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Full Length Research Paper

Influence of effective microorganisms and green manure on soil properties and productivity of pearl millet and alfalfa grown on sandy loam in Saudi Arabia

Saleh M. Ismail[1,2]

[1]Arid Land Agriculture Department, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Soil and Water Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.
E-mail: smibrahim@kau.edu.sa or smii2001@gmail.com. Tel: +966-596-068-380.

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A field experiment was conducted to study the effect of effective microorganisms and green manure on soil properties and yield of pearl millet (*Pennisetum glaucum* (L.) R. Br.) and alfalfa (*Medicago sativa* L.) grown on sandy loam soil under arid conditions at Agriculture Experimental Station of King Abdulaziz University (KAU), Hada Alsham, Saudi Arabia. Four treatments were investigated. In control (T1), the soil was only treated with the recommended dose of mineral fertilizers required for the cultivated crops. The second treatment (T2) was a diluted solution (1:1000) of effective microorganisms (EM1) sprayed on the soil surface. The third treatment (T3) was green manure added as soil mulch above the ground while the fourth treatment (T4) was mixture of EM1 and green manure together. Results indicated that, EM1 and green manure improved forage yield and soil properties. The best improvement in yield was found in T4 and T3 of alfalfa and pearl millet fields, respectively. These treatments resulted also in the great reduction in soil bulk density and saturated hydraulic conductivity, high water retention and soil organic matter. Total nitrogen, phosphorus, potassium, iron and magnesium were also increased under these treatments. Little enhancement in physical and chemical properties of soil was pronounced in T2 treatment since the improvements were not significant compared with the control. The results suggested that using green manure alone or mixed with EM1 is a practical method to enhance soil properties and productivity of coarse texture soils under dry land conditions.

Key words: Effective microorganisms, pearl millet, green manure, alfalfa.

INTRODUCTION

Soil properties play an important role in crop production and water use efficiency especially in light texture soils of arid regions. Enhancing physical and fertility properties of sandy soil will contribute to fight against hunger by increasing crop production by adding new reclaimed soil to cultivated areas. One of the best methods to enhance soil properties is using biofertilizers or green manures. One of the world's most commonly used biofertilizers is effective microorganisms (EM), (Higa, 1991; Mickan and Muller, 2008). EM is commercially available with the name of EM1. It is suspension contains "a selection of groups of microorganisms, in particular, lactic acid bacteria, yeast, photosynthetic bacteria". All of these are mutually compatible with one another and can coexist in liquid culture. EM1 may improve the biological status of soils, increase soil organic matter, improve germination and root development, increase photosynthesis, and increase plant growth and yield (El-Shafei et al., 2008).

Crop production was highly affected by the application of green manure. Wheat yields were increased from 500 to 1400 kg ha^{-1} with increasing N from 81 to162 kg ha^{-1} by using green manure (Ladha, 1996). Farm yard manure (FYM) with a rate of 10 t ha^{-1} applied in combination with chemical amendments enhanced rice and wheat yields in

sodic soil (Hussain et al., 2001). Use of legume green manures had a significant positive effect on wheat yield, (Huxham et al., 2005). Narayan and Lai (2006) studied the effect of green manure of several soil depths on the growth characteristics and yield of sunnhemp (*Crotolaria juncea*). They found that, the growth in terms of plant height, root length, number of nodules/plant and biomass yield increased under increasing soil depth. Effect of green manure was distinct on productivity of wheat in all investigated depths. The highest grain was recorded under green manuring of sunnhemp in deep soil depth.

Physical properties like bulk density, porosity, void ratio, water permeability and hydraulic conductivity significantly improved when FYM (10 t ha^{-1}) applied in combination with chemical amendments. Other organic materials like rice straw, wheat straw, rice husk and chopped salt grass also improved the physical properties of a saline sodic soil, (Hussain et al., 2001). Increasing organic matter in soil as a result of green manure improved soil structure, increased aggregates stability, enhance infiltration rate, led to higher water-holding capacities and increased water use efficiency (Tanaka et al., 2005; Narayan and Lai, 2006). Using legumes green manure and crop rotation increased the level of Nitrogen, Phosphorus, and potassium in soil especially Nitrogen. Moreover, crop nutrient use efficiency was improved by green manure application. Legumes are superior green manure crops as they fix atmospheric nitrogen and add it to the soil nitrogen pool. That nitrogen is released gradually with long-term decomposition of organic matter, thus decreasing the risk of leaching nutrients and the need for nitrogen fertilizer application in subsequent seasons (Leinonen, 2000; Mayer et al., 2003; N'Dayegamiye and Tran, 2012).

Green manure and animal waste increase absorption of non-solution phosphorus in soil and increases phosphorus absorption from phosphorus fertilizer. Sometimes green manure alone do not provide enough essential nutrients for maximizing crop yield, so one of the best strategies is using green manure with a blend of chemical fertilizers. This mixture reduces the application of mineral fertilizer and decreases environment pollution (Fageria, 2007). Barley manure increased available potassium in the soil. Increasing soil organic carbon by green manure application increases microbial numbers and activity, which help to releases many essential nutrients for plants, (Carlsson and Huss-Danell, 2003; Bremer et al., 2008). The effects of EM and green manure on the yield of subsequent seasons were investigated by many researchers but their effects on growth yield directly after addition has not been studied. Therefore, the main objectives of this study were to investigate single and multiple effects of EM and different types of green manure on:

1. Forage production of pearl millets and alfalfa in subsequent cuts directly after application in the same growing season;

2. The change in physical and fertility properties after one year of EM and green manure application.

MATERIALS AND METHODS

Location and experimental design

The experiment was carried-out at Agriculture Experimental Station of King Abdulaziz University (KAU) located at Hada Alsham village (21° 79' N, 39° 70' E and Altitude of 226 m), 110 km north east of Jeddah, KSA. The Climate of the area is arid with high temperatures during summer season. The soil texture is sandy loam. Two forage crops, alfalfa and pearl millet were cultivated in this experiment. Alfalfa was sown manually in one half of the experimental site using seed rate of 60 kg ha^{-1}. The crop was sown on December 2009 and continues until December, 2010. Thirteen cuts were obtained during the period of the experiment. Pearl millet was grown twice in the second half of the area. One cultivated in December, 2009 to April, 2010 and the other was through May to October. 2010. Pearl millet was sown manually in rows with 20 cm apart in a rate of 4 seeds for each hill with a distance of 20 cm between each consecutive hill. Two and three cuts were harvested from the first and second growing period respectively. The experimental field was subjected to sprinkler irrigation system during the whole growing seasons.

Treatments

Four treatments were investigated in the current study. The soil was only treated with the recommended dose of mineral fertilizers required for the used crops. In the second treatment (T2), suspension of stock cultures of effective microorganisms (EM1) was diluted 1:1000 with irrigation water and applied four times at a rate of 50 m^3 ha^{-1} sprayed on the soil surface directly after second, third, fourth and fifth cuts of alfalfa. However, EM1 was added three times with the same previous rate for pearl millet directly after first and second cuts of the first growing period and after the first cut of the second growing period. The third treatment (T3) was green manure from alfalfa for alfalfa field and from pearl millet for pearl millet field. In the proper age, the plants were cut, then left on surface as mulch with a rate of 7.5 ton/ha for each time. The distribution was exactly as explain in the second treatment. The fourth treatment (T4) includes EM1 and green manure together added with the same rate and method explained in T2 and T3. The total applied green manure was 30 and 22.5 ton/ha for alfalfa and pearl millet respectively. The design of the experiment was complete randomized plot (6 m^2 size) with 4 replications. The investigated soil properties were measured before cultivation and after one year from the starting of the experiment for both fields. At the end of the experiment, obtained data were statistically analyzed using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by the least significant differences (LSD) at P = 5%.

Measured parameters

Total fresh and dry yield

Fresh and dry yield was measured by cutting one square meter from the center of the plot of each replicate for each crop during each cut and the fresh yield was determined, then yield per hectare was calculated for each cut. Sub-samples were drown into paper bags and kept in a forced air oven at 80°C for 72 h and then the dry yield per hectare was calculated. Total fresh and dry yields for the

Table 1. Effects of green manure and EM1 treatments on total fresh and dry yields of alfalfa and pearl millet (t/ha) during the whole growing season.

Treatments	Pearl millet		Alfalfa	
	Fresh	Dry	Fresh	Dry
T1 (Control)	170.8^{c}	27.2^{d}	109.2^{c}	29.7^{c}
T2 (EM1)	180.2^{c}	29.8^{c}	122.9^{b}	32.0^{b}
T3 (Green manure)	224.5^{a}	42.8^{a}	125.4^{b}	32.8^{b}
T4 (Green manure + EM1)	192.6^{b}	34.1^{b}	131.5^{a}	35.2^{a}
L.S.D.$_{05}$	10.3	1.89	4.56	1.18

Means with different superscripts differ significantly, $P < 0.05$).

whole growing season were calculated by summation of all cuts for each crop.

Physical properties

Soil core with length and diameter of 5 cm was used to collect undistributed soil samples from the upper 30 cm of soil surface. The samples were collected from each replicate for both crops before and after one year of cultivation for measuring physical parameters of soils as follows:

Soil organic matter (SOM): Determination of SOM involved the reduction of potassium dichromate ($K_2Cr_2O_7$) by organic carbon compounds and subsequent determination of the unreduced dichromate by oxidation-reduction titration with ferrous ammonium sulfate (Pansu and Gautheyrou, 2006).

Bulk density: Bulk density was measured by the core method in the undisturbed soil sample as explained in Blake and Hartge (1986).

Saturated hydraulic conductivity: The saturated hydraulic conductivity was measured with a constant head method and was calculated suing Darcy's formula (Klute, 1986).

$$Q/AT = -K (L+h/L) \quad (3)$$

Where:
K = saturated hydraulic conductivity (cm ha $^{-1}$)
Q = volume of water (cm^3) passed through the soil in time T (h)
L = length of soil column (cm)
h = total head (cm)
A = cross section area of the cylinder in cm.

Soil water retention: Soil water curves were determined by the pressure plate apparatus (Klute, 1986). Undisturbed soil samples were collected in steel rings 5 cm in diameter, then saturated for 24 h. The saturated samples were subjected to matric potentials of -0.0, -0.06, -0.1, - 0.33, -0.66, -1, -2, and -3 bars. After equilibrium the retained moisture was calculated for each sample in each treatment.

Fertility properties

Soil fertility parameters were determination of total Nitrogen, available phosphorus; potassium and micro-element include Fe, Mn, Cu and Zn.

Total nitrogen

Determining of total nitrogen involves the digestion and distillation steps according to the Kjeldahl method (Jackson, 1973) using Kjeltec auto 1030 analyzer.

Phosphorus

Available P, was determined as described in Olsen and Sommers (1982), the phosphorus was measured by ammonium molybdate colorimetric methods.

Potassium

Available K, was detemined as described in Carson (1980), and was measured using flame emission spectrophotometry.

Micro-nutrients

Available Zn, Mn, Cu and Fe were extracted using DTPA extraction method as described by Miller et al. (2000) and measured by ICP-AES method.

RESULTS

Total fresh and dry yields

Results of total fresh and dry yields of pearl millet and alfalfa are presented in Table 1. Results indicated that EM and green manure treatments significantly increased total fresh and dry yield for both crops. The highest fresh and dry yields of pearl millets crop obtained from T3 is followed by T4, T2 and T1, respectively. However, the differences in fresh yield between T2 and T1 were not significant. The highest fresh and dry yields of alfalfa crop were obtained from T4, followed by T3, T2 and T1, but the differences in fresh and dry yield between T3 and T2 were not significant.

Physical properties

Soil organic matter (SOM)

Result of soil organic matter before and after one year of

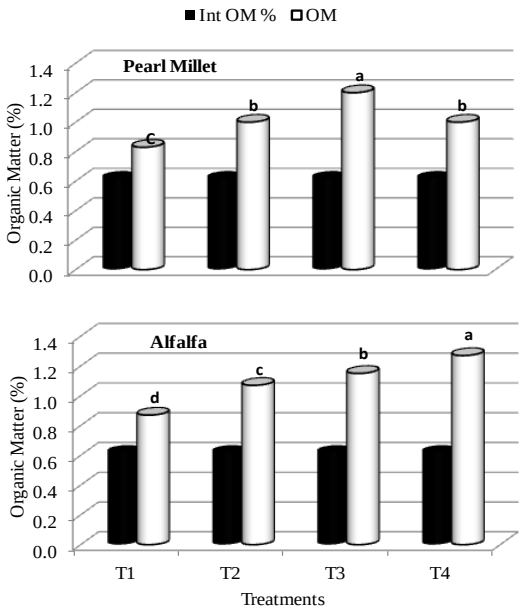

Figure 1. Effect of green manure and EM1 treatments on soil Organic matter (means with different superscripts differ significantly, P < 0.05).

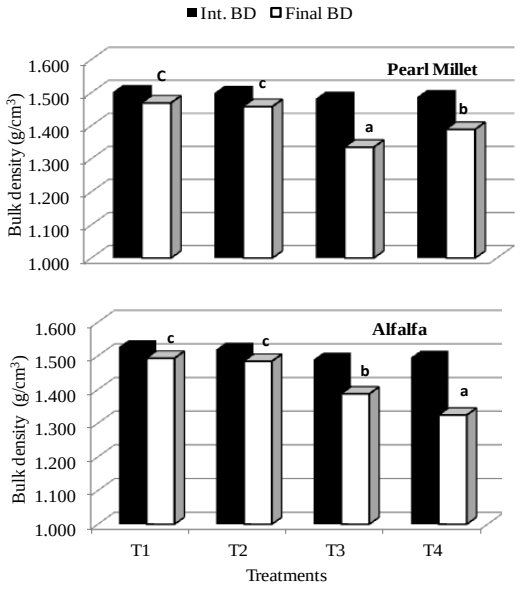

Figure 2. Effect of green manure and EM1 treatments on soil bulk density g/cm^3 (means with different superscripts differ significantly, P < 0.05).

cultivation is presented in Figure 1. The results indicated that, green manure and EM increased SOM after one year of cultivation compared with before cultivation in both fields. The highest SOM for pearl millet field obtained from T3 followed by T4 and T2 however, the differences between T4 and T2 were not significant. The least SOM was recorded in T1. Significant differences

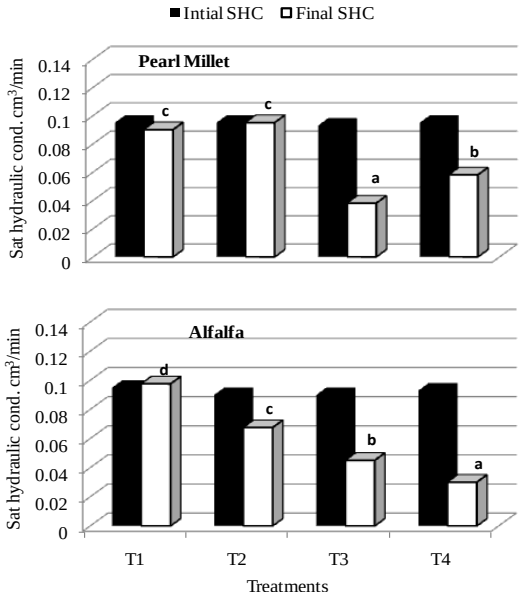

Figure 3. Effect of green manure and EM1 treatments on Saturated hydraulic conductivity (means with different superscripts differ significantly, P < 0.05).

were found among treatments in alfalfa field. The highest SOM obtained from T4 then gradually decreased in T3, T2 and T1, respectively.

Soil bulk density (BD)

Results of soil bulk density are presented in Figure 2. Soil BD was decreased by using green manure and EM application. The reduction was significant only in T3 and T4 but was not significant in T2 and T1 for both fields. The highest reduction in BD was obtained from T3 for pearl millet field followed by T4, T2 and T1, respectively. For alfalfa field, the least reduction obtained from T1 followed by gradual increase up to T4 where the highest reduction was obtained (Figure 2).

Saturated hydraulic conductivity and moisture retention

Results of saturated hydraulic conductivity are shown in Figure 3. Results revealed that, saturated hydraulic conductivity was significantly decreased as a result of green manure and EM application especially in alfalfa field. The least saturated hydraulic conductivity obtained from T3 of pearl millet field followed byT4. The differences between T2 and T1 were not significant. For alfalfa field, the least saturated hydraulic conductivity obtained from T4 and gradually increased up to T1.

Results of soil moisture retention for investigated treatments are presented in Figure 4. Results of soil

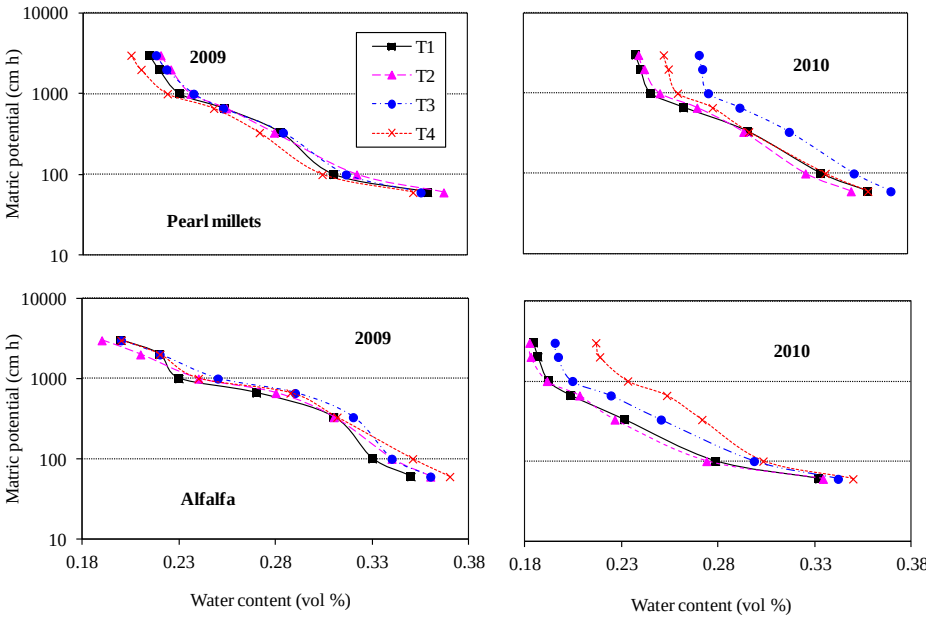

Figure 4. Effect of green manure and EM1 treatments on soil water tension for pearl millet and alfalfa fields before and after one year from cultivation.

Table 2. Effect of green manure and EM1 treatments on macro - nutrients of soil after one year of cultivation.

Cultivated crop	Treatment	Total nitrogen (ppm)	Available phosphorus (ppm)	Available potassium (ppm)
Pearl Millet	Before cultivation	613^{e}	30.1^{d}	197.6^{e}
	T1 (Control)	750^{d}	64.5^{c}	218.1^{d}
	T2 (EM1)	962b^{c}	69.9^{b}	223.8^{c}
	T3 (Green manure)	1663^{a}	83.8^{a}	297.5^{a}
	T4 (Green manure + EM1)	1313^{b}	80.8^{a}	261.9^{b}
	L.S.D.$_{.05}$	70	7.8	16.2
Alfalfa	Before cultivation	613^{c}	30.1^{c}	197.6^{d}
	T1 (Control)	1350^{b}	60.9^{b}	217.2^{c}
	T2 (EM1)	1425^{b}	78.8^{a}	245.3^{b}
	T3 (Green manure)	1855^{a}	82.8^{a}	289.1^{a}
	T4 (Green manure + EM1)	1990^{a}	85.9^{a}	293.5^{a}
	L.S.D.$_{.05}$	205	9.0	12.7

Means with different superscripts differ significantly, $P < 0.05$.

water retention before cultivation (2009, indicated that, no significant differences were recorded among treatments for both fields. However, significant differences were found in soil water retention among treatments after one year of cultivation (2010). Application of green manure and EM resulted in considerable increase in soil water retention. In pearl millet field, the highest retained water obtained from T3 followed by T4. The amount of retained water was almost similar in T1 and T2. Results of soil water retention for alfalfa field were almost similar as in pearl millet field however, the highest retained water obtained from T4 followed by T3.

Fertility properties

Macro-nutrients

The results of macro-nutrients include N-P-K are presented in Table 2. The results indicated that, all treatments include control significantly increased total Nitrogen, available phosphorus and potassium compared with those before cultivation for both fields. The highest total nitrogen, available phosphorus and potassium in pearl millet field obtained from T3 followed by T4, T2 and T1, respectively. In alfalfa field, the highest N, available P

Table 3. Effect of green manure and EM1 treatments on available micro- nutrients of soil after one year of cultivation.

Cultivated crop	Treatment	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
Pearl Millet	Before cultivation	0.40^{b}	7.58^{b}	14.3^{d}	13.3^{d}
	T1 (Control)	0.55^{ab}	8.47^{a}	15.5^{c}	16.4^{c}
	T2 (EM1)	0.56^{a}	8.85^{a}	16.3^{b}	22.5^{b}
	T3 (Green manure)	0.69^{a}	8.75^{a}	18.2^{a}	25.3^{a}
	T4 (Green manure + EM1)	0.63^{a}	8.68^{a}	18.4^{a}	24.8^{a}
	L.S.D.$_{.05}$	0.15	0.56	1.7	2.2
Alfalfa	Before cultivation	0.41^{b}	7.58^{b}	14.3^{e}	13.3^{c}
	T1 (Control)	0.58^{a}	8.72^{a}	15.8^{d}	17.7^{b}
	T2 (EM1)	0.59^{a}	8.72^{a}	16.7^{c}	20.8^{b}
	T3 (Green manure)	0.63^{a}	8.75^{a}	17.4^{b}	26.6^{a}
	T4 (Green manure + EM1)	0.71^{a}	8.42^{a}	19.0^{a}	29.1^{a}
	L.S.D.$_{.05}$	0.14	0.40	1.3	3.5

Means with different superscripts differ significantly, $P < 0.05$.

and K obtained from T4 followed by T3 however the differences between these treatments were not significant. Also, T2 significantly increased the availability of phosphorous, potassium and total nitrogen compared to T1.

Micro-nutrients

The results of available micro-nutrients include: Cu, Zn, Fe and Mn are presented in Table 3. Results revealed that all investigated treatments significantly increased the availability of micro-nutrients compared with those before cultivation in both field. Results also showed that EM and green manure treatments increased the availability of Cu and Zn in soil however the differences among them were not significant in both fields. The availability of Fe and Mn was significantly increased by the application of EM and green manure. The highest available concentrations of Fe and Mn under pearl millet field were found in T3 and T4 however, the differences between them were not significant, then followed by T2and T1, respectively. For alfalfa field, there was gradual significant increase in the availability of Fe. The highest available concentration of Fe obtained from T4 followed by T3, T2 and T1, respectively. Similar behavior was found in Mn however the differences between T4 and T3 were not significant.

DISCUSSION

Increasing yield of the treatments treated with EM1 might be due to two reasons. Firstly, the increase in number of nodulation as a result of the incensement in rhizobial population due to EM1 application especially in alfalfa field (Javaid et al., 2000). Secondly, the increase in nutrient availability in soil as a result of organic matter decompositions (Konoplya and Higa, 2001). In green manure or green manure mixed with EM1 treatments, the increase in crop yield for both crops could be due to the increase in organic matter contents. Increase organic matter enhanced soil physical properties such as, bulk density, infiltration rate, water holding capacity. Soil chemical properties has also main role in increasing crop yield due to the abundant availability of nutrients released from organic matter decomposition or due to the increase in nutrients solubility because of the presence of organic acids produced form organic matter decomposition. Similar results were reported by (Mandal et al., 2003; Sing et al., 2007; N'Dayegamiye and Tran, 2001).

Application of green manure and EM1 increased SOM. The increase in SOM in the treatments of T3 and T4 in both fields could be due to the fact that, the quantity of added green manure in these treatments is higher than that of T1 and T2. In pearl millet field T3 significantly increased SOM compared to other treatments. The results might be due to low quality of added green manure. Application of low quality green manure (high in lignin or poly phenolics and low in N) like pearl millet may increase soil organic carbon and consequentially SOM (Sanchez, 1989). The decrease in SOM of T4 compared with T3 could be attributed to the fact that adding EM1 to the green manure increased the decomposition activity. Microorganisms in EM1 decomposed green manure in order to get their requirement of N, which was low in pear millet manure. Decomposition of green manure led to decrease the accumulation of organic matter in soil. In alfalfa field the highest SOM found in T4. The results could be due to the fact that, alfalfa green manure is rich in N content as well as the growing alfalfa fix Nitrogen from air via Rhizobium bacteria. As a result, the

microorganisms presented in EM1 may obtain their N requirement from the penalty of N presented in soil. That led to increase SOM accumulation because of the slow decomposition of alfalfa green manure. Similar results were reported by (N'Dayegamiye and Tran, 2012).

The significant decrease in soil bulk density due to the application of EM1 and green manure is probably due to the greater amount of organic matter deposition. Increasing the deposition of OM influence soil structural properties by enmeshing soil primarily particles and micro-aggregates into macro-aggregates. Constituting of macro-aggregates decreased active pore space in soil. The direct effect of decreasing active pores is a reduction in bulk density (Singh et al., 2007; Sultani et al., 2007). Increasing organic matter in soils encouraged granulation, then reduced buck density. The reduction in bulk density is met by a reduction in large pore space which led to a gradual reduction in saturated hydraulic conductivity. The reduction in saturated hydraulic conductivity in coarse textured soil treated with organic matter could be also attributed to the reduction in effective mean pore radius as a result of the soil expansion (Mustafa et al., 1988; Al-Darby et al., 1992; Ismail, 1996; Brandsma et al., 1999).

Application of green manure and EM1 in both fields increased the retained water in treated soils. In fact, the obtained results of SOM, BD and saturated hydraulic conductivity may explain the results of soil water retention. Increasing the soil moisture retention capacity might be due to the enhancement in physical parameters of soil including the increasing of SOM, deceasing of bulk density and saturated hydraulic conductivity, (Yönter and Yağmur, 2011; Egodawatta et al., 2012). Total N and the availability of P, K, Fe and Mn in peral millet and alfalfa fields were increased by green manure application. The increase of these nutrients might be due to the decomposition of applied green manure. The significant differences among treatments could be due to the type and quantity of green manure as well as the additive of EM1. The highest N, P and k under pearl millet field (T3 and T4) in pear millet field coud be due be due the type of applied green manure. The green manure from pearl millet characterized by high C: N ratio, high lignin and high polyphenol contents. As a result, it decomposes and release nutrients slowly. Such type of green manure has a low direct nutrient effect and a high indirect mulching effect on crops (Tian et al., 1992; Achakzai and Bangulzai, 2006). Presence of EM1 with green manure (T4) increased the rate of decomposition and release large amount of nutrients. In spite of the greater release of nutrients their concentrations were less than that in T3. That might be due to the participation of plants and microorganisms in consuming the released nutrients. Total nitrogen in alfalfa field was higher than that in pearl millet field. The increase could be due to the nitrogen fixation by Rhizobium bacteria. Alfalfa green manure is characterized by low C:N ratio, lignin and polyphenol contents so it decomposes rapidly, and increase nutrient availability and concentration (Sangakkara and Weerasekera, 2001).

The increase in available P in soil for both fields could be due to the accelerated mineralization of P by the organic acids resulted from the decomposition of organic manure (Egodawatta et al., 2012). Incorporation of green manure with soil showed a tendency of immobilizing available P (Somado et al., 2007). Green manure increase the concentration of K, Mn, Fe, either by increasing their solubility from soil insoluble mineral fraction or releasing these nutrients from the decomposition of organic matter (Kapland and Estes, 1985; Mosavi et al., 2009). Karami et al. (2012) found that, soil Mn concentration was increased when applying different organic matter sources. Easily decomposable residue may add substantial amounts of Fe, Mn, Cu, and P nutrients to the growing plants by increasing their availability (Walters et al., 1992).

Conclusion

Results of this study revealed that, EM1 and green manure treatments improved forage yield and soils properties. The best enhancement found in the treatments of green manure with and without EM1 in both fields. These treatments resulted in the highest yield, the greatest reduction in bulk density and saturated hydraulic conductivity, and the highest water retention and soil organic matter. Moreover, total nitrogen, available P, K, Fe and Mn were also increased under these treatments. Little improvement in physical and chemical propertied of soil was pronounced in EM1 treatment however the improvements were not significant compared with the control. In conclusion, green manure or green manure mixed with EM1 is recommended with almost equal benefits to use in coarse textured soil to improve soil properties and consequently yields especially under dry land conditions.

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