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Inhibitory effect of some plant extracts on mycelial growth and sporulation of *Curvularia eragrostidis* isolated from pearl millet

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ABSTRACT: The efficacies of extracts from five plants (*Khaya senegalensis, Allium sativum, Zingiber officinale, Vitellaria paradora* and *Azadirachta indica*) were evaluated for their inhibitory effect on mycelial growth and sporulation of *Curvularia eragrostidis* isolated from pearl millet. Each plant material was air dried, powdered and surface sterilized with ethanol. These were then soaked in sterile water for 24 hours and filtered through double layer of muslin cloth and incorporated in potato dextrose agar amended with streptomycin (PDAS) under aseptic environment. The potato dextrose plant extracts agar were compared with Benomyl as standard check and untreated control. Mycelial growth was lowest in benomyl treated Petri dishes both at 7 and 14 days after inoculation (DAI) and highest on control Petri dishes. All the plant extracts except *Allium sativum* did not differ significantly with control at 14 DAI. The sporulation was highest in control and lowest in benomyl treated PDAS. *Azadirachta indica, Khaya senegalensis* seeds and *Allium sativum* bulb stimulated the production of significantly fewer spores than control and did not differ from each other. Spore size (length and width) was significantly reduced by the plant extracts evaluated.

Introduction

Pearl millet, *Pennisetum glaucum* (L.) R. Br., is an important cereal crop in Nigeria. It occupies 5 million hectares, which is about 57% of the total land area (8.7 million hectares) devoted to cereal production within the savanna and Sahel zones. Diseases constitute an important production constraint resulting in substantial grain yield losses (Selveraj, 1987). *Curvularia* midrib spot (*Curvularia eragrostidis*) occurs throughout the millet growing areas, infecting all the three types of pearl millet grown in Nigeria (Zarafi *et al.*, 2004). On farmers fields, variable disease incidences (27 – 98% and severity index (0.25 – 0.63) have been reported (Bawa, 1992; Zarafi *et al.*, 2004).

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The wide occurrence/spread and high incidences and severities of this disease calls for the sourcing of control measures, hence Bawa (1992) reported that benomyl, metalaxyl + carboxin, carbendazim and mancozeb significantly inhibited mycelial growth and sporulation of *C. eragrostidis*. Great advances are being made in controlling plant disease. Control strategies which have been adopted include cultural methods, fungicides, and breeding for varietal resistance (Ikwelle *et al.*, 1990). Biological control of plant pathogens using antagonistic microorganisms has proved to be reliable alternative to use of chemicals in the management of some diseases (Enoghene and Okigbo, 2001; Okigbo, 2002; Okigbo and Ikediugwu, 2000).

The presence of anti-fungal compounds in higher plants has been recognized as an important factor in disease resistance (Qasem and Abu Blan, 1966). Such compounds being biodegradable and selective in their toxicity are considered valuable for controlling some plant diseases (Singh *et al.*, 1983). Some higher plants and their constituents have shown success in plant disease control and have proved to be harmless to non-target organisms and non-phytotoxic unlike chemical fungicides (Appleton and Tansey, 1975; Misra and Dixit, 1976; Singh *et al.*, 1983).

Fungicides are not identical in their action, even among species of the same group. Therefore selected agents must be known to be effective against the target organism. There is therefore, a strong need to investigate as many plants as possible for the control of *Curvularia eragrostidis* so as to come up with control package that is affordable, durable and free of chemicals that can pollute the environment. This work therefore examines anti-fungal effect of neem leaf and seed (*Azadirachta indica*), garlic bulb (*Allium sativum*), mahogamy seed (*Khaya senegalensis*), ginger rhizome (*Zingiber officinale*) and shea butter leaf (*Vitellaria paradora*) extracts on *Curvularia eragostidis*, the causal organism of midrib spot disease of pearl millet.

Materials and Methods

Pathogen isolation: The leaves of "gero" millet with midrib spots were collected from farms in Samaru – Zaria, Nigeria. In the laboratory, midrib portions with typical spots were cut into pieces and surface sterilized for 5 minutes in 0.5% solution of sodium hypochloride. The sterilized pieces were plated in 9-cm Petri dishes containing potato dextrose agar with streptomycin (PDAS) after rinsing twice with sterile water. The plates were incubated at room temperature ($28 \pm 2^{o}C$). Fungal outgrowth was sub-cultured in fresh media to obtain pure culture.

Preparation of leaf extracts agar: The various plant materials were air-dried and powdered separately using blender (Benatone blender model BLG – 40L). Mixing 20 g of each powder with 5 ml ethanol for 5 minutes did surface sterilization. Cold-water extracts were obtained by infusing 20 g of each powder in 100 ml of sterile water for 24 hours. The suspensions were filtered through double layer of sterile muslin cloth and 10 ml of each suspension was mixed in 100 ml of PDAS to obtain potato dextrose plant extract agar (PDAS – plant extracts). Petri dishes were dispensed with 20 ml of PDAS – plant extract and allowed to solidify. Inoculum discs of 4 mm diameter obtained from the edge of a seven day old culture of *C. eragrostidis* on PDAS were inoculated face downwards at the centre of each of the different plant extract plates. PDAS without any plant extract served as control, while PDAS with benomyl served as standard check.

Four replicate plates of plant extracts were completely randomized on laboratory bench at room temperature ($28\pm2^{o}C$). Mycelial growth was determined by measuring culture size along two diameters at 7 and 14 days after inoculation (DAI). The culture on each plant extract agar was harvested 14(DAI) by blending in 150 ml of water and sieved through double layer of muslin cloth. The spore concentration was determined with the aid of a haemocytometer at x 40 magnification. Spore counts were done thrice from each Petri dish – suspension and means of the three readings were recorded. Slides made from each Petri dish suspension was viewed under a microscope with micrometer and spore sizes (length and width) of 50 randomly selected spores were measured for each replicate. Data collected was subjected to ANOVA and means were separated using Student Newman –Kuels (SNK) ranking at 5% level of significance (SAS Institute, 1990).

Results

The performances of the plant extracts on mycelial growth are presented in Table 1. At 7 DAI, mycelial growth was lowest on PDAS incorporated with benlate (benomyl), garlic and neem leaf which did not differ from each other, significantly had the highest inhibitory effect on mycelial growth. This was followed by neem seeds extract, which did not differ significantly from garlic and neem leaf extracts as well as ginger. Mahogany leaf extract and shea butter leaf extract, which did not differ from each other had the least inhibitory effect on mycelial growth and were at par with the control

At 14 DAI, benomyl performed significantly better than all the other treatments. This was followed by garlic, which was significantly different from all the other plant extracts. Neem seed and leaf, mahogany seed, ginger rhizome and shea butter leaf did not performed significantly better than the control. The inhibitory effect of these extracts on mycelial growth was short lived.

There were significant differences in the sporulation among the different treatments (Table 1). Sporulation in the control was significantly the highest while benomyl was the least. Among the plant extracts, the greatest inhibitory effect on sporulation was recorded in *K. senegalensis* and *A. indica* seeds, which did not differ from each other. *K. senegalensis* seeds also did not differ from the standard check (benomyl). This was followed by *A. sativum*, which did not vary from *A. indica* leaf. *Z. officinale* and *V. paradora* leaf were at par with one another and had the lowest inhibitory effect in sporulation although they were significantly better than the control.

Spore size was also reduced by plant extracts (Table 2). Spore length varied significantly with plant material. Control had significantly largest spores. This was followed by PDAS- amended with *Z. officinale*, *A. indica* leaf and benomyl respectively, without significant difference. The shortest spores were obtained from neem seeds, mahogany seeds, shea butter leaf and garlic bulb.

Table 1: Effect of plant extracts on mycelial growth and sporulation of *Curvularia eragrostidis in vitro*

Plant	Part used	Mycelial growth (cm) at		Sporulation
		7 DAI	14 DAI	
Garlic (*Allium sativum*)	Bulb	6.06^{c}	7.87^{b}	3.50^{cd}
Sheabutter (*Vitellaria paradora*)	Leaf	8.24^{a}	9.00^{a}	4.50^{b}
Neem (*Azadirachta indica*)	Leaf	6.11^{bc}	8.72a	4.00^{bc}
Mahogany (*Khaya senegalensis*)	Seed	8.58^{a}	9.00^{a}	3.00^{de}
Neem (*Azadirachta indica*)	Seed	7.04^{bc}	8.74^{a}	3.25^{d}
Ginger (*Zingiber officinale*)	Rhizome	7.10^{b}	8.13^{a}	4.67^{b}
Benlate (benomyl)	Powder	4.10^{d}	5.61^{c}	2.67^{e}
Untreated control	-	8.64^{a}	9.00^{a}	5.59^{a}

Values in the same column carrying the same letter(s) are not significantly different (P=0.05) using student –Newman Keuls Test (SNK).

Table 2: Effect of plant extracts on the size of *Curvularia eragrostidis in vitro*

Plant	Spore length (μm)		Spore width (μm)	
	Mean	Range	Mean	Range
Garlic (*Allium sativum*)	0.67^{c}	0.68-0.76	0.33^{b}	0.32-0.34
Sheabutter (*Vitellaria paradora*)	0.67^{c}	0.63-0.74	0.33^{b}	0.28-0.34
Neem leaf (*Azadirachta indica*)	0.73^{b}	0.68-0.78	0.32^{bc}	0.30-0.34
Mahogany (*Khaya senegalensis*)	0.66^{c}	0.62-0.70	0.29^{c}	0.28-0.34
Neem seed (*Azadirachta indica*)	0.66^{c}	0.64-0.68	0.29^{c}	0.26-0.30
Ginger (*Zingiber officinale*)	0.74^{b}	0.70-0.76	0.32^{bc}	0.31-0.33
Benlate (benomyl)	0.70^{bc}	0.62-0.78	0.33^{b}	0.28-0.34
Untreated control	0.80^{a}	0.70-0.84	0.35^{a}	0.32-0.36

Values in the same column carrying the same letter(s) are not significantly different (P=0.05) using Student –Newman Keuls Test (SNK).

The width of the spores also varied with plant extracts. The control significantly had the largest spores compared with other treatments. This was followed by benomyl, shea butter leaf, garlic bulb and ginger rhizome, which were statistically the same. Neem and mahogany seeds extracts were at par and produced the smallest spores. The inhibitory effect of plant extracts on mycelial growth was not significant at 14 DAI, but these plant extracts reduced sporulation and spore size significantly.

Discussion

The advantages of using plant extracts in the control of plant disease have been emphasized by several workers (Appleton and Tansey, 1975; Misra and Dixit, 1976; Shinfe, 1984; Awua, 1989; Obagwu, 1997; Amadioha, 2000; Okigbo, 2002; Okigbo and Enoghene, 2002/03).

Inhibitory effect of garlic and neem extracts in mycelial growth and sporulation of many plant pathogenic fungi such as *Colletotrichum* spp, *Fusarium oxysporum, Aspergillus flavus, Mycosphaerela* sp., *Rhizoctonia solani*, *Pyricularia oryzae, Sclerotium rolfsii*, have been reported by many scientists working independently in different regions (Qasem and Abu Blan. 1966; Misra and Dixit, 1976; Thind and Dahiya, 1977 ; Agrawal, 1978; Gupta *et al.*, 1981; Singh *et al.*, 1983; Shinfe, 1984; Tawari and Dath, 1984; Kodera *et al.*, 1989; Forcke *et al*., 1990.; Tawari and Nayak, 1991; Obagwu, 1997; Amadioha, 2000; Onifade, 2000). The short lived inhibitory effect on mycelial growth of the tested plant extracts agrees with an earlier report by Obagwu (1997) who found that neem and garlic extracts inhibited mycelial growth of *Colletotrichum* sp for 2 to 7 days depending on the concentration. *K. senegalensis* although have not been tested on fungi, its toxic effect in insects has been reported (Bamaiyi *et al.*, 2007). The antifungal activity of most plants has been attributed to the presence of phytoncides, allicin, and essential oils (Gupta *et.al*., 1981).

Conclusion and recommendation

The results of the experiment showed that *K. senegalensis*, *A. indica* and *A. sativum* reduced sporulation and spore size, which compared favourably to benomyl. These plant extracts could be good substitutes for synthetic fungicides for the control of *C. eragrostidis.* This approach to plant disease management, which is economically and environmentally sound, has good prospect, however, extensive work on the appropriate concentration need to be worked out. There is also the need for identification, isolation, characterization and formulation of the active ingredients responsible for the toxicity exhibited by these plant materials. Other effective extraction methods need to be tested. These plant materials also need further field evaluation for recommendation to farmers.

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