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Full Length Research Paper

Effects of Super Absorbent Polymer and *Azotobacter vinelandii* on Growth and Survival of *Ficus benjamina* L. Seedlings under Drought Stress Conditions

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ABSTRACT

The effect of 0.0 (control), 0.1, 0.2, 0.4, and 0.6% of “STOCKOSORB” hydrogel polymer, with or without *Azotobacter* inoculation, on survival and growth of ficus (*Ficus benjamina* L.) seedlings grown in sandy soil under drought stress was investigated. The ability of the soil to retain water increased with increasing polymer concentrations particularly in the presence of *Azotobacter*. The highest level of the polymer was capable of changing the typical sandy soil to a loam or even silty clay in terms of water potential and water content. The highest STOCKOSORB polymer concentration plus *Azotobacter* prolonged the time of water loss from the soil by about 46% more than the control soil. During drought stress, the seedlings grown in 0.6% STOCKOSORB-amended soil survived three times as long as those grown in the control soil. Shoot and root growth increased significantly in the polymer amended soil as compared with non-amended soil. *Azotobacter* inoculation improved the effect of the polymer since it gave an additional survival time to seedlings. Plant water potential increased significantly with polymer application and *Azotobacter* inoculation, thus both treatments aided in the establishment and growth of ficus seedlings under water stress conditions. There were no significant differences between 0.4% and 0.6% Stocksorb. The results indicated that 0.6% of the hydrophilic polymer “STOCKOSORB” can be used in arid and semi-arid areas to enhance the drought tolerance of Ficus tree seedlings.

Keywords: *Ficus*, hydrogel polymer, survival, water potential.

INTRODUCTION

Drought is one of the most important abiotic stress factors limiting plant growth and crop production in arid and semi-arid regions (Polle *et al.*, 2006). Dramatic limitation in the water availability in such regions affects the existing tree species and their ability to cope with water stress. Tree species and provenances that are most capable of tolerating water stress are options for the future of forestation (Bredemeier, 2011, Sánchez-Gómez *et al.* 2013). Ornamental and/or forestation species must be adapted to growing conditions in the central region of Saudi Arabia, with lack of water, hot climate, low relative humidity, and high evapotranspiration states. Ficus (*Ficus benjamina* L.) of family Moraceae, is an evergreen tree and was found to tolerate desert heat and grow in soil of low fertility (*Krause et al.*, 2010). These trees deserve attention because they grow fairly rapidly, can endure the unrelenting, tropical sun, and can survive the high salinity levels if it is adequately supplied with water. These trees are widely planted as an ornamental evergreen in yards, parking lots, streets, parks, and the potted plants are used to form bonsai (Abedi-Koupai and Asadkazemi, 2006).

Unfortunately, under the conditions described above, *F. benjamina*, like many other ornamental trees raised in nurseries, are subjected to water stress soon after planting because of poor root to soil contact and high evaporative demand. Moreover, most *Ficus* species are relatively susceptible to water limitations (Ghehsareh *et al.*, 2010). Therefore, their plantations will be at risk in future climates. Even plants grown in containers with a limited quantity of media require frequent irrigation to maintain adequate moisture around roots. This problem is often worse for seedlings because they typically experience optimal nutrient and water supply regimes before planting, which can promote poor plant response to more demanding conditions experienced in the field, particularly under conditions of water deficits (Pazderů and Koudela, 2013). Therefore, prolonged drought stress, due to insufficient rainfall and irrigation water in arid and semi-arid areas, can be detrimental for plant growth and development (Wang *et al.*, 2003).

Recently, water-retentive chemicals (hydrophilic polymers) are used as a possible solution for conserving irrigation and rainwater in such desert regions. Hydrophilic polymers become gels and absorb many times their weight of water and store it for relatively long period of time. Hydrogels were found to promote the soil water-holding capacity and to reduce watering requirements for plants, particularly in sandy soils (Ekebafe *et al.*, 2011). However, the effects of hydrogels on seedling survival have been inconsistent. Increasing in seedling survival and growth was reported with several plant species (Nnadi and Brave, 2011; Moslemi *et al.*, 2012); . Moreover, hydrogels have been used to establish tree seedlings and transplants in the arid regions and to increase plant survival and overall dry weights, particularly for less drought tolerant tree species (Orikiriza *et al.*, 2013). The ameliorative effect of hydrogels for drought-stressed seedlings was demonstrated for *Citrus* (Arbona *et al.* 2005), *Quercus* (Chirino *et al.* 2011), *Fagus* (Beniwal *et al.* 2011) and *Populus* (Shi *et al.* 2010). However, Heiskanen (1995) reported that hydrogels provided no benefit in the establishment of plant seedlings under field conditions.

STOCKOSORB is a highly cross-linked polyacrylamide polymer with the ability to absorb a high volume of water due to network spaces in its cross-linked structure (Chirino *et al.* 2011). The application of STOCKOSORB polymer can provide more uniform substrate moisture, prevent leaching of water from soil and increase the water storage capacity of soil substrates to avoid or reduce drought-stress damage (Chirino *et al.* 2011). Moreover, STOCKOSORB resists soil temperature fluctuations, and remains relatively a longer time without being degraded than other types of hydrogels and its applications in forestry has shown encouraging results with respect to plant biomass production and survival (Chen *et al.*, 2008; Luo *and* Polle, 2009).

Azotobacter is an obligate aerobic diastrophic soil-dwelling organism with a wide variety of metabolic capabilities, including the ability to fix atmospheric nitrogen by converting it to ammonia. Among the free-living nitrogen-fixing bacteria, those from the genus Azotobacter have an important role, being broadly dispersed in many environments such as soil, water and sediment. Research has shown that inoculation with these bacteria has a positive effect on plant growth (Hasanpour *et al.*, 2012).

The objective of this study was to investigate the effect of a new hydrophilic polymer, STOCKOSORB-500 M (Chemische Fabrik Stockhausen GmbH and Co. KG, B¨akerpfad, Krefeld, Germany), on soil water content, period of water conservation in the soil, transpiration rate, survival, and growth of *F. benjamina* seedlings grown under drought stress conditions.

MATERIALS AND METHODS

Field experiments were conducted at the central region of Saudi Arabia during May – August, 2013. The weather during the experimental period was characterized by sunny, hot, dry days, and warm nights (25–27^{O}C). The average daily maximum temperature was 35.6◦C with little variation. The daily minimum temperature during the experiment ranged from 16.2^{O}C to 27.5^{O}C. No rain had fallen during the experimental period.

Soil used

Sand soil was used in the study. The soil was obtained from a field located in the central region of Saudi Arabia. Soil samples were air dried, sieved through a 2 mm sieve and their physical chemical characteristics were analyzed. A pH meter was used to determine the soil pH [Anderson and Ingram, 1993] while organic C and N were deter- mined by the Walkley-Black [Nelson and Sommers , 1982] and Kjeldahl [Bremner, 1965] methods. Available phosphorus was analyzed using the Bray method [Bray and Kurtz, 1945]. Exchangeable cations (Ca2+, Mg2+, K+ and Na+) were extracted by shaking the soil sample for 2 hours with 1 M Ammonium acetate [Okalebo *et al.*, 2000]. Concentrations of K and Na were then determined by a flame photometer whereas Ca and Mg were determined by Atomic Absorption Spectrophotometry [Bray and Kurtz, 1945]. The characteristics of the soils are presented in Table 1.

Hydrogel polymer

STOCKOSORB -500M hydrogel manufactured by the Chemische Fabrik Stockhausen GmbH und Co. KG, B¨akerpfad, Krefeld, Germany, was used to amend the soils at rates of 0.1% – 0.6% hydrogel concentrations (w/w). The control had no hydrogel added. The hydrogel concentrations were made by mixing the hydrogel powder with the soil prepared in a concrete mixer. The amount of hydrogel and the mixing procedure followed previous studies [Orikiriza *et al.*, 2013] and recommendations by the manufacturer.

Table 1. Physical and chemical analyses of the soil used in the experiment.

Physical properties	Chemical properties	
Particle size distribution:	CaCO3 (%) 0.41	Soluble anions (meq/l)
Sand (%) 92.3	OM (%) 0.26	CO_3^{2-} 0.22
Silt (%) 6.2	Ec (dsm^{-1}) 0.53	HCO_3^- 0.86
Clay (%) 1.5		Cl^- 1.83
Soil texture (Sandy)		
	Soluble cations (meq/l)	
	Ca^{2+} 2.96	
	Mg^{2+} 1.68	Avail. elements (mg/kg)
	Na^+ 2.04	N 19.2
	K^+ 0.21	P 8.3
pH 8.01		Fe 2.4
Soil suspension (1 soil : 2.5 water)		

Treatments and experimental design

Six-month-old Ficus (*Ficus benjamina* L.) seedlings were transferred from nursery soil in the greenhouse to 30-L plastic containers filled with 30 kg sandy soil each. The soil was previously mixed with 0.0, 0.1%, 0.2%, 0.4% or 0.6% (w : w) soil conditioner STOCKOSORB-500 Micro hydrogel per pot. Treatments were assigned as HG0, HG1, HG2, HG3 and HG4. Each pot was inoculated or not with the free living nitrogen fixing bacteria *Azotobacter vinelandii*, in a rate of 3 kg bacteria per hectare according to Amiri and Rafiee (2013), to make a sum of 10 treatments.

Each treatment was applied to 6 seedlings. In addition, 6 pots without seedlings were used to monitor evaporative water loss from the soil surface throughout each treatment. The seedlings, averaged 60 ± 3.5 cm tall with a caliper of 2.2 ± 0.3 cm at the soil line, were grown outdoors under natural conditions in a shade-free location. Containers were sunken in the ground such that the surface of the potted soil was at approximately the same level of the surrounding ground surface. Empty containers were used as sleeves to line the holes so that the plant-holding containers could be removed and replaced easily. The tops of the containers were covered with white polyethylene film to minimize evaporation.

A completely randomized design with 6 replicates for each treatment was used in this experiment. Seedlings of uniform height (one seedling per pot) were located in lines with spacing of 2 m between lines and 1 m between pots to avoid mutual shading. The ground surface between and surrounding the trees consisted of bare soil. At the time of transplanting, all trees were fertilized with the complete water-soluble fertilizer “Sangral” (William Sinclair Horticulture Ltd, England) compound fertilizer (20N-20P-20K, plus micronutrients) at the rate of 600 kg ha^{-1}. Each tree received a total of about 10 g fertilizer.

For the first 4 weeks, all seedlings were watered to field capacity (FC), supplying an amount of water equal to transpiration losses as pots were weighed every other day, to ensure the establishment of seedlings and to allow adaptation to the field conditions before drought treatments were imposed. By the end of this period, the watering was discontinued and survival and growth variables of the seedlings were determined. The field capacity of the experimental soil was determined gravimetrically and found to be 12%.

Measurements

The following measurements were registered:

a- Soil water content at field capacity in each treatment as kg water/pot and as percent water content.

b- Daily water consumption, as the total amount of water applied to replace that absorbed or transpired by plants, was measured gravimetrically. Then weekly water loss was determined.

c- The survival of the seedlings was monitored every other day by appearance. The tree was considered to be dead when their leaves became yellow dry and dropped. Besides, upon re-watering, dead seedlings cannot grow again.

d- Leaf water potential was determined in six randomly chosen leaves from each treatment, using a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA) as described by Scholander *et al.* (1965). The sampled leaves were enclosed in a polyethylene bag just before detaching them from the plant and conserved in a thermal isolated box. The measurements were made as soon as possible using a pressure increment of 0.1 MPa per 2 or 3s.

e- Shoot length, leaf area per plant (measured with a leaf area meter LI-COR Model 3100, Lincoln, Nebraska; USA), and dry weight of plant parts were recorded at harvest. The decision to harvest any particular treatment was based on the need to do so at the beginning of death symptoms, before death of seedlings began to occur. Dry weights were determined after drying at 70^{O}C till constant weights. Leaves which dropped during stress treatments were included.

Data Analysis

The experiment was arranged in a completely randomized design and one-way Analysis of variance (ANOVA) was used to test the effect of hydrogel on the measured parameters according to Snedecor and Cochran (1980) with statistical COSTAT software. Differences among means were tested with LSD at 5% level of significance.

RESULTS AND DISCUSSION

Soil-Water Status

Data recorded in Table 2 showed that soils amended with STOCKOSORB polymer were able to store much more water than those of control soil without polymer treatment. The *Azotobacter* inoculation gave an additional improvement to the soil and made it store more water. It was obvious also that the high concentrations of the polymer were much better than the low concentrations in storing water by the soil. In this regard, at field capacity, pots with 0.6% HG (HG4) and without *Azotobacter* were found to hold more than 200% of that recorded for control. While the corresponding value in the presence of *Azotobacter* was about 250% as compared to control treatment. There were no significant differences between soils treated with 0.6% and 0.4% in their capacity to absorb water in the absence of *Azotobacter*, but there was a significant increase in soil water content at 0.6% in the presence of *Azotobacter* as compared with that at 0.4% concentration.

Data recorded in Table 1 showed, also, that the time needed for losing 50% of the initial water in the STOCKOSORB polymer amended-soils increased as compared with the un-amended control soil. Moreover, *Azotobacter* inoculation prolonged the time of water loss in inoculated soils as compared with un-inoculated soils. Collected data indicated that there was a linear relation between HG polymer concentration and time of water loss. In this regard, the time of 50% water loss at HG3 (0.4%) and HG4 (0.6%) treatments was much longer than that recorded for the control or low HG polymer-concentration treated soils. In addition, *Azotobacter* improved the time of water loss and made it longer.

At harvest (end of experiment), the recorded data indicated that, the amount of water remained in control pots (HG0) was as low as 13% and 15% of the field capacity (approximately 13% and 15% of the initial water content) in the absence and the presence of *Azotobacter*, respectively. This amount of water was linearly increased with increasing HG polymer concentrations in the amended soils (R2 = 94%). An interesting observation was that, at HG3 and HG4 the amounts of water remained in the soil by the end of the stress period were as much as 41% and 43% of the field capacity, respectively, without *Azotobacter* inoculation. The corresponding values with *Azotobacter* inoculation were 44% and 46%, respectively. This may indicate that a portion of the irrigation water was captured to the polymer material and may not be easily available for the plant at high STOCKOSORB polymer concentrations.

In this regard, the present study showed that the application of STOCKOSORB polymer caused an increase in water content and a decrease in the water loss. These results confirmed the earlier findings of Landis and Haase (2012) who used the applications of hydrogels in the nursery and during out planting and found that the main use of hydrogels has been to retain water for plant growth especially when irrigation isn't provided. Another application which has been widely tested is the incorporation of hydrogels into growing media prior to sowing as a mean to hold more water and reduce moisture stress. In another study, Orikiriza *et al*. (2013) reported that when hydrogels are added to the soil the water holding capacity is increased. Hydrogels also reduce nutrient loss from soils as runoff is prevented or minimized (Agaba *et al.*, 2011).

In addition to hydrogels, soil microorganisms were found to have beneficial effects on drought exposed woody plants. In this regard, Beniwal *et al.* (2010) found that hydrogel performance in decreasing the water loss from treated soils was improved by the addition of mycorrhiza and *Azotobacter*. It was found also that the role of su

per absorbent polymers increased by the addition of *Rhizobacteria* that increased the capacity of water storage in the soil and reduced the waste water (Moslemi *et al.*, 2012). The results in Table 2 indicated that an amendment of soils with STOCKOSORB would greatly enhance the ability of soil to contain more water than that of untreated soil. In addition, *Azotobacter* inoculation increased the storage of water in soil, may be through increasing nitrogenous compounds in soil colloid (Hayat *et al.*, 2010), thus decreases the water loss from soil, and prolongs the time of 50% water loss. Therefore, STOCKOSORB polymer treatment in the presence of *Azotobacter* could improve drought tolerance of the

Table 2. Effects of STOCKOSORB polymer amendment (HG) and *Azotobacter* (AZ) inoculation on soil-water budget during stress period.

HG treatments	AZ	Soil water content (kg/pot)	Time of 50% water loss (day)	Total loss of water during stress (kg)	% water loss
HG0	-	03.75±0.88	05.2±1.11	3.27±0.56	87.2%
	+	03.88±0.93	06.4±1.24	3.31±0.85	85.2%
HG1	-	04.38±1.22	07.4±1.87	3.30±0.65	75.3%
	+	05.46±1.71	08.7±2.01	3.95±0.72	72.4%
HG2	-	06.76±1.63	10.2±2.12	4.75±0.93	70.3%
	+	07.65±1.95	12.2±2.23	5.11±1.04	66.7%
HG3	-	09.97±1.48	19.3±2.54	5.86±1.13	58.8%
	+	11.14±1.91	22.2±2.65	6.27±1.45	56.3%
HG4	-	11.63±2.11	26.1±3.11	6.64±1.38	56.9%
	+	13.22±2.24	30.5±3.24	7.16±1.26	54.2%
LSD 5%		1.76	2.44	1.25	

* Values represent means of 6 replicates (means are followed by ±SD).

seedlings grown in the treated soil.

Both results on the survival of the seedlings under stress in the different soils and the data on the water potentials (discussed later) revealed that the amendment of the sandy soil with 0.4% and 0.6% STOCKOSORB, with *Azotobacter* inoculation, changes completely the water retention properties of the soil. The containers with 0.4% (HG3) and 0.6% (HG4) in *Azotobacter* treated soil absorbed as much as threefold more water than the control soil (nearly 11 and 13 kg water/pot, respectively, instead of 4 kg). While with 0.1% (HG1) and 0.2% (HG2) of STOCKOSORB in the soil, only about 5 and 7 kg water were absorbed, respectively. This dramatic effect of the HG polymer application is in agreement with the observations of Ekebafe *et al.* (2011) and Pazderů and Koudela (2013), who found an exponential increase in the water content of a sandy soil with increasing hydrophilic soil conditioners. In the soil STOCKOSORB particles swells to a hydrogel, where 1 kg of STOCKOSORB is able to absorb up to 250 liter of demineralised water (EVONIK Industries, 2014).This high ability of the soil to hold a great amount of water and increase its retention capacity as hydrophilic amendment concentration increases may also explain why, in several cases, no water collection of HG polymer amended soils was found (Olszewski *et al.*, 2012).

Weekly loss of water

Data recoded in Figure 1 showed a gradual decrease in the amount of water lost weekly as the time of drought stress increases, particularly in the absence of STOCKOSORB treatment. The disappearance of evapo-transpiration process was also obvious at low concentrations of STOCKOSORB treatments either in the absence (Figure 1A) or in the presence (Figure 1B) of *Azotobacter* inoculation. It is clear that, at all times of measurements, the kinetics of the weekly loss of water shows that soils with higher STOCKOSORB concentrations and, consequently longer plant life, released more water during the stress period (after the last watering) than the control or low STOCKOSORB treated-soils. This finding is true either with *Azotobacter* inoculation or not. Therefore, the ratio of soil water content at the beginning of stress treatment and at harvest time was significantly higher at 0.4% and 0.6% HG polymer concentrations than that recorded at lower concentrations or at control treatment (Figure 2). Data in the same figure indicated that at the end of stress period the water content% of *Azotobacter* inoculated-soil was significantly higher than that of un-inoculated soils. Data showed also that water content% of hydrogel + *Azotobacter* (HG+AZ) treatment was higher than that of HG polymer treatments alone. In this respect, soil water contents at HG3 (T3) and HG4 (T4) amended soils were about 14% and 17%, respectively, in the absence of *Azotobacter*; and about 16% and 20%, respectively, in the presence of *Azotobacter*. While, at the beginning of stress, the corresponding values were 33.2% and 38.8% with HG alone and about 37% and 44% with HG and *Azotobacter*.

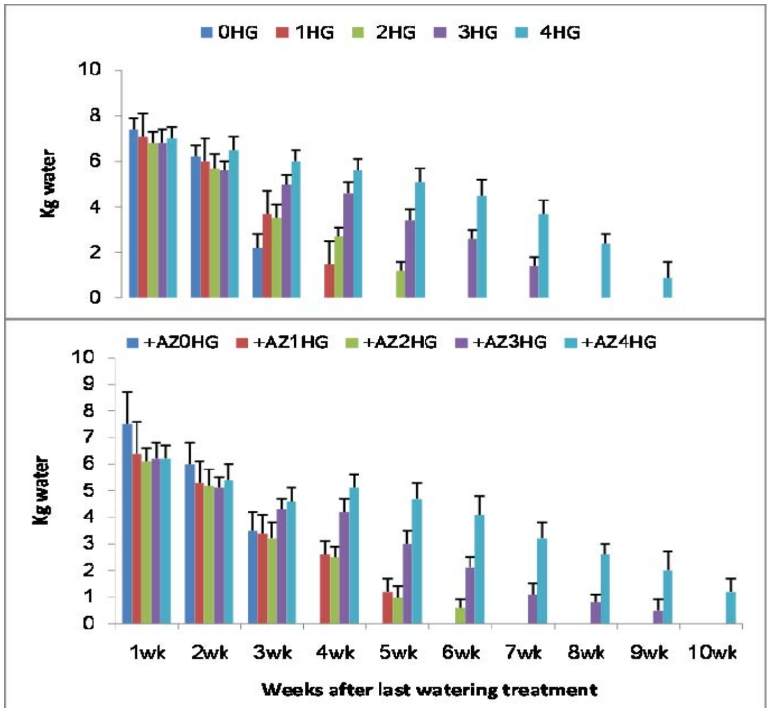

Figure 1. Effects of STOCKOSORB hydrogel (HG) alone (Top) or STOCKOSORB +*Azotobacter* inoculation (HG+AZ) together (Bottom) on weekly evapo-transpiration after the last re-watering of *F. benjamina* seedlings (vertical bars indicate ±SD of the mean (n = 6 seedlings).

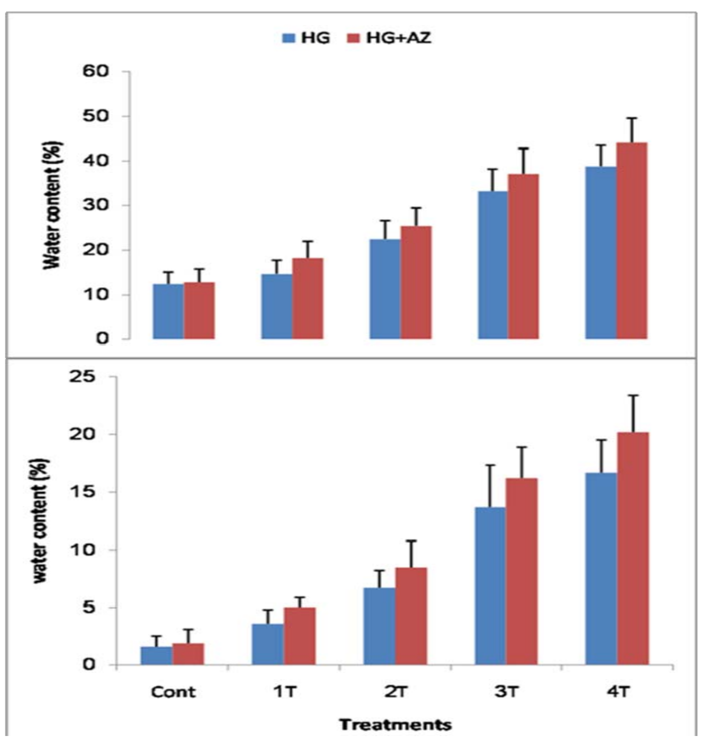

Figure 2. Soil water contents (%) at the beginning (Top) and at the end (Bottom) of stress period as affected by STOCKOSORB (HG) treatments and *Azotobacter* (AZ) inoculation. (Vertical bars indicate ±SD).

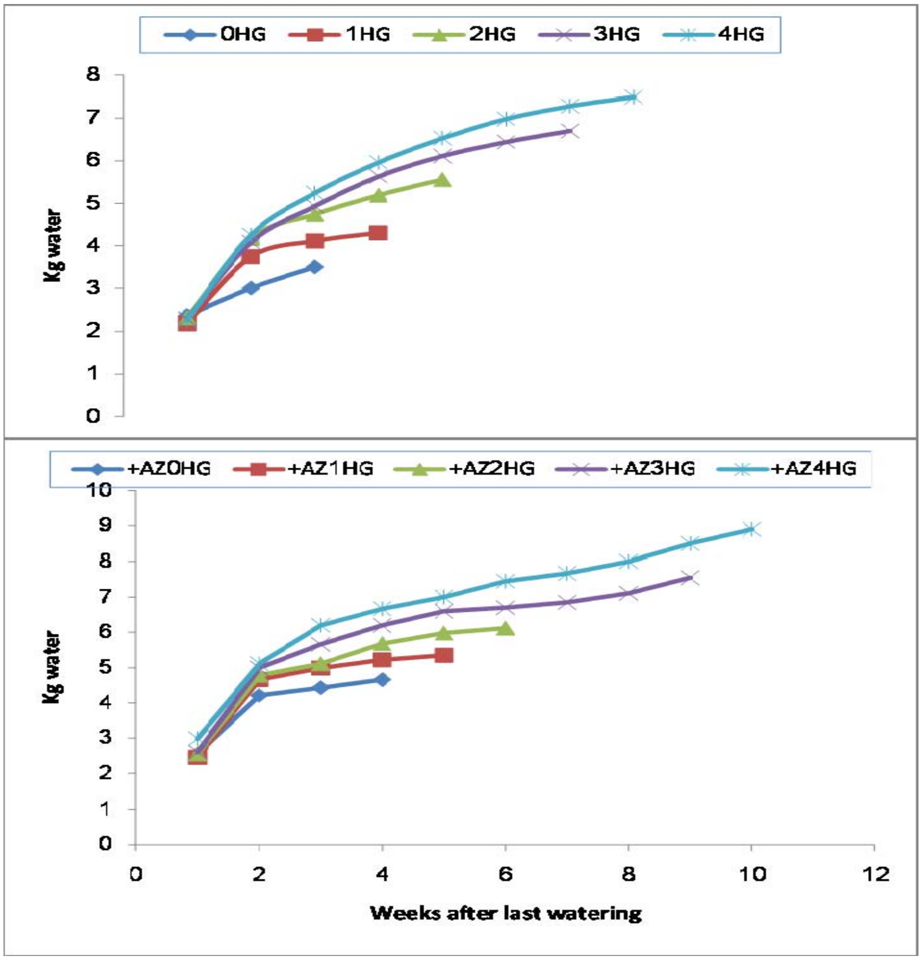

Figure 3. Effects of STOCKOSORB hydrogel (HG) alone (Top) or STOCKOSORB +*Azotobacter* inoculation (HG+AZ) together (Bottom) on cumulative water loss (kg/week) after the last re-watering of *F. benjamina* seedlings (n = 6 seedlings).

Cumulative water loss

As a result of the weekly water loss through the evapo-transpiration in each treatment through the experimental period, recorded data indicated that cumulative water loss for HG-treated seedlings, particularly at high STOCKOSORB concentrations, was higher than that for the control seedlings (Figure 3). It seems that overall transpiration of plants was affected by the use of the polymer in the media since it potentially increased water availability for plants. *Azotobacter* inoculation also seemed to improve water availability therefore collected data showed that the cumulative water loss in HG+AZ treatments was more than that observed in HG treatments alone. Specht and Harvey-Jones (2000) observed an increase in overall water uptake and stomata activity increase in many tree seedlings including queensland maple (*Flindersia brayleana*), gum trees (*Eukalyptus grandis*), and oak (*Quercus rubra*) when polymers were incorporated into the media. They found also that, the seedlings showed an increase in overall plant mass associated with the increase in transpiration and carbon dioxide intake. In contrast to results obtained by Anna *et al.* (2011), no reduction in evaporation was observed in the HG-amended soils during the present study. In fact, the reverse was found, and the soil with the highest concentration of HGs lost more water than the control soils because of the adequate soil water content available for plants and the extended time of seedling survival at the high concentrations of STOCKOSORB particularly with the presence of *Azotobacter* inoculation (Table 2 and Figure 1).

The water harvested by the STOCKOSORB HG was readily transferred both to the seedlings and to the atmosphere. This could be confirmed by the findings of Specht and Harvey-Jones (2000), who found that the amendment of the soil with different polymers prolonged the time until 50% of the soil water was evaporated. In this connection, HGs were found to potentially influence soil structure, compaction, soil texture, and evaporation rates (Geng, *et al.*, 2011). In the present study, since the soils amended with the higher concentrations of STOCKOSORB HG retained much more water during the irrigation period than the control soils, they could afford to lose more water and still retain more soil moisture than the other treatments.

An additional effect of *Azotobacter* is to prime the plants' defense systems for increased stress tolerance involving different signaling pathway required for the recruitment of osmolytes (Beniwal, 2010). Furthermore, *Azotobacter* was found to improve the level of nitrogenous compounds in soils, which may have facilitated water uptake (Hayat *et al.*, 2010). Although osmo-regulation is important to maintain water uptake when the soil water potential decreases, the present study provides evidence that hydrogel, in the presence of *Azotobacter,* primarily afforded a strategy of drought avoidance. This finding was also corroborated by the maintenance of higher rates of N2 assimilation and transpiration in Ficus seedlings. Most importantly, the protective effect was much stronger when both STOCKOSORB HG and *Azotobacter* were present suggesting that, together water can be tapped or can be retained and transferred to plants more efficiently than STOCKOSORB HG alone. This finding clearly has implications for forest plantation on regions affected by periods of drought. It is likely that applications of hydrogel in natural ecosystems will also be influenced by soil microorganisms. However, it is unknown if all soil microorganisms can afford these beneficial effects.

Seedling survival and growth

Data recorded in Table 3 showed that the survival time after the last watering increased in STOCKOSORB treated-seedlings as compared with the untreated control plants. *Ficus* seedlings were able to grow for nearly 2 months without watering, when the soil was amended with 0.6% HG (HG4 treatment) while the control seedlings grew for about 3 weeks only. *Azotobacter* inoculation made a further positive effect and increased the ability of the hydrogel treated seedlings to withstand drought stress and to survive for a longer period of time than un-inoculated seedlings.

It was clear also that some growth increments of the stressed shoots were noticed in spite of the water stress conditions at which seedlings were growing. It was evident that as the STOCKOSORB concentration increased, shoot growth and root and shoot dry weights as well as leaf area (Figure 4) increased to reach their maximum values at 0.6% (HG4 treatment). In the absence of *Azotobacter*, there were no significant differences between growth rates or dry weights of 0.6% and 0.4% STOCKOSORB treated seedlings. However, the application of *Azotobacter* improved the effect of the hydrogel and enhanced the growth of treated seedlings more than those without inoculation. In this regard, data revealed that the 0.6% of STOCKOSORB treated seedlings showed significantly higher values for growth attributes than those recorded at 0.4% treatment.

With reduced irrigation rates for some tree seedlings, Abedi-Koupai *et al.* (2010) reported that hydrogel amendments increased the survival rate by 1.5 times than seedlings without hydrogel treatments. In the present satudy, when irrigation was withheld for seven days, all control tree seedlings died compared to the 57% and 71% survival rate of the STOCKOSORB amended soil in the absence and in the presence of *Azotobacter*, respectively. These results confirm those obtained by Orikiriza *et al.* (2013) who found that 0.4% HG amendments doubled the survival rate and prolonged water availability for *Pinus halepensis* seedlings when irrigation was stopped. They found also that, the hydrogel media allowed for several weeks to pass before seedlings started to die whereas in the control with no hydrogel plants started to die after some days of drought. In sandy soils, Luo *and Polle* (2009) and Shi *et al.* (2010) found that incorporation of hydrogels in sandy soils increased the survival time of some tree seedlings, because Hydrogels were found to enhance the water holding capacity of soils and thus provide supplementary plant available water to plant root zones in dry soils, thus survival time increases as a consequence.

Comparable to the present study, the increased soil water storage with increased HG concentration in pot experiments with the seedlings, in principle, is also found in the measurements of the suction potential in pressure plate tests in other study (Demitri *et al.,* 2013). This suggests that the total amount of water stored in the hydrogel should be available for the plants when grew on this substrate (Guan *et al.*, 2014). Results of the present experiment with *Ficus* seedling indicate that the plants do not have full access to this water reservoir. Plants in the control soils died only after the water content was reduced to about 2% in the presence or absence of *Azotobacter*. While the tree in the soils amended with 0.6% HG died even at water content of about 17% and 20% in the absence or the presence of *Azotobacter*, respectively. Therefore, a significant part of the soil water seemed to be stored by the hydrogel and apparently was not available for the plants.

In an early study, Agaba *et al.* (2011) reported that the hydrogel amended sandy soil was found to increase the shoot and root biomass of *Agrostis stolonifera* plants by 2.2 and 4 times respectively compared to the control. They found also that the 0.4% hydrogel amendment in sandy soil increased the water use efficiency of the plants eight fold with respect to the control. The hydrogel stimulated development of a dense root network and root aggregation that increased contact of the roots with moisture thus improving water use efficiency of hydrogel

Table 3. Effects of hydrophilic polymer amendment on survival, shoot growth, root and shoot dry weights of *F. bingamina* seedlings at harvest time.

HG treatments	AZ	Survival (days)	Shoot growth increment (cm)	Root dry weight (g)	Shoot dry weight (g)
HG0	-	21.4±2.11	3.8±0.98	3.86±1.11	8.77±2.14
	+	28.2±2.66	4.4±0.87	4.75±1.23	9.92±2.33
HG1	-	28.5±2.23	5.6±1.22	4.32±1.09	11.23±2.24
	+	35.5±3.12	7.5±1.65	5.45±1.32	13.22±2.45
HG2	-	35.2±3.08	7.9±1.43	6.11±1.67	14.45±3.15
	+	42.4±3.36	9.5±1.23	7.68±1.87	16.85±3.54
HG3	-	49.4±3.45	10.7±2.34	8.75±2.21	21.25±3.23
	+	63.2±3.52	12.4±2.45	10.15±2.32	23.46±3.65
HG4	-	56.5±3.26	11.6±2.65	9.54±2.06	24.15±3.76
	+	70.3±4.28	14.8±2.58	12.85±2.65	28.42±3.68
LSD 5%		6.75	2.16	2.25	5.45

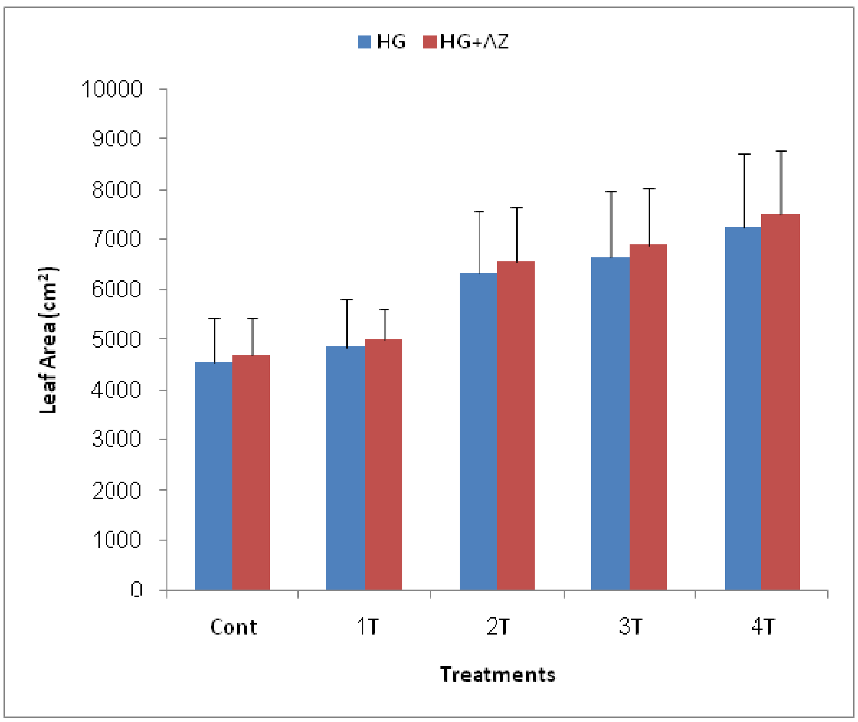

Figure 4. Effects of STOCKOSORB hydrogel (HG) alone or STOCKOSORB +*Azotobacter* inoculation (HG+AZ) together on leaf area (cm^2) of *F. benjamina* seedlings (Vertical bars indicate ±SD).

amended soil. The results suggest that hydrogels can improve sandy soil properties for plant growth by absorbing and keeping water longer in the soil matrix thus reducing watering frequency. In this connection, earlier studies by Landis and Haase (2012) showed that, hydrogels definitely increased the water holding capacity of the growing medium and seedlings grown in hydrogel-amended medium averaged lower moisture stress than those grown in un-amended control media when subjected to desiccation following lifting. Besides, hydrogels have been shown to retain nutrient ions against leaching especially in growing media with low cation exchange capacities such as the sandy soils used in this study. Therefore, seedling growth was improved in the STOCKOSORB amended soils.

The beneficial effects of the soil free-living organisms, such as *Azotobacter* and *Azospirillum* on plants, were found to be attributed to improvements in root development, increases in the rate of water and mineral uptake by roots, and to biological nitrogen fixation (Parmar et al., 2011; Hasanpour *et al*., 2012). An interesting finding was that the growth of both shoots and roots during the water stress period did not stop but was continuing in a slow rate (Table 3). It has been shown in several studies that roots of many tree species are rather sensitive to water stress conditions and subsequently elongation growth declines and finally, when photosynthesis becomes strongly limiting, root growth also stops (Fischer and Polle, 2010; Orikiriza *et al.*, 2013).

Early studies showed that hydrophilic polymers reduced nutrient losses from soils, reduced soil salinity, especially in sodic soils, and enhanced plant growth by allowing nutrients, incorporated into the hydrogel matrix, to release to the plant as needed (Shakesby, 2000). When hydrogels were incorporated to saline sandy soils, the plants had increased pigments, photosynthesis activity, amino acids, and protein contents of corn plants and kept roots cooler during hot summer warm days (El Sayed and El Sayed, 2011). It has been found that, treatment of seeds with *Azotobacter* and mycorrhiza can help to control incidence of disease, to improve nutrient uptake efficiency, to produce thiamin, riboflavin, indole acetic acid and gibberellins and to promote plant growth (Miri et al., 2013). It was reported that these microorganisms results in the secretion of vitamins and amino acids and production of siderophores and auxins which are among the direct mechanisms of increasing root development and plant growth [Akbari *et al.*, 2007)]. *Azotobacter* might increase early vigor of seedlings and result in faster growth of roots enabling them to exploit deeper soil layers and to produce more adventitious roots in the topsoil. The latter may be important for using water and nutrients before water loss through evaporation from the topsoil. Moreover, Azotobacter can make it possible by providing N nutrient to roots (Solanki et al., 2011). In this regard, Ponmurugan *et al.* (2012) found that multiple plant growth promoting activities such as IAA production, NH3 release, PO4 solubilization, HCN and siderophore were produced from Azotobacter isolates.

Plant-Water Status

The effects of STOCKOSORB hydrogel and *Azotobacter* inoculation on the leaf water potential (ψ_w) of *Ficus* seedlings are shown in Figure 5. It is noticeable that water potential of HG treated seedlings is higher than that of untreated ones, and ψ_w of *Azotobacter* inoculated seedlings is higher than that of un-inoculated ones grown at the same concentration of STOCKOSORB. It is obvious also that ψ_w of drought-stressed control plants dropped rather fast after about 15 days of stress treatment, with death occurring one week later. The seedlings that grew at 0.1% and 0.2% HG showed the same pattern of decline as control plants, in respect to leaf ψ_w, starting 7 and 15 days later, respectively. Plants grown in the high concentrations of STOCKOSORB polymer, however, exhibited nearly similar pattern except that seedlings toke longer time to death. At both treatments (HG3 and HG4), the decline markedly in leaf ψ_w started on day 35 and 42, respectively, and they took 15 and 30 days until the same minimum value of about – 4.7 MPa was reached just before plant death.

Leaf water potential of *Azotobacter* inoculated seedlings, grown under stress conditions and STOCKOSORB treatments, showed almost the same pattern of decline as that of un-inoculated seedlings grown under same conditions. However the drop in leaf ψ_w in HG0+AZ started after 3 weeks of the last watering while the corresponding drops in HG1+AZ, HG2+AZ, HG3+AZ and HG4+AZ treatments were observed after 4, 5, 7 and 8 weeks after last watering. Another observation was that the drop occurred in the absence of *Azotobacter* was sharp and sudden while it was gradual for *Azotobacter* treated seedlings.

It was clear that STOCKOSORB treatments improved water potential of the seedling compared to untreated seedlings. Moreover, *Azotobacter* inoculation made a further improvement in water potential and also prolonged the time for water potential to drop. In this regard, seedlings of *Ficus* maintain their water potential high for 42 days at HG4 and for 56 days at HG4+AZ treatments before the plants were finally dead. This change in water retention and plant water potential pattern is similar to the one described for the differences between sandy and loamy soils. This view is strongly supported by the water budget of the 30 L container during the stress experiment containing 0.4% to 0.6% of HG. The soil amended with 0.6% concentration of HG and HG+AZ treatments, for example, had a substantial percentage (about 39% and 44%, respectively) of soil water content at the beginning of the desiccation experiment, which was reduced to about 17% and 20%, respectively, when the seedlings finally died. According to the figures given by Orikiriza *et al.* (2013), the soil might, thus, be converted by the polymer amendment from a typical sandy soil to a loam or even silt clay soil with regard to its hydraulic properties. It was reported that addition of hydrogels to a sandy soil changed the water holding capacity to be comparable to silt clay or loam and increasing overall water holding capacity (Shi *et al.*, 2010).

In an early study, Anna *et al.* (2011) found that the 0.4% hydrogel amendment in sandy soil increased the water use efficiency of the turf grass species eight fold

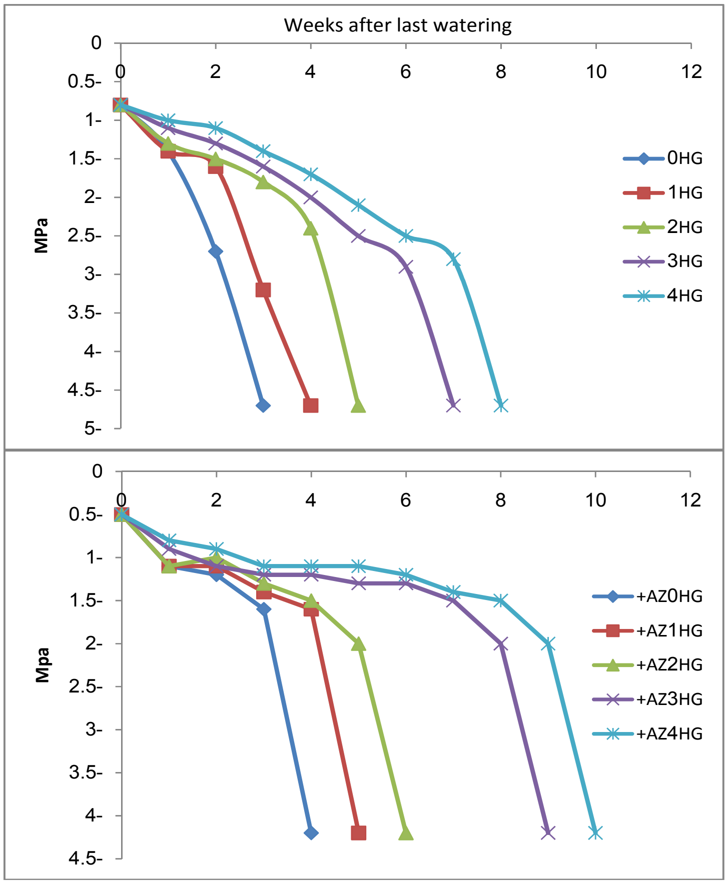

Figure 5. Effects of STOCKOSORB hydrogel (HG) alone (Top) or STOCKOSORB +*Azotobacter* inoculation (HG+AZ) together (Bottom) on leaf water potential (MPa) of *F. benjamina* seedlings (n = 6 seedlings).

with respect to the control. The hydrogel stimulated development of a dense root network and root aggregation that increased contact of the roots with moisture thus improving water use efficiency of hydrogel amended soil. The results suggest that hydrogels can improve sandy soil properties for plant growth by absorbing and keeping water longer in the soil matrix thus reducing watering frequency. This conclusion is in agreement with that reported by Agaba *et al.* (2011) who found that hydrophilic polymers reduced irrigation frequencies without affecting the growth of ornamental plants. According to Beniwal et al. [2010], plants growing in hydrogel-amended soil have more water available (reflected higher leaf water potential) for longer period of time than control plants thus the frequency of irrigation may be reduced.

CONCLUSION

The present study indicated that the use of hydrogel polymers may be a useful tool playing a significant role in the early establishment and growth of landscape tree species under arid and semi-arid conditions. Hydrogels have the potential to improve the growth of *Ficus* seedlings in the critical days after planting out. Enhanced leaf retention and accelerated roots extension, are important factors. The longer survival of *Ficus* seedlings under water stress, together with the growth of shoot and root systems, will considerably increase the fitness of the seedlings and enable the plants to grow fairly well under water deficit conditions during forestation. This can be achieved, to some extent, by the application of STOCKOSORB soil amendments.

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