INTRODUCTION

Bambara nut (Vigna subterranea L. Verde.) is a leguminous seed crop of African origin (Nwanna et al., 2005), that is highly utilized and been found to have a high nutritious value and drought tolerance (Anchirirah et al., 2001 and Ocran et al., 1998). It is considered to be a famine culture crop probably because it associates with mycorrhiza. Synergy between mycorrhizal fungi and rhizobia micro symbionts (nitrogen fixers) in legumes has been studied by (Jesus et al., 2005; Kaschuk et al., 2010) and their association described as a tripartite (Vega et al., 2010), where the Mycorrhizal help to increase the absorption and solubilisation of phosphorus to rhizobia in plant nodules (Scotti, 1997), while Rhizobia fix nitrogen provide it as ammonia to the plant, which provides carbohydrate to microsymbionts (Silveira et al., 2001; Gross et al., 2004). The benefit of these microorganisms to the host plant depends on the compatibility between the rhizobial strain and mycorrhizal fungi inoculated. Positive symbiosis formed with mycorrhizal fungi (Frey-Klett et al., 2007), when found to be synergistically effective they are called “mycorrhiza helper bacteria” (MHB) (Garbaye; 1994). Fungal-rhizobial inoculant has been able to increase N2 fixation in soybean by 30% as compared to conventional use of rhizobia (inoculant) (Jayasinghearachchi et al., 2010) and their association described as a tripartite (Vega et al., 2010), when found to be synergistically effective act as “mycorrhiza helper bacteria” (MHB) when both were co-inoculated in Bambara plant.

MATERIALS AND METHODS

Preparation of broth for inoculation

Pure cultures of the rhizobia isolates was obtained and inoculated into 100ml Erlenmeyer flasks containing 50 ml of yeast-mannitol. The inoculated broth was incubated at 28°C on a Rotary shaker for 7 days after which the bacterial count was determined to be about 10^{9} cfu/ml. Further treatment was then applied and inoculated to RM plants plants 1 week of growth (Woomer et al., 2012).

Pot Experiment

Sea sand was washed repeatedly with water to remove debris and to reduce pH to between 6.6 - 6.8 which is most suitable for rhizobia growth. The crushed gravel and medium sized gravel were also washed till the water was clean. The sea sand, crushed gravel and peat were mixed in a ratio 6:6:1 and mixed until it was evenly distributed. The mixture was then sterilized at 121°C and 1.05 kg cm^{-2} for 15 minutes. The medium sized gravel was then sterilized (Woomer et al., 2012). Sterilized 500 ml pots were filled with the sterile sand and sterilized seeds were planted in them and allowed to germinate. One week after planting (WAP), the BG plants were thinned to one viable plant per pot.1 ml of the inoculums which were already prepared as described above, was introduced into the cowpea plants. Bambara groundnut seeds were sterilized and planted in sterilized soil in pot under screen house conditions and allowed to germinate. Dual inoculation of B. japonicum (USDA110 strain) (1ml) (Somasegaran, and Hoben, 2012) using sterile pipette and 10 g and 20g of mycorrhiza (G. mossea) (Carine et al., 2017 and Gomoung et al., 2017) was applied to plants and limited amount of water (10ml, 20ml, 50ml) was also applied. A completely randomized block experimental design was used treatments (see Table 1) (including three controls, KNO3 treatment to which nitrogen was applied, Rhizobial application alone and the un-inoculated control) were replicated in each of the 4 blocks.

Application of nutrient and water to Plants

Cowpea plants were allowed to grow for 8 week during which they were given 20 ml of nutrient solution consisting of both micro and macro nutrient. To prepare nutrient solution given to plant, the stock solutions were mixed using 100 ml of agriculture on the environment and may be used to address the current challenge of meeting the fast-growing demand for agricultural products worldwide.
macro- stock solution and 10 ml micro- stock solution made up to 10 liters using distilled water. The nutrient solution was sterilized at 121°C and 1.05 kg cm\(^{-2}\) for 15 minutes and was aseptically given to the plants weekly. The solution for the N\(\delta\) treatment (control containing nitrogen) was prepared using 5% of N in KNO\(_3\); this was sterilized at 121°C and 1.05 kg cm\(^{-2}\) for 15 mins after which 50 ml of the solution was added to the plant weekly. Water application was done by giving 10ml, 20ml and 50ml of water (according to the treatment) every other day to the plants (Table 1).

**Harvesting**

Plants were allowed to grow for 10 weeks, after which they were harvested by cutting at the base with a secateur. The shoots and roots were placed in labelled paper bags in an oven where they were dried at 68°C for 72 hours until constant weight was obtained. The shoot dry weights were recorded.

**Determination of chlorophyll**

Chlorophyll readings were taken from leaves using a Spad meter at the 2\(^{\text{nd}}\) 4\(^{\text{th}}\) and 6\(^{\text{th}}\) weeks after planting.

**Determination of % N and % P in plant shoots**

The total % Nitrogen in the shoots of the plants were determined using the micro Kjeldahl method (Kjeldahl; 1883) while phosphorus was determined by the molybdenum blue method (Murphy and Riley, 1962). The three control treatments plants were used as a reference plant.

**Statistical Analysis**

The data collected was analysed for correlation (Pearson’s) using SPSS version 20 and Fisher’s least significance was used to compare means at p ≤ 0.05. **RESULTS**

3.1 %Nitrogen

The %N in the shoot increased with increase in the amount of water in both 10g and 20g G. mossae applications. 10g/10ml treatment had a lower %N than the treatment that had only 10g with no rhizobia inoculation, but the 10g/20ml and 10g/50ml treatments had higher %N than the 10g only treatment. All 10g G. mossae had lower %N than the USDA110 only treatment but were higher than the un-inoculated control and the KNO\(_3\) control while the 20g/10ml, 20g/20ml, 50g/ml all had higher %N than the KNO3 and un-inoculated control but only 20g/10ml had a %N that was lower than that obtained in the USDA110 only treatment.

**Figure 1** Uptake of Nitrogen in shoot of plants

3.2 %Phosphorus

%P increased with increase in the amount of water at both 10g and 20g G. mossae applications. The %P of USDA110 only was lower than that of all other treatments except that of the un-inoculated control. While the 10g/50ml and 20g/50ml treatments had higher %P than their corresponding treatments that had only G. mossae with no USDA110 applied in them.

**Figure 2** Uptake of Phosphorous in shoot of plants

Leaf chlorophyll

Leaf chlorophyll reduced steadily from the 2\(^{\text{nd}}\) week to the 6\(^{\text{th}}\) week in all treatments and also decreased with increased application of water.

**Figure 3** Leaf chlorophyll of experimental plants

Shoot weight

Shoot weight increased with increased application of water in both 10g and 20g G. mossae treatments but plants with USDA110 inoculation had higher weights than their counterparts with no USDA110 inoculation.

**Figure 4** Dried weight of plant shoot
Mycorrhizal Inoculation Efficiency (MIE)

MIE increased significantly with increase in the amount of water application when both 10g and 20g of G. mosseae. When 20g G. mosseae was applied there was no significant difference in the MIE on application of 10ml, 20ml and 50ml of water while there was significant difference for the 10g application.

Correlation

There was a positive correlation between leaf chlorophyll and shoot dry weight, %P and negative correlation between %N and MIE which were not significant. Significant negative correlation was found between the % N and shoot dried weight, and no significant correlation between the MIE phosphorous but n and other parameters although it had a negative correlation with the leaf chlorophyll at the 2nd and 6th week after planting.

Table 2 Correlation between plant parameters

| Treatments | Water (ml) | Inoculum amount (ml) | G. mosseae (g) | MIE(%) |
|------------|-----------|----------------------|----------------|--------|
| 10g        | 100       | -                    | 10             | 15.14  |
| 10g/10ml   | 10        | 1                    | 10             | 18.9   |
| 10g/20ml   | 20        | 1                    | 10             | 34.1   |
| 10g/50ml   | 50        | 1                    | 10             | 43.78  |
| 20g        | 100       | -                    | 20             | 24.89  |
| 20g/10ml   | 10        | 1                    | 20             | 24.89  |
| 20g/20ml   | 20        | 1                    | 20             | 27.57  |
| 20g/50ml   | 50        | 1                    | 20             | 69.73  |
| R          | 100       | 0                    | 0              | 0      |
| KNO3       | 100       | 0                    | 0              | 0      |
| control    | 100       | 0                    | 0              | 0      |

Table 1 Mycorrhizal Inoculation Efficiency (MIE) in plants

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|------------|-----------|----------------------|----------------|--------|
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| 10g/20ml   | 20        | 1                    | 10             | 34.1   |
| 10g/50ml   | 50        | 1                    | 10             | 43.78  |
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| 20g/10ml   | 10        | 1                    | 20             | 24.89  |
| 20g/20ml   | 20        | 1                    | 20             | 27.57  |
| 20g/50ml   | 50        | 1                    | 20             | 69.73  |
| R          | 100       | 0                    | 0              | 0      |
| KNO3       | 100       | 0                    | 0              | 0      |
| control    | 100       | 0                    | 0              | 0      |

*Correlation is significant at the 0.05 level

DISCUSSION

Availability of water had effect on the amount of Nitrogen and phosphorus uptake and shoot weight which all increased as the availability of water increased similar to the findings of Carine et al., 2017. Higher amounts of G. mosseae also had effects on the N, P, shoot weight and MIE (Fig 1, 2, 3 and Table 1) this is similar to the findings of Moila; 2018. The Mycorrhizal Inoculation Efficiency increased with the amount of water and amount of mycorrhizal when dual inoculation was used in plants and is similar to the report given by Esale et al., 2015 and Tsoata et al., 2015.

There was no significant increase in leaf chlorophyll when dual inoculation was applied differing from the result obtained by Kaschuk et al., 2010 there were there was an increase in the photosynthetic rate. Although Bambara did not necessarily depend on mycorrhiza for satisfactory growth and nodulation with rhizobia as observed by Jesus et al., 2005, its presence enhanced the development, growth, %N, %P, and MIE especially under conditions of limited water. The treatment with 20g/50ml of water had the highest value for all the parameters taken. The Mycorrhizal Inoculation Efficiency increased with the amount of water and amount of mycorrhizal when dual inoculation was used in plants and is similar to the report given by Esale et al., 2015, Tsoata et al., 2015.

CONCLUSION

Although Bambara groundnut did not necessarily depend on mycorrhiza for satisfactory growth and nodulation with rhizobia, its presence enhanced the development, growth, %N, %P, and MIE especially under conditions of limited water. In addition, B. japonicum was able to positively influence and establish symbiosis with G. mosseae and synergistically effectively act as “mycorrhiza helper bacteria” (MHB) when both were co-inoculated in Bambara plant.

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