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Effects of Bio-Pesticides on the Management of Ginger Leaf Spot at Umudike, South-East Nigeria

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Abstract
Some bio-pesticides were evaluated for the management of ginger leaf spot caused by *Phyllosticta zingberi*. The experiment was conducted at the laboratory and screen house of National Root Crops Research Institute, Umudike during cropping seasons of 2020 and 2021. The objective of the study was to determine the most effective bio-fungicide used in the control of *phyllosticta zingberi* infecting ginger rhizomes. All bio-fungicides fungicides (Neem Leaf, Neem and fruit Garlic) used were tested for efficacies against leaf spot disease of ginger caused by *phyllosticta* spp. at Umudike. The experiment was laid out in complete randomized design (CRD) involving two ginger varieties and three bio-pesticides in three replications. Results for pathogen virulence showed that isolates were virulent and the extracts efficacy results showed that crude Garlic extract treated plates gave a mean growth inhibition of 96.02%, Neem Leaf (91.31%) and Neem fruit (64.27%) were found to be significantly effective ($P<0.05$) better than control in inhibiting the growth *Phyllosticta* spp. isolates in the laboratory indicating that garlic bio-pesticide has the highest inhibitory potency across the most of the virulent isolates obtained and treated in the laboratory. Spraying of garlic bio-pesticide twice on ginger plants at 8 and 14 weeks after planting completely prevented leaf spot disease incidence on the treated ginger with a severity score of 1 while spraying of neem seed bio-pesticide twice on ginger plants at 8 and 14 weeks after planting gave the least protection and control of ginger leaf spot disease on ginger with a severity score of 3.

Key words: Bio-pesticide, leaf spot, Azadiracta indica, Allium sativum, Protectant Curative

Introduction

Leaf spot disease caused by *Phyllosticta zingiberis* (Stevens and Ryan) is of common occurrence in different parts and agro-ecologies of Nigeria. The fungus attacks the leaves only, forming round to oval light-yellow spots which may coalesce to form large discoloured areas, often drying up in the centre to form a hole (Meenu and Kaushal, 2017; Meenu and Jebasingh, 2019), the plants become stunted and the development of the rhizomes is adversely affected. This disease has the potential to cause serious damage to ginger crop especially when the environmental conditions favour disease development. The disease attacks all known varieties of ginger in Nigeria as all the varieties appear to be susceptible. There is no known control measure yet, except removing and affected parts as some farmers do in Nigeria (Ekefan *et al*., 2018). This method reduces the leaf area of the plant which in turn results in reduced photosynthesis, stunt growth and the development of the rhizomes adversely affected (Waraich *et al*., 2011). Ginger is a crop of great importance to Nigeria and the world for its spicy and medicinal values (Nair, 2013; Deme *et al*., 2021). Unfortunately, the crop is being threatened by many diseases of importance among which include leaf spots. The major constraints (yields, the postharvest and culinary uses of the crop) that limit production of ginger are leaf spot, yellows, rhizome rot complex, soft rot and storage rots (Dohroo, 2016).

The history of yellow leaf spot in ginger started with a report by Meenu and Jebasingh (2019) who stated that Ramakrishnan mentioned leaf spot disease for the first time in Godavari and Malabar regions of India in 1942. Later on, the disease was reported from Sarawak (Deme *e. al*., 2021) and it also occurs widely in Kerala State. Singh (2015) reported the disease from Chhatisgarh and then in Nigeria by Nwankiti and Arene (1982). This disease is now widespread in most ginger-growing countries of the world. Leaf spot disease of ginger leads to heavy reduction in rhizome yield, through the destruction of chlorophyll (Ayodele *et al*., 2018). Symptoms are observed on leaves as oval to elongated spots that later turn whitish surrounded by dark brown margin with yellow halo (Xizhen *et al*., 2016). Knowledge on the reaction of various ginger germplasm to leaf spot disease is very scanty (Arunakumara and Satyanarayana, 2015). Concerted efforts therefore

should be made to identify the pathogenic organism(s) responsible for ginger leaf spot and methods of managing the pathogens using simple bio-pesticides that are environmentally friendly and readily available in the regions where ginger is grown. Plant-based fungicides have proven potentials, and can serve as alternatives to synthetic fungicides (Lengai *et al*., 2020).

Non-judicious use of synthetic fungicides for controlling plant diseases has ultimate negative effects on human and animal health and, the agroecosystem. Eco-friendly control measures including plant extracts and organic materials, which act directly on the plant pathogens or indirectly by inducing disease resistance in plants (Mishra and Raja, 1999), have gained considerable attention as alternative means to synthetic fungicides. Efforts have been made to control plant diseases using plant extracts (Sallam *et al*., 2001). They show evidence that the plant extracts are effective against wide range of fungal, bacterial and viral pathogens. Plant extracts of neem have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish *et al*., 1999). This study aimed to isolate and identify the pathogenic organisms responsible for leaf spot disease of ginger and to determine the appropriate bio-fungicide for its management *in vitro* and *in vivo* .

Materials and Methods

A laboratory diagnosis and screen house screening were carried out at National Root Crops Research Institute (NRCRI), Umudike to identify the ginger leaf spot pathogen and investigate the fungitoxicity of the bio-pesticides. Pathogenicity tests were conducted with isolate samples collected from ginger farms that were naturally infected with ginger leaf spot disease without inoculation at Umudike in Abia State in 2010.

Laboratory diagnosis

Isolation of pathogenic ginger leaf spot organisms from ginger leaf samples obtained from ginger fields in Umudike and the environs

First in this stage was culturing of collected infected ginger leave samples in Petri plates in plant pathology laboratory of NRCRI, Umudike. In the experiment, leaf spot infected ginger leaves were collected from different ginger fields in Umudike and the environs. The leaf samples were individually packaged in separate sterile nylon bags and taken to the laboratory. Isolation of possible pathogenic organisms present in the obtained infected ginger leaves and soils of the fields where the ginger plants were planted. The trial was carried out as a part of the experiment, but a pre-experiment prior to establishment of the experiment in the laboratory and in the screen house. In the pre-experiment, the culturing and isolation methods according to Nwadili *et al*. (2015) were adopted. The experimental was CRD involving eight isolate and two ginger varieties. Another isolation from infected leaves of grown ginger plants in the screen house to get out the organisms inoculated on the leaves of the ginger plants in the screen house to proof or disproof pathogenicity test. The experiment was carried out in completely randomized design (CRD) both in the laboratory and in the pathogenicity test in the screenhouse. Three virulent isolates of *P. zingiberis* were selected for the fungitoxicity tests of the plant extracts. Pour plate method was used to determine the fungitoxicity of the plant extracts on the pathogen by rating the percentage growth inhibition of each of the extracts on the pathogen responsible for ginger leaf spot disease. The growth inhibition was estimated using the formula thus

$$\text{Growth inhibition of isolates (\%)} = \frac{C-T}{C} X \frac{100}{1}$$

Where; C = Control experiment where no treatment was applied in the Petri plate medium, T = Treated experiment where a type of extract was applied in the medium

Screen house assay of bio-pesticides on ginger leaf spot disease in the screen house

The trial on control of ginger leaf spot using bio-pesticides was carried out in the screen house in NRCRI, Umudike on 12 weeks old ginger pants inoculated with spore suspensions (1 x 10^6) of each of the three isolates of *P. zingiberi* isolated and confirmed to be pathogenic. The experimental design was CRD involving three isolates of *phyllosticta* and three crude water extracts. The spore suspensions were prepared by introducing 10ml of sterile distilled into Petri dishes containing each isolate and gently scrubbing the surface of culture with a wire loop to dislodge the spores of the spores. The suspensions were passed through Wattman No.1 filter paper and the concentration reduced through serial dilution. The inoculum suspensions from each isolate (1 x 10^6) were used to inoculate the three varieties of ginger in CRD experiment involving four ginger plant stands in a replicate and three replicates which were used for the evaluation. Crude water extracts of neem leaf (*Azadiracta indica* leaf), Neem fruit (*Azadiracta indica* seed) and Garlic (*Allium sativum*) were prepared using cold water extraction. The extracts were applied on the inoculated ginger plants in the screen house once at 3 months after planting, twice at 12 and 14 weeks after planting. The plants were scored for disease incidence and, severity according to Nwadili *et al*. (2015).

$$\text{Disease incidence} = \frac{\text{No .of plants infected by a particular pathogen}}{\text{Total number of plants}} X \frac{100}{1}$$

The disease severity was scored on a 4-point scoring scale (Nwadili *et al*., 2015) in the following order:
1 = No symptom, 2 = spots on at least two lowest leaves, 3 = spots on at least four lowest leaves, and 4 = spots on apical leaves and eventual wilting. Data were collected on the incidence and severity of ginger leaf spot disease as stated above and subjected to analysis using R-statistics softwere, 3.0 and means were separated using least significant difference (LSD).

Results and Discussion

Results

Isolation of pathogenic organisms from ginger leaf samples

Results from the laboratory analysis showed that eight isolates were obtained and studied for pathogenicity of leaf spot disease of ginger. However, among the isolates, three (Um4, Um7 and Um8) out of the eight isolates were more virulent than the others and were selected for inhibitory effects of the bio-pesticides. Table 1 shows the virulent abilities of the isolates on two varieties of ginger tested in the laboratory. The results showed that Um7 gave the highest mean leaf spot disease severity of 4.26 on a scoring scale of 5 points. This was followed by Um4 with a mean disease severity of 4.25 and then Um8, which gave a mean leaf spot disease severity score of 3.67. The selected leaf spot virulent isolates were then tested on two ginger varieties (Ug1 and Ug2) in the screen house for their virulence on the ginger varieties when treated with some bio-pesticides at different frequencies of application.

Effect of crude bio-pesticides on growth inhibition of P. zingiberi isolates in the laboraory

The Table 2 showed growth inhibition results of the three virulent isolates of *P. zingiberi* and their responses to management by the crude bio-pesticides of neem leaf (*Azadiracta indica* leaf), Neem fruit (*Azadiracta indica* seed) and Garlic (*Allium sativum* depending on the potency of that individual crude bio-pesticide. The results showed that garlic gave the highest growth inhibition of 96.28% on Um4 (*P. zingiberi* isolate), followed by neem leaf extract which gave 91.56 growth inhibition on Um4 and neem fruit extract gave the least result of 64.21% on Um4. These were significantly better ($P \leq 0.05$) than the control where no treatment was applied. Similar trend of growth inhibition was expressed by the different plant extracts on the other two isolates of *P. zingiberi.* The mean results of the plant extracts showed that garlic is the most effective plant extract among the ones investigated with the potency of inhibiting the growth of the different isolates of the pathogen responsible for leaf spot disease of ginger. Isolate Um7 had the lowest mean growth inhibition of 82.73% across the three plant extracts showing that some of the isolates of the pathogen cannot be controlled easily as the others due to their resistance to all the bio-pesticides. This is one of the reasons why an array of eco-friendly bio-pesticides should be evaluated for the control of the disease caused by this important pathogen of ginger.

Screen house assay of bio-pesticides on ginger leaf spot disease in the screen house

The effects of spray application and spray application frequencies of the bio-pesticides on the control of ginger leaf spot disease are shown in Table 3. The effects of different spray applications and spray application frequencies investigated on the ginger variety UG1 which is susceptible to the leaf spot disease is presented in Table 3 and this showed that garlic (*Allium sativum*) was the most effective bio-pesticide in the control of ginger leaf spot disease with a mean leaf spot disease severity of 1.33 across three spray frequencies. The bio-pesticide (garlic) was effective in managing the leaf spot disease even at a single spray frequency at 8 weeks after planting (WAP). When the garlic extract was applied once at 8 weeks after planting, it was able to control the leaf spot disease giving a low disease severity score of 2, which was significantly ($P<0.05$) different from control. The bio-pesticide (garlic) when applied twice or at two application frequencies (8 and 12 WAP), there was a complete protection of the plants from the leaf spot disease giving a disease severity of score 1 which was significantly ($P<0.05$) different from control and the other two crude extracts, even at a single or double frequency. This shows that Garlic is both a curative and a protective bio-pesticide. Neem leaf (*A. indica*) bio-pesticide was effective at three application frequencies while neem fruit bio-pesticide did not show any complete leaf spot management even at three application frequencies.

Discussion

Management of ginger leaf spot disease by using plant extracts is modern, advanced and risk-free alternative method of managing leaf spot of ginger. Several plant extracts are known to play important role in the management of plant diseases (Singh *et al*., 2010). They act directly or indirectly against plant pathogens, either to inhibit the growth, multiplication or by inducing resistance in crop plants (Yasser *et al*., 2017). In this study, in vitro-tested plant extracts inhibited radial growth of the isolates up to 96% or more. Garlic extract caused 96.28% growth inhibition showing that it is highly effective in the control of the pathogen. This corroborates the work of Singh (2015) which states that there is efficiency in using fungicides in the control of leaf spot disease of ginger under the field conditions of India. Garlic or Neem leaf extracts are efficacious in the control of ginger leaf spot disease and can be adopted for the control of ginger leaf spot.bThe isolation and identification of virulent isolates reveals that even among *phyllosticta* spp, there are some isolates that are more virulent than others and some others that con be virulent only under some conditions. This can make management controls difficult and expensive supporting the results of Ewuziem and Alleluyanatha (2019) that controlling yellow leaf spot increases cost of production but its economic implications depends on severity and level of applications of selected treatment.

This work reveals that among the tested bio-pesticides, Garlic (*Allium sativum*) was found to be the most statistically and significantly effective in comparison to the others tried in the experiment and the effectiveness of neem leaf [(*Azadirachta indica*) leaf and neem fruit (*A. indica*)], for the reduction of the disease severity, confirms results of Deme *et al*. (2021). *Phyllosticta* leaf spot of ginger can be managed by one or two sprays of garlic (*Allium sativum*) and two or three sprays of neem leaf (*A. indica* leaf) bio-pesticide. The successive application confirms the bio-pesticide is not totally protectants or curative fungicides.

Although, *A. indica* fruit extract did not show any significant difference in comparison to the control sprayed with water, it was found effective as compared to other tested controls at other spraying frequencies. This shows that the bio-fungicide possess some levels of active ingredients that can be effective on some other strains (isolates) of *phyllosticta* spp. Deme *et al*. (2021) had showed that *Phyllosticta* leaf spot can be managed by one and two sprays of Bordeaux mixture (Copper fungicide) and also diphenamidone (Dithane M-45). This confirms that better control performance can be achieved by spraying more than once which is also the general information from this work.

The control of ginger leaf spot disease in ginger cultivation is a difficult task because the disease has a rapid emergence, high rate of recurrence, and high virulence. Most studies have focused on the composition and efficacy of fungicides used as alternative to different chemical fungicides. The reason for this is because leaf spot disease which undermines production efficiency, has become a serious threat to the production of ginger. In addition, it has also been reported that most chemical fungicides have serious negative impacts on the environment. Based on all these issues, it would be useful to develop appropriate alternative options that are easily bio-degradable and completely safe to the environment.

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Table 1: Virulence of *P. zingiberi* isolates on Ug1 and Ug2 ginger varieties in laboratory at Umudike under ambient temperatures

	Ginger Varieties		
Isolates	**Ug1**	**Ug2**	**Means**
Um1	4.6	4.2	1.42
Um2	4.1	4.5	1.33
Um3	3.3	4.1	2.70
Um4	1.5	1.0	4.25
Um5	3.7	3.2	2.45
Um6	4.8	4.2	1.05
Um7	1.2	1.4	4.26
Um8	1.4	1.1	3.67
LSD	1.8	2.01	

Um1 – Um8 = P. zingiberi isolates. Virulence scores: 1 = No symptom, 2 = spots on at least two lowest leaves, 3 = spots on at least four lowest leaves and 4 = spots on apical leaves and eventual wilting

Table 2: Effects of plant extracts on the growth inhibition of virulent isolates of *P. zingiberi* in laboratory in Umudike at ambient temperature

	Efficacy of plant extract (%) (Growth inhibition) isolates			
Treatment (Plant extract)	**Um4**	**Um7**	**Um8**	**Mean**
Neem Leaf	91.56	90.24	92.14	91.31
Neem fruit	64.21	62.18	66.41	64.27
Garlic	96.28	95.77	96.00	96.02
Mean	84.12	82.73	84.85	
LSD	3.50	4.44	3.61	

Um4 = P. zingiberi isolate 1. Um7 = P. zingiberi isolate 2. Um8 = P. zingiberi isolate 3

Table 3: Effect of foliar spraying frequencies of plant extracts on ginger leaf spot disease severity under screen house condition on UG1 ginger variety

	spray application frequencies			
Treatment (Plant extract)	**Once**	**Twice**	**Thrice**	**Mean**
Neem Leaf	3	2	1	2.00
Neem fruit	4	3	2	3.00
Garlic	2	1	1	1.33
Control	3	4	4	3.67
LSD (0.05)	0.31	0.11	0.00	

Disease severity category where: 1 = No symptom, 2 = spots on at least two lowest leaves, 3 = spots on at least four lowest leaves and 4 = spots on apical leaves and eventual wilting