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Priming of *prosopis cineraria* (L.) *druce* and *acacia tortilis* (forssk) seeds with fulvic acid extracted from compost to improve germination and seedling vigor

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ABSTRACT: Composting of waste plant materials and its use in agriculture and landscape sites is an environmental friendly way of reducing waste material and conserving the environment. In this perspective, we have taken the initiative at the Dubai based International Center for Biosaline Agriculture to compost the plant based waste material (lawn cuttings-grass) to compost. The material was inoculated with a consortium of microbes leading to the formation stable and mature compost with high organic matter (38%). In order to conduct seed germination tests, fulvic acid was extracted from the compost. A pot experiment was conducted over a period of 30 days in the green house to study the effect of fulvic acid on the seed germination, and plant growth of *Prosopis cineraria* (L.) *druce* (Ghaff) and *Acacia tortilis* (Forssk.) Hayne. Seeds of both trees were treated with fulvic acid at 0.5% and 1% concentrations water treatment was used as control. Generally seed germination and biomass were increased at both rates of fulvic acid application, However, a pronounced increase was found in seed germination when fulvic acid was used at 1.0% (*Prosopis cineraria* 27%; *Acacia tortilis* 20% increase over control). Similarly biomass (shoot and root) of *A. tortilis* and *P. cineraria* showed an increase of increase 34% and 94% respectively.

Keywords: *Acacia tortilis, Biomass, Pant growth, Prosopis cineraria, Seed germination*

INTRODUCTION

The *Prosopis cineraria* (L.) Druce is native to the United Arab Emirates (UAE) and its Arabic name is Ghaff. A large and well known example of the species is the Tree of Life in Bahrain – approximately 400 years old and growing in a desert devoid of any obvious sources of water. It is also the national tree of the United Arab Emirates. The citizens of the UAE are urged to plant it in their gardens to combat desertification and to preserve their country's heritage. It has many uses, such as but not limited to the wood a good fuel source, and provides excellent charcoal. The leaves and pods are consumed by livestock and are beneficial forage (Robertson *et al.*, 2012; Malik *et al.*, 2013). Dried pods also form rich animal feed, which is liked by all livestock. The tree is well-suited for an agroforestry setting, because it has a single-layered canopy, it is a nitrogen fixer (thus enriching the soil), and its deep roots avoid competition for water with arable crops. In the UAE, among other measures, prevention of shifting sand dunes is accomplished through plantations of *Acacia tortilis* (Forssk.) Hayne. There are few local tree species suitable for planting in the desert region and these are slow growing. *Acacia tortilis* has proved to be the most promising species for desert greening. It has been observed in the literature that the germination of seeds of both the trees is low to moderate, and this is an area which needs further attention to increase seed germination and plant growth, ultimately leading to optimize desert rehabilitations. In *Prosopis cineraria*, for example, the seed germination may vary from 5 to >70% and a similar is true for *Acacia* species (Arya *et al.*, 1993). Considering the importance of such need, we have attempted to prime the seeds with fulvic acid extracted from the compost prepared at

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International Center for Biosaline Agriculture (ICBA) to improve seed germination.

Inherent soil fertility of sandy soils in the UAE is very low due to low organic matter and clay contents, poor structure, unfavorable air-water balance, and low nutrient content. Ability of such soils to support agriculture can be improved by the addition of inorganic and organic amendments, which can either be obtained from mined material (inorganic) and biomass grown *in situ* (green manure), or through the incorporation of composts in the soil. Composts are known to improve the productivity of sandy soils on a sustainable basis (Tester, 1990; Leifeld *et al.,* 2001). Besides, the composts serve as a carrier as well as an enrichment medium for microorganisms.

The addition of organic matter (including composts) in soil has number of benefits. It has positive impact on soil fertility and productivity (Carter, 2002; Mohammadi *et al.,* 2011), physical and chemical properties of sandy soils (Debosz *et al.,* 2002), soil aggregation (Wortmann and Shapiro, 2008), nutrient supply (Hargreaves *et al.,* 2008), water holding capacity (Werner, 1997), soil pH (Crecchio *et al.,* 2001), air-water balance for root development and proliferation (Werner, 1997), microbial population and functions (Stamatiadis *et al.,* 1999), and stable organic matter content of the soil (Rivero *et al.,* 2004).

Besides multifarious beneficial effects of composts on soil fertility/structure and plant growth/nutrition (Lee and Bartlett, 1976; Wang *et al.,* 1995; Piccolo *et al.,* 1997; Nardi *et al.,* 2000), humic compounds in compost can increase seed germination and seedling vigour. The positive effect of humates (humic acid and fulvic acid) on soil-plant system is widely accepted and recognized (Smidova, 1962; Schnitzer and Poapst, 1967; Azam and Malik, 1983; Reynolds *et al.,* 1995; Kelting *et al.,* 1998, Nardi *et al.,* 2000; Arancon *et al.,* 2006; Edwards *et al.,* 2006; Ferrara *et al.,* 2007). In composts, the humates originate mainly from the fungal activities including synthesis of phenolic compounds that subsequently polymerize (Felbeck, 1965; Haider and Martin, 1967; Mayhew, 2004).

In general, humic compounds are obtained from naturally occurring lignite (brown coal) and leonardite (Youngs and Frost, 1963). It is very seldom to extract these growth promoting substances from composted materials to establish their role in seed germination and seedling establishment. At the Dubai based ICBA, a long term program is underway to standardize the composting process (in terms of quality and time required) and uses to improve soil quality of sandy soils.

The objective of this preliminary study is to determine the effect of fulvic acid extracted from compost on seed germination and seedling vigor of *P. cineraria* (L.) Druce and *A. tortilis*. (Forssk.) Hayne. The test plants were selected with the realization that these trees are suitable for arid zones, and the seeds are difficult to germinate. Their seed coat is impervious to water leading to dormancy that may extend over months or years. Species of *Acacia* and *Prosopis* are of interest to United Arab Emirates. In *Prospis cineraria* for example the seed germination may vary from 5 to >70% and the same is true for *Acacia* species (Arya *et al.,* 1993). Not only is the percent seed germination low, it takes a long time (up to several weeks) for seeds to germinate. For mass plantation of both trees species, it is important to secure efficient and rapid seed germination. Therefore, pre-sowing seed treatment is essential to break dormancy to increase seed germination. Numerous techniques have been used to make the seeds permeable to water (Cavanagh, 1980; Bonner *et al.,* 1974). Fulvic acid is also reported to substantially enhance seed germination and seedling vigour (Rauthan and Schnitzer, 1981; Piccolo *et al.,* 1993).

The objective of this study was to study the extent to which recently synthesized humic compounds in composts facilitate seed germination and seedling vigour of *P. cineraria* (L.) Druce and *A. tortilis* (Forssk.) Hayne.

Hypothesis to be tested

It is hypothesized that the seed germination and biomass of *P. cineraria* (L.) Druce and *A. tortalis* (Forssk.) Hayne. can be improved when the seeds are primed with different concentrations of fulvic acid extracted from compost.

MATERIALS AND METHODS

Raw material (feedstock) for compost preparation

The feedstock used for composting was lawn clippings and tree trimmings, such as grasses (*Distichlis spicata* (L.) Greene , *Paspalum vaginatum* (Sw.) Griseb, *Sporobolus virginicus* (L.) Kunth, *Sporobolus arabicus* (Boiss.), herbs (*Sesuvium portulacastrum* (L.), *Ipomea pescarpea (L.), Portulaca grandiflora* (Hook.), *Wedalia tribulata* (L.) A.S. Hitchc.), shrubs (*Atriplex nummularia* (Lindl.), *A. halimus* (L.), *A. lentiformis* (Torr.) S. Wats. and trees (*Phoenix dactylifera* (L.), *Azadirachta indica* (A. Juss.), *Plumeria alba* (L.). At ICBA a landscape has been established (Shahid, 2014) for educational purposes, from this landscape feedstock (lawn and tree trims) is available once a week in summer (March-September) and fortnightly in winter (October –February).

During the composting process, the heaped feedstock (approximately 500 kg) was moistened (50-60%) and covered with polyethylene sheet to minimize moisture loss. The material was manually aerated weekly by turning/mixing to initiate and hasten the decomposition process. After 8 weeks, dark brown to blackish compost was ready for use in the experiment. Standard analytical methods (Burt, 2004) were used for all the parameters while humate content was determined by extracting an aliquot of the material with 0.1N NaOH, drying the extract and determining the dry matter as a percent of the compost sample used. The final compost had pH (7.7) at (1:10, compost:water; electrical conductivity (8.47 mS/cm); organic matter (38.15%); organic C content (21.94%); total N (0.67%); humates content (4.36%); and moisture content (9.89%). Compost characteristics were essentially similar to those reported by other workers (Daldoum and Ameri, 2013; Sanchez-Monedero *et al., 2001*; Anonymous, 2014).

Soil Taxonomic class

According to the United Arab Emirates Keys to Soil Taxonomy (Shahid *et al.*, 2014) the soil used in the present study is classified as *carbonatic, hyperthermic Typic Torripsammens*. The *Typic torripsamments* means typical sandy soil; *Carbonatic* is mineralogy class, means, any particle-size class and more than 40 percent (by weight) carbonates (expressed as $CaCO_3$) plus gypsum, either in the fine-earth fraction or in the fraction less than 20 mm in diameter, whichever has a higher percentage of carbonates plus gypsum; *Hyperthermic* is temperature regime, the mean annual soil temperature is 22 °C or higher, and the difference between mean summer and mean winter soil temperatures is 6 °C or more either at a depth of 50 cm below the soil surface or at a densic, lithic, or paralithic contact, whichever is shallower.

The recently completed soil survey of the United Arab Emirates (EAD, 2009a,b) revealed sandy soils (Entisols) are dominant in the UAE. Therefore, the findings of the present study will have wider application not only in the UAE, but other GCC countries (Bahrain, Kuwait, Oman, Qatar, Saudia Arabia) where these soils have been reported to dominate the landscape (MAW, 1985; MAF, 1990; KISR, 1999; MMAA, 2005; Scheibert *et al.*, 2005; Shahid and Abdelfattah, 2008; EAD, 2009a,b; Omar and Shahid, 2013).

Physical and Chemical Characteristics

Air-dried and sieved (<2mm) soil was analyzed for selected physical (soil texture, color) and chemical characteristics. The analytical methods used are from Burt, (2004) except where otherwise specified. The results revealed the soil is fine sand in texture (sand 98%, silt 1%, clay 1%), slightly alkaline (pHs 7.6), non-saline (ECe 1.54 dS/m), saturation percentage (26); strongly calcareous (calcium carbonate equivalents 53%), very low organic matter content (0.22%) and very low total N (0.007 mg/kg).

Extraction of fulvic acid from compost

To the compost a known volume of 0.1 N KOH was added to develop 1:10 (compost: extractant) suspension, and the suspension was shaken (150 rpm) at room temperature (20 °C) on a to-and-fro shaker for 4 hours. The suspension was centrifuged and the supernatant containing humic and fulvic acids was collected and acidified to pH 1.5 using 6N H_2SO_4 with constant stirring following by heating at 90 °C for 30 minutes. Acidified extract was centrifuged to separate humic acid (settled) and to collect fulvic acid as supernatant (Asing *et al.*, 2004). The fulvic acid was neutralized to pH 7.0 using 2N KOH solution.

Seed treatment with fulvic acid

Seeds were softened by immersing in concentrated sulfuric acid for 15 minutes followed by repeated washings with distilled water to completely remove acidity. Subsequently, the seeds were soaked for 24 hours in the following media:

1- 0.1N KOH acidified to pH 1.5 and then neutralized to pH 7.0 (Control)

2- 0.5 % solution of fulvic acid fraction in water (1:200 dilution of FA fraction)

3- 1.0 % solution of fulvic acid fraction in water (1:100 dilution of FA fraction)

0.5% and 1.0% concentration of fulvic acid were used based on the findings of Khang (2011), who reported the higher concentration than 1% decreased plant height.

Pot experiment

A greenhouse experiment was conducted in July 2014 for four weeks. The plastic containers (400 cm^3) were filled with three hundred g portions of the air-dried and sieved (<2 mm) soil and moistened to field capacity (12% by weight). Five seeds each of *Acacia tortalis* and *Prosopis cineraria* were sown in each container and the treatments were triplicated. The seeded containers were kept under greenhouse conditions to study the seed germination and different seedling vigour parameters including i) root and shoot length, ii) fresh and dry weight of root and shoot and iii) number of leaves.

Seed germination was recorded after every 24 hours until germination ceased completely and the data collected was used to calculate four germination indices i.e. total germination (GT- percent viability of seeds germinated), speed of germination (S-how fast the seeds were germinated), accumulated speed of germination over time (AS) and coefficient rate of germination (CRG).

The data obtained was subjected to basic statistical analyses using microsoft excel software.

Fig. 1 shows seed germination in plastic containers with *Acacia tortilis* and *Prosopis cineraria*.

RESULTS AND DISCUSSION

Analyses of the soil used in seed germination experiment revealed native soil at ICBA is fine sand in texture, non-saline, moderately alkaline and strongly calcareous. Organic matter is very low (<0.5%) and the Munsell soil color-dry (Gretag Macbeth, 2000) is 10YR 6/4 pale brown, which is a composite reflection from the dominance of carbonates and sand, with insignificant contribution of organic matter to color composition. The high $CaCO_3$ (53%) can cause soil buffering capacity and affect nutrient availability to plants. Available water capacity is low (4-5%), suggesting careful water management plan to offset plant requirements and to avoid pressure on drainage system.

Treatment of compost with alkali and acidification of the alkaline extract revealed a total of 13.75% humate i.e. 8.7% fulvic acid and 5.05% of humic acid. These results suggest composts to be a good source of microbial synthesized humate. Binner *et al.*, (2011) reported a broad range (2.5 – 47%) of humate in composts and attributed this variation to the chemistry of feedstock and the composting conditions. The humate in composts result from the synthetic and transformational activities of microorganisms, particularly fungi (Felbeck, 1965; Haider and Martin, 1967). Large varieties of fungi are reported to synthesize phenolic compounds from simple as well as complex carbonaceous materials followed by polymerization into complex and high molecular weight compounds (Martin and Haider, 1971; Haider *et al.*, 1977).

Data presented in Table 1 shows soaking of seeds in fulvic acid solution improved seed germination (at 1.0% fulvic acid) which is recorded as 20% (*Acacia tortilis*) and 27% (*Prosopos cineraria*) increase over control.

Positive effect of both the humic acid and fulvic acid on the efficiency, pace of seed germination and overall plant growth has been reported in earlier studies (Katkat *et al.*, 2009; Azam and Malik, 1983; Malik and Azam, 1984; Smidova, 1962; Reynolds *et al.*, 1995).

The improvement in seed germination is attributed to enhance permeability of the seed coat, the cell membranes and thus moisture uptake necessary for activating the metabolic activities (Merlol *et al.*, 1991). Humic compounds are also reported to affect enzymes involved in cell division and cellular enlargement (Piccolo *et al.*, 1993).

Results (Table 2 and Fig. 2) show that the application of fulvic acid at both concentrations (0.5 and 1.0%) has significantly stimulated the seedling growth in both plant types, however, seedling growth was better at 1% fulvic acid application over other treatments (0.5% fulvic acid and control). Maximum root length of 12.27 cm and 12.53 in *Acacia* and *Prosopis* was recorded (at 0.1% fulvic acid) against 0.87 cm and

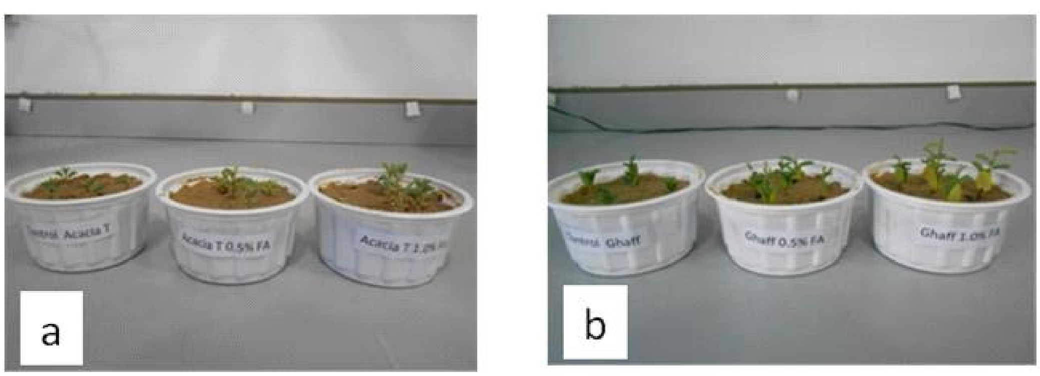

Fig. 1: Seed germination in plastic containers, a) *Acacia tortilis*, b) *Prosopis cineraria*. Plastic containers from left to right, control, 0.5% fulvic acid, and 1.0% fulvic acid

Table 1: Effect of various concentrations of fulvic acid (0.5 to 1.0%) on the germination of *Acacia tortalis* and *Prosopis cineraria* seeds

Treatments	Germination(%)	Speed of germination	Accumulated speed of germination (AS)	Coefficient of rate of germination
		Acacia tortalis		
Control	73.3b	0.58c	1.61c	35.2c
Fulvic acid 0.5%	93.3a	0.81b	2.25b	40.9b
Fulvic acid 1.0%	93.3a	1.06a	2.72a	51.9a
		Prosopis cineraria		
Control	73.3c	0.53b	1.10c	30.2c
Fulvic acid 0.5%	93.3b	0.56b	1.61b	33.8b
Fulvic acid 1.0%	100.0a	1.19a	3.00a	52.7a

The values sharing a similar letter in a column for each plant species do not differ significantly at 5% probability level.

Table 2: Effects of fulvic acid on seedling growth of *Acacia tortalis* and *Prosopis cineraria*

	Length (cm)		No. of leaves
Treatment	Shoot	Root	
		Acacia tortalis	
Control	2.53c	0.87c	3.07c
Fulvic acid 0.5%	3.10b	11.20b	4.80b
Fulvic acid 1.0%	3.53a	12.27a	6.13a
		Prosopis cineraria	
Control	1.87c	5.27c	3.33c
Fulvic acid 0.5%	3.20b	8.93b	5.27b
Fulvic acid 1.0%	3.67a	12.53a	6.73a

The values sharing a similar letter in a column for each plant species do not differ significantly at 5% probability level.

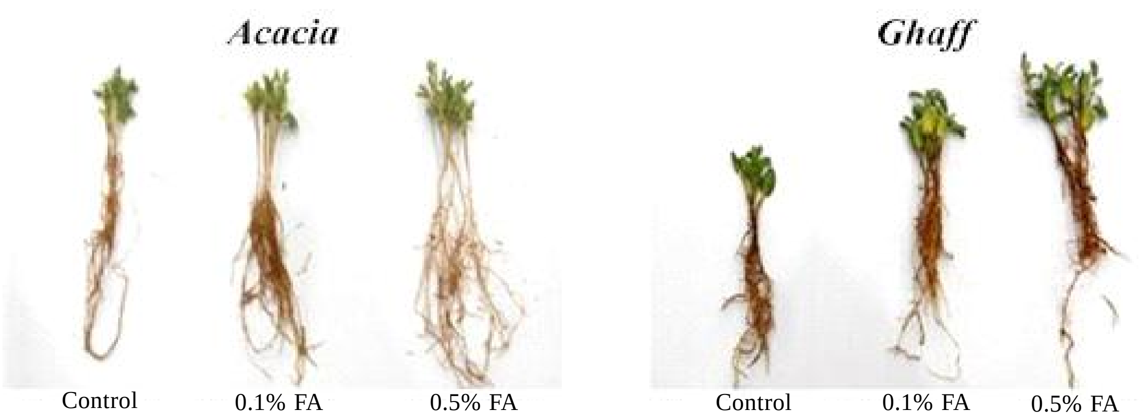

Fig. 2: Relative sizes and densities of *acacia* and *ghaff* grown with different treatments to seeds

5.27 cm in control treatment, respectively. Similar trend is observed in shoot length where 3.53 cm and 3.67 cm shoot length was recorded against 2.53 cm and 1.87 cm recorded in control treatment (water soaking) after 30 days of *Acacia* and *Prosopis*, respectively. The effect being statistically significant. A positive effect of compounds on plant growth is reported by Ferrara *et al.*, (2007). An increase in root growth after the application of humic compounds was reported by Arancon *et al.*, (2006). Fulvic acid also stimulated the emergence of leaves and the number increased significantly from 3.07 and 3.33 in respective controls to 6.13 and 6.73 (1% fulvic acid treatment) in *Acacia* and *Prosopis*, respectively. The treatment with 1% fulvic acid has shown better effect on plant growth relative to where 0.5% fulvic acid was applied and the control treatment (water soaking only). The statistical analyses revealed significant difference among various treatments. Fulvic acids are reported to increase the permeability of seed coat, plant cell membranes and enhance enzymatic activity of the root system leading to increased root proliferation (Trevisan *et al.*, 2010). It is visualized from the present study that the fulvic acids influences plants to grow stronger and healthier.

Table 3 clearly shows that fresh and dry biomass of both *Acacia* and *Prosopis* plants is significantly increased due to fulvic acid treatment of seeds; the positive effect was better at higher concentration (1% fulvic acid treatment). Positive effect of humic compounds and specifically fulvic acid fraction on plant growth has been reported in many studies (Adani *et al.*, 1998, Arancon *et al.*, 2006; Azam and Malik, 1983; Chen *et al.*, 2004; Katkat *et al.*, 2009; Merlol *et al.*, 1991; Patil, 2011; Rauthan and Schnitzer, 1981; Vaughan and Linehan, 2004; Reynold *et al.*, 1995; Trevisan *et al.*, 2010). The increase in root proliferation (Table 2) translates into enhanced seedling vigour in different crops (Piccolo *et al.*, 1993; Adani *et al.*, 1998). Some of these effects are also manifested in an increase or decrease in the activity of crucial enzymes (Ladd and Bulter, 1971; Malcolm and Vaughan, 1979a,b; Vaughan and Malcolm, 1979). In fact, humic compounds act more like plant growth regulators or hormones (Vaughan and Malcolm, 1985; Muscolo *et al.*, 1998, Nardi *et al.*, 2000).

CONCLUSION

Results of this preliminary study suggest that fulvic acid extracted from composts can be used as a seed soaking treatment for improving efficiency and pace of seed germination. This is particularly of significance for crops/trees with seeds that are hard to germinate and have a long dormancy period. Although sulfuric acid treatment has conventionally been used for breaking the dormancy and to soften the seed coat, fulvic acid will have added advantage as suggested by the results of this study. Based on the results it is concluded that the hypothesis is proved and hence fulvic acid can be used to improve seed germination and increase plant vigor. In view of the results obtained, it could also be suggested that use of composts in raising the seedlings may be beneficial. However, fulvic acid is only soluble in dilute alkali; therefore, it may be more beneficial to extract fulvic acid from composts prepared from locally available feedstock and prime the seeds to improve seed germination. With the improvement in seed germination, more plants can be produced in specified time; this will have significant impact on the pace of desert rehabilitation with plantation of *Prosopis cineraria* and *Acacia tortilis*.

Table 3: Effect of fulvic acid treatments on fresh and dry weight of shoot and root of *Acacia tortalis* and *Prosopis cineraria* seedlings

	Fresh weight (g)			Dry weight (g)		
Treatment	Root	Shoot	Total	Root	Shoot	Total
		Acacia tortalis				
Control	0.27b	0.38c		0.04c	0.09c	0.13c
Fulvic acid 0.5%	0.54a	0.62b		0.05b	0.16b	0.21b
Fulvic acid 1.0%	0.59a	0.93a		0.06a	0.19a	0.25a
		Prosopis cineraria				
Control	0.33c	0.85c		0.05c	0.16c	0.21c
Fulvic acid 0.5%	0.55b	0.92b		0.12b	0.24b	0.35b
Fulvic acid 1.0%	1.02a	1.27a		0.20a	0.30a	0.50a

The values sharing a similar letter in a column for each plant species do not differ significantly at 5% probability level.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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