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Date: 7 July 2021.

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Letter of Acceptance

Your paper was peer reviewed and I am directed by the Editorial Board that, your article titled: **Performance of Indian spinach (*Basella alba* L.) as affected by water stress and different sources of plant nutrients by Adediran, O.A., Adesina, O. A., Ibrahim, H., Yakubu, Z. and Odofin, A. J** had been accepted for publication in the International Journal of Organic Agriculture Research & Development. The article will be published in one of the issues which will be coming out shortly in the volume 17 (2021).

Thank you for your patience in the Editorial process.

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PERFORMANCE OF INDIAN SPINACH (*Basella alba* L.) AS AFFECTED BY WATER STRESS AND DIFFERENT SOURCES OF PLANT NUTRIENTS

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Abstract

Basella alba L. is an important leafy vegetable of enormous nutritional and economic benefits but it is underutilized in Nigeria partly because it has received very little research attention. This work aimed to determine the effects of different sources of nutrient and water stress on the performance of the crop. The treatments consisted of 4 water stress levels (daily, 2, 4 and 6 days watering intervals) and 4 nutrient sources (control, NPK 20-10-10, poultry manure and sapropel). These were arranged in Complete Randomized Design (CRD) with four replicates. Growth and yield data were recorded. The results revealed that water stress significantly reduced number of leaves, vine length, stem diameter and biomass yield. Water stress up to 4 days reduced the biomass yield by 82% in NPK treated plants and 26% in poultry manure treated plants but there was no significant difference between the shoot yield of daily and 2 days interval watered plants. NPK fertilizer and poultry manure significantly increased all the growth and yield parameters measured compared to sapropel treated plants which had statistical similar performance with the control plants. Application of NPK fertilizer and poultry manure significantly increased the shoot fresh yield by 137% and 82% respectively while application of sapropel only increased the yield by 6%. In the control and sapropel treated plants, water stress did not significantly affect the biomass yield up to 6 days watering interval. It can therefore be concluded that application of organic or inorganic fertilizer is needed for optimum performance of *B. alba*. Use of sapropel alone may not be a viable alternative to chemical fertilizer in the cultivation of the crop unlike poultry manure. Water stress sets in after 2 days watering interval causing significant reduction in growth and yield. The plant can tolerate up to 6 days watering interval, but when there is improved soil nutrition leading to better growth, watering at two days interval is optimum.

Key words: Indian spinach, water stress, water use efficiency, sapropel, poultry manure, NPK fertilizer.

Introduction

Indian spinach or Malabar spinach (*Basella alba* L.) is a leafy green vegetable that belongs to the *Basellaceae* family. This vegetable is popular in tropical and sub-tropical areas, where young stems and tips are used in cooking and making salad. The matured fruit and root are also useful (Kumar *et al.*, 2013). The water rich leaves have a mild flavor and slightly mucilaginous making it a good alternative to water leaf. It is reported to be low in calories and fat but very high in vitamins, minerals and antioxidant Kumar *et al.* (2015). It's anti-inflammatory and anti-bacteria properties was also reported (Azad *et al.*, 2013), thus, it has been described as functional food that lower the risk of various diseases (Kumar *et al.*, 2015). It is widely used in traditional medicine to treat ulcer, fever, hormonal imbalance, constipation, inflammation, wound and neutralize poison (Kumar *et al.*, 2013). *Basella* species are

endowed with various industrially important chemicals such as acacetin, anthraquinone, basellasaponins A, B, C and D, betacyanin, ferulic acid (Kumar *et al.*, 2013).

There are more than twenty leafy vegetables consumed in Nigeria but fewer than six are actually grown for commercial purpose, while others such as *B. alba* are underutilized plant with great potential (Olgorite, 2006). These underutilized vegetable which offer good domestic marketing opportunities and are often adapted to our tropical environment are yet to receive the desired research attention. Research on vegetable crops tends to be limited to a small number of crops for which there is an assured market, such as tomato, pepper, cabbage, onion, beans, and cucumber (UNCTAD, 2003). It is not usually cultivated conventionally but grows spontaneously in non-hygienic places in Nigeria. It is commonly grown close to drains from lavatories or other house liquid waste. This is to ensure constant water supply as it is believed that the plant requires relatively high amount of water. This may be the reason why it is called 'flowing water vegetable'. It is often found in wet localities (Abukutsa-Onyango, 2004). To attain food security in developing countries, it is important to maximize the potential of these vegetables.

Global warming and associated climate change have already negatively impacted the availability of critical natural resources; there is increasing scarcity of irrigation water (Zhang *et al.*, 2014). It is therefore important to practice climate smart agriculture in which just the adequate amount of water is made available to the plant. Adoption of a suitable irrigation water management practice is necessary to reduce cost of production and eliminate many associated problems.

Successful commercial vegetable production cannot be achieved without fertilizer application in the tropics as tropical soils are deficient in major nutrient element (Kostov, 2016). Chemical fertilizer though effective for vegetable growth, has posed lots of environmental pollution and health risk due to excessive use. With the advocate for organic farming, it is imperative to test alternative organic fertilizers which are more environmentally friendly. Use of animal manure has been reported to improve the productivity of crop among which poultry manure has been reported to be effective by many researchers (Al-gaadi *et al.*, 2019). Use of sapropel as bio-fertilizer is a relatively new technology for growing vegetables in Nigeria. Sapropels are subaqueous layers formed at the bottom of nutrient rich water under anaerobic condition (Ismail-Meyer *et al.*, 2018). It is a clean and ecologically friendly natural material obtained from remains of plankton, water plants and other water dwelling organism used as biofertilizers and in soil conditioning (Marunga *et al.*, 2020). Considering the bulkiness and lack of hygiene associated with use of animal dung, sapropel could be a better alternative. Hence this work was conducted to compare the effect of chemical fertilizer, poultry manure and sapropel on *B. alba* under water stress.

MATERIALS AND METHODS

The experiment was conducted between August and October, 2019 at the screen house of Federal University of Technology, Minna, Nigeria. It was a 4 x 4 factorial experiment comprising 4 water stress levels (daily watering, 2, 4 and 6 days watering intervals) and 4 nutrient sources (control, NPK 20-10-10, poultry manure and sapropel biofertilizer). These were arranged in Complete Randomized Design (CRD) with four replicates. Four seeds were

sown per pot filled with 3.5 kg of top soil at a depth of 5 cm. The seedlings were thinned to two stands per pot at two weeks after sowing. The water stress schedule was introduced at 3 weeks after sowing following the treatments. At each watering day, the soil was watered to field capacity with 40 cl of water per pot.

NPK 20-10-10, sapropel and poultry manure were applied at the rate of 625 kg ha⁻¹, 1580 kg ha⁻¹ and 3 ton ha⁻¹ according to the recommendations of Pujari (2017), Salami and Babajide (2015). The sapropel used (Emerald fertilizer sapropel) contained 7.91% N, 17.37-mg/kg phosphate and 7.76 mg/kg potassium.

Data were collected on vine length, number of leaves and branches, stem diameter (cm), leaf area (cm²). At 9 WAS, the shoots were harvested and weighed on a Mettler balance to obtain the fresh weight. These were oven dried at 70°C until constant weight was obtained to get the dry weight. The roots were severed and weighed on a Mettler balance to obtain the below ground biomass (g/plant). All the data collected were subjected to analysis of variance (ANOVA) using Statistical Analytical System (SAS). Means were separated using Least Significant Difference (LSD) test at P= 0.05. Where significant interaction exists, result of main effect are not presented.

Results

Effect of water stress on the performance of *Basella alba*

Number of leaves, vine length, stem diameter, shoot fresh yield and above ground biomass were significantly affected by water stress (P<0.05) (Table 1). Plants that received daily and two days watering interval had statistical similar vine lengths which were significantly higher than the vine length of plants watered at 4 and 6 days interval which were also at par. Water stress of 4 and 6 days significantly reduced the vine length of *B. alba* by 42 and 37% respectively compared to daily watering (Fig 1).

Plants watered daily and those watered at two days interval had similar stem diameter which were significantly higher than stem diameter of plants watered at 6 days interval which had the least value (Fig 2). Plants that received daily and two days watering interval had statistical similar shoot fresh yield which were significantly higher than the shoot fresh yield of plants watered at 4 and 6 days interval which were at par. Water stress of 4 and 6 days reduced the shoot fresh yield by 22 and 21% respectively (Fig 3).

Effect of nutrient sources on the performance of *Basella alba*

All the parameters measured were highly significantly affected by nutrient sources (P<0.01). Application of NPK fertilizer significantly increased the leaf area by 128% over the control (unfertilized) plants. This was statistically similar to the increase of 101% recorded in poultry manure treated plants. Application of sapropel only increased leaf area by 6% which was not significantly different from the value recorded in the control plants (Fig. 4).

Application of NPK and poultry manure significantly increased the vine length by 74% and 67% respectively over the control plants but there was no significant difference between the vine length of the sapropel treated and control plants (Fig. 5). Similar trend was recorded for number of branches (Fig. 6).

Plants fertilized with NPK had significantly thickest stem followed by the stem of plants fertilized with poultry manure. Sapropel treated and control plants had similar stem diameter that were significantly thinner than the stem of poultry manure and NPK treated plants (Fig 7). Application of NPK produced plants with significantly highest shoot fresh yield with 137% increase over the control plants. This was followed by plant treated with poultry manure which had 82% over the control plants. Application of sapropel only increased the biomass yield by 6% which was not significantly different from the yield of the control plant (Fig 8). Similar trend was recorded for root weight (Fig 9).

Interaction effect of Water Stress and Nutrient sources on the performance of *Basella alba*

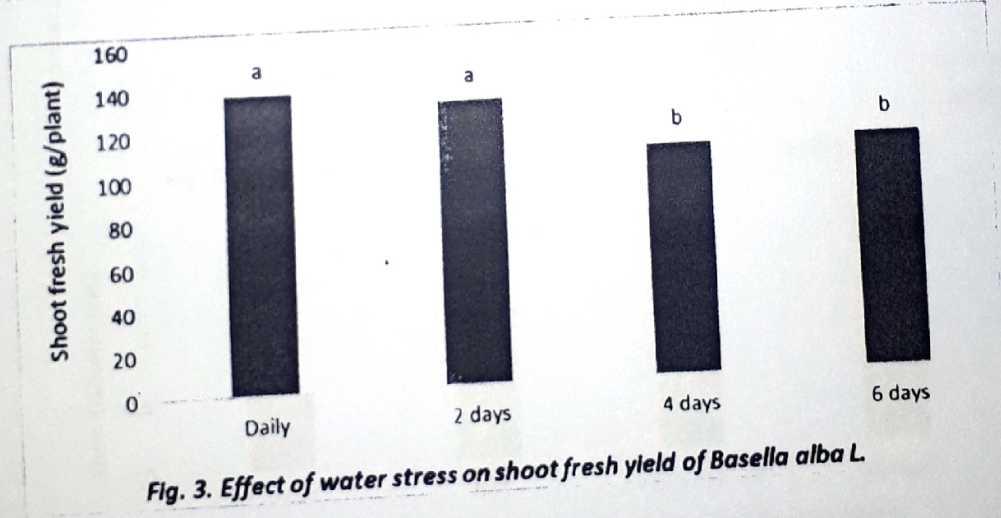
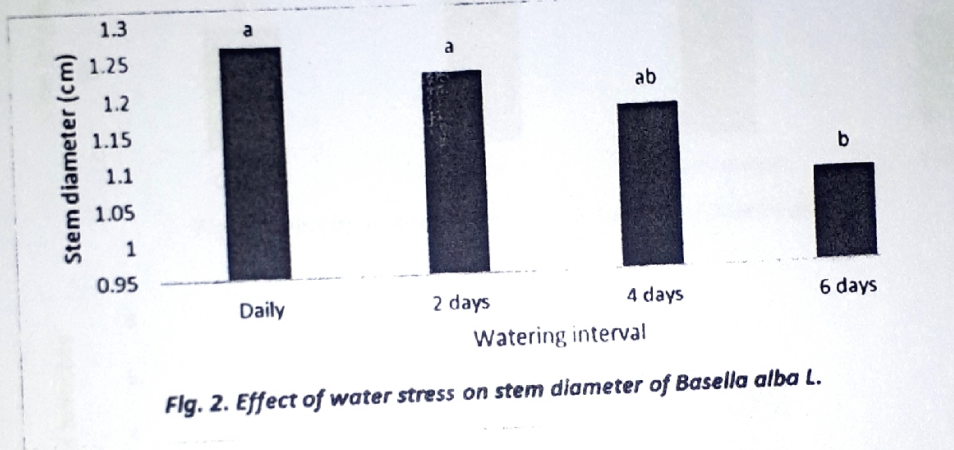
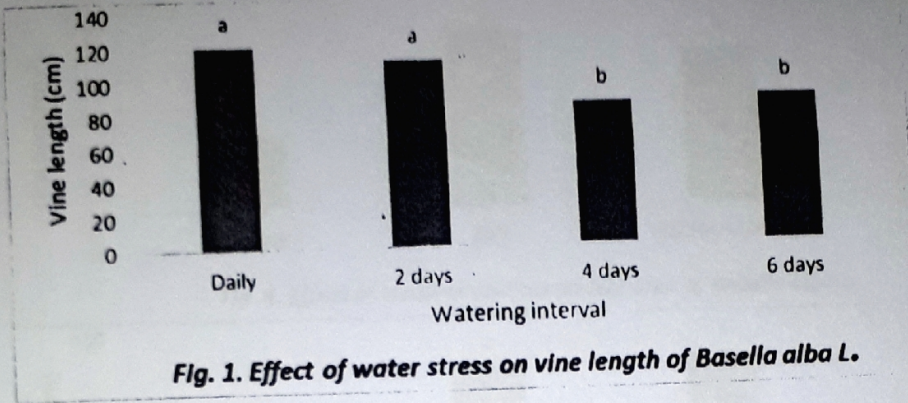
Number of leaves and above ground biomass was significantly affected by water stress, nutrient sources and interaction between the two ($P < 0.05$) (Table 1). The highest number of leaves was obtained in NPK fertilized plants watered daily. This was however statistically similar to the number recorded in poultry manure treated plants watered daily. When plants were fertilized with NPK, the number of leaves obtained in daily watered plants was significantly higher than in plants watered at two days interval. In poultry manure fertilized plants however, there was no significant difference between the number of leaves of daily and two days interval watered plants. The lowest number of leaves were recorded in sapropel treated and control plants irrespective of the watering interval (Table 2).

The highest above ground biomass yield (24.40g/plant) was obtained in NPK fertilized plants watered at 2 days interval similar to NPK fertilized plants watered daily (21.33 g/plant), The least value (7.22 g/plant) was recorded in the control plants watered at 6 days interval. When plants were fertilized with NPK, the highest biomass weight was obtained at 2 days watering interval which was similar to the value recorded in the daily watered plants. In poultry manure treated plants however, the highest biomass yield was recorded in daily watered plants. The value was however similar to those recorded in 2 and 4 days interval watered plants. Water stress up to 4 days reduced the biomass yield by up to 82% in NPK treated plants compared to the two days interval. In poultry manure treated plants, water stress of up to 4 days reduced the biomass by 26% compared to the two days interval. For the Sapropel treated and control plants, though the least biomass yield was obtained at 6 days watering interval plants, there was no significant difference between the biomass yield of the different watering schedule (Table 3).

Table 1: Mean square values for response of *Basella alba* L. to water stress and nutrient sources

Source of variation	Number of leaves	Leaf area (cm ²)	Vine length (cm)	Number of branches	Stem diameter (cm)	Shoot fresh yield (g/plant)	Above ground biomass (g/plant)	Below ground biomass (g/plant)
Water stress (W)	185.72***	724.51	4930.74***	18.52	0.12*	3806.77***	74.20**	0.30
Nutrient source (N)	1311.10**	15492.37***	17372.25***	150.39***	0.39***	40977.64***	369.70***	1.44***
W x N	57.52*	536.45	815.51	11.88	0.02	875.72	27.01*	0.11
Error	24.71	370.06	535.95	7.32	0.04	535.18	12.49	0.13

*, **, ***- significant at 5, 1 and 0.1% respectively



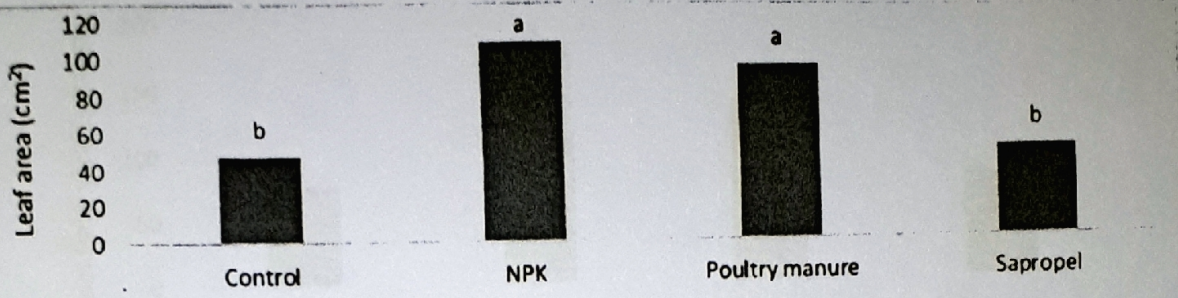


Fig. 4. Effect of nutrient sources on leaf area of *Basella alba* L.

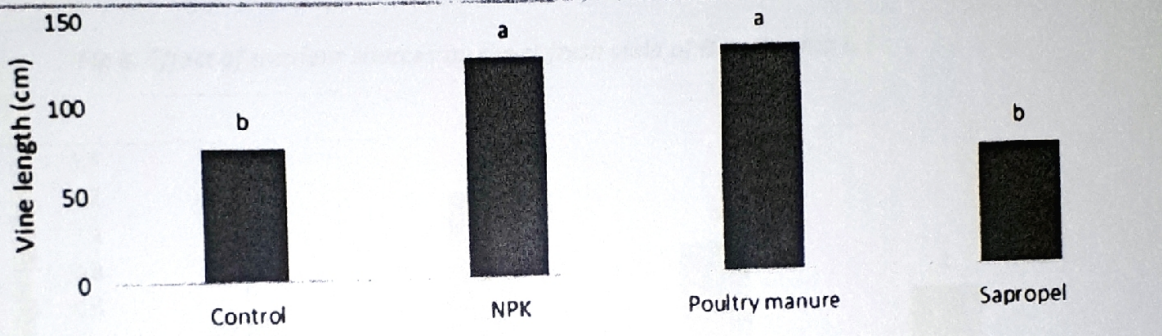


Fig. 5. Effect of nutrient sources on vine length of *Basella alba* L.

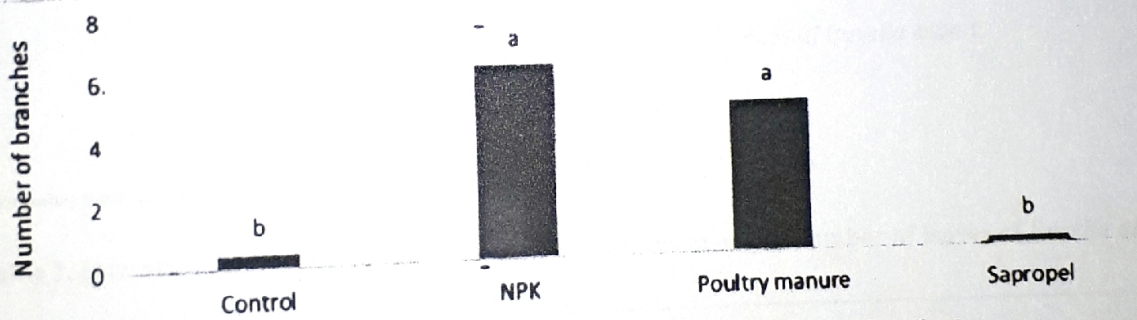


Fig. 6. Effect of nutrient sources on number of branches of *Basella alba* L.

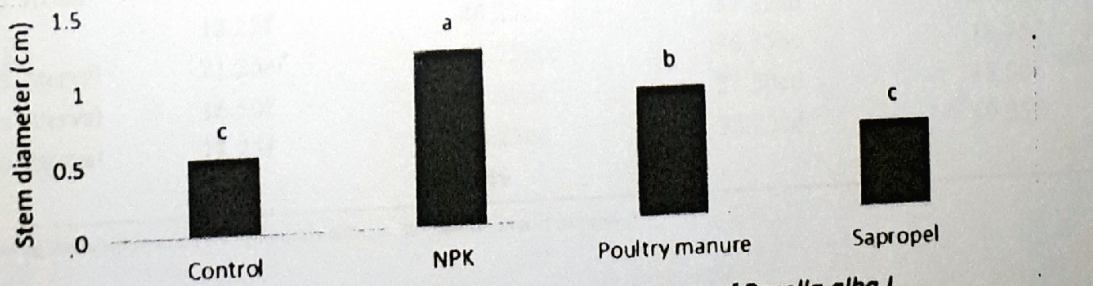


Fig. 7. Effect of nutrient sources on stem diameter of *Basella alba* L.

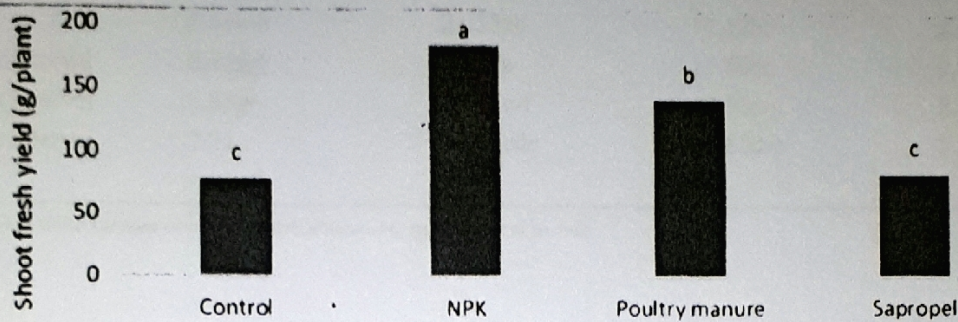


Fig 8. Effect of nutrient sources on shoot fresh yield of *Basella alba L.*

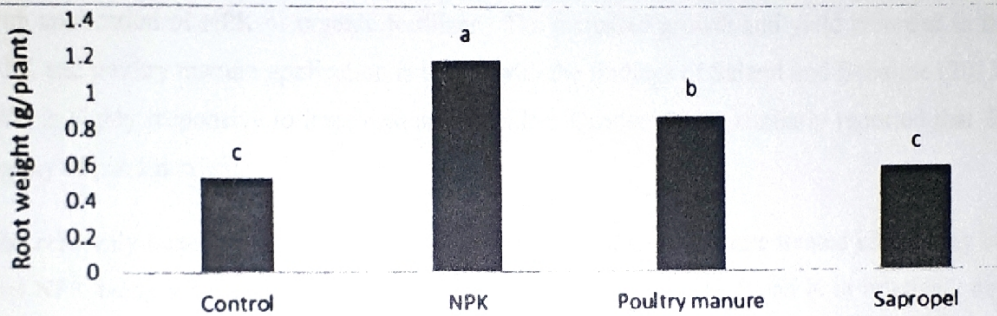


Fig 9. Effect of nutrient sources on below ground biomass of *Basella alba L.*

Means having similar alphabets are not significantly different using LSD (P=0.05)

Table 2. Interaction effect of water stress and nutrient sources on the number of leaves of *Basella alba L.*

	Control	Nutrient sources		
		NPK	Poultry manure	Sapropel
Water stress				
Daily	18.25f	46.25a	39.75ab	21.00ef
2 days interval	21.25ef	32.75bcd	36.75bc	18.75f
4 days interval	16.50f	27.50de	31.50cd	18.50f
6 days interval	18.25f	30.25cd	32.25cd	19.25f
SE ±		2.49		

Means having similar alphabets are not significantly different; SE- standard error of the mean

Table 3. Interaction effect of water stress and nutrient sources on the above ground biomass yield of *Basella alba L.*

Water stress	Control	Nutrient sources		
		NPK	Poultry manure	Sapropel

Daily	8.89e-h	21.33ab	17.12bc	8.86e-h
2 days interval	8.42fgh	24.40a	15.80cd	9.38e-h
4 days interval	8.05gh	13.37c-f	12.56c-g	8.38fgh
6 days interval	7.22h	13.45cde	11.85d-h	7.69gh
SE \pm		1.77		

Means having similar alphabets are not significantly different; SE- standard error of the mean

Discussion

Application of NPK and poultry manure remarkably improved the *B. alba* performance in this study. Abukutsa-Onyango (2004) similarly reported that *Basella* can grow under moderate soil fertility but its production is enhanced with application of NPK or organic fertilizer. The increased growth and yield recorded in this study as a result of NPK and poultry manure application is in line with the findings of Salami and Babajide (2015) who reported that *B. alba* is highly responsive to improved soil nutrition. Quader (2007) similarly reported that *B. alba* requires proper supply of plant nutrient.

The relatively better performance recorded in NPK than poultry manure treated plants may be attributed to the fact that NPK being a chemical fertilizer, has higher concentration of N, P and K in relatively equal proportions unlike organic manure. N, P and K are the primary essential nutrients required by plants. Poultry manure contains an average of 3-5% N, 1.5-3.5% P, and 1.5-3.0 K (Amanullah *et al.*, 2010) compared to the 20% N, 10% P and 10% K in the NPK fertilizer. Furthermore the nutrients in chemical fertilizers are readily available for plant growth than organic fertilizer which releases their nutrients slowly. Most of the nitrogen (75-80%) in poultry manure is in the organic form and need time to mineralize before becoming available to plant. The full benefit of organic manure may not be reaped in just one season of cropping. Quader, 2007 similarly reported that *B. alba* plants that received NPK produced higher vine length, number of leaves and dry matter than poultry manure treated plants. Similar result was reported by Oyedeji *et al.* (2014). In this study, sapropel only increased the shoot yield by 6%. This is close to the 9% yield increase reported by Aganofora *et al.* (2015). The nutrients in the sapropel fertilizer may not also be readily available for plant immediate use. Aganofora *et al.* (2015) further reported that sapropel's positive effect is manifested in subsequent years of crop cultivation. This may suggest that when using sapropel as the nutrient source, little quantity of chemical fertilizer need to be added to the soil especially in the first season for short-lived crops. At the long run, it may cut down on the quantity of chemical fertilizer used. The fresh shoot yield values obtained in NPK fertilized and poultry manured plants in this study (183.61 and 141.13 g/plant respectively) is within the range of the highest value (177.40 g/plant) reported by Salami and Babajide (2015) when they applied poultry manure.

Contrary to the daily irrigation recommended by Abukutsa-Onyango (2004), there was no significant difference between the performance of daily and 2 days watering interval in this study. The NPK and poultry manure treated plants had the highest above ground biomass at 2 days watering interval though statistically similar to daily

watering. Furthermore, plants that received 6 days watering interval did not reach permanent wilting point in this study. This may be an indication of good water use efficiency in the plant which is an important crop quality under water deficit. This contradicts the general believe that the plant requires constant supply of water and indicates that the crop could tolerate short period of drought. In this study, the significant reduction in the growth and yield of NPK and poultry manure treated plants from 4 days watering interval indicates that moisture stress sets in after 2 days watering interval. Water stress affects growth and yield by inhibiting nutrient uptake and reducing photosynthesis. At initial stages, the plant try to cope with adverse water stress condition by altering (increasing) abscisic acid (ABA) and proline content, enzymatic and non-enzymatic antioxidants (Deshmukh and Gaikwad, 2016). Water stress induces a decrease in leaf water potential and in stomatal opening, leading to down-regulation of photosynthesis-related genes and reduced availability of CO₂ for carbon fixation during photosynthesis. Osakabe *et al.* (2014) reported that in response to water stress, ion- and water-transport systems across membranes function to control turgor pressure changes in guard cells and stimulate stomatal closure. Endogenous ABA is rapidly produced during drought, triggering a cascade of physiological responses, including stomatal closure, which is regulated by a signal transduction network.

In this study, water stress up to 4 days reduced the biomass yield by 82% in NPK treated plants while it reduced by 26% in poultry manure treated plants. The wide disparity may be attributed to the fact that poultry manure increases the water holding capacity of the soil being rich in organic matter. The reduced growth of sapropel treated and control plants may be responsible for the non-significant water stress recorded in them at 4 and 6 days watering interval. They produced shorter plants, fewer number of leaves and branches and slimmer stem, hence the water demand in these plants will be lesser than their NPK and poultry manure treated counterpart which will need more water due to increased transpiration rate as a result of larger surface area and maintenance of balance.

Conclusion

Application of organic or inorganic fertilizer is needed for optimum performance of *B. alba*. Use of sapropel alone as soil amendment may not be a viable alternative to chemical fertilizer in the cultivation of the crop unlike poultry manure. Water stress sets in after 2 days watering interval causing significant reduction in growth and yield. Though the plant can tolerate up to 6 days watering interval but when there is improved soil nutrition leading better growth, watering at two days interval is recommended.

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