Assessment of Two Varieties of Okra Grown on Spent Mushroom Substrate Amended Soil and their Associated Fungi

Sallet I., Chuku E. C. and Ajuru M. G.
Department of Plant Science and Biotechnology,
Rivers State University, Port Harcourt.

D.O.I: 10.56201/ijaes.v8.no3.2022.pg37.44

Abstract

Assessment of two varieties of okra grown on spent mushroom substrate amended soil and their associated fungi were carried out at the River State University Teaching and Research Farm. Spent mushroom Substrate collected from the Diplomat Farm was treated and three different concentrations (6.0, 12.0 and 18kg) were prepared and used as amendments on 12.5 kg soil, with the untreated soil as control. A total of four treatments were established Viz A1B1, A2B1, A3B1 and B as control for the field and green house experiments respectively. The local (C1) and improved (C2) varieties of okra were grown in a randomised complete block design in triplicates for the various treatments in the field. Fruiting of the treatment plants began at week 8 after planting in the field experiment for A1B1C1 and A2B1C1 (local variety) and A1B1C2, A2B1C2, A3B1C2 and BC2 (improved variety) treatments. Highest number of fruits (8.33±0.58) was recorded for the improved variety grown on A3B1C2 treatment. Meanwhile, the local variety on A2B1C1 treatment had highest fresh weight (9.35±0.07g) and dry weight (3.35±0.07g) of total weight of harvest. Fungi investigation on harvested fruits revealed the occurrence of three fungal organisms (Aspergillus sp., Mucorsp. and Penicilliumsp.). The local variety recorded more fungal organisms including Aspergillus and Mucor at incidence of 40% and 60% respectively. However, only Penicillium was recorded for the improved variety at an incidence of 100%. Generally, both varieties of okra completed their life cycle, although the amended soils supported better performance compared to those grown in the control soil. The improved variety had higher yield and less fungi contaminants than the local variety. Therefore, the cultivation of the improved variety should be encouraged.

Keywords: *Okra variety, yield and fungi*

INTRODUCTION

Spent mushroom substrate is the leftover of wastes after different flushes of mushroom have been harvested. It has been found that stems and other mushroom tissues removed from the beds during harvest were useful for organic fertilizers to supply plants with nutrients for maximum growth or to produce the best crops, (Forbes *et al* 2021, Gonani *et al*, 2011).

Forbes *et al* (2021), revealed that the spent mushroom substrate left over are of no use, its unplanned disposal caused land, water and air pollution together with the nuisance in the surrounding. It has been reported that, environmental legislation has forced mushroom growers to think about more amicable ways of spent mushroom substrate disposal.

Kwiatkowski *et al*, (2021) reviewed that farmers in different countries are using spent mushroom substrate as manure for various field crops and horticulture without any support of fertilizer. They further reported that, it has high organic matter, thereby making it desirable for use as a soil amendment or soil treatment. Spent mushroom substrate is used for growing media, for cultivation of different economical crops which includes (Pepper, Tomatoes, Cabbage and other vegetables) (Jasinaka, 2018; Roy *et al.*, 2015). Lou *et al.*, (2017) reviewed that, mushroom growers pasteurize the spent substrate with steam to kill any pests or pathogens that may be present in the substrate and also, the final pasteurization kills weeds, seeds, insects and organisms that may cause diseases. Research conducted by mushroom biotechnologists showed that treated spent mushroom substrate as a casing material reduces the incidence of diseases and also found effective in controlling apple scab and late blight diseases.

Eleamer (2020), reported that spent mushroom substrate is a by-product of mushroom cultivation with the potential to be used in the cultivation system to suppress plant pathogens, enhance water holding capacity, increase soil water aeration and mprove the soil structure through the input of organic matter or nutrient contained in the substrate, (Kadiri, and Mustapha 2020).

According to Elsakhawy, (2020) the advantage of using spent mushroom substrate as a soil fertilizer over chemical fertilizer include the fact that it achieve a slow-release of nutrients that will not burn crops upon application. Gbogolade *et al* (2012) explained that, spent mushroom substrate has a low bulk, density that indicates its porous medium that can enhance the structure of the soil it is amended with.

Orluchukwu *et al.*, (2018) repored that, application of spent mushroom substrate, which consists of degraded cellulose and lignin is considered to be important for the improvement of soil and safe for human consumption.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the Department of Plant Science and Biotechnology, Faculty of Science at the Rivers State Institute of Agriculture and Training (RIAT) farm, Rivers State University, Port Harcourt. (See appendix 1) The piece of land is situated at longitude 40 48”18, 50”N and Latitude 60 58”39, 12”E measuring 20m x 20m with a total area of 400m^2.

Sources of Okra Seeds Used for the study

The dried seeds of improved and local okra seeds (*Abelmoschus esculentus*) were utilized for this study. The improved seeds, okra Hire, were purchased at fruit garden park, Port Harcourt. Local seeds were also purchased locally at the Mile 3 market, Port Harcourt in Rivers State. The local okra seeds pods were collected in sterile polythene bags while the improved okra seeds were canned and collected using sterile polythene bags. Spent mushroom substrates of grown *Pleurotus ostreatus* were obtained from Dilomat Farms and Service Limited in Rivers State University.

Soil and Treatment Mixture

Spent mushroom substrates were the treatment used to enrich the soil nutrient before planting. The first treatment was 6.0kg of spent mushroom mixed with 12.5kg of soil (A_1B_1). The second treatment was 12.0kg of spent mushroom substrate mixed with 12.5kg of soil (A_2B_1) and the third treatment was 18.0kg of spent mushroom substrate mixed with 12.5kg of soil (A_3B_1). Finally, 12.5kg of soil (B_1) was also weighed alone which served as control. The ratio of soil treatment used is 1:1 (A_1B_1), 2:1(A_2B_1), 3:1(A_3B_1) that is, 1:2:3, before bagging.

Agronomic Practice

A land area of 20mx20m (400m^2) was marked out for the experiment. The plot was partitioned into 10m x 10m (200m^2) for the study.

The land was cleared, ploughed, harrowed (tilled) and leveled. The area was marked into blocks and plots in a randomized complete block design (RCBD). Each treatment had three replicates, each of the plot distance was 10m, and the subplots were 2m by 2m with 0.5m between each plot and 1m walkway between the plots. Each subplot in between was three subplots dimension of 3m by 3m with 0.5 distance for each of the subplots (Mbazu, 2021).

Harvesting

Harvesting was done manually with the use of a sharp knife when the okra pods were greenish every 2-3days, when the pods had reached 6.5-9.5cm in length and width. Harvesting of fresh pods continued until after 18 weeks.. Dried and fresh fruits were weighed using, the multi-meter muii56232740YSIODO optical D0176102826 device.

Mycological Studies

Preparation of Mycological Medium

Sterilization of conical flask, slides, petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass waves were sterilized in the oven at 120^{0}C for an hour after washing with soaps while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Chuku, 2009).

Inoculating loops and scalps were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was sabouraud dextrose agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wood and wrapped with aluminum foil. The conical flask containing the mycological medium was autoclaved at 121^{0}C and pressure of 101kg cm^{-3} for 15 minutes. The molten afar was allowed to cool to about 40^{0}C and dispensed into petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of Fungi

The direct plating method of Mehrotra and Aggarwal (2003) was adopted where samples of spoilt okra seeds were inoculated into Sabouraud dextrose agar in petri dishes containing Ampicillin to hinder the growth of bacteria and this was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25^{0}C ± 3^{0}C. The entire set up was observed for 7 days to ensure full grown organisms pure culture of isolates were obtained after a series of isolations. (Plate 3.10)

Identification of Fungi Organisms

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology mycelia structure and other associated structure using the keys of (Barnett and Hunter, 1998).

Determination of Percentage Incidence

The percentage incidence of fungal occurrence was determined by the formular stated below (Chuku *et al,* 2019).

$$\frac{X}{Y} \times \frac{100}{1} = \% \text{ Incidence}$$

Where;

X – Total number of each organism in a variety

Y – Total number of all identified organism in a variety.

Data Analysis

Data obtained from the above studies were subjected to analysis of variance (ANOVA). Duncan multiple range test was also used for mean separation with the aid of SPSS version 25.

RESULTS AND DISCUSSION

Table 1: Number Of Okra Fruits Harvested From Field (g)

Treatments	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18
A1B1C1	2.00±1.46^{a}	2.67±0.62^{a}	1.67±0.88^{a}	3.00±0.19^{b}	2.00±0.46^{b}	1.67±0.88^{b}
A2B1C1	1.33±0.30^{a}	2.33±0.04^{a}	2.33±0.04^{b}	1.33±0.31^{b}	2.33±0.04^{b}	2.67±0.61^{b}
A1B1C2	5.33±1.53^{c}	6.33±1.53^{c}	5.67±2.08^{c}	5.67±0.58^{cd}	4.33±2.52^{c}	3.00±1.00^{c}
A2B1C2	4.00±1.00^{b}	4.00±1.00^{b}	3.67±1.16^{b}	4.67±2.08^{c}	5.67±1.53^{c}	2.33±0.58^{c}
A3B1C2	5.67±2.52^{c}	8.33±0.58^{d}	7.00±2.65^{d}	7.67±0.58^{d}	6.00±1.00^{c}	4.00±2.00^{c}
B1C2	1.33±0.58^{a}	2.00±1.00^{a}	1.33±1.13^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

*Mean with the same superscript across the coloums are not significantly different (P ≤

A1B1C2 Improved Okra grown on soil treated with 6.0 kg of Spent Mushroom Substrates

A2B1C2 Improved Okra grown on soil treated with 12.0 kg of Spent Mushroom Substrates

A3B1C2 Improved Okra grown on soil treated with 18.0 kg of Spent Mushroom Substrates

B1C2 Improved Okra grown on control

Table 2: Total Weight Of Okra Harvest From Field

Treatments	Fresh (g)		Dry (g)	
	Local	Improved	Local	Improved
A1B1C1	4.35±0.07	-	0.75±0.07	-
A2B1C1	9.35±0.07	-	3.55±0.07	-
A1B1C2	-	4.45±0.07	-	0.23±0.00
A2B1C2	-	2.55±0.07	-	0.05±0.00
A3B1C2	-	0.42±0.00	-	0.20±0.14
B1C2	-	0.65±0.07	-	0.01±0.00

A1B1C1	Local Okra grown on soil treated with 6.0 kg of Spent Mushroom Substrates
A2B1C1	Local Okra grown on soil treated with 12.0 kg of Spent Mushroom Substrates
A1B1C2	Improved Okra grown on soil treated with 6.0 kg of Spent Mushroom Substrates
A2B1C2	Improved Okra grown on soil treated with 12.0 kg of Spent Mushroom Substrates
A3B1C2	Improved Okra grown on soil treated with 18.0 kg of Spent Mushroom Substrates
B1C2	Improved Okra grown on control

Table 3: Fungal Percentage Incidence of harvested fruits

Fungal Isolate	Local variety (%)	Improved variety (%)
Aspergillus sp	40	-
Mucor sp	60	-
Penicillium sp	-	100

The improved and local okra grown in the field produced fruits as shown in Table 1. Both improved and local okra flowered at 4 WAP and continued to flowered at 18 WAP in the field.

The present study, revealed the number of fruits harvested after planting. The improved okra had highest fruits recorded (8.33 ± 0.58) from plot treated with 18.0kg of SMS at 10 WAP and local okra treated with 12.0kg of SMS at 10-12 WAP recorded (2.00 + 1.00) at 10 WAP.

From the data collected, the improved okra had highest fruits length value (7.50) cm from plots treated with 12.0kg of SMS at 14 WAP and local okra recorded (2.53) cm at 14 WAP. While improved okra grown on control recorded (4.80) cm and local okra grown on control recorded (0.00) cm at 12 WAP.

Also, the improved okra had highest width value (9.43) cm from plots treated with 6. 0kg of SMS at 14 WAP and local okra recorded (3.67) cm from plots treated with 6.0kg of SMS at 12 WAP. While improved okra grown on control recorded (3.33) cm at 10 WAP and local okra grown on control recorded (0.00) cm at 10 WAP.

Generally, improved okra had highest fruits than local okra. Improved okra grown on 6.0kg, 12.0kg, and 18.0kg of SMS had total number of fruits (29, 22, and 41), respectively and local okra grown on 6.0 kg and 12. 0 kg of SMS had fruits number (13 and 12) respectively. While improved okra grown on control recorded (14) fruits and local had (0) fruits.

Table 2 showed weight of okra harvested from field. Local okra had high fresh and dry weight (9.35g and 3.55g), respectively from plots treated with (12.0kg) of SMS and improved okra had (4.45g), fresh and (0.23g) dry. While improved okra grown on control recorded 0.65g fresh and (0.01g) dry and local okra recorded (0.00g). Therefore, improved okra had the highest yield on plot treated with 18.0kg, 6.0kg and 12.0kg of SMS respectively followed by local okra grown on 6.0kg and 12. 0kg of SMS only. While control had the lower yield for improved okra and for local no yield at 18 WAP. This is an indication that soil treated with high organic matter promote growth of plant and yield as reported by Aliyu (2001), Mbazu (2001) and Parcede *et al*., (2020).

Table 3 showed the fungal pathogens isolated from the fruits of local and improved okra. Form the result shown, it was observed that local okra were infected with *Aspergilus niger (40%)* and *Mucor (60%) spp* while improved okra were infected with *Penicillium italicum (100%).* The number of fungi identified in this table is lower than that reported by Ezekiel and Sombie (2014), in Ogun State Nigeria where eleven (11) fungal Pathogens were identified on okra fruits.

Ocimati *et al*, (2021) showed that spent mushroom substrates suppressed pathogens when used to grow crops. The substrate of spent mushroom has been reported to contain a wide diversity of microorganisms with strong antagonism towards pathogenic fungi (Schwartz, 2021).

CONCLUSION

Both varieties of okra have shown different potentials when cultivated on spent mushroom amended soils. However, the amended soils supported higher yield than the control soil. The improved variety performed better than the local both in terms of yield and fungi contamination.

REFERENCES

Aliyu, L. (2001). Effect of Nitrogen & Phosphorus on growth, & yield of sweet pepper *Capsicum annuum L. Journal of Agriculture and Environment*, 2 (2),243 – 251.

Bamett, H. L. & Hunters, B. B. (1998). Illustrated general of imperfect fungi, 4th edition. American pytopathological society press, St Paul Minnesota, 218.

Cheesebrough, M., (2000). *Distinct laboratory practice in tropical countries part 2.* Cambridge University Press London. 143 – 156.

Chuku, E. C., Agbagwa, S.S. & Worlu, C., (2019). Nutrient quality and associated spoilage Fungi of English Pear *(Pyrus communis L). International journal of Agriculture, Environment and Bioresearch,* 4 (6), 317-325.

Chuku, E. C. (2009). Fungi responsible for the spoilage of Plantain (Musa paradisiacal at various ripening stages. *Acta Agronomica Nigerian,* 9 (1 & 2), 35 – 40.

Eleamer, H. (2020). *The effect of spent mushroom compost on the indigenous rhizorspheremicrobiota in strawberry cultivation. Swedish University of Agricultural Science. Special Liason unit*, 4 -7.

Elsakhawy, J. A., & Abed, EL- Rahem, W. T, (2020). Evaluation of spent mushroom substrates extract as a biofertilizer for growth improvement of rice (Oryza sativa L.). *Egypt Journal of Soil Science,* 60,131-412.

Ezekiel, C. N. & Sombie, J. I. (2014), Survey of Aflatoxins and fungi in some commercial breakfast cereals and pastas retailed in Ogun State, Nigeria. *Journal of Natural Science* 6.27,32.

Forbes, B.M., Charity, P., Moses, M.C. G. & wonder, N., (2021). Effect of spent bulton mushroom substrate on yield and quality of Baby Spinach (*Spinaciaoleracea*). *International of Agronomy, 9, (https/idoi org 10.1/55/2021/6671647).*

Gbogolade, S.I., Lawal. M.M. & Oyetu, J. O. (2012). Effects of spent mushroom compost. *International Journal of Agriculture and Earth Science,* 4, (20),2489 - 2492.

Gonani, R. Z., Riabi, H. & Sharif, K. (2011). Impact of using leached spent mushroom compost as a partial growing media for horticultural plant. *Journal of Plant Nutrition* 34, (3),337– 348.

Jasinaka, A. (2018). "Spent mushroom compost, (SMS) – Retrieved added value product closing loop in Agricultural production". *Acta Agarar Debeceniesis,*150, 185 – 202.

Kwiatkowshi, C.A., Harasim, E. (2021). The effect of fertilization with spent mushroom substrate and traditional methods of fertilization of common thyme *(Thymus vulgaris L.)* on yield quality and Antioxidant properties of Herbal material. *Journal of Acta Scientarumpolononum Technologia Alimentaria,*1644-1730**.**

Luo, Z., sun, Y., Zhou., x, Biag., B, Hu., & Xu., X., (2017). " Composition variability of spent mushroom substrates during continuous cultivation, composting process and their effects on mineral Nitrogen transformation in soil. *Geoderma*, 307, 30-37.

Mbazu, C. (2021). Okra cultivation guide. The step – by – step okra farming procedure Africa publisher 31.

Mehrotra, R. S. & Aggawal A. (2009). *Phythopathologicaltechniques in plant pathology in plant pathology* 2nd edition. Tata Mcaraw-Hill Publishing Company Limited, 821.

Ocimati, W., were, E., Tazube, A.F, Dita, M., Zheng, S., & Guy, B. (2021). Spent pleurotus ostreatus substrate has potential for managing Fusarum Wilt of banana. *Journal of Fungi*, 7, 946-949.

Orluchukwu, J.A. & Okosa EU., (2018). Effect of spent mushroom substrate poultry manure on growth and yield of okra crop. *International Journal of Agriculture & Earth Science*, 2 (23) 2489-2492.

Parcedes, C. M., Moral, E., Perez- Murcia, M.O. & Bustamante, M.A., (2020). Effects of spent mushroom substrate and poultry manure on growth and yield of okra (*Abelmoschusescuientus L. moench)* in Port Harcourt Rivers State, *Journal of Soil Science*, 15, 78-95.

Roy, S., Shibu, B., Chakraborty, U. & Charaborty, B., (2015). "Evaluation of spent mushsroom substrate as Biofertilizer for growth improvement of *Capsicum nannum L*". *Journal of applied Biology and Biotechology*, 3, 3, 22-27.

Schwartz, H. F. (2021). *Foot roots of dry beans fact sheet crop series diseases*. Colarado state University Cooperative, Extension Service. 2938 – 2940.