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Aqueous Leaf Extract of *Duranta repens* Promotes Seed Germination and Seedling Growth of Salinity-Stressed *Solanum lycopersicum* Seedlings

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Abstract

Aqueous extract of *Duranta repens* leaves was investigated for its allelopathic effect on *Solanum lycopersicum* seedlings germinated and grown under salinity condition. The study was carried out using both laboratory petridish and pot assays to simulate the plant's immediate environmental conditions. The experiment consisted of 5 groups (1-5) each containing 4 replicates (of 10 seeds). Group 1 was treated with distilled water; Group 2 was treated with 5 mM NaCl; Group 3 was treated with *Duranta repens* aqueous leaf extract, Group 4 was treated with a mixture of 5 mM NaCl and the extract (2:1 v/v) and Group 5 was treated with a mixture of 5 mM NaCl and the extract (1:2 v/v). Results from the studies revealed that treatment with NaCl caused significant ($p < 0.05$) reduction in percentage germination and growth parameters (plumule and radicle lengths) of *S. lycopersicum* seedlings, when compared to those treated with distilled water and *D. rupens* aqueous leaf extract. NaCl treatment (Group 2) also caused an increase in malondialdehyde (MDA) and also increased the activities of superoxide dismutase (SOD) and catalase (CAT) while reducing chlorophyll concentration of the plants. However, in the presence of the extract, the adverse effects of the NaCl were attenuated, implying that the extract improved tolerance of *S. lycopersicum* seedlings. In conclusion, the findings of this study indicate that the constituents of the extract may play a positive role in plant survival under saline conditions and could be employed in the management of soil salinity problem in agriculture.

Keywords: *Duranta repens; Solanum lycopersicum; Salinity Stress; Allelopathy; Aqueous Extract*

Introduction

In recent years, research is focused on the development of eco-friendly compounds which could be used in industrial and agricultural sectors of the economy. For instance, phytochemicals with growth-inhibitory or stimulatory effects on other plants are explored in development of bio herbicides or bio fertilizers (Sunmonu and Van Staden, 2014), and to enhance the tolerance of some cultivated plants exposed to unfavorable environmental conditions such as salt stress, drought (Chang *et al.*, 2010) and diseases.

Salt stress is one of the major factors affecting crop production in the world (Zhu, 2001). Many arid and semi-arid regions in the world have soils containing high levels of salt, thus unfavorable for plant survival, the result of which is decrease in

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agricultural yield (Sekmen *et al*., 2007). Some plants including tall wheatgrass, alkali sacaton and Nuttall's saltgrass are, however, naturally resistant to high salt concentration (Niu, 2006). Salinity may arise when there is excessive evaporation and capillary rise, thus bringing salts into the root zone. This problem which exists in the coastal and semi-arid regions of Nigeria (Etuonovbe, 2009) can be managed by establishing permanent vegetation with deep-rooted plants that reduce surface evaporation and utilize excess water (Seelig, 2000). Salinity inhibits plant growth and development by inducing osmotic stress where uptake of water with other important nutrients is limited. In addition, it causes excessive uptake of ions, particularly Na^+ and Cl^- that ultimately interfere with various metabolic processes (Munns, 2002). Plant tolerance to salinity has also been reported to improve following treatment with phytochemicals (Sunmonu *et al*., 2017).

Phytochemicals have been reported to promote growth of these plant species in unfavorable environmental conditions. For instance, extracts from saponin-rich *Camellia* sp. seeds were reported to have stimulatory effects on the yield of tomatoes, cucumbers, and strawberries exposed to salinity stress (Wagentrisl, 2003). These effects are however attributed to many compounds present in the extracts. Thus, these extracts may play multifunctional roles, improving uptake of nutrients from soil, protecting against fungal infections and generally stimulating plant growth in such stress condition.

Duranta repens Linn. commonly called Golden dewdrop (English) appears as either a sprawling shrub or a small tree and grows up to 6 m tall (Said, 2016). It belongs to the family Verbenaceae and it is distributed throughout Mexico, South America and the Antilles (Whistler, 2000). It is widely cultivated as an ornamental plant in tropical and subtropical gardens around the world (Sousa *et al*., 2011) and also exhibits potentials as therapeutic agent (Johnson *et al*., 2018). Studies have reported the plant's antioxidant (Dressler *et al*., 2014), antifungal (Sharma *et al*., 2012), antibacterial (Ogbuagu *et al*., 2015) and antimalarial (Majunatha *et al*., 2013) activities. Phytochemicals including flavonoids, alkaloids, durantosides (I, II, III, IV), saponins, steroidal glycosides, beta-sitoserol, naringenin, sucrose, raffinose, acetosides, triterpenes have been reportedly isolated from the plant (Anis *et al*., 2002; Ahmed *et al*., 2009; Singh *et al*., 2016).

Owing to important phytochemicals present in *Duranta repens* (particularly flavonoids and alkaloids), this study investigated the allelopathic potential of its aqueous leaf extract on *Solanum lycopersicum* seed germination and growth under saline conditions.

Materials and Methods

Plant Materials

Leaf samples of *Duranta repens* were collected within the premises of Fountain University, Osogbo, Nigeria. The leaves were rinsed afterwards and air-dried for two weeks. The dried leaves were then blended to fine powder using an electric blender and stored in a dry air-tight container until required for use. *Solanum lycopersicum* seeds were obtained from JK Agric. Genetics Ltd (Hyderabad, India) with label no. 066638 and lot no. 1213/40331.

Seed Viability Test

Seeds collected from the tomato fruits were kept in a bowl of water enough to enable floating. Non-viable seeds floated on the water and were removed by scooping while the viable seeds were air dried for 24 h (Sunmonu *et al*., 2017).

Preparation of Leaf Aqueous Extract

Powdered leaf sample (50 g) was extracted in 500 mL of distilled water for 24 h at room temperature with intermittent shaking. The mixture was then passed through Whatman No.1 filter paper and the resulting filtrate was concentrated using a water bath. Afterwards, it was kept in the refrigerator at 4^0C until required for use.

Phytochemical Screening

Qualitative phytochemical screening of the plant extract was carried out following the methods described by Trease and Evans (1999) for tannins and alkaloids; Harbourne (1984) for saponins, glycosides, terpenoids; and Akpuaka (2009) for steroids.

Allelopathy Study

The experiment was carried out in two phases; germination and the post-germination phases. Both consisted of 5 groups; Group 1 served as the control and was treated with 5 ml of distilled water; Group 2 was treated with 5 ml of 5 mM NaCl (Sunmonu *et al.*, 2017); Group 3 was treated with 5 ml of 1% Extract; Group 4 was treated with 5 mM NaCl and 1% extract (2:1 v/v); Group 5 was treated with 5 mM NaCl and 1% extract (1:2 v/v). Each experiment had four replicates that were laid out as a four factor experiment in completely randomized design.

The petri-dish assay was carried out following the method described by Sunmonu and Van Staden (2014). Randomly picked viable seeds were assigned into five groups (1-5) of five replicates (A-E) of petri dishes containing 10 seeds each. Each petri dish was lined with whatman No. 1 filter paper which was used as the medium for growth and was soaked in the different mixtures of the extract and salt while distilled water was used as control. The petri dishes were then kept in a dark room 7 days under laboratory conditions.

The pot assay was carried out following the method described by Fasaanu *et al.* (2013). The treatment groups were also divided into five as with the germination test. Tomato seeds (n=10) were grown on small perforated pots filled with loamy soil. After germination, the seedlings were wetted for fourteen (14) days with different mixtures of the salt, extract and distilled water as applicable. On the 15^{th} day, the seedlings were uprooted, thoroughly washed, wrapped in foil paper, labeled accordingly and kept in the refrigerator at 4^0C until required for use.

Percentage Germination and Seedling Growth of Salinity-stressed *Solanum lycopersicum* Seedlings

Germination count was done on the 4^{th} day of the petri-dish assay and percentage germination was obtained using the following expression:

$$\text{Germination (\%)} = \frac{\text{Germinated seeds} \times 100}{\text{Total seed}}$$

Plumule and radicle lengths were also measured on the 8^{th} day using a piece of thread and a calibrated ruler.

Oxidative Stress Markers and Chlorophyll Concentration of Salinity-stressed *Solanum lycopersicum* Seedlings

Lipid peroxidation as measured by malondialdehyde (MDA) content, superoxide dismutase activity and catalase activity were assayed following methods described by Heath and Packer (1968), Kakkar *et al.* (1984) and Luck (1974). Chlorophyll concentration was evaluated following the method described by Comb et al. (1985).

Statistical Analysis

Data were expressed as Mean ± SEM for 10 determinations and were subjected to one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparison using the Statistical Package for Social Sciences (SPSS) version 17.0 for windows software package. Values were considered statistically significant at $p < 0.05$.

Results

Phytochemical Composition of Aqueous Extract of *Duranta repens*

Phytochemicals including alkaloids, flavonoids, terpenoids, saponins, phenolics, tannins were present in the extract and these were in high amounts except for tannins (Table 1).

Table 1: Phytochemical Constituents of Aqueous Extract of *Duranta repens* Leaves

Phytochemical	Presence/Absence
Alkaloids	+++
Flavonoids	+++
Terpenoids	+++
Saponins	+++
Phenolics	+++
Tannins	+
Steroids	-

- Absent; + Present; + Low concentration; +++ High concentration.

Percentage Germination and Seedling Growth of Salinity-Stressed Tomato Seeds:

Treatment of the tomato seeds with NaCl caused a significant ($p < 0.05$) reduction in the percentage germination, roots and shoot lengths of tomato seeds when compared to the control. However, these parameters were significantly ($p < 0.05$) increased by the extract either alone (Group 3) or in the seedlings raised in the saline environment (Groups 4 and 5) (Table 2). Data are expressed as mean ± SEM for 10 Determinations. Values with different superscripts are significantly different ($P < 0.05$).

Oxidative Stress Markers of Salinity-Stressed Tomato Seeds:

MDA concentration was significantly ($p < 0.05$) increased in the seedlings treated with NaCl while those treated with the extract showed non-significant ($p > 0.05$) change in MDA concentration, either alone or in the saline condition (Table 3).

SOD and CAT activities were increased ($p < 0.05$) compared to the control. Following the extract treatment however, only the catalase activity was significantly ($p < 0.05$) increased. Treatment with both extract and NaCl however, caused significant ($p < 0.05$) reductions in only SOD when compared to the NaCl-treated seeds (Table 3).

Chlorophyll Concentration of Salinity-Stressed Tomato Seeds:

Concentrations of chlorophylls a, b and total chlorophyll were reduced following NaCl treatment. Significant increases were however seen with the extract either alone or in the presence of saline condition (Table 4).

Data are Mean ± SEM of 10 determinations. Values with different superscripts along the same column for each parameter indicate significant difference at $p < 0.05$.

Table 2: Percentage Germination and Seedling Growth of Salinity-Stressed Tomato Seeds Exposed to Aqueous Extract of *Duranta repens*

Treatment	Germination (%)	Root Length (cm)	Shoot Length (cm)
Control	81.20 ± 0.49^{a}	2.74 ± 0.02^{a}	3.50 ± 0.03^{a}
3 ml NaCl	55.20 ± 0.49^{b}	1.44 ± 0.02^{b}	1.90 ± 0.03^{b}
3 ml Extract	83.60 ± 0.40^{c}	2.77 ± 0.02^{c}	3.58 ± 0.04^{a}
2 ml NaCl+1 ml Extract (2:1 v/v)	71.60 ± 0.40^{d}	1.86 ± 0.02^{d}	2.78 ± 0.04^{c}
1 ml NaCl+2 ml Extract (1:2 v/v)	73.20 ± 0.49^{e}	1.86 ± 0.02^{d}	3.04 ± 0.02^{d}

Table 3: Oxidative Stress Markers of Salinity-Stressed Tomato Seedlings Exposed to Aqueous Extract of *Duranta repens*

Treatment	Malondialdehyde concentration (nMol/g)	Superoxide dismutase activity (Unit/mg protein)	Catalase activity (Unit/mg protein)
Control (3ml)	0.51 ± 0.01^{a}	55.96 ± 0.06^{a}	0.19 ± 0.01^{a}
3 ml NaCl	2.81 ± 0.01^{b}	74.82 ± 0.19^{b}	0.28 ± 0.01^{b}
3 ml Extract	0.56 ± 0.01^{a}	58.18 ± 0.26^{a}	0.21 ± 0.00^{b}
2 ml NaCl+1 ml Extract	1.86 ± 0.01^{c}	59.16 ± 0.27^{a}	0.23 ± 0.00^{b}
1 ml NaCl+2 ml Extract	1.83 ± 0.01^{c}	57.96 ± 0.19^{a}	0.23 ± 0.00^{b}

Data are Mean ± SEM. Values with different superscripts along the same column for each parameter are significantly different at $p < 0.05$.

Table 4: Chlorophylls a, b and Total Chlorophyll Concentrations of Salinity-Stressed Tomato Seedlings Exposed to Aqueous Extract of *Duranta repens*

Extract concentration	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Control	42.16 ± 0.20^{a}	19.14 ± 0.12^{a}	61.52 ± 0.07^{a}
3 ml NaCl	37.70 ± 0.10^{b}	12.96 ± 0.11^{b}	50.40 ± 0.14^{b}
3 ml Extract	45.06 ± 0.24^{a}	19.86 ± 0.15^{a}	61.80 ± 0.10^{a}
2 ml NaCl+1 ml Extract	39.92 ± 0.12^{b}	15.48 ± 0.04^{a}	57.80 ± 0.10^{a}
1 ml NaCl+2 ml Extract	39.92 ± 0.12^{b}	15.70 ± 0.08^{a}	57.98 ± 0.11^{a}

Discussion

Soil salinity is a major threat to global food security, affecting about 20% of the world's cultivated land, and resulting into 50% loss of agricultural yield (Bartels and Sunkar, 2005). It is a major problem confronting crop production in the southern part of Nigeria, and has adversely affected economic development in the area (Etuonovbe, 2009). Plant extracts show promising stimulatory roles towards the survival of other plants grown in salinity stressed conditions. This is attributable to their constituent allelochemicals such as flavonoids and alkaloids which tend to improve tolerance of plants in such conditions (Wagentrisl, 2003).

Allelochemicals are a group of phytochemicals which exhibit either stimulatory or inhibitory effect on the growth and survival of other plant species in the environment (Khalaj *et al.*, 2013). Allelochemicals including saponins and polyphenols have been reported to attenuate or prevent the adverse effects of stress conditions on plant growth (Wagentrisl, 2003). In this study, phytochemicals such as saponins and polyphenols detected in the extract (Table 1) may be responsible for the growth-stimulatory effects exhibited on tomato growth, and conforms to an earlier study of Sharma *et al*. (2012) which reported the presence of high amounts of these compounds in aqueous extract of *D. repens*.

The significantly reduced percentage germination of the tomato seeds following NaCl treatment is in line with earlier studies that reported inhibited seed germination caused by high levels of salinity (Xue *et al*., 2004; Li, 2008). Salt-induced inhibition of seed germination could be attributed to decreased water uptake or specific ion toxicity which may change the activity of certain enzymes or hormones present in the seeds (Huang and Redmann, 1995). Thus, exposure to high salt concentration might cause significantly reduced seed germination rates and a subsequent reduction in root and shoot lengths (Neumann, 1997; Li, 2008). Also, a significant reduction in root length may be due to a reduction in the turgor of the root cells (Bewley and Black, 1994). Salt stress damages hypocotyls of germinating seedlings, resulting into significant reduction in its length (Li, 2008).

Studies have reported stimulatory effects which plant extracts have on germination and growth of other plant species (Kadioglu and Yanar, 2004; Avilés-Tamayo *et al*., 2018). These effects are attributable to growth enhancing phytochemicals present in the extracts. Thus, the significantly increased seed germination, root and shoot lengths of the tomato seedlings following treatment with the plant extract may be attributed to saponins and polyphenols present in the extract (Wagentrisl, 2003). Growth-enhancing plant extracts may perform their stimulatory roles by altering hormonal or enzyme actions (Cedergreen, 2010). Thus in this study, the extract may have stimulated certain enzymes responsible for nutrient mobilization in the seeds, and eventually leading to improved germination (Cedergreen, 2010). This however conforms to an earlier study of Kadioglu and Yanar (2004) that reported stimulatory effects of some weed extracts on germination of *D. Sophia*.

In addition to promoting germination and growth in normal conditions, plant extracts may attenuate the adverse effects of unfavorable conditions thereby promoting plant survival in such conditions (Sunmonu *et al*., 2017). Thus, in this study, the aqueous extract of *Duranta repens* may have stimulated enzymes needed for mobilization of stored nutrients, leading to improved seed germination and seedling growth. This is in line with an earlier study of Farooq *et al*. (2011) that reported growth-enhancing effects of sunflower extract on salinity-stressed rice seedlings.

Salinity stress causes oxidative stress in plants through the generation of toxic reactive oxygen species (ROS) in such plants (Parida and Das, 2005; Saeedipour and Moradi, 2014). These toxic ROS are highly reactive molecules that can cause oxidative damage to cellular membranes as well as DNA, proteins, photosynthetic pigments and lipids (Jithesh *et al*., 2006). In response to free radicals generated, plants produce a number of antioxidant enzymes most notably SOD and CAT so as to counter their effects (Wang *et al*., 2014). In addition, Malondialdehyde (MDA) concentration in such plants is increased, being an end product of the lipid peroxidation caused by the ROS (Del Rio *et al*., 2005). An earlier study by Cavalcanti *et al*. (2004) reported high levels of malondialdehyde coupled with growth retardation in cow peas grown under salt stress. In this study, the significantly increased

MDA concentration in *S. lycopersicum* seedlings treated with NaCl, compared to the control is a clear indication of stress condition. Also, the increase in the activities of SOD and CAT also indicates an increased production of these enzymes in order to combat the generated free radicals (Ashraf and Ali, 2008). This however conforms to an earlier study of Zhang *et al*. (2013) that reported alterations in the antioxidant enzyme activity of plants exposed to salinity stress.

The significantly reduced concentrations of chlorophyll a, b and total chlorophyll are indications of the damaging effects of NaCl on photosynthetic apparatus (Jithesh *et al*., 2006). ROS which are generated during salinity stress may cause oxidative damage to photosynthetic pigments (Verma and Mishra, 2005; Stepien and Klobus, 2006), thus reducing the plant's ability to photosynthesize. These findings are consistent with previous studies which reported reduction in chlorophyll content of plants exposed to salinity stress (Heidari, 2012). In the presence of *D. repens* extract however, the harmful effect NaCl was prevented, thus resulting into an increased concentration of chlorophyll in the tomato plants, further indicating a growth stimulatory role played by the extract in an environmental stress condition.

In conclusion, results from this study have shown that aqueous leaf extract of *D. repens*, under laboratory petridish and pot assays, was able to attenuate the negative effects of salinity stress such as poor germination and growth, oxidative stress and reduced chlorophyll contents in an allelopathy model plant, *S. lycopersicum*. This is an indication that the constituent phytochemicals of the extract improved tolerance of *S. lycopersicum* seedlings in saline conditions and thus may play important roles in improving the growth of seedlings in salt-stressed environments.

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