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Effects of Plant Extracts and Synthetic Fungicides on the Radial Growth of *Phoma oryzae* on Rice (*Oryza sativa L.*) in Some Rice Growing Areas of South Eastern Nigeria

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Abstract

An *in-vitro* experiment was conducted at the Plant Pathology Laboratory of National Root Crop Research Institute, Umudike, Abia State. The aim of the experiment was to test the effect of some plant extracts and synthetic fungicides on the radial growth inhibition of some fungi isolated from rice plant materials sampled from rice growing areas of South Eastern Nigeria. These pathogenic fungi cause serious yield as well as economic losses in rice production in the region. The treatments were arranged in a Completely Randomized Design (CRD) with three replicates. The experiment was conducted using both water and alcohol extracts of test plants and the synthetic fungicides (Benomyl and Apron plus). These were tested on the radial growth of three fungal pathogens namely; *Fusarium moniliforme*. The 3 mm disc of each test fungus was placed in the center of 9 cm Petri dish containing 10, 15, 25% of the plant extract mixed thoroughly in a molten PDA. The crude aqeous extract of Azadiractha indica gave the highest mycelial growth inhibition of *F. moniliforme* (52%) at extract concentration of between 10-30% while ethanol extract of Garcinia cola had the best mycelial growth inhibition of *Fusarium moniliforme* 50%. Aqeous extract of *Azardiractha indica* (neem) had the highest mycelial growth inhibition of *Helminthosporium oryzae* up to 52.80% while *Zinginber officinale* (Ginger) in ethanol extract gave the best inhibiting effect in the same organism. Also aqeous extract of *A. indca* had the best inhibitory effect (60.90%) in *Phoma oryzae* while *Piper guineensis* (Alligator pepper) performed best in radial growth inhibition of *Phoma oryzae* (69.30%) with ethanol extract. The plant extracts were as effective as the synthetic fungicides in inhibiting the radial growth of the test fungi. Therefore, extracts of the test plant materials which are readily available to the farmers should be used rather than depending on the synthetic fungicides which are not always available and expensive for rice farmers.

Keywords: *In-vitro* experiment; Pathogenic fungi; Plant extracts; Rice plants; South-Eastern states

Introduction

Problems of diseases are one of the major challenges facing Nigeria farmers as in other areas in the world where rice is cultivated. The disease problems could be tackled by the applications of either plant sourced fungicides or synthetic fungicides According to Olaifa et al. [1] plant extracts may exhibit any one of the following effects: contact, stomach or systemic poison, fumigant, anti-feedant, retardants and growth inhibition, with the following advantages over synthetic chemicals. Some indigenous plants have been used in the control of important fungal diseases of crop plants. *Cymbogan citrates, Azadirachta indica* (neem) and *Ocimum grastissimum* (Nchuanwu) leaf extracts have been used in the control of tuber rot of potato caused by *Rhizopus Oryzae* [2] and *Piper guinensis* and Citrus lemon have been used for the control of *Rhizotonia solani* and *Collectotrichum lindermeithianum* in cowpea [2]. Other plants that have been tested for their fungitoxicity in the recent time include *Garcinia kola* (bitter kola), *Zingiber officinale* (ginger) and *Allium sativum* (Garlic). This last group of plants has been found effective against bacterial spot disease of Solanum caused by *Xanthomonas compestris* in Abia State. Bowers and Locke [3] have also demonstrated that some botanical extracts such as Neem (*Azadirachta indica*) were very effective in reducing the population density of *Fusarium oxysporum* in soil and wilt diseases of tomato [4]. Okigbo and Ogbonnaya [5] working on antifungal effects of two tropical plant leaf extracts found out that *Ocimum gratissimum* and *Aframomum melengueta* were effective in reducing post-harvest yam rot caused by some storage fungi in Umudike, Abia State, Nigeria. They discovered that ethanol extracts were most effective followed by cold-water extracts and hot water extracts of the test plants. Hossanein et al. [4] reported leaf extracts of neem (*Azadirachta indica*) and China berry (*Melia azedrach*) were effective against wilt disease of tomato. They found that both ethanol and ethyl acetate of extracts of neem leaves assayed at 20%, completely suppressed the growth of *F. oxysporium* and inhibited *Alternaria solani*. Rushda et al., [6] observed that the leaf extracts of *Lontana camara*, *Gnaphalium purpureum, Solanum nigrum, Amaranthus spinosus* and *Veronica anagallis* aquatic had inhibitory effect on germination and sporulation of fungi. Nwachukwu and Umechuruba [7] reported that leaf extracts of *Ocimum basilicum, Venonia amygladina, Cymbopogon citratus, Azadirachta indica* and *Carica papaya* significantly reduced the incidence of seed-borne fungi of African yam bean seeds and increased seed germination and seedling emergence. Neem extract was the most effective while lemon grass was the least. Sudheer et al., [8] also reported that aqueous extracts of leaves, bark, stem and seeds of *Strychnos nux-vamica,* bulb of *Allium sativum*, and rhizome of *Zingiber officinale*, leaves of *Ocimum bascillicum* and fruits of *Azadirachta indica* had significant inhibitory effects on the seed-borne fungus of *Oryza sativa* seeds. According to them stem, bark and seed extracts (20% w/v) of *S. nux-vormica*, were more effective than

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other plant extracts and 0.3% of Mancozeb in controlling the fungus. They also posited that none of the plant extracts was phytotoxic at the tested concentration.

Materials and Methods

Preparation of plant extracts

Extract were prepared from fresh leaves of *Azardiractha indica* (Neem leaves), *Piper guinensis* (seeds), *Garcinia cola* (Bitter cola seeds), *Ocimum gratissimum* (leaf) and *Vernonia amygdalina* (leaf). The leaves or seeds were thoroughly washed in running tap water and distilled water and air dried at 27°C and ground to obtain one kilogram of paste of each test material. Water extract was obtained by adding each paste of 10 g, 25 g and 30 g to 100 ml of sterile water in 250 ml beaker and stirred vigorously and allowed to stand for 1 hour. Each plant material was so extracted one after the other. The supernatant liquid was passed through a Whatman No 1 filter paper. The filtrate was also passed through a membrane filter (0.22 cm) to avoid any bacterial or fungi contamination. The filtrate was later concentrated by autoclaving and then allowed to cool down before it was stored in the refrigerator under aseptic condition in Mac Carthney bottles properly corked and labeled. The interface between the corks and the bottles were sealed with masking tapes to prevent.

Alcohol extraction

For alcohol extraction each plant material was oven dried at 45°C in oven for 24 hours after being washed three times with distilled sterile water and air-dried at room temperature. Each plant material was ground with warring blender to obtain 1 kg powder each. Each powder 10 g, 25 g and 30 g were extracted with 95% ethanol (100 ml) and concentrated through a rotary vacuum pump flash evaporator to syrup form weighing 26 g from each powder. The syrup residue thus obtained was diluted to 2.5 g/l. These preparations were used in all the experiments.

Effects of plant extracts and synthetic fungicides on the growth of *Phoma oryzae* pathogens of rice in South Eastern States of Nigeria

Effect of the extracts on radial growth of the test fungi was separately investigated by placing one disc (3 mm diameter) of 8 day-old culture of each fungus in each of three petri dishes (9 cm diameter) containing 20 ml of potato dextrose agar (PDA) medium and 3 ml of leaf extract. Three concentrations of the extracts were used. These were 10, 25, 30 g/100 ml of distilled water and 0 g/100 ml which served as control. Each of these concentrations was replicated three times. These were tested on the radial growth of three fungal pathogens namely; *Fusarium moniliforme, Heminthosporium spp* and *Phoma spp*. Each of the fungus was tested separately. The 3 mm disc of each test fungus was placed in the centre of 9 cm Petri dish containing 10, 15, 25% of the plant extract mixed thoroughly in a molten PDA. Water (distilled sterile) was used as control. Twelve Petri dishes were used for each plant extract against one fungus at a time. Another twelve dishes were used for the synthetic fungicides. The Petri dishes were incubated at 27°C with 12 hours of alternating light and darkness. Daily observation and measurement of the radial growth were carried out. The diameter of the fungal colony was determined by measuring from the back sides of the plates with the use of meter rule. The colony growth was measured taking the average of the largest and shortest diameter of the same colony. This is because the colony growth of fungus is not always a regular circle. The fungitoxicity of the extracts was calculated in terms of percentage colony inhibition using the formula [9].

$$\%\ growth\ inhibition\ = \frac{dc-dt}{dc} \times \frac{100}{1}$$

Where dc is the average diameter of fungal colony with control, dt is average diameter of fungal colony with treatment. The treatments were arranged in a Completely Randomized Design (CRD) with three replicates. The experiment was conducted using both water and alcohol extracts of test plants and the synthetic fungicides (Benomyl). The control experiments were the sterile distilled water and alcohol alone in place of the extract of the test plant materials.

Results

Effects of water and ethanol extracts of plant materials and synthetic fungicide on the radial growth of *Phoma oryzae* in culture

Results in Table 1 showed that on first day, there was a significant difference in the effect of the five plant extracts by water extraction, where neem performed significantly better than the rest with radial growth reduction value of (60.90%) followed by piper with a value of (28.62%) which is statistically better than bitter cola (23.83%), Ginger (27.12%) and Ocimum (5.69%) in reducing radial growth of Phoma. The least was Ocimum with reduction value of (5.69%). The five plant extracts performed better than control in reducing radial growth of Phoma. There was also a significant difference among the three concentration levels where the concentration at 10 g was better than the rest with a reduction value of (45.60%). This is not consistent with the observations on other experiments where 10 g concentration most at times had the least reduction value. This was followed by 30 g with a value of (40.38%), which is statistically different from the other two levels while the least was at 25 g with a value of (30.99%). The three-concentration level performed better than the control (Table 1). For bitter cola, there was a significant difference among the three levels, where the highest interaction effect was obtained in 30 g with a value of 48.06%. This is significantly higher than the other two interaction levels. This is followed by 47.24% obtained in 25 g while the least was 45.73% observed in 10g interaction level. The three interaction levels were better than the control (Table 1). For Neem, the interaction effect at 30 g (81.44%) was significantly better than the other two levels. Interaction levels at 10 g and 25 g are statistically the same with radial growth reduction values of 81.09% each (Table 1). For ginger, the interaction effect showed a significant difference among the three levels, where 10 g had the highest reduction value of 48.07% which is significantly higher than the rest. This result was consistent with previous observations. This was followed by 35.07% in 20 g while the least was 25.33% in 25 g concentration level. For Ocimum, the highest interaction effect 10.59 was obtained in 30 g which is significantly higher than the rest. This was followed by 6.86% observed in 10 g interaction level while the least was 5.33% in 25 g levels. It should be noted that the reduction level in ocimum was correspondingly low compared to others. Nevertheless, the three interaction levels performed better than the control (Table 1). The results in Table 1, for Piper also showed the same trend, where the highest reduction value was observed in 10 g interaction level with a value of 46.23% followed by 41.67% in 25 g while the least was 26.75% in 30 g interaction level. Result in Table 1 showed that on the second day, there was a significant interaction effect among the five plant extracts. Neem had the highest radial growth reduction effect with a value of 60.75% which is statistically different from the rest followed by Piper with a value of 31.78% which is significantly higher than bitter cola, ginger and Ocimum. The plant extract that had the least reduction value was

Ocimum with a value of 6.48% which was comparatively lower than the rest. The five plant extracts were better than control in terms of reducing radial growth of *Phoma spp*. The best concentration was 10 g with a value of 41.67% followed by 30 g with a value of 41.43% while the least was 25 g with a radial growth reduction value of 31.61%. This result was not consistent with the previous observations where the reverse was the case. This should call for further investigation to understand properly the chemistry of the actions of the plant extracts (Table 1). For bitter cola, interaction effect at 10 g level produced the highest reduction value of 47.13% which is significantly higher than the rest followed by 37.88% in 30 g level while the least was 14.68%. This is comparatively lower than the other two (Table 1). For Neem, the highest reduction value was obtained in 30 g level with a value of 84.52%. This is statistically higher than the rest, followed by 81.97% obtained in 25 levels while the least was 76.50% in 10 g interaction level (Table 1). For ginger, the best interaction effect was observed in 30g concentration level with value of (37.05%). This is statistically different from the rest following (25.95%) in 25 g level while the least was (14.84%) in 10 g interaction level. For Ocimum, there was a reverse trend in this plant extract where highest reduction level (24.07%) was obtained in 10g followed by (1.85%) while the least was (1.00%) in 25 g. These values were also comparative low compared with previous observations (Table 1). For Piper, interaction effect at 10 g and 30 g are statistically the same but significantly higher than interaction effect at 25 g with a value of (35.45%) while the other two had value of 45.85% each (Table 1). The result in Table 1 showed that on the third day, most of the plant extracts could not show any effect in reducing the radial growth. Only Neem (55.04), piper (26.60) and bitter cola (15.30%) could exert some effect in reducing the radial growth of Phoma where bitter cola at concentration level of 30 g could not show any significant effect. The reverse was the case at interaction levels of 10 g and 25 g which had values of 41.19% and 1.59% respectively. The interaction effect of 10g was statistically higher than that at 25 g. For Neem, the interaction effect at 10 g 41.19% was statistically higher than the other two interaction levels followed by 1.59% at 25 g while the least 0.00% at 30 g interaction level. There was no interaction effect in Ginger and Ocimum (Table 1). The result in Table 1 also showed that on the first day, there was a significant difference among the effects of plant extracts by alcohol extraction, their concentrations and synthetic fungicides. Ocimum had the highest radial growth reduction 73.60% followed by Neem 71.60%) each of which were significantly higher than Bitter cola 54.60%) which was the least in growth reduction effect. Others were statistically the same Table 1). Concentration effect showed that there was a significant difference among the three levels. Concentration level of 10g had the least reduction effect 71.50% which is significantly lower than concentration levels of 25 g and 30 g with 86.40% and 85.30% respectively (Table 1). The best concentration at day one was 25g with percentage reduction of 86.40%. There was no significant interaction effect among the three botanic extracts and the synthetic fungicides. For Bitter cola, 25 gx Bitter cola had the least interaction effect 83.40% followed by 30gx bitter cola 70.10% while the least was 10 gx Bitter cola (Table 1). For Neem, 25 gx Neem and 30 gx Neem had the same percentage growth reduction of 98.50% each and also were higher than 10 gx Neem with 89.30%. For Ginger the same trend was repeated as in Neem (Table 1). For Ocimum, all the interaction levels had same growth reduction value 98.20%). For Piper, the highest reduction 98.20% was obtained in 25 gx Piper and 30gx Piper respectively which was statistically different from 80.10% obtained in 10g x Piper which was the least. Benlate generally had lower radial growth reduction percentage compared to phytochemicals used. The best interaction level for Benlate was 30 gx Benlate followed by 25 gx Benlate having 48.10% and 41.10% respectively (Table 1). The result in Table 1 showed that on the second day, there was a significant effect among the plant extracts where piper 67.30% performed significantly better than bitter cola, and neem with percentage reduction of 40.90% and 37.30) respectively but not from ginger 49.20% and Ocimum 47.20% respectively. There was no significant difference among the three concentration levels though concentration level of 25 g had the best performance 61.90% followed by 10g 61.50% while the least was 30 g with percentage radial reduction of 54.60%. The interaction effect showed a significant difference among the three interaction levels in each plant extract. For Bitter cola, 25 gx Bitter cola had the highest growth reduction 67.20% which was significantly higher than 30gx Bitter cola Table 1) but that not with 10 gx Bitter cola with 59.30%. For Neem, there was a very significant difference among the three concentration levels. 10gx Neem extract had the highest growth

Variables							Incubation period (days)					Concentration (%) and growth inhibition (%)			
Treatment	1						2					3			
	0	10	25	30	mean	0	10	25	30	Mean	0	10	25	30	Mean
Garcinia cola															
Water extract (WE)	0	45.73	47.24	48.06	23.83	0	47.13	14.68	37.88	24.92	0	41.19	1.59	0	15.3
Alcohol extract (AE)	0	64.8	83.4	70.1	54.6	0	59.3	67.2	37	40.9	0	35.2	33.3	16.7	21.3
Azardiractha indica															
Water extract	0	81.09	81.09	81.44	60,90	0	76.5	81.97	84.52	60.75	0	65.3	74.02	80.85	55.04
Alcohol extract	0	89.3	98.5	98.5	71.6	0	82.6	47.6	19	37.3	0	50.7	40	14.8	26.4
Zingiber oficinale															
Water extract	0	48.07	25.33	35.07	27.12	0	14.84	25.95	37.05	19.46	0	0	0	0	0
Alcohol extract	0	71.1	98.5	98.5	68.8	0	58	62.2	76.5	49.2	0	45.6	52.2	53	37.7
Ocimum gratissimum															
Water extract	0	6.86	5.33	10.59	5.69	0	24.07	1	1.85	6.48	0	0	0	0	0
Alcohol extract	0	98.2	98.2	98.2	73.6	0	64.4	65.2	59.3	47.2	0	47.5	50.4	40	34.4
Piper guinensis															
Water extract	0	46.23	41.67	26.75	28.66	0	45.85	35.45	45.85	31.78	0	37.79	31.5	37.12	26
Alcohol extract	0	80.1	98.6	98.6	69.3	0	83.1	96.51	89.4	67.3	0	65.6	96.7	81.1	60.8
Benlate	0	18.1	41.1	48.1	26.9	0	21.8	32.5	46.5	25.2	0	21.1	22.6	42,20	21.5
LSD 0.05 (WE)		0.29	--	--	0.14	--	0.04	--	--	0.02	--	0.05	--	--	4.95
LSD 0.05 (AE & Benlate)		30.78	--	--	15.39	--	11.38	--	--	22.75	--	25.12	--	--	12.56

Table 1: Percentage growth inhibition of *Phoma oryzae* by water and alcohol extracts of plant materials and Benlate in culture.

reduction 82. 60% which is significantly higher than 25gx Neem 47.60% and 30gx Neem 19.00% (Table 1). For Ginger, there was a significant difference among the interaction levels where the highest reduction occurred in 30gx ginger 76.50%) followed by 25 g x ginger 62.20% while the least was 10 g x ginger 58.00% which is significantly different from 30gx ginger. Each of the interaction levels performed better them the control Table 1). For Ocimum, there was no significant difference among the interaction levels, but the highest growth reduction occurred in 25 gx Ocimum 65.20) followed by 10 gx Ocimum 64.40) while the least was 59.30% in 30 gx Ocimum, but the interaction levels performed better than the control (Table 1). For piper, there was a significant difference among the interaction levels where the highest growth reduction was experienced in 25 gx piper 96.51 which is significantly higher than 10 gx piper 83.10% but not form 30 gx Piper 89.40%. The three interaction levels did better than the control (Table 1). For Benlate the only synthetic fungicide the growth reduction result was significantly lower than those produced by plant extracted fungicides. 30 gx Benlate had the highest growth reduction 46.50% which is significantly higher than the rest. That was followed by 25 gx Benlate 32.60% while the least was in 10 gx Benlate 21.80%. The three interaction levels were better than the control (Table 1).

Discussion

Effects of plant extracts, their concentrations and synthetic fungicides in the management of *Phoma oryzae* diseases on rice in South Eastern Nigeria

This study showed that aqueous extract of the plant material had significant effect on the radial growth of *Phoma* spp. Neem extract consistently produced higher inhibitory effect on radial growth of the fungal pathogens in culture followed by by extracs of *Piper guineensis* whereas the least inhibitory effect was recorded with *Ocimum gratissimum*. This result collaborated the work of a number of scientists who have investigated the efficacy of botanicals as effective control measures for fungal pathoges. Eliot indicated that over 2000 higher plant species are known to have pesticidal properties. Amadioha [2] in his investigation on fungitoxic effect of some leaf extracts against Rhizopus oryzae causing tuber rot of potato, found that *Cymbopogon citratus*, *Azadirachta indica* and *Ocimum gratissimum* were very effective in controlling the growth of *Rhizopus oryzae in-vitro* and *in-vivo*. Extracts of *A. indica* was found to be the best in reducing the radial growth of the pathogen in culture followed by *Cymbopogon citratus* and *Ocimum gratissimum*. The result obtained in this study is also in agreement with Okigbo and Ogbonnanya [5] who reported that *Ocimum gratissimum* and *Aframonum Melongueta* were effective in reducing post-harvest yam rot caused by some storage fungi in Umudike, Abia State. Although, *O. gratissimum* in this experiment was not very effective in reducing radial growth of Phoma spp as compared to extracts of other plants. Also, Nwachukwu and Umechuruba [7] and Singh et al., [10] reported that leaf extracts of *Ocimum basilicun*, *Venonia amydalina*, *Cymbopogon citratus*, *Azadirachta indica* and *Carica papaya* significantly reduced the incidence of seed borne fungi of African yam bean seeds and increased seed germination and seedling emergence. According to them Neem extract was the most effective while Lemon grass was the least. The result of effect of plant extracts and synthetic fungicides on radial growth in this study showed that the botanics were effective in reducing radial growth of the test pathogens in culture which compared with synthetic fungicide Benomyl. Ethanol extract of *O. gratissimum* gave the highest inhibitory effect followed by Neem while the least was Benlate in reducing the radial growth of *Phoma oryzae* in culture. The reverse was the case with the aqueous extracts of *O. gratissimum* which gave the least inhibitory effect. The differential efficiency of the water and alcohol extracts may be due to the solvents (water and alcohol) with alcohol extracting more active ingredients from the plant materials. The view is shared with Mohana and Raveesha [11] who observed that petroleum ether had a better extraction of the active compound for the control of Decalepsis hamiltonii than when benzene, chloroform, methanol and ethanol were used as extraxting solvents. Chiejina et al., [12], also observed that the efficacy of plant extracts on *Collectotrichum kahawae* radial growth and disease development vary dependent on the type of plant species used, method of extraction and concentration of the extracts applied. *Ociumum gratissimum* extract has been found to be very effective in reducing mycelial growth of many fungal pathogens. Okigbo and Ogbonnanya [5] reported that *O. gratissimum* was very potent in reducing radial growth of postharvest yam rot fungal pathogens. It was very evident from the result of this study that extracts of Piper guineensis performed better than the rest of the botanicals followed by *Zingiber officinale*. This result is similar to Amadioha [13] and Okoi and Afuo [14], who reported that Piper nigrum, Ocimum sanctum and Citrus lemon were active against colletotrichum lindermuthianum in cowpea. Also, Chiejina et al., [12] reported that *A. melegueta* and *Z. officinale* extract at various concentrations reduced the radial growth of fungal pathogens of tomato fruits. They observed that at 25%, *Z. officinale* gave complete reduction of mycelial growth of the pathogens while at 30% concentration; both extracts completely reduced the mycelial growth of the pathogens.

Conclusion

Ethanol extract of *Piper guineesis* had the best inhibitory effect (60.80%) in *Phoma oryzae* on the third day of culture followed by aqueous extract of *Azardiractha indica* (55.04%) while the least were aqueous extracts of *Zingiber oficinale* and *Ocimum gratissimum* with inhibition values of 0.00%) respectively. The synthetic fungicides evaluated inhibited mycelial growth of the fungal pathogens up to 46%-50% on the second day. The plant extracts were as effective as the synthetic fungicide (Benlate) in inhibiting the radial growth of the test fungi, therefore, it could be recommended that extracts of the test plant materials which are readily available to the farmers should be used rather than depending on the synthetic fungicides which are not always available as well as expensive to local rice farmers.

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