BIOPROSPECTING NEGLECTED BOTANICALS AS PHYTO-FERTILIZERS FOR UNDERUTILIZED FOOD CROPS

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Abstract: Botanicals are part of the bioresources for sustainable growth of food crops, however, some botanicals have been neglected. This study investigates the potential effects of neglected botanicals on selected underutilized food crops (millet, sorghum and sesame) in Nigeria. Blotter technique and soil inoculation methods were used to evaluate the best botanicals that could be employed to enhance seed germination (SG) and seedling growth of underutilized food crops. With the exception of Newbouldia laevis, other botanicals enhanced seed germination of all tested crops. Ficus asperifolia and Parquetina nigrescens significantly ($p<0.05$) enhanced the radicle length (RL) while the plumule length (PL) remained unaffected. Specifically, observation shows that Newbouldia laevis completely inhibited sesame seed germination and seedling growth while Parquetina nigrescens enhanced millet height and sesame number of leaves. Generally, the effect of the botanicals on each crop was specific. Thus, these neglected botanicals stand the chance to enhance and sustain seedlings of underutilized food crops in Nigeria. Further study would be carried out to uncover the chemical components in the tested botanicals.

Key words: fertilizer, growth, botanical, neglected and underutilized crops, plants.

Introduction

As the world population increases, there is a need to re-strategize on how to feed nations (FAO, 2017), especially on the use of food crops. Apart from the fact that economically important food crops which include, but are not limited to, rice, maize, wheat, vegetables and leguminous crops (FAO, 2018; GIEWS, 2018) have overshadowed underutilized food crop species, they are also not enough to feed the world population (Li and Siddique, 2018). Of the 30,000 underutilized edible crop species, a mere 30 are used to feed the world. These underutilized crops can help to increase nutrient contents and diversification in food production. In addition to

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diversifying nutritional intake, underutilized crops provide not only economic and environmental benefits, but farmers can also grow them on their own (FAO, 2018; Li and Siddique, 2018).

Since these underutilized food crops are important to aid the global availability and nutri-beneficial foods, thus it is necessary to enhance their growth through the use of fertilizers. In addition to health implications (Southland Organic, 2012; Buckler, 2018), an excessive rate of chemical fertilizers also causes soil nutrient deterioration, soil structure deformation, heavy metal accumulation as well as the low yield of crops (Savei, 2012; Rahman and Zhang, 2018). On this note, environmental and health-friendly bio/phyto-fertilizers are the best alternatives. Among the phyto-fertilizers, botanicals have not been explored on seed germination and growth of underutilized crops in Nigeria.

Botanicals are substances extracted from plants which are used in pharmaceutical and cosmetic products, as food ingredients, and also as plant protection products (Okrikata and Oruonye, 2012; Seiber et al., 2014; Damalas and Koutroubas, 2018). Extracts from botanicals especially trees and crop residues have the potentials to influence crop growth and yield (El Atta and Bashir, 1999; Ahmed and Nimer, 2002; Farooq et al., 2008). Sesbania sesban, Leucaena leucocephala, Eucalyptus microtheca (Hussein and Abbaro, 1997) and Moringa oleifera are botanicals that have been established specifically to accelerate and strengthen the growth of young plants and crops. They also have the potentials to improve resistance to pests and diseases, increase leaf area as well as the number of roots and crop yield (Fuglie, 1999).

In Africa, especially Nigeria, many botanicals have been neglected and their potentials to enhance seed germination and seedling growth of crops have been relegated to the background (Murray, 2013). Apart from their pesticidal properties, there is little or no information regarding their phyto-fertilizer activities (Ertani et al., 2013; Ziosi et al., 2012). To ascertain the on-farm effect, the crude extracts of Ficus asperifolia (FA), Parquetina nigrescens (PN) and Newbouldia laevis (NL) were prepared exactly the way they are prepared by rural farmers and were evaluated on seed germination and seedling growth of millet, sorghum and sesame.

Material and Methods

Preparation of botanicals: Fresh leaves of FA, PN and NL were plucked from a local village around the Ibadan axis (Latitude – 7.30, Longitude – 3.38, Elevation – 176m) in Nigeria, and were properly washed in clean water. The samples were air-dried to obtain 0% moisture and blended in a clean electric blender (Qlink blender, Model number: QBL-20L 40, China). Exactly 10g of each botanical were added into 100ml of clean water and each sample was filtered twice through cheesecloth, collected in a flask and stored at 4°C to be used later. Pearl millet
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(Pennisetum glaucum – ExBronu), sorghum (Sorghum bicolor – NG/SA/JAN/09/088) and sesame (Sesamum indicum – white/raw) seeds were used for the experiment.

Blotter technique: Prior to inoculation, the Petri dishes were underlaid with sterilized moistened filter paper. This was followed by botanical inoculation (seeds were soaked in each crude extract for 3 minutes) and air-dried at room temperature (25±2°C). Briefly, 4 inoculated seeds of millet, sorghum and sesame were separately and appropriately placed at an approximate equidistant position to each other in 9-cm-diameter Petri dishes and incubated for 7 days at a temperature of 25±2°C (ISTA, 2003). At day 7, the germinated seeds were counted and percentage seed germination was determined using the formula: %G = n/Nx100. The radicle and the plumule lengths were measured with a thread and the precision was determined by the ruler. This experiment was done in three replicates and results were compared with the control (water).

Soil inoculation: Each pot (30 cm in diameter) contained 2.5 kg of sterilized soil; the soil was separately inoculated with 5 ml of each crude extract and homogeneously mixed with a sterile rod to maintain uniformity. After 24 hours, 4 seeds of millet, sorghum and sesame were sowed separately at the depth of 2 cm into the treated soil while the untreated seeds served as the control (seeds without botanicals). Using a completely randomized design, pots were arranged in three replicates. Watering was done throughout the experiment based on the requirement. At day 7, the interactive effect of each crude extract per crop was established. Percentage seed germination was determined using the formula: %G = n/Nx100. Data obtained were subjected to statistical analysis (SAS, 2009).

The effect of botanical extracts on seedling height and the number of leaves: Following the protocol of soil inoculation, 3 viable millet, sorghum and sesame seeds were separately planted in steam-sterilized potted (30-cm-diameter plastic pots) soil (2.5 kg of soil). The pots were completely randomized and replicated three times. After 24 hours, seeded pots were separately inoculated with 5 ml of PN, FA and NL crude extract, while sterile distilled water was used as the control (that is, negative control). NPK chemical fertilizer (15-15-15) was applied at a recommended dosage as positive control. Watering was done throughout the experiment based on the requirement. Data on seedling height and number of leaves were obtained after 4 weeks and subjected to statistical analysis (SAS, 2009).

Results and Discussion

Both blotter technique and soil inoculation methods significantly \( p \leq 0.05 \) enhanced seed germination, though, with a comparative variation. The soil gave better germination than the blotter technique (Hassan et al., 2012). Apart from the
fact that germination bioassays are excellent tools (Hoagland and Williams, 2003), it also provides the basis for comparison (Haq et al., 2014) which enables to determine the best botanicals in this study.

Apart from millet, PN and NL significantly ($p \leq 0.05$) demonstrated the phyto-fertilizer effect on sorghum. Similarly, FA enhanced germination of millet and sorghum seeds more in comparison to sesame that experienced slight inhibition. Islam and Kato-Naguchi (2014) had a similar observation and reported that Ocimum tenuiflorum at a concentration of $30\text{mg dry weight (equivalent extract mL}^{-1})$ relatively enhanced the germination of barnyard grass. PN demonstrated more pronounced positive effects on sorghum and sesame (Table 1). PN, NL and FA might have altered well-defined sequence of complex processes underlying a set of biochemical, physiological and morphological changes in millet and sesame (Bewley and Black, 1994; Bewley et al., 2013).

Table 1. The effect of extracts on crops (% seed germination, radicle and plumule lengths).

| Parameters | FA       | PN       | NL       | CT       |
|------------|----------|----------|----------|----------|
| Millet     |          |          |          |          |
| SG (%)-BT  | 99.5a (± 0.76) | 66.8b (± 0.07) | 66.5b (± 0.07) | 33.4c (± 0.14) |
| SG (%)-SI  | 99.9a (± 0.21) | 99.9a (± 0.07) | 99.9a (± 0.07) | 33.2b (±0.14) |
| RL (cm)    | 9.3a (± 0.35) | 4.8b (± 0.07) | 9.2a (± 1.98) | 9.4a (±0.21) |
| PL (cm)    | 4.1b (± 0.14) | 2.6c (± 0.92) | 5.5a (± 0.14) | 6.4a (± 0.14) |
| Sorghum    |          |          |          |          |
| SG (%)-BT  | 99.9a (± 0.14) | 99.9a (± 0.21) | 99.9a (± 0.14) | 33.2b (± 0.14) |
| SG (%)-SI  | 99.6a (± 0.57) | 99.9a (±0.14) | 99.7a (± 0.42) | 99.5a (± 0.78) |
| RL (cm)    | 9.7a (± 0.35) | 8.9a (± 1.91) | 5.8a (± 0.99) | 9.3a (± 0.07) |
| PL (cm)    | 5.9a (± 0.50) | 4.9a (±0.99) | 10.9a (± 3.25) | 6.3a (± 0.07) |
| Sesame     |          |          |          |          |
| SG (%)-BT  | 50.1c (± 0.07) | 83.4b (± 0.14) | NG       | 100.0a (± 0.07) |
| SG (%)-SI  | 99.5a (± 0.78) | 99.4a (± 0.92) | NG       | 99.9a (± 0.14) |
| RL (cm)    | 4.6a (± 0.14) | 4.5a (± 0.14) | NG       | 5.0a (± 0.64) |
| PL (cm)    | 3.6a (± 0.14) | 3.3a (± 0.35) | NG       | 3.8a (± 0.42) |

Mean values of three replicates are shown. Mean values followed by different letters in the rows are significantly different ($\alpha = 0.05$). BT = Blotter technique, SI = Soil inoculation, PN = Parqueïna nigrescens, FA = Ficus asperifolia, NL = Newbouldia laevis, CT = Control. NG = No germination/growth. SG = Seed germination, RL = Radicle length, PL = Plumule length.

The botanical extracts responded differently to seed germination (Chukwuka et al., 2014). Specifically, NL exhibited a laudable effect on sorghum and millet in comparison to sesame that was completely inhibited. Also, since sesame seeds treated with NL were unable to germinate, radicle and plumule lengths could not be determined. This suggests that NL may contain some phyto-toxic compounds.
which might have interacted with millet and sesame seeds, thus resulting in inhibition of plumule length and seed germination (Table 1, Figure 1). Killani et al. (2011) documented a similar observation with rice husk extract on cowpea. They found out that rice husk extract at 2% concentration level completely inhibited germination of cowpea seeds. In addition, the inhibitory effects of *Picrorhiza kurroa*, *Asparagus racemosus*, *Ocimum sanctum*, *Valeriana wallichii* (Rawat et al., 2016) and *Ocimum basilicum* L. (Verma et al., 2012) on germination and seedling growth of seed crops are in line with NL on sesame seeds. The effect of NA on sesame germination is detrimental, which suggests allelopathy. Thus, it could be deduced that NA exhibited an allelopathic effect on sesame. Therefore, this suggests that NA possesses allelochemicals that might have affected the enzyme responsible for plant hormone synthesis, inhibition of nutrient and ion absorption with a direct effect on plasma membrane permeability (Fujii et al., 1991). The practical implication of NL on sesame (if used by farmers) will lead to seed/ plant mortality.

Figure 1. The effect of botanicals on the plant height and the number of leaves of millet, sorghum and sesame seedlings. PN = *Parquetina nigrescens*, FA = *Ficus asperifolia*, NL = *Newbouldia laevis*, CT = Control. SG = Seed germination, RL = Radicle length, PL = Plumule length.
The botanicals exhibited variation on the radicle length of the tested crops. FA was more effective on the crop radicle length in comparison to PN and NL. The effect of NL was outstanding on plumule length of sorghum while other botanicals showed no effect (Table 1). The report of Hassan et al. (2012) has stated that botanical extracts have the potentials to inhibit the emergence of sorghum radicle than plumule. The basis for comparison between this work and that of Hassan et al. (2012) can be linked to the choice of botanicals. Obviously, different botanicals possess different phyto-chemicals (Ntonifor, 2011) and have the potentials to react differently to seed germination and seedling growth.

Apart from the effect of PN on plumule length of millet that was inconceivable, other observable parameters were satisfactory. The PN extract had a stimulatory effect on early growth of millet and sesame (Tadele, 2014). High germination capacity and early growth of millet and sesame indicated that PN extract exerted a positive action on the germination of seedling growth (Tadele, 2014). PN also relatively enhanced the seedling height and the number of leaves (Akanmu et al., 2013) of the tested crops. This is in comparison to FA and NL with respect to seedling height (Figure 1). This suggests the possibility of using PN as phyto-fertilizer to enhance seed germination and seedling growth of millet and sesame. In comparison to NPK chemical fertilizer used as the control, the effects of botanicals on seedlings were comparatively different from that of NPK chemical fertilizer. Apart from NL on sesame, millet and sorghum seeds planted in botanically treated soil developed into seedlings while those planted in NPK treated soil were unable to develop into seedlings. This agreed with Carter (1967) who reported that chemical fertilizers including NPK can cause germination damage to certain crops if influenced by banding properties and other soil factors.

**Conclusion**

Apart from NL, PN and FA are potential phyto-fertilizers due to their effect on seed and seedlings of the underutilized crops. This study proposed that there may be phyto-toxic compounds in NL contributing to inhibition of sesame seed germination. The laboratory and greenhouse evaluation has helped to establish NL, PN and FA as potential phyto-fertilizers (though with variation) to millet, sorghum and sesame. Our future research is to investigate the importance of these results under field conditions and to determine the actual mechanisms involved in the differential effects of the botanical extracts on different species of underutilized food crops.

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Bioprospecting neglected botanicals as phyto-fertilizers for underutilized food crops

BIOLOŠKO ISTRAŽIVANJE SA POTENCIJALNO KOMERCIJALNOM SVRHOM ZANEMARANIH BOTANIČKIH SUPSTANCI KAO FITODUBRIVA ZA NEISKORIŠĆENE PREHRAMBENE USEVE

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Resim

Botaničke supstance su deo bioloških resursa za održiv rast prehrambenih useva, međutim, neke botaničke supstance su zanemarane. Ova studija istražuje moguće uticaje zanemarenih botaničkih supstanci na izabrane neiskorišćene prehrambene useve (proso, sirak i susam) u Nigeriji. Tehnika filter papira i metode inokulacije zemljišta korišćene su da se procene najbolje botaničke supstance koje se mogu koristiti kako bi poboljšali klijanje semena i rast rasada neiskorišćenih prehrambenih useva. Sa izuzetkom Newbouldia laevis, druge botaničke supstance poboljšale su klijanje semena svih istraživanih useva. Ficus asperifolia i Parquetina nigrescens značajno (p<0,05) su povećale dužinu radikule, dok je dužina plumule ostala nepromenjena. Posmatranje posebno pokazuje da je Newbouldia laevis potpuno inhibirala klijanje semena i rast rasada susama, dok je Parquetina nigrescens povećala visinu prosa i broj listova susama. Uopšteno govoreći, uticaj botaničkih supstanci na svaki usev bio je specifičan. S tim u vezi, ove zanemarene botaničke supstance imaju mogućnosti da poboljšaju i održe rasad neiskorišćenih prehrambenih useva u Nigeriji. Dalje istraživanje bilo bi sprovedeno kako bi se otkrile hemijske komponente kod testiranih botaničkih supstanci.

Ključne reči: dubrivo, rast, botanička supstanca, zanemareni i neiskorišćeni usevi, biljke.

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