0.30	0.79^{cdef}±0.07	0.38^{cd}±0.02
T_{10}	0.1	5.0	10.60^{a}±0.51	5.26^{b}±0.48	0.98^{bc}±0.07	0.47^{be}±0.04
T_{11}	1.0	5.0	8.50^{c}±0.33	4.61^{bcde}±0.39	0.92^{cde}±0.07	0.42^{cd}±0.04
T_{12}	10.0	5.0	6.44^{e}±0.12	2.70^{f}±0.26	0.86^{cdef}±0.03	0.25^{e}±0.03
T_{13}	0.0	10.0	5.60^{e}±0.14	2.59^{f}±0.09	0.41^{h}±0.04	0.22^{e}±0.02
T_{14}	0.1	10.0	10.30^{ab}±0.27	4.09^{cde}±0.37	0.72^{ef}±0.04	0.44^{c}±0.02
T_{15}	1.0	10.0	11.20^{a}±0.17	5.12^{be}±0.20	1.15^{ab}±0.11	0.58^{ab}±0.02
T_{16}	10.0	10.0	7.87^{cd}±0.18	4.44^{cde}±0.19	0.69^{fg}±0.01	0.45^{c}±0.02

Each value represents mean of 8 replicates ± SEM.
Means sharing the different letters are significantly different from each other's at 0.05%.
SA, Salicylic acid; Cu, copper

Table- 5: Effect of different levels of salicylic acid and copper on copper accumulation in maize seedlings.

Treatment	SA(mM)	Cu(µM)	Cu accumulation (ppm)			
			15 DAS		30 DAS	
			Shoot	Root	Shoot	Root
T_1	0.0	0.5	0.0092^{a}±0.000	0.0425^{h}±0.0005	0.0095^{a}±0.0003	0.0535^{d}±0.0003
T_2	0.1	0.5	0.0100^{a} ±0.000	0.0418^{h}±0.0004	0.0100^{a}±0.0003	0.0518^{d}±0.0003
T_3	1.0	0.5	0.0101^{a} ±0.000	0.0410^{h}±0.0004	0.0101^{a}±0.0003	0.0462^{d}±0.0004
T_4	10.0	0.5	0.0098^{a} ±0.000	0.0522^{f}±0.0004	0.0098^{a}±0.0002	0.0687^{c}±0.0003
T_5	0.0	2.5	0.0095^{a} ±0.000	0.0415^{h}±0.000I	0.0098^{a}±0.0002	0.0462^{d}±0.0004
T_6	0.l	2.5	0.0099^{a} ±0.000	0.0420^{h}±0.0003	0.0096^{a}±0.0003	0.0532^{d}±0.0002
T_7	1.0	2.5	0.0095^{a} ±0.000	0.0415^{h}±0.0004	0.0101^{a}±0.0002	0.0470^{d}±0.0004
T_8	10.0	2.5	0.0098^{a} ±0.000	0.0622^{e}±0.0006	0.0102^{a}±0.0002	0.0715^{c}±0.0004
T_9	0.0	5.0	0.0099^{a} ±0.000	0.0735^{d}±0.0004	0.0099^{a}±0.0002	0.0818^{c}±0.0004
T_{10}	0.1	5.0	0.0099^{a} ±0.000	0.0435^{gh}±0.0003	0.0105^{a}±0.0002	0.0538^{d}±0.0004
T_{11}	1.0	5.0	0.0098^{a} ±0.000	0.0542^{f}±0.0004	0.0098^{a}±0.0002	0.0680^{c}±0.0005
T_{12}	10.0	5.0	0.0102^{a} ±0.000	0.0845^{c}±0.0003	0.0100^{a}±0.0003	0.0968^{b}±0.0004
T_{13}	0.0	10.0	0.0101^{a} ±0.000	0.1070^{a}±0.0006	0.0099^{a}±0.0002	0.1178^{a}±0.0005
T_{14}	0.1	10.0	0.0101^{a} ±0.000	0.0910^{b}±0.0005	0.0099^{a}±0.0003	0.1130^{a}±0.0004
T_{15}	1.0	10.0	0.0105^{a} ±0.000	0.0728^{a}±0.0005	0.0101^{a}±0.0002	0.0782^{c}±0.0003
T_{16}	10.0	10.0	0.0100^{a} ±0.000	0.0492^{fg}±0.0005	0.0105^{a}±0.0002	0.0542^{d}±0.0014

Each value represents mean of 8 replicates ± SEM.
Means sharing the different letters are significantly different from each other's at 0.05%.
SA, Salicylic acid; Cu, copper

Discussion

Salicylic acid is a natural plant hormone produced in response to adverse environmental condition (Hussein et al., 2007), and has also been applied exogenously to induce plants tolerance against biotic and abiotic stresses (Shakirova et al., 2003; Sawada et al., 2006; Zhou et al., 2009). Zhang et al. (2008) reported that excessive copper reduced plant root length, root dry weight, total dry weight, and root to shoot ratio and leaf area. The present study was conducted to observe the effect of copper with or without salicylic acid on maize plant growth. The higher concentration of SA (10.0 mM) reduced the growth of maize plants; while only the lower concentration (0.1 mM) was efficient in improving growth parameters. The Higher concentration of Copper (1 0.0 µM) is toxic and significantly reduces the growth of maize plants. In the present study, a negative relationship was observed between growth parameters and increasing copper concentration at both harvests (15 and 30 DAS).

The shoot length in 1.0 µM Cu alone treatment, was decreased by23.25% at15 DAS and 23.94 % at 30 DAS of the control (0.5 µM Cu). However, no significant effect was found at 2.5 µM Cu and 5.0 µM Cu. The higher concentration may have a negative effect on plant growth ultimately leading towards lower absorption of water. In a similar study, it was found that root length was decreased due to copper toxic concentration (Martín et al., 2006). The higher concentration of copper (10.0 µM) without salicylic acid showed toxic effect on root length which decreased by 36.11 % at 15 DAS. The main symptoms of excess copper can be detected in roots by a decrease in root growth (Martins and Mourato, 2006). Ouzounidou et al. (1995) also showed that excess copper in maize roots affected both root growth and ultra-structure. Shoot fresh weight and root fresh weight also decreased at higher level (10.0 µM Cu) by 51.50 % and 52.89 % respectively at 15 DAS and also decreased at higher level (10.0 µM Cu) by 38.33 % and 45.82 respectively at 30 DAS as compared of the control (0.5 µM Cu). Martins and Mourato (2006) observed that fresh weight reduced to 73 % of the control.

Shoot dry weight and root dry weight also decreased at higher level (l0.0 µMCu) by 63.33 % and 61.90 % respectively at 15 DAS and also decreased at higher level (10.0 µM Cu) by 56.38 % and 52.17 respectively at 30 DAS as compared of the control (0.5 µM Cu). The root dry weight also decreased at 5.0 µM Cu by 52.38 % and 17.59 % at 15 and 30 DAS respectively of the control. The number of leaves decreased only at 10.0 µM Cu by 9.09 % and 15.02 % at 15 and 30 DAS as compared of control. The copper accumulated in shoot has no significant result while root has great significant result. Copper is immobile and much accumulated in root as compared to shoot. The copper is highly toxic to root as compared to shoot. The shoot effect is due to root effect.

Restricted translocation of copper from root to shoot was also reported on observed in *Agrostis stolonifera* (Wu et al., 1975). Copper has no effect on copper accumulation in shoot. The copper accumulation in roots increased by 151.76 % and 120.19 % at 0.0mM SA + 1 0.0 µM Cu at 15 and 30 DAS respectively of sowing as compared to control (0.0mM SA + 0.5 µM Cu). Chen and Kao (2000) observed a progressively decreasing root length after increasing concentrations of copper from 20 to 50 µM. The accumulation of copper in root observed at 0.0 mM SA with 5.0 µM Cu 72.94 % and 52.90 % more respectively as compared to control (0.0mM SA + 0.5 µM Cu) at 15 and 30 DAS. The root has tendency to accumulate to higher quality of copper than aerial parts of the plants (Cathala and Salsac, 1975).

Salicylic acid acts as endogenous signal molecule responsible for inducing abiotic stress tolerance in plants. They emphasized that exogenous application of Salicylic acid increased plant growth (Bastam et al., 2013).

The present study showed that the application of Salicylic acid on growth of maize plant improved all growth characters i.e., shoot &root length, fresh and dry weight of both shoot and root and also area of green leaves. The highest increment was shown in dry weight of shoot and root at 30 DAS when treated with 0.lm mM SA by 26.60 % and 34.78 % respectively of the control (0.0 mM SA). Hussein et al. (2007) concluded that the highest increment was shown in stem dry weight and lowest in stem diameter when treated with Salicylic acid. The shoot length increased at 0.1 mM SA by 13 % of the control. Moreover the shoot & root length, fresh weight of shoot &root, dry weight of shoot & root and numbers of leaves similar response. The effect of Salicylic acid is reflected in higher fresh weight and dry weights in sunflower plants (El-Tayeb et al., 2006). The root length increased at 0.1 mM SA by 11.19 % at 15 DAS and 22.44 % at 30 DAS of the control (0.0 mM SA).

Shoot fresh weight and root fresh weight also increased at lower level of Salicylic acid (0.1 mM SA) by 3.59 % and 22.31 % respectively at 15 DAS and also increased at 0.1 mM SA by 23.35 % and 42.68 respectively at 30 DAS as compared of the control (0.0 mM SA). Shoot dry weight and root dry weight also increased at lower level (0.1 mM SA) by 3.33 % and 9.52 % respectively at 15 DAS and also increased at lower level (0.1 mM SA) by 26.60 % and 34.78 respectively at 30 DAS as compared of the control (0.0 mM SA). The number of leaves increased at 0.1 mM SA by 12.73 % and 16.45 % at 15 and 30 DAS as compared of control (0.0 mM SA). The area of green leaves increased at 0.1 mM SA by 10.52 % and 31.63 % at 15 and 30 DAS as compared of control (0.0 mM SA).

Salicylic acid has no effect on copper accumulation in shoot. The accumulation of copper in shoots at 15 and 30 DAS were not observed significantly different but in case of root higher concentration of salicylic acid and lower concentration of copper (10.0mM SA + 0.5 µM Cu) and (l0.0mM SA + 5.0 µM Cu) applied the accumulation of copper increased by 22.82 % and 98.82 % of the control (0.0mM SA + 0.5 µM Cu) at 15 DAS and higher concentration of salicylic acid and lower concentration of copper (l0.0mM SA + 0.5 µM Cu) and (10.0mM SA + 5.0 µM Cu) applied the accumulation of copper in roots increased by 28.41 % and 80.93 % of the control (0.0mM SA + 0.5 µM Cu) at 30 DAS. Hussein et al. (2007) reported that salicylic acid application increased number of leaves, leave area and plant dry matter. They also reported that salicylic acid improved dry matter accumulation under saline and non-saline condition. However salicylic acid was found more active in plants grown under normal condition. The interaction of salicylic acid and copper on growth plants were significant effect. The shoots length, roots length, fresh and dry weights of shoot and root, number of leaves and leaf area increased at when lower concentration of salicylic acid is applied (1.0 mM SA + 10.0 µM Cu) by 9.52 % 19.57 %, 23.35 %, 7.11 %,22.34 %, 26.09 % 10.96 % and 11.76 % respectively of the control (0.0 mM SA + 0.5 µM Cu). Sakhabutdinova et al. (2003) concluded that the salicylic acid treatment reduce the damage action of salinity and water deficit on wheat seedling growth. At Higher concentration of copper and salicylic acid (10.0mM SA + 10.0 µM Cu) no effect was observed on copper accumulation in roots at 15 and 30 DAS.

Conclusion

Contrasting results were observed by the application of different levels of salicylic acid. Application of lower concentration of salicylic acid (0.1 mM) significantly increased biomass production, shoot and root lengths, number and area of leaves, whereas application of higher level of salicylic acid (10.0 Mm) had inhibitory effects. Maize seedlings harvested at 15 and 30 DAS produced highest copper accumulations in roots when treated with 10 mM salicylic acid and 5 µM copper contamination. However, minimum copper accumulation was observed when maize seedlings were treated with 10 mM salicylic acid along with 0.5 µM copper. Copper accumulation in shoot remained uninfluenced by different levels of copper and salicylic acid. Application of salicylic acid at lower concentration can have potential to compensate reduction in plant growth under copper contaminated areas.

References

Adrees M, Ali S, Rizwan M, Ibrahim M, Abbas F, Farid M, Zia-ur-Rehman M, Irshad MK and Bharwana SA, 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environ. Sci. Pollut. Res. 22(11): 8148-8162.

Bashmakov DI, Lukatkin AS, Revin VV, Duchovskis P, Brazaitytë A and Baranauskis K, 2005. Growth of maize seedlings affected by different concentrations of heavy metals. Ekologija. 3: 22-27.

Bastam N, Baninasab B and Ghobadi C, 2013. Improving salt tolerance by exogenous application of salicylic acid in seedlings of pistachio. Plant Growth Regul. 69(3): 275-284.

Bennett LE, Burkhead JL, Hale KL, Terry N, Pilon M and Pilon-Smits, 2003. Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. J. Environ. Qual. 32(2): 432-440.

Borkert C, Cox F and Tucker M, 1998. Zinc and copper toxicity in peanut, soybean, rice, and corn in soil mixtures. Comm. Soil Sci. Plant Anal. 29(19-20): 2991-3005.

Brennan A, Jiménez EM, Puschenreiter M, Alburquerque JA and Switzer C, 2014. Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil. Plant Soil. 379(1): 351-360.

Brun L, Le Corff J and Maillet J, 2003. Effects of elevated soil copper on phenology, growth and reproduction of five ruderal plant species. Environ. Pollut. 122(3): 361-368.

Cathala N and Salsac L, 1975. Absorption du cuivre par les racines de mais (*Zea mays* L.) et de tournesol (*Helianthus annuus* L.). PlantSoil. 42(1): 65-83.

Chen L-M, Lin CC and Kao CH, 2000. Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. Bot. Bull. Acad. Sinica. 41:99-103.

Clemens S and Ma JF, 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. Annu. Rev. Plant Biol. 67(1): 489-512.

Duncan DB, 1955. Multiple range and multiple F tests. Biometrics. 11(1): 1-42.

Ehsan N, Nawaz R, Ahmad S, Khan MM and Hayat J, 2016. Phytoremediation of chromium-contaminated soil by an ornamental plant, vinca (*Vinca rosea* L.). J.Environ. Agric. Sci. 7: 29-34.

El-Tayeb M, El-Enany A and Ahmed N, 2006. Salicylic acid-induced adaptive response to copper stress in sunflower (*Helianthus annuus* L.). Plant Growth Regul. 50(2-3): 191-199.

Hayat Q, Hayat S, Irfan M and Ahmad A, 2010. Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot. 68(1): 14-25.

Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA, 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. J. Agron. Crop Sci. 194(3): 193-199.

Hussein M, Balbaa L and Gaballah M, 2007. Salicylic acid and salinity effects on growth of maize plants. Res. J. Agric. Biol. Sci. 3(4): 321-328.

Kopliku D and Mesi A, 2012. Correlative evaluation between experimental copper and lead Ion concentrations and root length of *Allium cepa* L. in some riverside points of NënShkodra lowland. J. Int. Environ. Appl. Sci. 7(5): 913.

Krämer U, 2010. Metal hyperaccumulation in plants. Annu. Rev. Plant Biol. 61(1): 517-534.

Lexmond TM, Van der Vorm P, 1981. The effect of pH on copper toxicity to hydroponically grown maize. Netherlands J. Agric. Sci. 29:217-238.

Liu JJ, Wei Z, and Li JH, 2014. Effects of copper on leaf membrane structure and root activity of maize seedling. Bot. Stud. 55(1): 47.

Liu Z, Ding Y, Wang F, Ye Y and Zhu C, 2016. Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Rep. 35(4): 719-731.

Lombi E, Zhao F, Dunham S and McGrath S, 2001. Phytoremediation of heavy metal–contaminated soils. J. Environ. Qual., 30(6): 1919-1926.

Macnicol R and Beckett P, 1985. Critical tissue concentrations of potentially toxic elements. Plant Soil. 85(1): 107-129.

Madejon P, Ramirez-benitez JE Corrales I, Barceló J and Poschenrieder C, 2009. Copper-induced oxidative damage and enhanced antioxidant defenses in the root apex of maize cultivars differing in Cu tolerance. Environ Exp Bot. 67: 415-420.

Maksymiec W and Krupa Z, 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in Arabidopsis thaliana. Environ. Exp. Bot. 57(1): 187-194.

Martín JAR, Arias ML and Corbí JMG, 2006. Heavy metals contents in agricultural topsoils in the Ebro basin (Spain). Application of the multivariate geoestatistical methods to study spatial variations. Environ. Pollut. 144(3): 1001-1012.

Martins LL and Mourato MP, 2006. Effect of excess copper on tomato plants: growth parameters, enzyme activities, chlorophyll, and mineral content. J. Plant Nutr. 29(12): 2179-2198.

Minnich M, McBride M, and Chaney R, 1987. Copper activity in soil solution: II. Relation to copper accumulation in young snapbeans. Soil Sci. Soc. Am.J. 51(3): 573-578.

Mocquot B, Vangronsveld J, Clijsters H and Mench M, 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil. 182(2): 287-300.

Najam S, Nawaz R, Ahmad S, Ehsan N, Khan MM and Nawaz MH, 2015. Heavy metals contamination of soils and vegetables irrigated with municipal wastewater: A case study of Faisalabad, Pakistan. J. Environ. Agric. Sci. 4: 6-10.

Noreen S and Ashraf M, 2010. Modulation of salt (NaCl)-induced effects on oil composition and fatty acid profile of sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid. J. Sci. Food Agric. 90(15): 2608-2616.

O'connor CS, Lepp N, Edwards R and Sunderland G, 2003. The combined use of electrokinetic remediation and phytoremediation to decontaminate metal-polluted soils: a laboratory-scale feasibility study. Environ. Monit. Assess. 84(1-2): 141-158.

Ouzounidou G, Čiamporová M, Moustakas M and Karataglis S, 1995. Responses of maize (Zea mays L.) plants to copper stress—I. Growth, mineral content and ultrastructure of roots. Environ. Exp. Bot. 35(2): 167-176.

Ouzounidou G and Ilias I, 2005. Hormone-induced protection of sunflower photosynthetic apparatus against copper toxicity. Biolog. Plant. 49(2): 223-228.

Rivas-San VM and Plasencia J, 2011. Salicylic acid beyond defence: its role in plant growth and development. J. Exp. Bot. 62(10): 3321-3338.

Rizwan M, Ali S, Qayyum MF, Ok YS, Adrees M, Ibrahim M, Zia-ur-Rehman M, Farid M and Abbas F, 2017. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. J. Hazard. Mater. 322: 2-16.

Sağlam A, Yetişsin F, Demiralay M and Terzi R, 2016. Chapter 2 - Copper Stress and Responses in Plants A2 - Ahmad, Parvaiz. Plant Metal Interaction. Elsevier. pp. 21-40.

Sakhabutdinova A, Fatkhutdinova D, Bezrukova M and Shakirova F, 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg. J. Plant Physiol. 21: 314-319.

Sawada H, Shim I-S, and Usui K, 2006. Induction of benzoic acid 2-hydroxylase and salicylic acid biosynthesis—modulation by salt stress in rice seedlings. Plant Sci. 171(2): 263-270.

Shakirova FM, Allagulova CR, Maslennikova DR, Klyuchnikova EO, Avalbaev AM and Bezrukova MV, 2016. Salicylic acid-induced protection against cadmium toxicity in wheat plants. Environ. Exp. Bot. 122: 19-28.

Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA and Fatkhutdinova DR, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci. 164(3): 317-322.

Shakoor MB, Nawaz R, Hussain F, Raza M, Ali S, Rizwan M, Oh S-E and Ahmad S, 2017. Human health implications, risk assessment and remediation of As-contaminated water: A critical review. Sci. Total Environ. 601: 756-769.

Spurgeon DJ, Hopkin SP and Jones DT, 1994. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): Assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. Environ. Pollut. 84(2): 123-130.

Steel RGD, Torrie JH and Dickey D, 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York.

Strobel NE and Kuc JA, 1995. Chemical and biological inducers of systemic resistance to pathogens protect cucumber and tobacco plants from damage caused by paraquat and CuCl2. Phytopathology. 85:1306–1310

Wang Q-R, Cui Y-S, Liu X-M, Dong Y-T and Christie P, 2003. Soil contamination and plant uptake of heavy metals at polluted sites in China. J. Environ. Sci. Health. Part A. 38(5): 823-838.

Wani AB, Chadar H, Wani AH, Singh S and Upadhyay N, 2017. Salicylic acid to decrease plant stress. Environ. Chem. Lett. 15(1): 101-123.

Wang C, Zhang S, Wang P, Hou J, Qian J, Ao Y, Lu J and Li L, 2011. Salicylic acid involved in the regulation of nutrient elements uptake and oxidative stress in Vallisneria natans (Lour.) Hara under Pb stress. Chemosphere. 84(1):136–142.

Weckx JEJ and Clijsters HMM, 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiol. Plant. 96(3): 506-512.

Wu L, Thurman D and Bradshaw A, 1975. The uptake of copper and its effect upon respiratory processes of roots of copper- tolerant and non- tolerant clones of Agrostis stolonifera. New Phytol. 75(2): 225-229.

Zhang LP, Mehta, Liu ZP and Yang ZM, 2008. Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 49(3): 411-419.

Zhao X-F, Chen L, Rehmani MIA, Wang Q-S, Wang S-H, Hou P-F, Li G-H and Ding Y-F, 2013. Effect of nitric oxide on alleviating cadmium toxicity in rice (*Oryza sativa* L.). J. Integ. Agric. 12(9): 1540-1550.

Zhou ZS, Guo K, Elbaz AA and Yang ZM, 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of Medicago sativa. Environ. Exp. Bot. 65(1): 27-34.