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Combined Effects of *Ocimum gratissimum* and Soil-borne Phytopathogenic Fungi on Seedling Growth of Quality Protein Maize

M. A. Abiala[1], A. O. Akanmu[2*], A. C. Oribhaboise[2] and T. Aroge[3]

[1]Department of Biological Sciences, Mountain Top University, Prayer City, Ogun State, Nigeria.
[2]Department of Botany, University of Ibadan, Ibadan, Nigeria.
[3]Laboratory of Plant Pathology, College of Plant Protection, Jilin Agricultural University, Changchun 130118, Jilin Province, P.R. China.

Authors' contributions

This work was carried out in collaboration among all authors. Author MAA designed the study and wrote the protocol. Author AOA performed the statistical analysis and wrote the first draft of the manuscript. Authors ACO and TA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The soil borne phytopathogenic fungi; *Fusarium verticillioides*, *Fusarium solani* and *Curvularia lunata* were investigated separately and as combinations for their pathogenicity and possible control with extracts of *Ocimum gratissimum* on the seedlings of Quality Protein Maize (QPM) varieties (*Zea mays* L.); ILE 1 and SW 5. The experiment was factorial based and laid out in a completely randomized design, replicated thrice. Data collected on the percentage germination, growth characters and disease severity were statistically analysed. The pathogenic effect of *F. solani, F. verticillioides* and *C. lunata* in their factorial combinations significantly ($p<0.05$) inhibited seed germination of the QPM maize varieties, and showed high disease severity on ILE1 (31.62%) than SW5 (30.37%). Application of *O. gratissimum* extract at 0.5 g/ml concentration significantly ($p<0.05$) enhanced seed germination and growth characters of both maize varieties.

**Corresponding author: E-mail: akinakanmu@gmail.com;*

More so, *O. gratissimum* extract selectively ($p<0.005$) reduced disease severity on SW5 but showed relative effect on ILE1 with respect to number of leaves, plant height and leaf area. The extract of *O. gratissimum* is therefore a potent phytofungicide in the management of phytopathogenic fungi of QPM.

Keywords: Phytopathogens; Quality Protein Maize (QPM) extracts; Ocimum gratissimum; germination; disease severity.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production [1]. The maize grain accounts for about 15 to 56% of the total daily calories in diets of peoples in about 25 developing countries, particularly in Africa and Latin America [2]. With its high content of carbohydrates, fats, proteins, some important vitamins and minerals, it is regarded as a major cereal crop for both livestock feed and human nutrition worldwide. Millions of people, particularly in the developing countries derive their protein and calorie requirements from maize [3]. However, maize contains on an average about 2% lysine, which is less than one-half of the concentration recommended for human nutrition by the Food and Agriculture Organization (FAO) of the United Nations [4]. Therefore, the need to genetically ameliorate the poor nutritional value of maize grains [5], has led to the successful development of QPM [3,6].

This protein-rich maize - QPM has been implicated with phytopathogenic infections [6,7]. Maize streak virus (MSV) transmitted by *Cicardulina* spp. and southern leaf blight caused by the fungus *Bipolaris maydis* are among the diseases of QPM in tropical environments [8]. The incidences of *Curvularia* leaf spot, caused by *Curvularia lunata,* maize ear rot, caused by *Fusarium moniliforme* and maize rust incited by *Puccinia polysora* have earlier been reported [9-12]. Therefore, this study evaluates the pathogenicity potentials of *Fusarium verticillioides, Fusarium solani* and *Curvularia lunata* most especially in their combinations and their possible control by *Ocimum gratissimum* extracts with respect to seedlings of QPM varieties.

2. MATERIALS AND METHODS

2.1 Sources of Planting Materials and Phytopathogenic Fungi

The seeds of two QPM varieties ILE 1 and SW 5 were obtained from the Institute of Agricultural Research and Training (IAR&T) Ibadan, Oyo state, Nigeria. While the isolates of phyto-pathogenic fungi; *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata*, were obtained from the Plant Pathology / Mycology laboratory, Department of Botany, University of Ibadan, Nigeria.

2.2 Source of Plant Extract

The fresh leaves of *Ocimum gratisimum* used for the biocontrol experiment were obtained from the Botanical Garden of Department of Botany and authenticated at the herbarium laboratory, also in the Department of Botany, University of Ibadan, Nigeria.

2.3 Preparation and Quantification of Inoculum

The isolates of phytopathogenic fungi; *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata*, were subcultured on Potato Dextrose Agar (PDA) and incubated at room temperature for 7 days. Fungi growths on the plates were harvested separately by flooding with sterile distilled water then brushed with carmel hair brush into sterilized conical flasks. The solutions were sieved with double folded cheese cloth to allow for the passage of fungal spores. The inoculums suspension were then quantified by spore counting using haematocytometer, all the spore suspension were re-adjusted to $1x10^6$ spores/ml.

2.4 Preparation of Plant Extract

The sorted clean leaves of *Ocimum gratisimum* were soaked in 5% NaOCl solution for 3 minutes for surface sanitization, rinsed in two exchanges of sterile distilled water then spread out on sterile surface for the water to dry up. One hundred grams (100 g) of treated leaves added to 200 ml of sterile distilled water were blended in a sterilized electric blender in the preparation, to produce a concentration of 0.5 g/ml. The blended slurry was aseptically sieved with sterilized muslin cloth.

Table 1. Scoring of disease severity

Scale	Symptoms observed
0	Apparently healthy root or mesocotyl or crown tissue
1	< 25% of tissue with disease rot symptoms
2	25–49% of tissue rotted
3	50–74%, of the tissue rotted
4	75% or greater of the roots rotted
5	Wilted or dead seedlings/completely rotted mesocotyl or crown tissue

2.5 Seed and Soil Sterilization

The viable QPM seeds were treated with 5% sodium hypochlorite solution for one minute, rinsed in two exchanges of sterile distilled water and air dried in laminar flow for 2 hours. The sandy loamy soil (0-15 cm depth) collected from the research farm of the Department of Botany; University of Ibadan, Nigeria was sterilized using an electric soil sterilizer.

2.6 Screen House Experiment

The perforated bags containing 500g of sterilized soil were arranged in a completely randomised factorial design experiment in three replications. Ten viable seeds were planted per bag with respect to varieties. The seeded bags were then inoculated with 10 ml of already quantified (1 x 10^6 spores/ml) *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* separately and as combinations, the combined fungal inoculums were proportionately added up into 10 ml that was inoculated into each bag. After 24 hours of pathogen inoculation, 10 ml of *Ocimum gratissimum* extract was applied to another sets of pots to give pathogen – extracts interaction alongside with the pathogenicity experiment. Sterile distilled water was used as the control experiment. Adequate management practices such as regular watering were carried out. Data were collected on the 7^{th} and 14^{th} days after planting on the percentage seed germination, number of leaves, seedling height (cm) and leaf area (cm^3) and numbers of infected seedlings to determine the disease severity.

2.7 Disease Assessment

The percentage germination of the QPM seeds was determined according to Saupe [13] as:

Percentage seed germination =

$$\frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

Infection caused by the phytopathogenic fungi was calculated by the method described by Khalid et al. [14].

Percentage disease incidence =

$$\frac{\text{Number of seedling infected}}{\text{Total Number of seedlings counted}} \times 100$$

2.8 Scoring of Disease Severity

The disease rating used in this study was according to the method described by Soonthornpoct et al. [15].

2.9 Statistical Analysis

The Data collected were subjected to Analysis of Variance with General Linear Model procedure using Statistical Analysis System software version 9.1 SAS [16]. Differences among mean of the treatments were separated with New Duncan Multiple Range Test at 5% level of probability [17].

3. RESULTS

The fungal pathogens significantly ($p<0.0001$) reduced the germination of QPM seeds most especially when compared to the control. *Fusarium verticillioides* was observed as the most virulent pathogen as was found to significantly ($p<0.05$) inhibits seed germination of both maize varieties. Similar observation was recorded for *C. lunata*. Combined effect of *F. solani* + *C. lunata*, *F. verticillioides* + *C. lunata* and *F. verticillioides* + *F. solani* + *C. lunata* showed higher pathogenic effect on both maize varieties. The treatment combinations of of *F. solani* + *C. lunata* (6.1 cm), *F. verticillioides* + *F. solani* (6.8 cm), and that of *F. verticillioides* + *C. lunata* + *F. solani* affected the seedling height of ILE 1 variety when compared to the control. On similar note, *F. verticillioides* alone, *F. solani* (8.9 cm) alone, combination of *F. verticillioides* + *C. lunata* (9.4 cm), and as well as treatment combination of *F. solani* + *C. lunata* (9.8 cm) reduced the seedling height of SW 5 when compared to the control (10.2 cm). However, all the pathogenic fungi with the exception of *F. verticillioides* and also *F. solani* showed a severe effect on the leaf area of ILE 1compared to SW5 and the control (Table 3). The antagonistic effect

of the extract of *Ocimum gratissimum* evaluated against the fungal pathogens of QPM varieties showed significant ($p<0.05$) increase in the germination rate, as higher germination rate was recorded in SW 5 variety. Similarly, the antagonistic activities of *Ocimum gratissimum* extract on the pathogenic fungi also showed an appreciable increase on growth characters of the seedlings. A significant ($p<0.05$) increase was recorded in the number of leaves of both ILE 1 and SW5 irrespective of the treatment combinations, while the seedling height of SW 5 showed a higher significance ($p<0.05$) than that of ILE (Table 2). The most reduced leaf area was recorded in the combined treatments of *F. verticillioides* + *F. solani.*

Generally, the inhibitory effect of the extract on the fungal pathogens showed significant ($p<0.05$) effect in the order; *F. solani* > *C. lunata* > *F. verticillioides* and *C. lunata* > *F. verticillioides*, *F. solani* and *C. lunata* respectively (Table 2). There was an increase in germination at day 14 in comparison to day 7. Plants treated with the extracts of *Ocimum gratissimum* (76.88%) at planting significantly ($p<0.05$) increased germination rate more than those treated with the fungal pathogens. The antagonistic effect of the extract of *Ocimum gratissimum* evaluated against the fungal pathogens of QPM varieties showed significant ($p<0.05$) increase in the seed germination, as higher germination rate was recorded in SW 5 variety (Table 4).

The pathogens negatively and significantly ($p<0.005$) correlated with percentage germination ($r = -0.15$). Meanwhile, the days of observation (day 7 and 14) and QPM varieties contributed significantly ($p<0.05$) to the germination rate. However, days of observation ($r = 0.27$) and QPM varieties ($r = 0.22$) were positive and significantly ($p<0.005$) related with percentage germination while the treatments of the fungal pathogens (separate and as combinations) and extracts produced highly significant ($p<0.0001$) correlation with percentage germination ($r = 0.22$) (Table 5).

Higher significant ($p<0.05$) disease severity was recorded for treatment combination of *F. verticillioides* + *F. solani* + *C. lunata* on ILE 1 as 50%, while its corresponding value in SW 5 was 39.4%, followed by treatment of *C. lunata* alone (48.3%, 36.1%) on ILE 1 and SW 5 respectively, then combination of *F. verticillioides* + *F. solani* on ILE 1 (44.4%) and SW 5 (34.7%). However, the treatment combination of *F. verticillioides* + *C. lunata* and *F. verticillioides* alone and as well as *F. solani* alone showed lower disease severity, although, only treatment combination of *F. verticillioides* + *C. lunata* showed significant ($p<0.05$) effect on ILE 1, when compared to other treatment combinations. Despite the application of *Ocimum gratissimum* to control the fungal pathogens, higher disease severity was still recorded for ILE 1 compared to SW 5. However, the control effect of *Occimum gratissimum* extract in the treatment of the pathogenic fungi was evident, as reduced level of disease severity was measured on both QPM varieties. Treatment combinations of *F. solani* + *C. lunata* showed the least significant ($p<0.05$) disease severity on ILE 1 (19.8%) and on SW 5 (21.7%) variety (Table 6).

4. DISCUSSION

Phytopathogenic fungi have been established to be associated with maize cultivation [18-21] as observed in this study. The fungal pathogens significantly inhibited the seed germination of QPM most especially ILE 1 variety

Table 2. Effect of phytopathogenic fungi on the growth of QPM seedlings

Treatments	ILE 1			SW 5		
	Number of leaves	Seedling height (cm)	Leaf area (cm^2)	Number of leaves	Seedling height (cm)	Leaf area (cm^2)
Control	4.00^a	8.50^a	31.70^a	4.00^a	9.20^b	27.20^a
Fv	3.90^a	6.60^a	35.50^a	3.90^a	9.70^{ab}	34.70^a
Fs	3.60^a	7.40^a	34.90^a	4.20^a	8.90^b	31.80^a
Cl	3.70^a	7.50^a	28.70^a	4.00^a	10.30^a	30.10^a
Fv + Fs	3.30^a	6.80^a	28.30^a	3.90^a	10.60^a	35.10^a
Fv + Cl	3.90^a	7.10^a	28.00^a	4.00^a	9.40^b	26.90^a
Fs + Cl	3.70^a	6.10^b	26.90^b	4.20^a	9.80^a	36.40^a
Fv +Fs + Cl	3.90^a	7.60^a	26.70^b	4.20^a	10.30^a	33.40^a

The significant difference ($p<0.05$) is indicated by different letters along each row
Fv = Fusarium verticillioides, Fs = Fusarium solani and Cl = Curvularia lunata

Table 3. Inhibitory effect of *Ocimum gratissimum* extract against the phytopathogenic fungi on the growth of QPM seedlings

Treatments	ILE 1			SW 5		
	Number of leaves	Seedling height (cm)	Leaf area (cm^2)	Number of leaves	Seedling height (cm)	Leaf area (cm^2)
Control	4.90^{ab}	9.00^{a}	41.70^{a}	4.70^{a}	9.60^{a}	47.50^{a}
Fv	5.00^{a}	8.50^{a}	42.90^{a}	4.90^{a}	10.10^{a}	49.10^{a}
Fs	4.90^{ab}	7.70^{b}	48.60^{a}	5.00^{a}	9.10^{a}	48.20^{a}
Cl	5.00^{a}	8.90^{a}	52.10^{a}	5.00^{b}	10.10^{a}	46.50^{a}
Fv + Fs	4.90^{ab}	7.10^{b}	30.00^{a}	4.00^{b}	8.70^{b}	41.70^{b}
Fv + Cl	5.00^{a}	9.10^{a}	42.90^{a}	4.00^{b}	9.10^{a}	45.70^{b}
Fs + Cl	4.90^{ab}	8.50^{b}	38.90^{a}	4.30^{ab}	10.10^{a}	44.90^{b}
Fv +Fs + Cl	4.80^{b}	8.50^{b}	38.10^{a}	4.70^{a}	9.00^{a}	46.60^{b}

The significant difference (p<0.05) is indicated by different letters along each row
Fv = Fusarium verticillioides, Fs = Fusarium solani and Cl = Curvularia lunata

Table 4. Effect of days of experiment, varieties and treatment applied on the seed germination

Observed characters	Seed germination (%)
Day 7	63.33^{b}
Day 14	71.98^{a}
Pathogen treatments	58.23^{b}
Effect of extract and Pathogen	76.88^{a}
ILE 1 variety	63.96^{b}
SW 5 variety	71.35^{a}

The significant difference (p<0.05) is indicated by different letters along each column

Table 5. Correlative effects of the pathogen, extracts, days and QPM varieties on percentage seed germination

Correlation	Organisms	Days	Treatments	Varieties	Germination (%)
Organisms					
Days	0.00ns				
Treatments	0.00ns	0.00ns			
Varieties	0.00ns	0.00ns	0.00ns		
Germination (%)	-0.15*	0.27*	0.57**	0.22*	

*Highly significant (p<0.001) = **, Significant (p<0.05) = *, ns = not significant, WAP = Week after Planting*

Table 6. Percentage disease severity of phytopathogenic fungi on QPM seedlings and the control effect of Occimum gratissimum extracts

Treatments	Pathogens alone		Extract + Pathogens	
	ILE 1	SW 5	ILE 1	SW 5
Fv	27.10^{ab}	31.90^{ab}	23.60^{ab}	29.30^{ab}
Fs	29.20^{ab}	33.10^{ab}	26.80^{ab}	21.70^{a}
Cl	48.30^{a}	36.10^{a}	31.70^{ab}	26.20^{ab}
Fv + Fs	44.40^{a}	34.70^{ab}	39.40^{a}	33.30^{ab}
Fv + Cl	39.30^{a}	21.40^{a}	20.80^{ab}	27.80^{ab}
Fs + Cl	35.50^{a}	29.20^{ab}	31.40^{ab}	19.80^{a}
Fv + Fs + Cl	50.00^{a}	39.40^{a}	35.90^{a}	22.60^{a}

The significant difference (p<0.05) is indicated by different letters along each row
Fv = Fusarium verticillioides, Fs = Fusarium solani and Cl = Curvularia lunata

(63.96%), as similarly reported by Akande and Lamidi [12], whereas, the combined treatment; *F. solani* + *F. verticillioides* + *C. lunata* resulted in the least germination rate and this could be associated to the interactive effect of the three pathogenic organisms [21,22]. Though, percentage seed germination of both QPM varieties was reduced in the treatments with

fungal pathogens but with the increasing days of observation SW5 recorded higher resistance of the pathogens and enhanced seed germination. This suggested that SW5 has the potentials to resist the fungal pathogens.

The treatment combinations of *F. verticillioides* + *F. solani*, and *F. verticillioides* + *F. solani* + *C. lunata* produced higher disease severity, while the least growth response was observed in the treatment of; *F. verticillioides* + *F. solani*. This could be associated with the variation in virulence of each of the pathogens involved as was documented on *F. verticillioides* [18,20,21, 23,24], *F. solani* [25] and *C. lunata* [11]. Hence, their interactions conferred higher virulence and in turn produced higher pathogenic effect on the host plant as was demonstrated on the inhibition of seed germination and infected seedling growths of the QPM in this study. Although, no significant difference was observed in the resistance of the two QPM varieties to diseases caused by the inoculated fungal pathogens but SW 5 showed better growth performances, this was in line with the result obtained by Akande and Lamidi [12] in which low disease severity of southern leaf blight, *curvularia* leaf spot and maize rust fungal diseases were reported on eight QPM varieties experimented (Mama-ba, Dada-ba and CIDA-ba, Obatampa, EV8363, EV8766, Pool-18-SR and Pool-15-SR). Effects of the phytopathogens correlated with growth disorderliness of the seedling, and also significantly associated with disease severity produced and this agreed with the report of Pataky et al. [26], on the resistance of sweet corn northern leaf blight.

The use of plant extract in the control of phytopathogens has been extensively studied [27-29], thus considering other plant extract like *O. gratissimum* will be of advantage towards management and sustainability of QPM maize varieties. Aqueous extracts of *O. gratissimum* in this study significantly enhanced the germination percentage of the QPM varieties when compared to the control, this agreed with the effects of extracts on promotion of seedling emergence reported [30,31].

The extracts of *O. gratissimum* applied at 0.5 g/ml concentration significantly antagonized the pathogenic effect of *F. verticillioides*, *F. solani, C. lunata* and their combinations, thereby reduced their severity from 37.52% in pathogens treated plants to 24.47% in extract control plants. This was in consonance with the findings of Awuah [32] who reported the efficacy of fungicidal effects of *O. gratissimum* extracts against 14 of the 20 fungi species tested. This was also substantiated by Fagbohun et al. [33] who reported that the extracts of *O. gratissimum* showed moderate antifungal activities against all the test fungi; *Botryodiplodia theobromae, Phytophthora palmivora, Aspergillus flavus, Aspergillus niger* and *Aspergillus glaucus*. However, Colpas et al. [34] linked the mode of activities of *O. gratissimum* extracts to its induction of plant defence responses. More so, the morphological growths of the extracts treated plants were significantly enhanced as was earlier reported by Akanmu et al. [29] on millet growths by the extracts of *Moringa oleifera, Senna alata* and *Manihot esculenta*. QPM variety SW 5, showed better germination and seedling growths than ILE 1, the reason for this could be associated to the work of Aliu et al. [35] on genetic variability in the phenotypic and genotypic traits of the grain quality.

5. CONCLUSION

With the current advances in the biocontrol research and rise in the adoption of plant extract in plant disease control, this study showed the aqueous extracts of *Ocimum gratissimum* as a promising and potential phytofungicide that when fully explored would contribute significantly to the practice of organic agriculture. More so, the SW 5 variety of QPM showed better resistance to diseases caused by the phyto-pathogenic fungi of maize, thus SW5 could be a choice for food security.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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