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CONTROL OF GROWTH AND SPORULATION FUNGI ASSOCIATED WITH ONION BULBROT USING FUNGICIDES

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Abstract

Onion is a crop that is consumed in almost every household in Nigeria for a wide variety of dishes. The crop has a fairly constant demand and uses not limited to any climate or associated with any nationality. Like any other crop, onion is subject to attack by a number of pathogens especially fungi. Onion bulbrot causes serious yield loss up to 10-50%. This has to be controlled for profitable production. Diseased onion bulbs from four different locations (Sokoto, Zaria, Kebbi and Kano) were obtained in order to isolate and identify the fungal pathogens that are associated with the onion bulbrot. Two pathogens (*Fusarium oxysporium* and *Aspergillus niger*) were isolated from all the samples collected from the four locations. Four different fungicides (Benlate, Rovrin, Delsene M and Rovral TS) were assessed *in vitro* to determine the most effective among them in controlling the mycelial growth of the two pathogens isolated. Delsene M completely inhibited the mycelial growth of the two pathogens at half the recommended rate. This was followed by benlate which also gave complete control of the mycelial growth at half the recommended rate of the two pathogens. Rovrin however gave complete inhibition of the growth of the two pathogens at the recommended rate, but had the least control in the three concentrations tested.

Key words: Bulbrot, fungicides, mycelial growth, pathogens

Introduction

Onion is one of the most commonly consumed vegetable crops in Nigeria and around the world. The crop is mainly grown for its bulbs (Cramer, 2000; Hussaini *et al*., 2000). In 2012 alone it was estimated that about 240, 000 tons of green onions and 1, 350, 000 tons of dry onions were produced in Nigeria (Anon. 2014). In comparison with other fresh vegetables, onions are relatively high in food value and antioxidant phytochemicals (Hussaini *et al*., 2000; Smith, 2003). The crop is second only to tomatoes in importance among the vegetables in Nigeria and fifth in the world market (Cramer, 2000; Hussaini *et al*., 2000). and Storage of onion is important in Nigeria as it is highly perishable. Onion is a crop that has a fairly constant demand regardless of any climate or nationality. Onion is consumed in almost every household daily mainly for seasoning in a wide variety of dishes. This is mainly due to its mildness, crispness, juiciness and sweatiness. Extracts from onion have potent antibacterial properties resulting from their organic sulphur compound content. Allicin, a sulphur-containing product of onion is used in order to increase the intake of thiamine from foods (Fujiwara, 1952). Onion like any other crop is subject to attack by a number of pathogens especially fungi. Bulb rotting caused by fungal pathogens is one of the major reasons for storage losses. It has been reported that bulbrot causes about 10-50 % of storage losses of different onion varieties (Metthananda, 1992). The objectives of this research were (i) to isolate and identify the fungal pathogens that are associated with the onion bulbrot; (ii) to confirm the causal agent(s) of the bulbrot; (iii) to evaluate the most effective fungicides that could inhibit the mycelial growth and sporulation of the bulbrot fungi; and (iv) to recommend the most effective fungicides for the control of the bulbrot to growers of onion.

MATERIALS AND METHODS

Diseased onion bulbs were sourced from Sokoto, Birnin Kebbi, Zaria and Kano. The experimental laboratory was first disinfected by spraying 4 liters of izal at 10% a.i/l using falcon knapsack sprayer. About 200g of Irish potato were peeled and cut into smaller pieces and then placed in 1000 ml of distilled water. These were boiled for 30 minutes. Clear liquid was decanted and the volume made up to 1000 ml with distilled water in order to compensate for loss due to evaporation. The mixture was transferred into conical flask and then covered with cotton wool and aluminum foil. This was then autoclaved for 20 minutes at a pressure for sterilization. About 10ml of streptomycin solution (1g/200ml of sterile distilled water) was added to the mixture at about 50^{o}C and allowed to cool for 24 hours on the laboratory bench.

Isolation of Pathogens

The samples from the four locations (Sokoto, Birnin Kebbi, Zaria and Kano) were treated independently. This was to avoid the transfer of pathogens from one source to another. The table tops, hands and petri dishes were then sterilized with alcohol. From each sample, an infected tissue was selected and cut into smaller pieces of about 2 to 5 mm and then transferred to sterile petri dishes. Pieces of sample from each location were put in McCartney bottle and then surface-sterilized with sodium hypochlorite (Milton) for some time ranging from 2 to 5 minutes depending on the hardness of the tissue. The samples were then rinsed three times with distilled water to remove excess Milton and to avoid the crystallization of its salt, a phenomenon that can affect the growth of the pathogens after plating. The samples were then transferred onto the surface of Ager media aseptically using flame-sterilized inoculation forceps in a culture room. The plates were then arranged inside culture hood and observed daily for growth.

Subculture

Subculture refers to the process of separating the different organisms growing from the plated samples and then growing each on separate fresh medium. This is to obtain the pure culture of each pathogen. Potato dextrose agar with streptomycin (PDAS) was prepared into which growing organisms on the onion samples were sub-cultured between 24 and 72 hours after plating as the organisms emerge. The fresh PDAS was then observed for further growth of the pathogens.

Identification

Identification of the isolates from the samples was done by putting drop of methylene blue-in-lactophenol on slides and putting small portion of each fungal colony growing from the plated samples in the stain. A portion of the culture was taken on the 7th day and spread on the stain with sterilized inoculation needle. The slides were then observed under x40 objective of light microscope.

Pathogenicity Test

This test was carried out in order to confirm that the isolated pathogens are the causal agents of the onion bulbrot. Fresh healthy onion bulbs were inoculated with the conidial suspension of the isolates (pathogens). Each isolate was inoculated separately through the stem and through the leaves on separate bulbs. The isolates were then inoculated together into other fresh bulbs, through both the stem and the leaves. There was a control to which distilled water was inoculated. The inoculated bulbs were then kept at a room temperature of 25^{o}C in the laboratory and observed for bulb rot after seven days.

Evaluation of Fungicides (*in-vitro*)

The effects of four chemicals (Delsene M, Rovrin, Benlate and Rovral TS) on the pathogens isolated were assessed *in-vitro* using three different concentrations viz: 0.5x, x and 1.5x, where "x" stands for the recommended weight of each of the

fungicides to be dissolved in 250ml of water for field spray. Three litres of PDAS was prepared in 12 flasks of 250ml by volume into which the four chemicals were proportionally weighed into each flask at three concentrations. Each concentration was then dissolved in 250ml of PDAS in a conical flask. The chemicals were first dissolved in 5ml of sterile water before adding to the 250ml PDAS. The fungicide plates were allowed to cool on the laboratory bench for 24 hours. A total of 12 PDAS- fungicides plates were used for each of the isolates together with 4 PDAS plates as control making a total of 28 treatments. Using sterilized inoculation needle, a small portion of each of the fungal pure culture was taken and placed at the centre of fresh PDAS-fungicides plate in a clean environment. The plates were then arranged in the laboratory at a room temperature of 25^{o}C and observed for the growth of the pathogens after the 7th day.

Results and Discussion

Identification of Association Fungi

Two fungi strains (*F. oxysporium* and *A. niger*) were isolated from all the bulbs from the four locations. The result showed that the two fungi strains were associated with onion bulbs collected from the four locations (Sokoto, Kebbi, Kano and Zaria). Similar reports were made by other workers, including Vigitha *et al.* (2014) who reported *F. oxysporium* to be associated with onion bulbrot, while Rajapakse and Edirimanna (2002) isolated *Fusarium* and *Aspergillus* from rotten and healthy onion bulbs.

Pathogenicity Test

The treatments differ with respect to the type of pathogen inoculated and the point of inoculation in rotting. Treatments inoculated through stem with *A. niger* got rotten much more slower than those inoculated with *F. oxysporium*. Treatments inoculated with *F. oxysporium* through the leaves showed no sign of rotting after 7 days. However, when cut, those inoculated through the stem were found to be rotten. Onion bulbs that were inoculated with the two pathogens combined together at the same point and time were all rotten after 7 days irrespective of the point of inoculation was stem or leaves. Although the bulbs inoculated with *F. oxysporium* started rotting earlier than those inoculated with *A. niger*, after 7 days, the rotting was more severe on those inoculated with *A. niger*. There was no any sign of rotting on the control after seven days as expected. These results are in agreement with the Report by Arden (1986) who showed that bulbs can be attacked by *F. oxysporium* directly through unwounded root tissues by direct penetration through root tissue into the stem. He added that although infected and healthy bulbs cannot be distinguished from each other, on cutting an infected bulb vertically, a brown discolouration of the stem is evident. The results obtained indicated that *F. oxysporium* is faster in causing the bulbrot and that it penetrates the bulb through the stem and progressed outwardly to cause the whole bulb to rot.

Effects of Fungicides on Mycelial Growth (in-vitro)

In-vitro assessment of the four fungicides (Table 1) indicated that Delsene M completely inhibited the growth of *F. oxysporium* and *A. niger* at all the three different concentrations tested. Half of the recommended rate for benlate gave complete control of *F. oxysporium* while complete control of *A. niger* was obtained from all the concentrations tested. Complete control of *F. oxysporium* was obtained with Rovrin at only the recommended rate while highest mycelial growth inhibition of *A. niger* was achieved at the recommended rate Rovral TS gave the least inhibition of the mycelial growth of the two pathogens by all the concentrations tested. However, the fungicide is practically better than observed in the control.

Conclusion

It is evident that onion production in these four areas (Kano, Kebbi, Sokoto and Zaria) is faced by the problem of bulbrot induced by *F. oxysporium* and *A. niger*. These pathogens respond to four different fungicides (Benlate, Delsene M, Rovral TS and Rovrin) at different rates. The four chemical fungicides control or inhibit the mycelial growth of the two fungi in this order: Delsene M > Benlate > Rovrin > Rovral TS > Control. Both Delsene M and Benlate controlled the mycelial growth of the two fungi in the 3 concentrations accessed. This indicates that using half the recommended rate of Delsene M or Benlate by farmers will be more economical. The choice between the two will depend on cost and availability. Rovrin is more effective at the recommended rate tested particularly on *F. oxysporium.* Rovral TS is the least effective although it is found to be better than control. Since these pathogens are soil-borne and not seed-borne, a pre-application of the fungicides as prophylactic treatment might be effective in their control. The growers of onion are therefore advised to use half of the recommended rate of Delsene M (0.312g/250ml of water) or Benlate (0.375g/250ml of water) for the effective control of onion bulbrot. This can be done by dissolving the proportionate weights in water and dipping the roots of onion seedlings in the solution for 24 hours before transplanting. It is hoped that these systemic fungicides will be absorbed and will serve to control the attack by the fungi from the soil environment.

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Table 1: Effects of three concentrations of four fungicides on the mycelial growth of two fungi that are associated with onion bulb rot

Fungicide	Concentration per 250ml PDAS (g)	Average growth of mycelium (cm)	
		F. oxysporium	*A. niger*
Rovrin	0.440	0.800	1.300
	0.880	0.000	1.100
	1.320	1.100	1.300
Benlate	0.375	0.000	0.000
	0.750	1.600	0.000
	1.125	0.200	0.000
Delsene M	0.312	0.000	0.000
	0.625	0.000	0.000
	0.938	0.000	0.000
Rovral TS	0.440	2.100	4.200
	0.880	3.200	3.100
	1.320	2.400	3.400
Control	-	5.200	9.000