FUNGICIDAL INHIBITION OF GROWTH AND SPORE GERMINATION OF *Rhizopus stolonifer* AND *Fusarium oxysporum* ISOLATED FROM ROTTEN IRISH POTATO TUBERS

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Abstract

In this study the effects of four chemicals fungicides on the growth and spore germination of Rhzopus stolonifer and Fusarium oxysporum were studied. The chemical fungicides used include carbendazim, hexconazole, benomyl and mancozeb. On the growth of the two fungal isolates hexaconazole showed the best inhibitory effects on growth of the two pathogenic fungi followed by carbendazim and least inhibition in growth was recorded in mancozeb. The results of spore germination indicated that hexaconazole yielded the highest percentage spore germination inhibition of R. stolonifer followed by benomyl and the lowest percentage spore germination inhibition was observed in mancozeb. The highest percentage spore germination inhibition of F. oxysporum was recorded in hexaconazole followed by carbendazim and the least was noticed in mancozeb. Inhibition in growth and spore germination increased with corresponding increase in concentrations of the fungicides. Therefore, it would be recommend that hexaconazole, carbendazim and benomyl should be used for control of Tubers rots of Irish Potato.

Keywords: Fungicides, Inhibitation, Growth, Germination, *Rhizopus stolonifer*, *Fusarium oxysporum*, Rots.

1.0 Introduction

Irish Potato belongs to the family Solanaceae (kudi, 2008). Irish Potato was introduced into Nigeria in the later part of 19th century by Europeans in Jos, plateau (Ifenkwe, 1981). Irish Potato is ranked fourth in importance after rice, wheat and maize. It has high nutritive value and grown for food, livestock feed and industrial purposes (Odebunmi *et al*., 2007).

Post-harvest loss of root and tuber crops has been a serious problem to the farmers as more than 40% of their harvest maybe lost to decay (Olurinola, 1992). Irish Potato like other tropical food crops is attacked by many pathogens which includes fungi, bacteria and viruses. Salami and Popoola (2008) reported that *Rhizopus oryzae, Fusarium rodolens, Butryodiplodia theobromae, fusarium oxysporum* and *Penicillium* species were responsible for post-harvest rot of irish potato tubers in Southern Nigeria. The finding of Kabeil *et al*., (2008) and Amadioha (2007) revealed that *Alternaria solani*, *Phytopthora infestans*, *Rhzopus stolonifer* and *Rhizoctonia bataticola* caused tuber rots of Irish Potato.

Several management strategies are available for the control of plant diseases. These include cultural techniques, biological control, resistant cultivars, crop rotation and chemicals. Many attempts have been made by researchers on the effects of fungicides on the growth and germination of fungi. Benomyl and hexaconazole were found to be effective in inhibiting the growth of *Stenocarpella maydis*, *Glomus mossae*, *Fusarium pallidoroseum*, *Fusarium oxysporum* fr. sp. *Lycopersici*, *F. Oxysporum*, and *Trichoderma hirzianum* (Viviana *et al.*, 2000; Marley and Gbenga, 2004; Taskeen *et al.*, 2011; Soumik *et al.*, 2010).

It was also discovered that carbendazim and mancozeb reduced the growth of *F. Solani, F. Proliferatum, F. avenaceum, F. oxysporum, Alternaria porri* and *Stemphyllum vesicarium* (Allen *et al.*, 2004, Kopachi and Wagner, 2006; Misra and Gupta, 2012). The fungicides benomyl showed significant inhibition in spore germination of *Metarrihizium anisopline* and *Glomus mossae* (Viviana *et al.*, 2010). Most *et al.* (2010) confirmed that rodimil and Dithene-M 45 inhibited spore germination of *Rhzopus artocarpi* and *Fusarium oxysporum*.

The aim of this paper is to determine the effectiveness of carbendazim, hexaconazole, benomyl and mancozeb in inhibiting the growth and spore germination of *R. stolonifer* and *F. oxysporum* associated with storage rot of Irish Potato tubers.

2.0 Materials and Methods

2.1 Sample collection

The fungal isolates; *Rhzopus stolonifer* and *Fusarium oxysporum* were obtained from the stock cultures of fungal isolates associated with the storage rots of Irish Potato tubers. The chemical fungicides; carbendazim, hexaconazole, mancozeb and benomyl were procured from Lokoja international market.

2.2 Effects of fungicides on growth of the isolates

The study was carried out using potato dextrose agar (PDA) supplemented with different fungicides. The fungicides used include carbendazim, mancozeb, benomyl and hexaconazole. The PDA was prepared by dissolving 39g in 1000ml of distilled water and autoclaved at 121°C for 15mins. After cooling to about 60°C fungicides were incorporated separately at the rate of 100, 200, and 300, 400 and 500mg/L. The PDA supplemented with fungicides was then poured into 9cm Petri dish and allowed to solidify. Each plate was inoculated singly at the centre with 4mm diameter agar disc containing 7 days old cultures of the isolates. Each treatment consists of three replications. The control experiment was PDA without addition of fungicides. All the inoculated plates were incubated at 28±2°C for 7 days. At the end of the incubation period percentage inhibition in growth was assessed.

2.3 Effects of fungicides on spore germination of isolates

It was carried out using potato dextrose broth (PDB). One millimetre of each fungicide at varying concentrations (100, 200, 300, 400 and 500 mg/L) was poured into 50ml conical flasks and 9ml of the PDB was added to each conical flasks. The PDB supplemented separately with fungicide was used to study spore germination of the isolates.

Spore suspension of *R. stolonifer* and *F. oxysporum* containing 20-30 spores per microscope Field was prepared from 7 days old cultures of the isolates. One drop of the spore suspension was put in cavity glass slide containing a drop of the fungicides. Hence slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of Petri plate. Each treatment consists of three replications. The control experiment was PDB without addition of

fungicides. All the inoculated period the percentage inhibition in spore germination was determined.

2.4 Statistical Analysis

The data obtained was statistically analysed using one-way analysis of variance (ANOVA) via minitab 16 version. Least significance difference (LSD) was used to compare the mean at 5% level of significance.

3.0 Results and Discussion

3.1 Effect of fungicides on growth of the isolates

Effect of different concentrations (100,200,300,400 and 500 mg/L) of each fungicide were evaluated against the growth of *R. Stolonifer* and *F. oxysporum*. All the fungicides used inhibited the growth of *R. stolonifer*. The percentage inhibition in growth increased with increase in concentrations of the fungicides. Hexaconazole has the highest inhibition in growth of *R. stolonifer* followed by carbendazim and least percentage inhibition in growth was recorded by mancozeb. The results are presented in table 1.

Table 1: Effects of fungicides on growth of *R. stolonifer*

Fungicide	Mycelia growth/ concentrations (mg)					
Hexaconazole.	22.6	19.5	15.5	8.2.	3.6.	90.0
Carbendazim.	28.5	22.2	18.6	12.5	8.5	90.0
Benomyl.	30.6	27.5	22.4	18.6	14.4	90.0
Mancozeb.	48.2	38.4	34.6	28.2	23.5	90.0

Table 2: ANOVA for the effects of fungicides on growth of *R. stolonifer*

Source	DF	SS	MS	F	P
Fungicides	3	16275.6	5425.2	88.365	0.000
Error	20	1227.9	61.355		
Total	23	17503.5			

The percentage inhibition in Mycelia growth of *F. oxysporum* also varied with the type of fungicides and their concentrations. Hexaconazole gave the higher inhibition followed by Carbendazim and Mancozeb has the lowest. All the fungicides gave the highest percentage inhibition of Mycelia growth at the highest concentrations (500mg) followed by 400mg, 300mg and 100mg respectively. The results are presented in table 1.

Table 3: Effects of fungicides on the growth of *F. oxysporum*

Fungicide	Mycelia growth/ concentrations (mg)					
Hexaconazole.	19.2	12.8	9.2	7.4	4.8	68.3
Carbendazim.	23.6	15.0	12.6	10.5	7.4	68.5

Benomyl.	26.2	17.4	14.5	11.6	9.7	67.4
Mancozeb	32.6	21.5	17.8	14.2	12.5	67.9

Table 4: ANOVA for the effects of fungicides on growth of *F. oxysporum*

Source	DF	SS	MS	F	P
Fungicides	3	10046.1	3348.7	289.42	0.000
Error	20	231.4	11.7		
Total	23	10277.5			

3.1.2 Effect of fungicides on spore germination of the isolates

The effect of hexaconazole, carbendazim, mancozeb and benomyl against spore germination of *R. stolonifer* are presented in the table 3. All the fungicides showed significant inhibition of spore germination of *R. stolonifer*. However the maximum inhibition in spore germination was observed in highest concentration (500mg) followed by 400mg 300mg 200mg and 100mg respectively. At the high concentration hexaconazole was found to be the most effective in inhibiting spore germination of *R. stolonifer* followed by highest concentration of Benomyl and Carbendazim was the least.

Table 5: Effect of fungicides on spore germination of *R. stolonifer*

Fungicide	% inhibition of spore germination / concentrations (mg)					
Hexaconazole.	61.2	58.4	42.5	36.2	18.6	99.0
Carbendazim.	68.0	65.7	58.6	44.4	26.5	98.2
Benomyl.	64.8	53.5	51.8	38.4	22.8	98.9
Mancozeb	78.6	68.5	62.6	54.2	29.7	98.6

Table 6: ANOVA for the effects of fungicides on growth of *R. stolonifer*

Source	DF	SS	MS	F	P
Fungicides	3	12518.2	4172.7	103.554	0.000
Error	20	805.9	40.29		
Total	23	13324.1			

It was revealed from the result in the table 4 that different concentrations of the fungicides caused significant inhibition in spore germination of *F. oxysporum*. The maximum inhibition in spore germination was found at higher concentration of 500mg followed by 400mg 300mg 200mg and 100mg respectively as compared to control which exhibited least inhibition in spore germination. The fungicides hexaconazole at higher concentration was found to be most effective in inhibiting the spore germination followed by highest concentration of carbendazim, benomyl and mancozeb respectively. The results are presented in table 3.

Table 7: Effect of fungicides on spore germination of *F. oxysporum*

Fungicide	% inhibition of spore germination / concentrations (mg)					
Hexaconazole.	54.6	42.8	29.5	16.7	8.2	90.8
Carbendazim.	68.4	61.7	52.3	42.5	18.9	92.7
Benomyl.	72.2	56.6	54.3	48.7	24.6	92.5
Mancozeb	76.5	59.2	57.5	52.8	28.4	91.6

Table 8: ANOVA for the effects of fungicides on spore germination of *F. oxysporum*

Source	DF	SS	MS	F	P
Fungicides	3	12030	4010	40.40	0.000
Error	20	1985	99.25		
Total	23	14014			

3.2 Discussion

The results of this study revealed that all the fungicides used proved effective in inhibiting growth and spore germination of *R. stolonifer* and *F. oxysporum*. The study shows that Hexaconazole was most effective in inhibitating growth of *F. oxysporum*. While mancozeb gave the least inhibition effect on the growth of *R. stolonifer* and *F. oxysporum*. This tally with findings of Mamza *et al*., (2008) that mancozeb gave the least inhbition in growth of *Fusarium pallidoroseum*. But contrarily, Obagwu (1997) recorded that mancozeb was most effective in reducing the growth of *Colletotrichum capsici*. Also, Soumik *et al.,* (2010) observed that hexaconazole was most effective in inhibitating the growth of *Trichoderma hirzianum*. Benomyl and carbendazim recorded the highest inhibition in growth of *Fusarium pallidoroseum, Glomus mossae, F. oxysporum* and *F. proliferatum* (Viviana et al., 2000, Allen *et al.,* 2004, Mamza *et al*., 2008). On the effect of fungicides on the spore germination of the isolates hexaconazole proved to have high inhibitory effect against spore germination of *R. stolonifer* followed by benomyl and mancozeb respectively. The highest percentage inhibition of spore germination was observed at highest concentration of all the fungicides. This agreed with findings of Yanez and France (2010) and Viviana *et al.,* (2010) that benomyl inhibited spore germination of Metarrihizium anisopliae and Glomus mossae in culture media. Most *et al*., (2010) discovered that Dithene-M 45 was most effective in reducing spore germination of Rhzopus artocarpi. The spore germination of *F. oxysporum* in this study was highly reduced by hexaconazole followed by carbendazim and the least inhibition was observed in mancozeb. But most *et al*., (2010) and Duran *et al.,* (2004) reported that rodimil was effective in reducing spore germination of *F. oxysporum* and *Beauveria brassiana*.

4.0 Conclusion

The presence of these pathogens in Irish Potato tubers will result in reduction in nutrients contents, market value and production of mycotoxins which are hazardous to the health of the consumers. The chemical fungicides used in the study showed significant inhibition in growth

and spore germination of *R. stolonifer* and *F. oxysporum*. The effects of hexaconazole and carbendazim are promising and can be used to control tuber rot of Irish Potato.

4.1 Recommendations

The following measures are recommended so as to reduce post-harvest rots Irish Potato tubers:

- Application of synthetic chemicals such as hexaconazole, carbendazim and benomyl to field soil to reduce the incidence of the pathogens
- Dipping the Irish Potato tubers in hot water at 60°C for 25 minutes will prevent the tuber from infection by the pathogens.
- Physical damage to the tubers should be avoided since they serve as Major entry points of pathogens into the tubers.
- Rodents which create wounds on the tuber should be kept away to prevent infection.
- Contact between healthy and infected tubers should be avoided to prevent re-infection.
- Tubers should be well spread out on wood or clean concrete floor.
- Farmers should use improved and resistant cultivars such as Ajax and Patista.
- Employ biological control measures through the use of organisms that are parasites or antagonistic to the pathogen
- Tubers should be surface sterilizer before storage.
- Use of storage clamps and raised platforms to prevent the tubers from getting contact with soils so as to avoid re-infection.

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