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EVALUATION OF POLYPHENOL EXTRACT FROM BIO-FORTIFIED *Pleurotus pulmonarius* MUSHROOM AGAINST OKRA (*Abelmoschus esculentus*) PLANT DISEASE

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ABSTRACT
Okra (Abelmoschus esculentus) is a popular vegetable crop belonging to the family of Malvaceae. Even though identification of Fusarium oxysporium as the major pest of Abelmoschus esculentus has been widely done, information about the healthy management of this pest in okra production remains scarce. Therefore, the objective of this study was to determine the effects of the polyphenol extract of Pleurotus pulmonarius mushroom against Fusarium oxysporium disease incidence and severity in two different okra varieties as well as to determine the effect of the extract on the agronomic responses, yield and yield components of the two okra varieties. The experiment was conducted at the Teaching and Research Farm of Department of Crop Science, University of Nigeria, Nsukka. It was a 5 × 2 factorial experiment laid out in a Randomized Complete Block Design (RCBD) with three replications and ten treatments, having 5 concentrations of the mushroom extract (100%, 75%, 50%, 25% and 0%) and 2 varieties of okra (hire (H) and clemson spineless (C)). A two-way analysis of variance (ANOVA) was used to evaluate the influence of the two independent variables (concentrations, varieties) on the disease incidence, severity, agronomic responses, yield and yield components of the two okra varieties. Result showed that the main effect of concentrations of the extract on the number of leaves were significantly higher (p<0.05) from weeks 1 to 8, except at week 3. The main effect of concentration of the extract on the plant height was significantly higher at 25%. The main effects of the two okra varieties on plant height were significantly higher (p<0.05) from week 2 to week 5, while the interaction of varieties by concentrations of extract on the plant height were significantly higher at 100% (33.6 cm) and 75% (32.9cm) for clemson spineless (C) and hire (H), respectively, at week 8. The main effects of the two okra varieties on the Fusarium oxysporium disease incidence were significantly higher (p) at weeks 3 and 4 while the interaction by concentration of extract on disease incidence was not statistically significantly at all stages of growth (from week 3 to week 8). The interaction of varieties by concentrations on yield (fruit weight per hectare) was significantly higher at 75% and 50% concentrations which gave 3984 ton/ha and 3692 ton/ha for hire (H) and clemson spineless (C) respectively. However, the difference in the yield and yield parameters at 75% and 50% concentrations of the extract was not statistically significantly. It is therefore recommended that 50% concentration of Pleurotus pulmonarius mushroom extract be used for development of bio pesticide for the two okra varieties since the plants had better responses at that very concentration.

Keywords: *Pleurotus pulmonarius,* evalution, *Abelmoschus esculentus,* extract

INTRODUCTION
Okra (*Abelmoschus esculentus*) is a popular vegetable crop from the *Malvaceae* family with an edible green seed pod. It is known as lady's finger in some English-speaking countries (Ayto, 2002). It is also called *ọ́kwùrù* in Igbo land in South Eastern Nigeria (Merriam-Webster Dictionary, 2020). Okra originated in East Africa in Ethiopia, Eritrea and Sudan (Muimba-Kankolongo, 2018). According to Goucher (2013), okra can be traced

back to Africa, where it is believed to have been domesticated more than 6,000 years ago. The plant then spread to other parts of the world through trade and migration. Okra was introduced to the Americas during the transatlantic slave trade and has since become a popular crop in many countries, including the United States, Europe and Asia (Okereke *et al*., 2017). Okra is cultivated in tropical, subtropical and warm temperate regions of the world where it is used in the cuisine of many countries (National Research Council, 2006). It is one of the oldest cultivated crops and at present grown in many countries and is widely distributed from Africa to Asia, southern Europe and America (Kumar *et al.,* 2013). As a member of the *Malvaceae* family, okra is related to such species as cotton, cocoa, and hibiscus (Satya *et al*., 2016) and therefore needs proper management practices to get optimum yield. Okra production is faced with many challenges but, the most prominent among them that affects drastically the fruit production in okra is the wilt disease. The most common wilt disease afflicting the okra plant in humid tropical environment are the fusarium wilt, caused by *Fusarium oxysporum***,** a soil-borne fungus that attacks the vascular system and the verticillium wilt, which causes the yellowing and wilting of the leaves.

Fusarium oxysporum has been reported to cause attacks of voracious and destructive nature in crops (Edmunds and Pottorff, 2012). Amuji (2013) had reported *Fusarium oxysporum* to cause greater percentage of losses in tomato yield in south eastern Nigeria. This is because *Fusarium oxysporium* attack the vascular system of plants, restricting water and nutrient flow, ultimately leading to wilting and death of the affected plant. Providentially, Chinekwu *et al.* (2023) had reported the efficacy of mushroom extract against *Fusarium oxysporium.* This is because mushrooms are rich in polyphenols which can kill or inhibit the growth of *Fusarium oxysporium* and many other fungi. Polyphenols are collective term for several sub groups of phenolic compounds (Tsao, 2010). Many mushroom species produce secondary metabolites which are polyphenolic compounds, polyketides, terpenes and steroids that have medicinal properties (Subbulakshmi and Kannan, 2016). Some of these secondary metabolites of mushrooms have antimicrobial properties and can be used against pathogens. Mushrooms have also natural polysaccharides which possess health-promoting properties and a lot of beneficial therapeutic effects (Omomowo *et al.*, 2016). Egwim *et al*. (2011) reported the presence of valuable pharmaceutical substances in mushroom which have a stimulating effect and act as anaesthetic in ophthalmology, powerful pain reliever and antipuretic action among other uses. Some mushrooms or mushroom extracts are used in the treatment of various diseases (Borchers *et al.,* 2008). This is why the polyphenol contents of *Pleurotus pulmonarius* mushroom were used in this research against *Fusarium oxysporium* that attacked two okra varieties. The *Pleurotus pulmonarius* mushroom polyphenol was first improved through biofortification. This was why *Gongronema latifolia leaves* which are rich in polyphenol were used as mushroom substrate to cultivate *Pleurotus pulmonarius* mushroom. The whole idea being that the *Pleurotus pulmonarius* mushroom will hydrolyse the *G.latifolia* leaves mushroom substrate and absorbs the *G.latifolia* polyphenol to add to its contents through bio-fortification.

Objective of the study

The main objective of the study was to evaluate polyphenol extract from bio-fortified *Pleurotus pulmonarius* mushroom against okra (*Abelmoschus esculentus*) plant disease.

The specific objectives of study were to:

- Determine the in-vivo activities of the *Pleurotus pulmonarius* mushroom extract against *Fusarium oxysporium* disease incidence and severity on the two different okra species.
- Determine the effect of the *Pleurotus pulmonarius* mushroom extract on the agronomic responses, yield and yield components of two okra species.

MATERIALS AND METHODS

Study location

The experiment was conducted at the Department of Crop Science, University of Nigeria, Nsukka Research Farm, from May to August, 2023. Nsukka is located in the derived savannah ecological zone on the latitude 06˚ 51'E, longitude 07° 29'N, 475m above sea level). It is characterized by lowland humid condition with bimodal annual rainfall distribution of 1155mm to 1955m (with peak in July and September with a short break in mid-August), 29˚C to 31˚C mean annual temperatures and a relative humidity ranging from 69% to 70% (Uguru *et al.,* 2011).

Field layout

The layout was a 5 × 2 factorial in a Randomised Completely Block Design (RCBD) with three replications. The factors were 5 different concentrations **(**100%, 75%, 50%, 25% and 0%**)** of extract of *Pleurotus pulmonarius* mushroom cultivated in *Gongronema latifolia* substrate and 2 different varieties of okra (clemson spineless (C) and hire (H)). The plots were separated by a path of 50cm while blocks were kept at a distance of 1m apart. Crop spacing was 60 × 45 cm. Okra seeds wer sown in holes containing 5mls suspension of a colony forming unit (CFU) of *Fusarium*

oxysporium. All cultural practices were observed before and after planting.

Preparation of *Pleurotus pulmonarius* Mushroom Extract

One kilogram of dried *Pleurotus pulmonarius* mushroom grown in *G. latifolia* substrate were harvested, pulverized and extracted with 2.5L of 80% absolute methanol. The extract was concentrated using rotary evaporator in the Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka.

Treatment application

Mushroom extract of 1g, 0.75g, 0.50g, 0.25g and 0% were placed in five different containers with labels. Thereafter, 10 ml of distilled water was poured into the various containers including the 0% labelled container (control). The containers were stirred to dissolve the extract and 36 seeds of each variety were immersed in the extract for 10 minutes before they were planted in the field.

Data collection

Data were collected on seedling emergence, stage, growth parameters; flowering, fruiting and yield.

Emergence stage: Data were collected on the following days: Days to 1st seedling emergence, days to 50% seedling emergence, and days to 100% seedling emergence.

Growth stage: agronomic data were collected during the growth, flowering and at harvest.

Disease incidence: The disease incidence and severity were assessed as described by Wokocha, (1990). The following disease index scale was used for disease severity.

0 = No visible disease symptom
1 = Less than 15% infested
2 = 15 – 35% infested
3 = 36 – 49% infested
4 = 50 – 74% infested
5 = more than 75% infested.

Statistical Analysis

All the data obtained were subjected to analysis of variance (ANOVA) according to the procedures outlined for randomized complete block design (RCBD) experiments using GenStat 10.4 analytical software. Separation of treatment means were done using Fisher's least significant differences (F-LSD) at 5% probability level

RESULTS

The result of the main effect of concentration of the extract on the number of leaves is shown in Table 1. There were significant differences on the number of leaves at weeks 1, 2, 4, 5, 6, 7 and 8. The number of leaves at week 3 was statistically the same in all treatments. At week 8, 75% concentration gave significantly higher number of leaves. The main effects of the two okra varieties on the number of leaves were significantly different at only week 1 with Clemson leading. There was no significant difference on the number of leaves in other weeks (Table 2).

The interaction of concentrations by variety on number of leaves was significant from week 1 to week 8 (all through the course of growth) as shown in Table 3. The result of the main effect of concentrations of the extract on plant height was significantly higher at week 8. Although, the plant height was significantly higher at 100%, it was statistically the same with that of 75% concentration (Table 4).

Table 1: Main effect of concentrations of the extract on the number of leaves

Concentration	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
0%	0.83	2.92	4.67	3.75	3.83	4.08	3.67	3.33
25%	2.50	3.75	4.92	6.00	4.83	6.00	5.00	3.92
50%	2.42	4.25	5.17	6.17	5.08	5.75	5.50	5.17
75%	2.42	4.92	5.67	7.25	7.00	7.08	7.00	6.33
100%	2.50	3.75	4.79	7.00	6.50	7.58	7.17	5.17
Lsd (p<0.05)	0.732	0.745	NS	1.659	1.725	1.823	1.811	2.310

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant

Table 2: Main effects of varieties on number of leaves

Variety	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C	2.47	3.97	5.03	5.63	5.47	5.70	5.37	4.23
H	1.80	3.87	5.05	6.43	5.43	6.50	5.97	5.33
Lsd(p<0.05)	0.463	NS	NS	NS	NS	NS	NS	NS

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant

Table 3: Interaction of concentration by variety on number of leaves

Conc*Var	WK1	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C0	1.17	3.17	4.50	4.00	3.83	4.17	3.17	2.33
C25	2.50	3.50	4.50	6.00	5.33	7.00	7.33	6.50
C50	2.33	3.50	4.33	4.67	3.83	4.00	4.00	2.83
C75	3.33	5.17	5.67	6.17	6.33	5.67	5.17	4.17
C100	3.00	4.50	6.17	7.33	8.00	7.67	7.17	5.33
H0	0.50	2.67	4.83	3.50	3.83	4.00	4.17	4.33
H25	2.50	4.00	5.33	6.00	4.33	5.00	2.67	1.33
H50	2.50	5.00	6.00	7.67	6.33	7.50	7.00	7.50
H75	1.50	4.67	5.67	8.33	7.67	8.50	8.83	8.50
H100	2.00	3.00	3.41	6.67	5.00	7.50	7.17	5.00
Lsd (p<0.05)	1.035	1.053	1.444	2.346	2.440	2.579	2.561	3.266

C = Clemson spineless, H = Hire, Var = variety, LSD = Least significant difference, WK = week, NS = Not significant.

Table 4: Main effect of concentration of the extract on plant height

Concentration	WK2	WK3	WK4	WK5	WK6	WK7	WK8
0	3.18	4.02	5.31	5.95	9.20	12.03	14.8
25%	5.16	6.15	7.86	9.11	13.28	15.24	17.0
50%	5.83	6.53	8.27	9.50	13.54	19.51	22.0
75%	6.38	7.44	9.52	11.19	16.83	21.50	28.0
100%	4.88	6.83	9.04	10.84	17.43	21.65	28.1
Lsd (p<0.05)	1.191	1.517	1.886	2.837	4.522	5.759	9.17

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant.

The main effects of the varieties on plant height were significantly higher in Clemson spineless variety at weeks 2, 3, 4 and 5, but statistically the same at weeks 6, 7 and 8. Clemson variety was higher than the hire variety at all the weeks except at week 8 (Table 5). The interaction of concentrations by variety on plant height was significantly higher at week 8 for Clemson spineless variety at 100% extract although statistically the same with that of 75%. The interaction of concentration by variety on plant height at 0% was significantly lower in both Clemson spineless and Hire varieties (Table 6). The main effect of concentration of the extract on disease incidence on leave had significant differences at weeks 3 and 4 of the plant growth while weeks 5 through 8 were statistically the same (Table 7). The effect of 75% concentration of the extract on disease incidence on leave at week 8 was significantly higher although, statistically the same with 50% and 25%.

The main effects of varieties on disease incidence on plant leaves were statistically non significant at weeks 3, 4 and 5 but, Clemson spineless variety was significant higher at weeks 6, 7 and 8 (Table 8). The interaction of variety by concentration of extract on disease incidence on plant was significantly higher in Clemson spineless at 75% concentration throughout the growth period of the plant but statistically lower in hire variety at 0% concentration (Table 9). Table 10 below showed the interaction of variety by concentration on fruit weight per hectare and average fruit weight per plant (Table 10). At 75% concentration, hire variety gave significantly higher weight of fruit per hectare of 3984 ton/ha while Clemson spineless variety produced 3692 ton/ha. The average fruit weight per plant was statistically the same at 25% and 50% concentrations for both hire and Clemson spineless varieties.

Table 5: Main effect of the two okra varieties on plant height

VARIETY	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C	5.94	7.18	9.43	10.95	15.12	18.37	21.2
H	4.23	5.21	6.54	7.70	12.99	17.61	22.8
Lsd (p<0.05)	0.753	0.959	1.193	1.794	NS	NS	NS

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant.

Table 6: Interaction of concentration by variety on plant height

Conc*var	WK2	WK3	WK4	WK5	WK6	WK7	WK8
C0	4.37	5.05	6.25	7.32	11.38	11.98	14.3
C25	5.80	7.27	9.80	11.88	15.98	20.15	19.8
C50	5.50	5.83	7.72	8.38	9.33	13.27	15.0
C75	7.97	8.93	11.13	12.17	16.92	19.47	23.1
C100	6.08	8.82	12.25	15.00	22.00	26.97	33.6
H0	2.00	2.98	4.37	4.58	7.02	12.08	15.3
H25	4.52	5.03	5.92	6.33	10.58	10.33	14.2
H50	6.15	7.23	8.82	10.62	17.75	25.75	29.0
H75	4.80	5.95	7.90	10.22	16.75	23.53	32.9
H100	3.67	4.83	5.83	6.75	12.87	16.33	22.7
Lsd(p<0.05)	1.684	2.145	2.668	4.012	6.395	8.144	12.96

C = Clemson spineless, H = Hire, Var = variety, LSD = Least significant difference, WK = week, NS = Not significant.

Table 7: Main effect of concentration of the extract on *Fusarium oxysporium* disease incidence on leave

Concentration	WK3	WK4	WK5	WK6	WK7	WK8
0	3.17	4.167	4.500	4.333	4.667	4.667
25%	2.58	3.917	4.333	4.000	4.833	4.833
50%	2.25	3.583	4.250	4.000	4.583	4.583
75%	3.67	4.167	4.500	4.417	4.667	4.667
100%	2.42	3.250	4.250	4.500	4.667	4.667
Lsd (p<0.05)	0.996	0.5277	NS	NS	NS	NS

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant

Table 8: Maineffect of the two okra varieties on *Fusarium oxysporium* disease incidence on plant leaves

VARIETY	WK3	WK4	WK5	WK6	WK7	WK8
C	2.75	4.067	4.467	4.500	4.800	4.800
H	2.90	3.567	4.267	4.000	4.567	4.567
Lsd(P<0.05)	NS	NS	NS	0.3315	0.1796	0.1796

C = Clemson spineless, H = Hire, LSD = Least significant difference, WK = week, NS = Not significant

DISCUSSION

The okra variety that had statistically the same number of leaves across the various growth stages (except at week 1) could be as a result of the same inherent trait in them. This similar trait might have led to the same adaptation pattern in each of the variety in terms of leaf development. The interaction of concentrations by variety on number of leaves showed that at 75% and 100% concentrations there was higher production of leaves in Clemson spineless, while that of hire variety at 50% and 75% concentrations had the best productivity. The bio pesticide effects of the polyphenol extract of *Pleurotus pulmonarius* mushroom can be harnessed at the range of 50 to 75% concentrations for economy of raw material in treatment of these two okra varieties. Eze and Ogonnaya (2010) had also reported the efficacy of *Physcia grisea* plant extract in the treatment of fungal pathogen.

The polyphenol extract of *Pleurotus pulmonarius* mushroom at the concentrations of 50%, 75% and 100% produced statistically the same effect on plant height at Week 8 after planting. This shows that these three concentrations could be considered in seed treatment for the two varieties of okra. However, since 50% concentration gave statistically the same effect with that of 75% and 100%, it is recommended that lesser concentration (75%) be used in bio-pesticide formulation for economy of raw material sake. The plant height generally increased progressively with age across the two okra varieties in all the concentrations of the extract. Dimkpa and Diepriye (2019) had similar finding in their field evaluation of some okra varieties in River State; they also observed that Clemson variety had significantly higher growth over hire and other varieties in River state, Nigeria. Clemson spineless treated with 75% concentration of extract (C75) and clemson spineless treatment with 100% concentration of extract (C100) gave the highest plant height

while hire treatment with 50% concentration of extract (H50) and hire treatment with 75% concentration of extract (H75) gave the best result in hire variety. For economy of raw materials, 50 to 75 percent formulation with polyphenol extract of *Pleurotus pulmonarius mushroom* will give optimum of bio pesticide activities for the okra varieties.The interaction of variety by concentration of extract on disease incidence on plant physiology that was significantly lower in clemson spineless treated with 25% concentration at week 3 after planting showed the efficacy of the extract and the phytotoxicity of extract at the plant juvenile stage. The interaction of variety by concentration of extract on disease incidence that were statistically the same at C50, C100, H50 and H100 showed that polyphenol extract of *Pleurotus pulmonarius* mushroom at the range of 50 to 100% concentration will produce effective bio-pesticide action in the treatment of *Fusarium oxysporium* disease in the okra varieties. Chinekwu *et al.*(2023) had also reported the in vitro efficacy of *Ganoderma lucidum* mushroom extract against *Fusarium oxysporium.* Overall, 75% concentration of the extract gave significantly higher fruit weight per hectare (3984 t/ha) and average fruit weight although, statistically the same with the yield of 3692 t/ha produced at 50% concentration.

Table 9: Interaction of variety by concentration of extract on *Fusarium oxysporium* disease incidence on plant leaves.

Conc*var	Wk3	Wk4	WK5	WK6	WK7	WK8
C0	3.33	4.333	4.500	4.500	4.833	4.833
C25	2.00	4.000	4.333	4.167	4.667	4.667
C50	2.00	3.500	4.500	4.333	4.833	4.833
C75	3.67	4.500	4.667	4.500	5.000	5.000
C100	2.67	4.00	4.333	5.000	4.667	4.667
H0	3.00	4.000	4.500	4.167	4.500	4.500
H25	3.17	3.833	4.333	3.833	5.000	5.000
H50	2.50	3.667	4.000	3.667	4.333	4.333
H75	3.67	3.833	4.333	4.333	4.333	4.333
H100	2.17	2.500	4.167	4.000	4.667	4.667
Lsd(p<0.05)	1.409	0.7462	0.5374	0.7413	0.4016	0.4016

C = Clemson spineless, H = Hire, Var = variety, LSD = Least significant difference, WK = week, NS = Not significant.

Table 10: Interaction of variety by concentration on fruit weight per hectare and average fruit weight per plant

Var × Conc	Weight per hectare (tons/ha)	Average fruit weight (g)
C0	381	25
C25	3127.5	42
C50	3692	38
C75	3084	30
C100	1848	31
H0	406	20
H25	1431.5	41
H50	1464	42
H75	3984	42
H100	3104	39
LSD (p<0.05)	295	5

C = Clemson spineless, H = Hire, Var = variety, Conc= concentration, LSD = Least significant difference

CONCLUSION/RECOMMENDATIONS

In conclusion, 75% concentration of polyphenol extract of *Pleurotus pulmonarius* mushroom gave significantly higher yield although, statistically the same with that of 50% concentration. It is therefore recommended that 50% concentration be used for development and production of bio-pesticide for treatment of these two varieties of okra. Information gained from this study may be used as a baseline for future pest control in related studies on other family members of *Malvaceae*.

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