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Effect of *Allium sativum* and*Ocimumbacilicum* in controlling rot and boosting sprouting of white yam setts

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ABSTRACT

The study assessed the effect of extracts from the foliage of two botanicals- Allium sativum L. andOcimumbacilicum L in controlling rot and boosting sprouting of white yam setts. Two white yam tubers cv. Giwa, were sectioned into minisetts treated with the botanical extracts and thereafter inoculated with the rot-fungi - Fusariumoxysporum and Rhizopusnigricans. The setts were planted first in potted bags then transferred onto yam heaps and data collected from 6 weeks after emergence of sprouts. Parameters measured included sprouting characteristic; the number of leaves per vine, mean leaf area (mm^2), length of shoot (cm), number of roots and rot index. Results showed that Yam setts inoculated with R. nigricans produced a mean of 11 roots while setts inoculated with F.oxysporum had a mean of 5 roots. Roots produced by the un-inoculated setts (the control) were statistically lower than those inoculated with R. nigricans but higher than those of F.oxysporum inoculated setts. Mean root length of the yam setts was not too different from that of root numbers. Mean leaf area in control subplots were larger (42.45 mm^2) compared to those inoculated with F. oxysporum inoculated setts while those inoculated with A. sativum had significantly lower leaf area (22.36 mm^2). It is implied that A. sativum may perhaps contain inhibitory properties that depresses the activities of many rot causing fungi than O. basilicum. , The yam setts treated with R. nigricans suffered a more severe rot (1.55) than those of F. oxysporum(1.00). The study indicated that the use of A. sativum and O. basilicum controlled rot in yam minisetts, thereby confirming the potential of plant extracts to substitute for chemical fungicides.

Key Words: *Alliumsativum; Ocimumbacilicum;* rotting, sprouting, yam minisett.

INTRODUCTION

White yam tubers (*Dioscorearotundata* L. Poir Fam. Dioscoreaceae)are exposed to several diseases in the field as well as in storage. Several of these diseases can be related with the yam planting material or ingenious to planting sites.*F. oxysporium* is the most economically important species in the *Fusarium* genus given its numerous hosts and the level of loss that can result when it infects a plant. *F. oxysporium*include many representatives that are pathogenic to plants, often causing vascular wilt diseases such as damping –off problems, and crown and root rots [1]. It was recently reported to cause secondarybasal node rot in snap beans, rice and in tomato [2].Rhizopus spp. has been implicated as one of the organisms responsible for soft rot by macerations of infected plant tissues such as that of the fruit mesocarp of almond [3]. It has been reported that microbes such as *Sclerotiumrolfsii, Trichodermalongi brachyatum* and *Penicilium oxalic* are the most pathogenic fungi responsible for losses ranging from 38.2 to 70.2 % among *D. rotundata* cultivars [4].

The effort to improve yam seed material resulted in the minisett technology that was developed by National Root Crops Research Institute, Umudike, Nigeria in the 1970's. This was necessitated by the scarcity and high cost of seed yam experienced by yam farmers in the country [5]. Prior to this time, most farmers had to set apart at least 30% of the harvested yam each year to be planted in the new season.

Other problems associated with yam planting material especially setts are their susceptibility to rots caused by microorganisms in both the sprouting media and on the mother seed yam. White yam production through the minisett technique has suffered a great set back in Africa because of rots [6]. Although the minisetts technology is expected to reduce the challenges of scarcity of seed material, it created increased additional harmful effects of microbial rot on yam minisett thereby increasing the problem of decreased sprouting and growth of the yam setts.

It becomes pertinent therefore, to search for novel protective pesticides thatcan be effective, cheap and non-synthetic in origin which can control yam rot. This study consequently investigated the efficacy of the use of *Allium sativum* L.and*Ocimumbacilicum* L.in minimizing rot and thus enhancing sprouting of seed yam.

MATERIALS AND METHODS

The experiment was conducted in the Teaching and Research farm, GidanKwanu Campus, Federal University of Technology, Minna, Niger State which is in the southern Guinea Savannah zone of Nigeria.

Collection and preparation of yam setts

Two healthy and rot free white yam tubers (4 kg each of cv. *Giwa*) were purchased from a Garatu town (local yam market) in Minna, Niger State, Nigeria. The yam tubers were sectioned into 36 yam setts in a semi-circular shape, each weighing about 100g. These were set on the laboratory bench of the Department of Crop Production and treated with the botanical extracts – *A.sativum*and *O.bacilicum* and thereafter inoculated with rot-fungi – *F.oxysporum and R.nigricans*.

Culturing of *Fusariumoxysporum* and *Rhizopusnigricans*inocula in Potato Dextrose Agar (PDA)

F.oxysporum and *R. nigricans* species from infected yam tuber were collected and cultured in Potato Dextrose Agar (PDA) medium. The two pathogens were isolated and sub-cultured in order to obtain pure cultures according to Sule*et. al.*,[7].

PDA (oxoid) [39 g L^{-1}] was dissolved in distilled water, homogenized and then poured into a 500 ml conical flask. The PDA was sterilized at 121^{o}C for 15 minutes in an autoclave. The sterilized media was allowed to cool to 45^{o}C before been dispensed into petri dishes and allowed to glitz[8]. The solidified PDA in Petri dishes were inoculated at the centre with hyphal strands of *F.oxysporum* and *R. nigricans* separately using sterilized inoculating needles.They were labeled respectively and moved into the laboratory in an inoculating hood under the ambient temperature setting of between 27^{o}C to 29^{o}C as described by Asare-Bediako *et al.*, [4]. The specimen were left for a week for fungi colonies to grow. The culture *and F. oxysporum* and *R. nigricans* were then weighed with the concentration of 0.05 g/100 ml of distilled water respectively before being used for inoculation.

Collection and preparation of botanical materials

Fresh and healthy *A. sativum* bulbs and *O. basilicum* leaves were purchased from Minna central market, Niger State, Nigeria. The outer cover of *A. sativum* was peeled off and then squashed into slurry. Fifty grams was mixed in 500 ml of distilled water and were thoroughly mixed together. *O. basilicum* foliage was chopped and then air-dried at 28^{o}C for a week. It was then ground into powder and sieved with 0.2 mm diameter mesh. Fifty grams (50 g) of its powder was dissolved in five hundred millimeters (500 ml) of distilled water before being used [9].

Application of botanicals and inoculation on yam setts

Each piece of yam sett (100 g) was immersed into the plant extract of *A. sativum* and *O. basilicum*(separately) and allowed to air-dry for 45 minutes before being inoculation with *F. oxysporum* and *R. nigricans*(also separately).

A Completely randomized experimental design was employed involving three replications of each treatment. The garden soil used as planting medium was sterilized (at 80-90^{o}C for 2 hrs) then cooled, and filled into four-litre plastic buckets (¾ depth of the buckets). The buckets were pre-labeled accordingly. The inoculated yam setts were planted by burying them 2-3 cm deep below soil surface.

Parameters measured

The parameters measured within the first six weeks included sprouting characteristics, the number of leaves, leaf area (mm^2), length of shoot (cm), number of roots and rot index. The mean of data collected from the three

replicates was used for all computation reported here. [Sprouting in this study also describes the period after dormancy when tuber starts to show signs of re-germination [10]. Also, indexing for root-rot was recorded on a 0 - 4 Likertscale according to Sidhu and Webster [11]. Data collected(x) were transformed using the formula $\sqrt{x+\frac{1}{2}}$ before the data was subjected to Analysis of Variance (ANOVA) while means were separated with Least Significant Difference (LSD) test at 5% level of probability.

RESULTS AND DISCUSSION

Effect of plant extract and inoculation with fungi on number of roots produced per plant.
The number of roots produced on the yam setts at six weeks after emergence (6 WAE) was not affected among the botanicals - *A. sativum*and *O. basilicum*(Table 1). The tubers treated with these plant extracts produced a mean of 9 and 7 roots respectively. The number of roots produced by fungi inoculated yam setts however, was statistically significant (P< 0.05). Yam setts inoculated with *R. nigricans*produced a mean of 11 roots while yam setts inoculated with *F.oxysporum*had a mean of 5 roots. Roots produced by the un-inoculated setts (the control) were statistically lower than those of *R. nigricans*inoculated setts but higher than those of *F.oxysporum*inoculated setts.

Yam setts inoculated with *R. nigricans*and treated with *A. sativum*produced the greatest number of roots (13 roots) followed by setts inoculated with *R. nigricans*and treated with*O. basilicum*with 8.8 roots. The trend was the same with the effect of *O. bacilicum* on the number of roots produced in setts inoculated with the fungi*R. nigricans*that had greater number of roots compared to that of*F.oxysporum*that had 3.5 roots. This result shows thatthe extract from*A. sativum*had a greater antifungal effect on the yam setts. The effectiveness of extract of*A. sativum*inhibiting the growth of fungi in this experiment is in agreement with the work by Ankri and Mirelman[12]who reported that the inhibitory activity of *A. sativum*was due possibly to the presence of the active ingredient – *allicin*.

Effect of plant extract and inoculation with fungi mean root length produced per plant.
The result of Yam setts inoculated with *R. nigrican* shad statistically longer roots - mean of 31.5cm., compared to those of *F.oxysporum* inoculated setts - 15.4cm. Roots produced by the un-inoculated setts (the control) were statistically lower than those of *R. nigricans* inoculated setts (25.7cm.), but higher than those of *F.oxysporum* inoculated setts.

Setts inoculated with *R. nigricans* and treated with *A. sativum* had longer roots (37.22cm) compared with setts inoculated with *R. nigricans* buttreated with*O. Basilicum* that hada mean length of 25.8cm.Yam setts inoculated with *F.oxysporum*and treated either *A. sativum*or *O. bacilicum*were shorter than the control. Like result of the number or roots produced, it appeared that *A. sativum* had a good potential in suppressing rot development in yam setts.This result is consistent with the report of Ogbebor *et al.*,[13]who reported that *A. sativum* and *O. basilicum* exhibited a high inhibitory effect on fungal growth *in vitro*.

Effect of plant extract and inoculation with fungi on leaf area produced per plant
Largest mean leaf area of 42.45 mm^2 was observed in yam setts of the control subplots. This was followed by that of yam setts inoculated with *F. oxysporum* and applied with *A.sativum* with significantly lower leaf area - 22.36 mm^2 (Table 3). It is implied that *A. sativum* may perhaps contain inhibitory properties that depresses the activities of fungi than *O. basilicum*.

Effect of plant extract and inoculation with fungi on number of leaves produced per plant
Table 4 shows that the yam setts inoculated with *F. oxysporum* had the lowest mean number of leaves (2.71). The highest mean number of leaves (7.63) was recorded from the control, which was closely followed by that of *R.nigricans* treated yam setts (5.84). Yam setts protected with *Allium* had the highest mean number of leaves (6.32) followed by that of *O. bracilicum* that had the lowest number of 4.45leaves. Result of the interaction between fungi used to inoculate the yam setts and botanicals used as protectants was statistically significance (P < 0.05).

This result shows that extract of .*A. sativum* had a higher antifungal effect than *O. basilicum*as it enabled the yam vines to produce highest number of leaves.

The result also shows that setts not inoculated or protected with the botanicals had a mean number of leaves. Although this may not be immediately explainable but there might be more explanations if more test are conducted to confirm this trend.

Effect of plant extract and inoculation with fungi on tuber root index per plant

The result in Table 5, shows that mean when the yam setts were inoculated with fungi and protected with the two botanicals, *R.nigricans* had the same mean rot score as the control while the *F.oxysporum* treated yam setts had a mean of 0.84. The interaction between the treatments (extracts and fungi) was not significant. Therot score was serious enough to deter germination of the yam setts.Rot in yam setts protected with *A. sativum* extract was less severe than that of yam setts protected with *O. basilicum*extract.

This infers that *A. sativum* may control rot in yam minisetts better than that of *O. basilicum*hencethe low rot score - *A. sativum* (1.00) in relation to *O. basilicum* (1.50).

The result attained with *A. sativum* in this study confirms its importance as was earlier reported by Ankri and Mirelman[12]. They suggested that the plant extracts from *A. sativum*may exhibit strong antifungal properties. Okigbo and Ogbonnaya[10] had also earlier observed and defined rotting as softening of tissues arising from activity of fungi or bacteria. As witnessed in this current study, the growth of the yam setts could cause tissue softening which may not necessarily be attributed to rotting but as a result of the wound created from cutting of the setts.

The intensity of rotting,may vary with the type of fungal activities formed by the inoculants. For example, the yam setts treated with *R. nigricans* suffered a more severe rot (1.55) than those of *F. oxysporum*(1.00). But the effect of the growth of the yam setts and low temperature may as wellcause some degree of softening. Wounding induces signals that elicit physiological and biochemical responses in both adjacent and distant tissues hence tissue softening. [15; 16].

Emehute*et al.*, [17]established that the fresh cut surface of yam setts serve as entry points for microbial organisms while the raw flesh of the host encourages the massive explosion of other microbial activity.Normal sprouting of yam setts can be affected by (i) the pressure of microbial activities in the rhizosphere of the setts; (ii) amount of the available food reserves for the initiation of the sprouting and (iii)how soon the healing of the fresh cut surface will be. To reduce the incidence of these disease organisms in yam minisetts, Otoo*et al.,* (1987) recommended that minisett should be treated with a mixture of synthetic fungicids and wood ash. Some plant extracts have been used successfully to control diseases in tuber crops [18; 19; 20]. It was observed by Osai and Ikotun[21] that microbial rot of yam mini setts had been identified to be responsible for poor sprouting and growth of yam minisetts. *Aspergillusflavus, A. ochraceus, A. tamari, Trichodermaspand, Penicillium sp.* among others, have been identified to cause rots on tubers [22; 23]. *Ocimum*can been used to inhibit the growth of fungi in the tuber especially yam minisetts. An experiment carried out by Okigbo and Obgonnaya[10]observed that *F. oxysporum, A. flavus and P. chrosysogenum* organisms have been associated with the roots of yam tubers. These organisms have been associated with postharvest rots too [24; 25; 26]. From their investigations it appears that *Ocimum* have proved effective against mycelia and spore germination of many rot-causing microorganisms.

Allium is considered one of the principal antimicrobial constituent in *Allium sativum* as it was used in the perseveration of tuber from rottening[27]. It was reported that the bioactive effect of *Allium* spp. are attributed to the sulphur containing molecules and other metabolic product breakdown of these molecules that have received increasing attention for the antimicrobial efficacy [28].

Table 1: Mean number of roots produced at 6 weeks after emergence.

	Plant Extracts		
Fungi	*A. sativum*	*O. basilicum*	Mean
F.oxysporum	6.9[b]	3.5[c]	5.20
R. nigricans	13.3[a]	8.8[b]	11.05
Control	8.0[b]	7.9[b]	7.95
Mean	9.4	6.73	-

** Mean in the same row and column followed by the same letter are significantly different (P> 0.05) LSD (5%).*

Table 2: Mean root length (cm) per plant at 6 weeks after emergence.

	Plant Extracts		
Fungi	*A. sativum*	*O. basilicum*	Mean
F. oxysporum	16.39[c]	14.30[c]	15.35**C**
R. nigricans	37.22[a]	25.80[b]	31.54**A**
Control	26.21[b]	25.25[b]	25.73**B**
Mean	26.61	21.78	

** Mean in the same row and column not followed by the same letter are not significantly different (P> 0.05) LSD (5%)*

Table 3: Mean leaf area (cm^2) per plant at 6 weeks after emergence.

	Plant Extracts		
Treatment	*A. sativum*	*O. basilicum*	Mean
F. oxysporum	17.62[c]	19.48[c]	18.6B
R. nigricans	36.12[a]	48.78[a]	42.5A
Control	22.36[b]	6.71[c]	14.5C
Mean	25.37	24.99	

** Means in the same row and column followed by the same letter are not significantly different (P> 0.05).*

Table 4: Effect of the treatment of yam setts with plant extracts (*A. sativum*and O.*basilicum)* and inoculation with fungi on mean number of leaves per plant at 6 weeks after emergence.

	Plant Extracts		
Fungi	*A. sativum*	*O. basilicum*	Mean
F. oxysporum	3.06[c]	2.36[c]	2.71
R. nigricans	7.39[a]	4.31[b]	5.84
Control	8.5[a]	6.67[a]	7.63
Mean	6.32	4.45	

** Means in the same row and column not followed by the same letter are not significantly different (P> 0.05).*

Table 5: Mean yam sett rot index per plant at 6 weeks after emergence.

	Plant Extracts		
Treatment	*A. sativum*	*O. basilicum*	Mean
F. oxysporum.	0.67	1.00	0.84
R.nigricans	1.33	1.58	1.46
Control	1.00	1.92	1.46
Mean	1.00	1.50	

CONCLUSION

It is implied from this study that *A. sativum* may perhaps contain inhibitory properties that depresses rotting activities of fungi than *O. basilicum*andthat the use of these botanicals has helped boost the sprouting of yam setts which can certainly contribute to superior yield of yam seeds for the farmers.

Worthy of note also is that the cut yam setts protected with botanicals and planted immediately (at zero hours) give a better results than when planting was delayed. These test botanicals are available to farmers in tropical Africa and can therefore be readily accessible hence cutting on the use of highly expensive and rarely available synthetic fungicides.

The use of *extracts.fromA. sativum* and *O. basilicum* could be used as protectants against rot in yam minisettfurther popularizing the technology but more studies are needed to elucidate on the usable dosage.

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