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Pathogenicity of fungi associated with brown spot and leaf necrosis of *Hydrangea macrophylla* (big leaf *Hydrangea*) in Uyo, South-South, Nigeria

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ABSTRACT:

Disease incidence surveys were carried out on brown spot and leaf necrosis of *Hydrangea macrophylla* between July and September; 2014.The symptoms observed in the field were brown spot which was circular or slightly irregular in shape and somewhat sunken on the flesh leaves of *H. macrophylla*. The center of the spot was about 1 inch in diameter. Symptoms observed in the field include circular brown spot found predominately at the base of the leaf of *H. macrophylla*. The spot slowly turns tan to somewhat light grey encircled by a purple halo. The spot was about one eighth to one fourth inch in diameter. The infected *H. macrophylla* samples were placed on the potato Dextrose Agar (PDA) and moist chamber. The results showed that *Colletotrichum gloeosporioides* was the most frequently isolated organism on the infected *H. macrophylla* leaves with percentage frequencies of occurrence of (90.0%), while *Cercospora hydrangea* had (10.0%) frequency of occurrence. Pathogenicity on susceptible *H. macrophylla* using all the fungal isolates, showed that *C. gloeosporioides* incited brown spot and leaf necrosis of *H. macrophylla* in this study. The other fungi isolated also may have synergized the disease development in this study.

Keywords:

Hydrangea macrophylla (big leaf *Hydrangea*), Brown spot and leaf necrosis, Pathogenicity test.

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INTORDUCTION

Hydrangea belongs to the plant family Hydrangeaceae. *Hydrangea* is a flowering shrub, easy to grow with different colours in the garden from mid-summer through fall (Fowler and Black, 2000). *Hydrangea* begins flowering from early spring to late autumn. There are firmly packed in bunches towards the end of the stems. They deliver vast sprouts that during late summer and autumn, they produce large blooms with bold and beautiful colour. (Carter and Locke 1994).

Hydrangea macrophylla is reported to have originated in Japan. The name *Hydrangea* originates from the Greek word ("Hydro" which implies jar or vessel') and is generally interpreted as "water barrel" which alludes to *Hydrange's* requirement for a lot of water and its cup shaped flower (Reed, 2000). The colour of *Hydrangea* could be blue, pink or purple and this is controlled by the acidity of the soil (Horst and Locke, 1998).

This plant is a hard and tender shrub and sometimes a woody climber. These are mostly deciduous plants, though a few tender species are evergreen. *Hydrangea* produces diverse flowers from the large globes of "mophead" to the discs of the "lacecaps", thick cones of oakleaf and panicle *Hydrangea*. (Reed *et al*., 2002).The height of *Hydrangea* ranges from dwarf (about2 - 3ft. high) to large bushes with stems of 9-10ft. (Reed, 1999; Reed, 2004). *Hydrangea* inclines toward well drained loamy and acidic soils for ideal productivity. It also thrives in the pH ranges of 4.5 – 6.5 enriched liberally with organic matter. Reed (2001). They requires very low temperature. (Jones and Reed, 2006)

Hydrangea macrophylla is a medicinally important plant (Reed, 2004). *Hydrangea* root is used as syrup with honey and sugar or simply steeped in water and drank as tea. *Hydrangea* bark can be used for the treatment of burns, bruises sparing and sore muscles (Reed, 1998; Reed and Rinehart, 2007). Wild plant is *Hydrangea* used as tonic to the entire genito-urinary system which includes the prostrate and also it generally improves the health of the lungs, stomach and the prostrate (Reed, 1999; 2000). It also cools the body and the circulatory system (Pippa *et al*., 2000). *H. macrophylla* is affected by several fungal diseases which inflict heavy losses in its production. One of such fungal disease is the leaf spot disease. The field symptoms were brown spot which was circular or slightly irregular in shape. The disease spread rapidly during frequent summer rains. The leaf spot disease showed significant impact on the appearance, spectacular floral display and market value of *H. macrophylla*. The disease attack also reduces medicinal potential of the plant. This research work is to study the brown spot and leaf necrosis of *H. macrophylla* (big leaf *Hydrangea*) with the following objectives:

- To isolate and identify organisms responsible for brown spot and leaf necrosis of *Hydrangea macrophylla*.
- To determine pathogenicity test against microorganisms.

MATERIALS AND METHODS

Altogether two experiments were carried out,

Table 1. Mean disease incidence and severity of the leaf spot disease of *H. macrophylla*

S. No	Days	Sample plant	Infected plants	Disease incidence	Disease severity
1	3	100	17	17	1
2	6	100	34	34	1
3	9	100	45	45	4
4	12	100	67	67	4
5	15	100	70	70	5
6	18	100	73	73	5
7	21	100	75	75	5
8	24	100	80	80	5
9	27	100	83	83	5
10	30	100	89	89	5

Disease incidence: (%)

Table 2. Fungi associated with brown spot and leaf necrosis of *Hydrangea macrophylla* and their percentage frequencies of occurrence

S. No	Fungi	Number of pathogens	Percentage frequencies of occurrence (%)
1	*Colletotrichum gloeosporioides*	9	90.0
2	*Cercospora hydrangea*	1	10.0

one at shade house and one at the laboratory at the department of crop science, Faculty of Agriculture, University of Uyo. Uyo is located in the tropical rainforest zone of Nigeria which is located in the 5º03' N, 07º 56' E and altitude 38m above mean sea level (Meteorological Garden, 2008).

Disease survey for the incidence and severity of leaf spot disease of *Hydrangea macrophylla*

Disease survey was conducted on the infected *H. macrophylla* field during the wet season (July to September) of 2009. A total of 100 plants were tested. The disease incidence was acquired by counting the total number of diseased plants in the examined zone over the total number of plants inspected. The disease incidence and severity rate and seriousness were recorded for 30 days Disease incidence and severity were assessed based on Wokocha (1990).

$$\text{Disease incidence (I)} = \frac{\text{Number of plant unit affected x 100}}{\text{Total number (Healthy and infected of unit assessed)}}$$

Disease severity was also recorded on the following scale:

0 = No disease observed

1 = 14% of leaves is infected

2 = 15- 36% of leaves is infected

3 = 37- 50% of leaves is infected

4 = 51 – 75% of leaves is infected

5 = 75 -100% of leaves infected. Wokocha (1990)

Collection and handling of samples

The samples (*H. macrophylla*) were randomly collected from D – line Ewet Housing Estate, Uyo, Akwa Ibom State. These samples were carefully collected (with clean surgical blades), placed in clean specimen bags and taken to the laboratory for investigation.

Isolation of associated fungi

Infected *H. macrophylla* were washed in sterile water to remove contaminant. The washed leaves were cut with sterile surgical blade into sections of 3-5cm long, surface disinfected in 1% sodium hypochlorite solution for 10 minutes and was rinsed in the distilled water for several times. The cut leaf sections were plated on the fresh Potato Dextrose Agar (PDA) and wet chamber. The dishes were incubated at 28+ 2ºc for five (5-7) days. Successive sub-culturing of the isolates on fresh PDA medium was done to obtained pure cultures. The pure culture was stored as stock cultures on PDA slants inside McCartney bottles in the refrigerator at 15^0C until needed (Mbadianya *et al*., 2013).

Identification of pathogens

Pure cultures were wet mount and observed under compound binocular microscope at 100 x and 40 x amplifications for fungal growth. Based on their morphological and cultural characteristics, fungal isolates identified (Barnett and Hunter, 1987). The percentage frequencies of occurence of all the isolated fungi was calculated (Table 5). The presence of fungal growth was recorded each time (Wokocha and Aduo, 2011). The

Table 3. Pathogenicity test of the isolated fungi

S. No	Fungi	Pathogenicity	D.I	D.S
1	*Colletotrichum gloeosporioides*	+	84	5
2	*Cereospora hydrangea*	-	0	0

D.I: Disease incidence; D.S: Disease severity; +: pathogenic; -: Non- pathogenic

Table 4. Disease incidence and severity of leaf spot disease of *H. macrophylla* caused by *Colletotrichum gloeosporioides*

S. No	Days	Disease incidence (%)	Disease severity
1	7	3	1
2	14	16	1
3	21	54	4
4	28	84	5

percentage frequency of occurrence of each isolate was done as per Ebele (2011).

Frequency of occurrence of different types of fungi=

$$\frac{\text{Number of times a fungus was encountered x100}}{\text{Total fungal isolations}}$$

Preparation of PDA

Potato Dextrose Agar (PDA) was prepared, autoclaved and poured into sterile petridishes. PDA utilized in this investigation was sourced from Center Biomedical Laboratories Ltd. Umoren Lane, Uyo (Mbadianya *et al*., 2013).

Preparation of inoculum suspension

The isolates obtained were grown on potato dextrose agar. Hyphal mat from 5-7 day old PDA cultures of the pathogens were scraped aseptically on to a fine cheese cloth, filtered and washed in several changes of sterile distilled water to remove traces of stalling materials. The mat were then transferred aseptically into 200ml of distilled water solution in a warring blender and homogenized for one minute at a low speed in order to get the inoculate ready for pathogenicity test on *H. macrophylla* (Wokocha *et al*., 1986).

Characterization and identification of isolates

The identification of isolated organism was done by comparing the characteristics of the culture with the characteristics of the known taxa. Barnett and Hunter (1987); Frank (2006).

Study on the brown spot and leaf necrosis of *H. macrophylla* (fungal causative agent: *Colletotrichum gloeosporioides)*.

After knowing the reason for the disease, we repeated shade house pathogenicity test with *Colletotrichum gloeosporioides* on *H. macrophylla* and disease incidence and severity was resolved from 100 plants. Data was collected for 30days after inoculation and the symptoms were deliberately noticed.

RESULTS

The mean disease incidence at 30 days and severity of the brown spot and leaf necrosis of *H. macrophylla*.

Hydrangea macrophylla (Table 1) demonstrated that the rate of infection of the pathogen increased as sampling days expanded. The results demonstrated that for each 100 plant unit inspected, 17 plants were infected as at the 3rd day of the disease survey while 89% of plants were infected at the 30th day of the study. The disease severity (degree of damage) likewise follow similar pattern with the disease incidence. From the 18th day to the 30th day, the severity was over 70% of infec-

Table 5. The cultural, morphological and differential characteristics of fungal isolate

S. No	Source of isolate	Isolate	Frank (2006)	Barnett and Hunter (1987)
1	Colour of colony in mass	Black	Black	Black
2	Type of soma present	Setae	Setae	Setae
3	Nature of the hyphae	Aseptate	Aseptate	Aseptate
4	Special vegetative structure	Broom like	Broom like	Broom like
5	Asexual spore (shape)	Oblong	Oblong	Oblong
6	Special vegetative structure	Erect wall	Erect wall	Erect wall
7	Conidia head	Disc shape	Disc shape	Disc shape
8	Probable organism	*C. gloeosporioides*	*C. gloeosporioides*	*C. gloeosporioides*

tion. This results affirm that *Hydrangea macrophylla* is susceptible to fungal attack.

Fungi associated with Brown spot and leaf necrosis of H. *macrophylla*

A total of two (2) different fungi were isolated from infected *H. macrophylla* leaves (Table 2). They were *Colletotrichum gloeosporioides* and *Cercospora hydrangea.* The isolated fungi were identified following macroscopic and microscopic observation of their morphological characteristics and the help of identification guide (Barnett and Hunter, 1987), Frank (2006). *C. gloeosporioides* has the highest percentage frequency of occurrence (90.0%) while *Cercospora hydrangea* had the lowest (10.0%) percentage frequencies of occurrence.

Pathogenicity test

The pathogenicity test results of all the fungal isolates (Table 3) demonstrated that only *Colletotrichum gloeosporioides* was pathogenic on *Hydrangea macrophylla* plants since it produces a replica of brown spot which was circular or marginally irregular in shape and to some degree sunken on fleshy leaves of *H. macrophylla*. The re-appearance of these symptoms and reisolation of the pathogen from the infected *H. macrophylla* plant showed that *C. gloeosporioides* is the culprit pathogen in this study. *Cercospora hydrangea* also isolated may have played a synergistic role in the disease development and progression (Table 4) shows the highest incidence (84%) and severity that were recorded at 28 days after inoculation.

DISCUSSION

The result of the disease survey on *H. macrophylla* taken for a period of 30 days indicated that the infection rate of the pathogen increased progressively as the sampling days increased. The degree of damage (severity) also increased remarkably in the same manner. Two different fungi were isolated from the infected *H. macrophylla* leaves. They were *C. gloeosporioides* and *Cercospora hydrangea. C. gloeosporides* had the highest percentage frequency of occurrence (90.0%) while *C. hydrangea* had the lowest frequency of occurrence (10.0%). *C. gloeosporioides* may be the primary inoculum causing primary infection. The results of the pathogenicity test of all the fungal isolates showed that only *C. gloeosporioides* was pathogenic on *H. macrophylla*. It produces the characteristics symptoms of brown spot with alternating dark and slightly lighter rings of dead tissue which often give the spot a bull-eye target spot appearance which produce the most severe effect at 28 days after inoculation. This was similar to the characteristics symptoms described by (Pirone, 1978). *C. gloeosporioides* was reisolated from infected leaves of *H. macrophylla*. The observation of the characteristic symptoms of brown spot with alternating dark and slightly lighter rings on the leaves and the reisolation of the pathogen from the infected *H. macrophylla* showed that *C. gloeosporioides* incited brown spot and leaf necrosis found on the leaves of *H. macrophylla* in this study. Other fungi isolated may have been secondary invaders on the lesion caused by the culprit pathogen and thus could not cause spot on the leaves. This observation is in agreement with the report of Flentje (1965) and Wilhelm (1967) that such organisms, when inoculated on the host in the absence of the primary pathogen would have no effect.

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