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EXPLOITATION OF EM.1-TREATED BLEND OF ORGANIC RESOURCES AND HUMIC ACID FOR ORGANIC BERSEEM (*Trifolium alexandrinum* L.) PRODUCTION

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ABSTRACT

A field trial was conducted to evaluate blends of organic resources and humic acids, in order to enhance organic berseem forage production. The objective of this study was to compare an EM.1 (effective microorganisms)-treated blend of organic resources with an untreated blend of organic resources, and thereby evaluate the usefulness of humic acid as an organic fertilizer. The two types of blends,each with 4 application levels(0, 15, 30, and 45 t·ha^{-1}), and humic acid (4 application levels: 0, 15, 30, and 45 kg·ha^{-1}) were evaluated using a randomized complete block design with a split-plot arrangement (blend types were the main plot factor and humic acid a sub-plot factor). Except for the difference in EM.1 inoculation, both blends were prepared by co-composting cow manure, poultry manure, and kitchen waste (2:1:1 ratio by volume, respectively).

The differences among treatments with respect to fresh and dry yield, as well as mineral composition were recorded. The blend treated with effective microorganisms was found to be a more effective fertilizer than the untreated blend. Additionally, heavier application of both types of organic resource blends and humic acid enhanced both fresh and dry yields, and improved the mineral composition of the crop. These findings are valuable for organic growers and it may open a new avenue for researchers worldwide.

Key words: Effective microorganisms, Organic fertilizers, Composting, Mineral composition

INTRODUCTION

Organic farming practices are rapidly becoming more popular globally, because the chemicals used in conventional agricultural systems have led to increasing concerns about food quality, sustainability, and other environmental consequences. Conversely, organic agriculture holds promise for the production of high-quality food, increased sustainability, and better environmental protection (Gomiero et al., 2011). Because of the growing demand for organic dairy products from consumers in Saudi Arabia and many other parts of the world, farmers and agricultural companies are looking for a method of sustainable organic feed production. The most challenging difficulty for producers attempting to meet this demand is the lack of an appropriate organic fertilization plan, as the organic fertility plans suitable elsewhere, such as cover cropping and crop rotation, are not universally suitable. For example, in Saudi Arabia,it is not possible to grow more than a single crop per year, and organic fertilizers such as fish meal and bone meal are costly and not readily available. In Saudi Arabia and many other parts of the world, cow manure (CM), poultry manure (PM), and kitchen waste (KW) compost seems to be a good alternative choice for organic fertility management, because CM and PM are by-products of most farms, and KW is a waste product that is generally collected and piled outside cities as municipal solid waste. Furthermore, HA as an alternative source of organic amendment is compact, easy to ship, readily available, and its producers claim that 1 kg of HA provides as much fertilization benefit as 1 ton of manure (Humintech, 2012).

In this study, we treated plants with a blend of organic resources (BOR) and EM.1 (a group of co-existing microorganisms including lactic acid bacteria, yeast, and phototrophic bacteria) that is produced by EMRO (http://www.emrojapan.com),and is used for waste degradation and fermentation. The objective of this study was to increase the quantity and value of local organic fertilizer resources, because organic fertilizers are bulky and difficult to import. In accordance with the project's objectives, an attempt was made to utilize KW as some ratio of the BOR, because it is generally discarded as waste in Saudi Arabia. The BOR was treated with EM.1 to increasing its fertilizing potential. Additionally, this study explored the use of HA as an organic fertilizer, a purpose for which it is not generally used.

MATERIALS AND METHODS

Site and treatment of the experiment

A field trial was conducted from 2013 to 15 at the agriculture research station of King Abdulaziz University in the Hada-A'Sham area of Jeddah. In each growing season, a new section of the study field with the soil initial properties shown in Table 1 was used. Climatic conditions of the experimental site are shown in Fig. 1.

Table 1. Soil properties (0–30 cm) of the experimental site

Soil properties (0–30 cm)	2013	2014
pH	7.8	7.7
EC ($dS\cdot m^{-1}$)	2.2	2.3
Soil texture	Sandy Loam	Sandy Loam
Organic matter (%)	0.65	0.66
N (%)	0.09	0.10
P($mg\cdot kg^{-1}$)	124	140
K ($mg\cdot kg^{-1}$)	184	178
Ca ($mg\cdot kg^{-1}$)	3108	3111
Mg ($mg\cdot kg^{-1}$)	121	144
Fe ($mg\cdot kg^{-1}$)	64.0	62.2
Cu($mg\cdot kg^{-1}$)	1.20	1.55
Zn ($mg\cdot kg^{-1}$)	2.16	2.28
Mn ($mg\cdot kg^{-1}$)	6.98	7.55

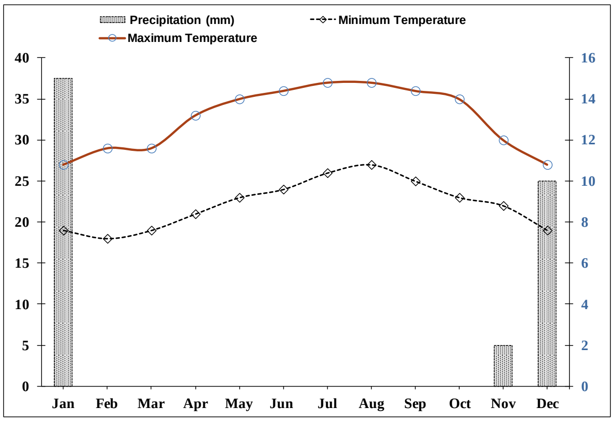

Fig.1. Climatic conditions of the experimentalsite during 2013-15 (Hada-Al Sham, Jeddah-Saudi Arabia)

The experiment consisted of treating plots with two types of BOR (BOR_{EM} and BOR_{plain}), each with 4 levels: 0, 15, 30, and 45 $t\cdot ha^{-1}$) in combination with humic acid (HA) (4 levels: 0, 15, 30, and 45 $kg\cdot ha^{-1}$). Both BOR types were evaluated using a randomized complete block design with a split-plot arrangement (BOR as the main plot factor and HA as a sub-plot factor).

Preparation of BOR

BOR was prepared by composting cow manure (CM), poultry manure (PM), and kitchen waste (KW), in a ratio of 2:1:1 by volume for 3 months: one was without the addition of EM-1® to prepare the BOR_{plain}, and other with the addition of EM-1® to prepare the BOR_{EM}. EM-1® was provided by the EMRO (http://www.emrojapan.com) Saudi partner, Rashed Establishment for Trading and Agriculture (http://www.rashedagri.com.sa). The BOR_{EM} was produced by activating theEM-1® by adding a mixture of 1 part molasses and 20 parts water, and apply it to the BOR after 1 week, at a rate of 1 $L\cdot t^{-1}$ BOR.

Agronomic practices and data collection

The organic fertilizers (BOR and HA) were applied to the experimental field every year, two weeks prior to sowing. The crop was sown each year in the first week of October, and the 1st harvest was carried out 45 days after sowing (DAS), while the subsequent 3 harvests were carried out at 1 month intervals. All other agronomic practices were carried out uniformly in all treatments.

To assess the effects of the treatments on the crop production, data on crop yield and mineral composition were compiled. Fresh forage and dry matter yields were determined according to the method described by Daur and Tatar (2013). Subsequently, the dry matter samples were analyzed for their mineral composition.

Mineral Analysis

Each year, the oven-dried samples from all cuts were combined separately according to treatments structure and powdered. The powdered samples were used for mineral analysis. Nitrogen content was determined according to Bremner (1996), using the Kjeldahl method. Phosphorus was determined colorimetrically following protocol described by Ryan et al. (2001), and all other elements were determined according to Bakhashwain et al. (2013), using Varian ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy).

Statistical Analysis

Data were analyzed using MSTATC software, following the procedure for RCBD with a split-plot arrangement, and a climatic graph was drawn using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Fresh (Table 2) and dry matter (Table 3) yields both varied significantly ($p < 0.05$) among all treatments. However, their interactions were non-significant. Yield increased significantly with BOR (BOR_{plain} or BOR_{EM}) and HA treatment levels. Previous studies support our results, which indicate that organic manure has beneficial effects on the physical and chemical properties of soil, and supplies plants with important nutrients (Abusuwar and Daur, 2014; Ali et al., 2014, Ouni et al., 2014a). Moreover, humic acids improve soil structure and soil fertility, the metabolisms of soil microorganisms, and nutrient uptake. In addition, it enhances the water-holding capacity of the soil, leading to exceptional plant growth and micronutrient uptake (Ouni et al., 2014b; Plaza et al., 2015).

Table 2. Fresh yield ($t \cdot ha^{-1}$) of berseem under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	15	30	45	**$Means_{BOR}$**
		Interaction (BOR x Humic acid)				
0	**BOR_{plain}**	16.80	18.32	19.60	20.00	**18.68^{d}**
	BOR_{EM}	16.88	18.32	19.92	20.16	**18.82^{cd}**
15	**BOR_{plain}**	19.64	19.20	19.76	20.40	**19.75^{c}**
	BOR_{EM}	20.44	20.96	20.40	21.20	**20.75^{b}**
30	**BOR_{plain}**	20.80	20.88	20.72	21.68	**21.02^{ab}**
	BOR_{EM}	20.88	20.72	20.80	21.68	**21.02^{ab}**
45	**BOR_{plain}**	21.60	21.68	22.16	22.32	**21.94^{ab}**
	BOR_{EM}	21.60	21.68	22.00	22.72	**22.00^{a}**
$Means_{HA}$		**19.83^{c}**	**20.22^{b}**	**20.67^{bc}**	**21.27^{a}**	

ANOVA Results: BOR = Significant p< 0.05; Humic acid Levels = Significant p< 0.05; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR_{plain} = BOR without EM.1; BOR_{EM} = BOR with EM.1; HA = Humic acid

Table 3. Dry matter yield ($t \cdot ha^{-1}$) of berseem under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	15	30	45	**$Means_{BOR}$**
		Interaction (BOR x Humic acid)				
0	**BOR_{plain}**	5.50	6.05	6.46	6.60	**6.15^{d}**
	BOR_{EM}	5.54	6.07	6.57	6.65	**6.21^{cd}**
15	**BOR_{plain}**	6.56	6.36	6.52	6.74	**6.55^{c}**
	BOR_{EM}	6.76	6.64	6.73	7.00	**6.78^{bc}**
30	**BOR_{plain}**	6.86	6.80	6.80	7.10	**6.89^{bc}**
	BOR_{EM}	6.89	6.84	6.86	7.18	**6.94^{b}**
45	**BOR_{plain}**	6.94	7.10	7.26	7.46	**7.19^{ab}**
	BOR_{EM}	7.15	7.15	7.28	7.66	**7.31^{a}**
$Means_{HA}$		**6.53^{c}**	**6.63^{b}**	**6.81^{ab}**	**7.05^{a}**	

ANOVA Results: BOR = Significant p < 0.05; Humic acid Levels = Significant p < 0.05; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR_{plain} = BOR without EM.1; BOR_{EM} = BOR with EM.1; HA = Humic acid

Table 4 illustrates the statistically significant ($p < 0.05$) differences among all BOR and humic acid (HA) levels on soil nitrogen (N) composition. Our findings are on par with those of Tahir et al. (2011) and Antoniadis et al. (2015), which indicate that nitrogen (N), phosphorus (P), and potassium (K) uptake increased in response to augmentation of organic material and humic acid. However, the interaction between BOR and HA appeared to have a non-significant ($p < 0.05$) effect on N composition. N composition increased with increasing levels of BOR and humic acid, but plant N content was higher when plants were treated with BOR_{EM} than when plants were treated with BOR_{plain}. This supports the claim that EM.1 inoculation improves organic matter composting without nitrogen loss as in other methods of composting occurs (Himanen and Hänninen, 2009). Findings related to the function of EM.1 in composting have not been previously reported, because EM.1 has generally been used only for either waste degradation or fermentation. However, previous studies confirm that microorganisms play a primary role during the composting of organic material, with the aid of a multi-enzyme system. EM.1 includes anaerobic bacteria and some other microorganisms that produce unique multi-enzyme complexes, called cellulosomes. These multi-enzyme complexes are responsible for the rapid degradation of organic materials (Schwarz, 2001; Kato et al. 2004; Wilson, 2011)

The differences in phosphorus (P) content (Table 5) were statistically non-significant ($p < 0.05$) among all BOR and HA treatments. However, Cimrin and Yilmaz, (2006) reported that humic acid improves phosphorus availability. This could be because adequate phosphorus content was already present in the soil. The differences in the potassium (K) content of berseem were also non-significant ($p < 0.05$) among BOR levels, but varied significantly ($p < 0.05$) among the humic acid levels (Table 6). Our results are comparable with those of Nikbakht et al. (2008), where the nitrogen (N), potassium (K), and magnesium (Mg) composition of plants were significantly improved with the application of higher levels of HA.

Table 4. Nitrogen composition (mg·g^{-1}) of berseem dry biomass under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	15	30	45	Means$_{BOR}$
		Interaction (BOR x Humic acid)				
0	BOR$_{plain}$	31.0	36.2	38.1	40.0	**36.3^{c}**
	BOR$_{EM}$	31.2	36.6	38.5	40.3	**36.7^{bc}**
15	BOR$_{plain}$	38.1	38.0	40.1	40.0	**39.1^{b}**
	BOR$_{EM}$	39.2	40.3	40.4	40.4	**40.1^{b}**
30	BOR$_{plain}$	39.4	40.4	40.2	40.4	**40.1^{b}**
	BOR$_{EM}$	41.3	42.3	42.3	42.3	**42.1^{ab}**
45	BOR$_{plain}$	41.3	41.2	41.4	41.6	**41.4^{ab}**
	BOR$_{EM}$	42.2	43.3	43.2	43.2	**43.0^{a}**
Means$_{HA}$		**38.0^{b}**	**39.8^{ab}**	**40.5^{ab}**	**41.0^{a}**	

ANOVA Results: BOR = Significant $p < 0.05$; Humic acid Levels = Significant $p < 0.05$; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR$_{plain}$ = BOR without EM.1; BOR$_{EM}$ = BOR with EM.1; HA = Humic acid

Table 5. Phosphorus composition (mg·g^{-1}) of berseem dry biomass under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	15	30	45	Means$_{BOR}$
		Interaction (BOR x Humic acid)				
0	BOR$_{plain}$	3.3	3.6	3.4	3.3	**3.4**
	BOR$_{EM}$	4.0	3.4	3.4	3.8	**3.7**
15	BOR$_{plain}$	3.4	3.5	3.6	3.3	**3.5**
	BOR$_{EM}$	3.8	3.3	3.3	3.4	**3.5**
30	BOR$_{plain}$	3.4	3.5	3.4	3.5	**3.5**
	BOR$_{EM}$	3.6	3.6	3.4	3.4	**3.5**
45	BOR$_{plain}$	3.3	3.3	3.6	3.3	**3.4**
	BOR$_{EM}$	4.0	3.4	3.3	3.6	**3.6**
Means$_{HA}$		**3.6**	**3.5**	**3.4**	**3.5**	

ANOVA Results: BOR = non-significant; Humic acid Levels = non-significant; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR$_{plain}$ = BOR without EM.1; BOR$_{EM}$ = BOR with EM.1; HA = Humic acid

Table 6. Potassium composition (mg·g^{-1}) of berseem dry biomass under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	15	30	45	Means$_{BOR}$
		Interaction (BOR x Humic acid)				
0	BOR$_{plain}$	2.2	2.5	2.4	2.7	**2.5**
	BOR$_{EM}$	2.2	2.7	2.8	2.7	**2.6**
15	BOR$_{plain}$	2.2	2.5	2.7	2.7	**2.5**
	BOR$_{EM}$	2.2	2.5	2.6	2.8	**2.5**
30	BOR$_{plain}$	2.4	2.4	2.7	2.7	**2.6**
	BOR$_{EM}$	2.4	2.5	2.6	2.5	**2.5**
45	BOR$_{plain}$	2.4	2.4	2.6	2.8	**2.6**
	BOR$_{EM}$	2.6	2.6	2.8	2.8	**2.7**
Means$_{HA}$		**2.3^{a}**	**2.5^{ab}**	**2.7^{b}**	**2.7^{b}**	

ANOVA Results: BOR = non-significant; Humic acid Levels = Significant $p < 0.05$; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR$_{plain}$ = BOR without EM.1; BOR$_{EM}$ = BOR with EM.1; HA = Humic acid

Calcium (Ca) content was statistically similar in all BOR and HA levels (Table 7). The probable reason for this is that the soil in Saudi Arabia has high calcium content, and thus it is not a limiting factor (Al-Juhaimi et al., 2014). Magnesium (Mg) content (Table 8) varied significantly ($p < 0.05$) across humic acid levels. This could be because humic acid itself contains reasonably high concentrations of Mg. The results of Nikbakht et al.(2008) and Ananthi and Vanangamudi (2014) support our study results that HA improves Mg uptake.

Table 7. Calcium composition ($mg \cdot g^{-1}$) of berseem dry biomass under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	**15**	**30**	**45**	**Means$_{BOR}$**
		Interaction (BOR x Humic acid)				
0	**BOR$_{plain}$**	1.7	1.9	1.9	1.8	**1.8**
	BOR$_{EM}$	1.6	1.6	1.7	1.7	**1.7**
15	**BOR$_{plain}$**	1.7	1.6	1.7	1.6	**1.7**
	BOR$_{EM}$	1.7	1.7	1.7	1.6	**1.7**
30	**BOR$_{plain}$**	1.9	1.9	1.8	1.7	**1.8**
	BOR$_{EM}$	1.7	1.7	1.7	1.7	**1.7**
45	**BOR$_{plain}$**	1.9	1.7	1.8	1.9	**1.8**
	BOR$_{EM}$	1.7	1.9	1.8	1.7	**1.8**
Means$_{HA}$		**1.7**	**1.8**	**1.8**	**1.7**	

ANOVA Results: BOR = non-significant; Humic acid Levels = non-significant; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR$_{plain}$ = BOR without EM.1; BOR$_{EM}$ = BOR with EM.1; HA = Humic acid

Table 8. Magnesium composition ($mg \cdot g^{-1}$) of berseem dry biomass under different levels and types of BOR, integrated with different levels of humic acid.

BOR Levels/BOR Types		Humic Acid Levels				
		0	**15**	**30**	**45**	**Means$_{BOR}$**
		Interaction (BOR x Humic acid)				
0	**BOR$_{plain}$**	2.0	2.3	2.4	2.4	**2.3**
	BOR$_{EM}$	2.1	2.3	2.3	2.4	**2.3**
15	**BOR$_{plain}$**	2.1	2.2	2.2	2.5	**2.3**
	BOR$_{EM}$	2.2	2.2	2.4	2.5	**2.3**
30	**BOR$_{plain}$**	2.2	2.3	2.4	2.4	**2.3**
	BOR$_{EM}$	2.2	2.3	2.4	2.5	**2.4**
45	**BOR$_{plain}$**	2.2	2.3	2.4	2.4	**2.3**
	BOR$_{EM}$	2.2	2.3	2.4	2.5	**2.4**
Means$_{HA}$		**2.2^{a}**	**2.3^{ab}**	**2.4^{ab}**	**2.5^{b}**	

ANOVA Results: BOR = non-significant; Humic acid Levels = Significant $p < 0.05$; BOR x Humic acid = non-significant

Mean values with different superscript letters differ significantly ($p < 0.05$)
BOR = Blend of Organic resources; BOR$_{plain}$ = BOR without EM.1; BOR$_{EM}$ = BOR with EM.1; HA = Humic acid

CONCLUSION AND RECOMMENDATION

In conclusion, EM.1 inoculation increases the fertilizer value of compost. Additionally, this study confirmed that increasing levels of organic manure and HA enhance nutrient uptake and increase crop yield. Further study is recommended, in order to investigate the effects of EM.1 and HA on biochemical reactions and the ultra-structures of cells.

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