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# Inhibition of some phytopathogenic fungi by spear grass *Heteropogon contortus* (L.) Buauv extract

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**Abstract**

The use of chemical herbicides for the control of phytopathogenic fungi is environmentally undesirable, hence the search for a more eco-friendly approach. This research is intended to determine the inhibitory potential of *Heteropogon contortus* (L.) Buauv, on some phytopathogenic fungi. Soil samples from fifty (50) farmlands in Ethiope east Local Government Area of Delta State, were aseptically collected and assessed for the presence of phytopathogenic fungi. By serial dilution, spread plate technique, cultural characteristics as well as microscopic examination, *Fusarium* sp., *Alternaria* sp. and *Aspergillus* sp. were isolated in percentage occurrences of 34%, 42%, and 64% respectively. Fresh leaves of *Heteropogon contortus* (L.) buauv were collected, rinsed in running water and air dried and then grounded to fine powder, which was extracted using methanol and water. Phytochemicals recovered included; Flavonoids, Alkaloids, Tannins, Phenols, Terpenoids, and Saponins. Poisoned plate technique was used for percentage inhibition of fungal mycelia growth using the aqueous extract, after three and five days. *Alternaria* sp. had a 100% inhibition, at days three and five, using a concentration of 7µL/mL and 5µL/ respectively. *Aspergillus* sp. exhibited a 100% mycelia inhibition at days three and five, using concentrations of 5µL/mL and 3µL/mL respectively. *Fusarium* sp. on the other hand, had a 100% mycelia inhibition at days three and five, using concentrations of 8µL/mL and 6µL/mL respectively. This study reveals inhibition of the phytopathogenic fungi and this inhibition increases with increasing concentrations and period of exposure. Spear grass may prove useful after all as an inhibitor of phytopathogenic fungi.

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**Introduction**

A weed which is often referred to as a grass is a plant considered undesirable or unwanted in a particular location, "a plant in the wrong place" (Ainit, 2015). The term weeds or grass (as mostly used), applies to any plant that grows or reproduce aggressively, or is invasive outside its native habitat. More broadly, "weed" occasionally is applied pejoratively to species outside the plant kingdom, species that can survive in diverse environments and reproduce quickly; in this sense it has even been applied to humans (Athul, *et al.*, 2017). The outstanding ability of weeds to resist pest and pathogens in their environment, have however penciled them out as potential producers of antimicrobials. There is growing interest in herbal fungicides because of their eco-friendly attributes (Dwivedi and Sing, 1998; Karnwal and Sing, 2006). Medicinal plants are used by many ethnic groups as a source of medicine for the treatment of various ailments in both humans and domestic animals. Antifungal activity of grass juice against *Candida albicans* was tested by Vishnu *et al*. (2016) using agar well diffusion method. These plants produce secondary metabolites that have antimicrobial properties, thus screening of medicinal plants provide another alternative for producing chemical fungicides that are relatively non-toxic and cost-effective.

The antimicrobial activities of a number of plants have been reported by many researchers (Bhavya, *et al.*, 2012; Muhammad, *et al*., 2012; Ainit, 2015). However the studies of antimicrobial activities of weeds generally are very limited towards pathogenic bacteria and fungi. Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. Fungi are ubiquitous in the environment, and infection due to fungal pathogens has become more common. In the developing countries, more than 800 million people do not have adequate supply of food and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005).

Lots of quality problems related to aspect of nutritional value, organoleptic characteristics, and limited shelf life in fruit and vegetables are caused by a wide variety of fungal genera (Agrios, 2004). As compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses.

The most important method of protecting the plants against the fungal attack is the use of fungicide. However, many fungicidal agents available in the market are toxic and have undesirable effects on other organisms present in the environment. Some synthetic fungicides are non-biodegradable, and hence can accumulate in the soil, plants and water, and consequently affect humans through the food chain. Also, the development of resistance of pathogenic fungi towards the synthetic fungicides is of great concern. Therefore, it is desirable to use some eco-friendly measures for the management of plant diseases. Environmental problems caused by the synthetic pesticides can be sorted out through the application of natural product and many researchers are at the point of identifying effective natural products to replace the synthetic pesticides (Kim, *et al.*, 2005). Similarly, the use of natural products for the control of diseases in plants is considered as an alternative source to synthetic pesticide due to their lower negative impacts on the environment. Besides being harmless and non-phytotoxic it has been proved that plant extracts exhibit inhibitory effect on pathogens. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and non-phytotoxic, unlike chemical fungicides.

There are evidences from earlier works that several plant species possess antifungal and antibacterial properties. Large numbers of researchers have studied the effects of plant extracts on bacteria and fungi in different parts of the world (Nagamani, *et al*., 2012; Dhale 2013).

There are however, not much work done on the use of weeds. The present investigation is therefore, undertaken to test the efficacy of spear grass, *Heteropogon contortus* (L.) Buauv. extracts against some phytopathogenic fungi. This will not only be a suitable alternative to chemical pesticide use against pathogenic fungi but, a form of "waste to wealth" initiative as the weeds will be put to good use.

**Materials and methods**

*Source and collection of plant materials*

Fresh and healthy leaves of spear grass plant were collected from fallow undeveloped site of the Delta State University main campus, Abraka in December 2018. Plant materials were washed thoroughly under running tap water to remove dust particles. Leaves were left to air dry at room temperature of about $30^0$C for 7 days and ground into fine powder before the extraction process was carried out.

*Isolation and identification of phytopathogenic fungi*

Soil samples (50) were collected from different farmland using a sterile trowel to a depth of 5cm into a sterile container and conveyed to the Microbiology laboratory for further analysis. Samples were serially diluted and plated on sabouraud dextrose agar (SDA) plates, using spread plate technique. Individual colonies from the mixed culture were subcultured to fresh SDA plates. Pure culture of phytopathogenic fungi was identified based on cultural characteristics and lactophenol cotton blue microscopic observation (Connell *et al.,* 2006; Rebecca *et al.,* 2012).

*Preparation of Extract*

For plant extracts preparation, 20g of leaf powder was extracted in a soxhlet apparatus using 200ml of solvent. Two solvents were originally used, methanol and water and the extract were concentrated using a rotary evaporator. The resultant extract were weighed and kept at $4^o$C until required for use when it was reconstituted to specific concentrations (Udaya Prakash, 2011, 2012).

*Phytochemical analysis*

A. *Alkaloids:* To 0.5g of each extract sample, was added 5ml of 2N HCl and filtered. Dragndoff's reagent was added and formation of red precipitate was used to indicate the presence of alkaloids.
B. *Phenols:* About 3-4 drops of ferric chloride solution was added to the extract. The presence of phenol was indicated by formation of bluish black colouration.
C. *Terpenoids:* Two (2)ml of chloroform and 3ml concentrated $H_2SO_4$ was added to 5ml of extract. This was done carefully to form a layer. Positive result for the presence of Terpenoids was indicated by reddish brown colour formation.
D. *Saponins:* To a small quantity of the extract was added distilled water up to 20ml and shaken vigorously. The formation of 1cm layer of foam stable for 10minutes indicates a positive result for the presence of saponin.
E. *Flavonoids:* Sodium hydroxide (10%) was added to the plant extract. The formation of yellow colouration indicated the presence of Flavonoids.
F. *Phenolics and Tannins:* A 10% Lead acetate solution was added to little quantity of the extract already dissolved in distilled water. The presence of Tannin was indicated by formation of a white precipitate (Borkataky *et al*., 2013).

Further, titration was used for the quantification of the various phytochemicals.

*Determination of antifungal activity of extract*

This was undertaken using the Poisoned plate method (Agjou *et al*., 2012). Concentrations of 1-10µL/mL were prepared and dispensed into sterile petri dishes which were then overplayed with molten PDA at $45^o$C. This was done carefully to avoid formation of bubbles. After solidification, agar discs with 6mm diameter mycelia were cut from the periphery of actively growing, 7 day old fungi cultures.

Control plates without the plant extract were also inoculated. All fungi species isolated served as replicates for this experiment. Plants were incubated at $28^0$C. After 3 and 5 days of incubation, the fungi colony diameter readings were taken. By measurement of mycelia growth, percentage inhibition of test fungi was determined and the means recorded (Gakuubi *et al*., 2017).

Inhibition of mycelia growth (%) = $\frac{dc-dt}{dc}$ x 100

Where dc =mean diameter of colony in control sample.

And dt = mean diameter of colony in treated sample.

**Results**

Results from this study reveal a high prevalence of some phytopathogenic fungi in the farmlands soil surveyed. *Fusarium* sp. *Alternaria* sp. and *Aspergillus* sp. had a number (and percentage occurrence) of 17 (34.0), 21 (42.0), 32 (64.0) respectively, from a total of 50 farmlands surveyed (Table 1). Phytochemicals detected in the spear grass extract were; Flavonoids, Alkaloids, Tannins, Phenols, Terpenoids and Saponins. Quantification by alcohol and aqueous extraction reveals that aqueous extract had significantly higher ($P \leq 0.05$) yields, apart from the Alkaloids (Table2).

Concentrations of 1-10µL/mL of extract were used totest mycelia growth inhibition. For *Alternaria* sp. percentage inhibition at day 3 range from 71.2 to 100 at concentrations of 1 and 10µL/mL respectively. Percentage mycelia inhibition range 75.2 to 100 at concentrations of 1 and 10µL/mL respectively, after day 5. At day 3, *Aspergillus* sp. experienced a 100% inhibition at a concentration of 5µL/mL while at day 5, the concentration of 3µL/mL exerted a 100% mycelia inhibition (Table 3 and 4). *Fusarium* sp. seem to be the least inhibited, with a 100% mycelia inhibition using 8µL/mL and 6µL/mL at days 3 and 5 respectively.

**Table 1.** Percentage occurrence of phytopathogenic fungi farmland soil samples (N=50).

| Phytopathogenic fungi isolated | Fungal prevalence number (%) occurrence |
|---|---|
| *Fusarium* sp. | 17 (34.0) |
| *Alternaria* sp. | 21 (42.0) |
| *Aspergillus* sp. | 32 (64.0) |

**Table 2.** Quantification of phytochemicals present based on solvent used.

| SN | Phytochemical | Solvent used Methanol | Water |
|---|---|---|---|
| 1 | Flavonoids | 1.97g/kg | 4.02g/kg |
| 2 | Alkaloids | 16.92g/kg | 9.05g/kg |
| 3 | Tannins | 5.51g/kg | 8.72g/kg |
| 4 | Phenols | 5.09g/kg | 9.20g/kg |
| 5 | Terpeniods | 0.32mg/kg | 0.52mg/kg |
| 6 | Saponins | 0.57mg/kg | 0.63mg/kg |

**Table 3.** Percentage of mycelia growth inhibition after three days.

| Conc. of extract (µL/ml) | *Alternaria* sp. | *Apergillus* sp. | *Fusarium* sp. |
|---|---|---|---|
| 1 | 71.20 ± 1.10 | 81.20 ± 1.06 | 63.40 ± 1.09 |
| 2 | 72.61 ± 0.68 | 90.20 ± 0.96 | 66.12 ± 1.40 |
| 3 | 75.12 ± 1.20 | 92.42 ± 1.10 | 67.30 ± 1.12 |
| 4 | 80.10 ± 0.77 | 95.22 ± 0.88 | 68.20 ± 1.10 |
| 5 | 82.10 ± 0.98 | 100 | 71.15 ± 0.78 |
| 6 | 91.42 ± 1.09 | 100 | 82.20 ± 1.10 |
| 7 | 100 | 100 | 95.30 ± 0.98 |
| 8 | 100 | 100 | 100 |
| 9 | 100 | 100 | 100 |
| 10 | 100 | 100 | 100 |

**Table 4.** Percentage of mycelia growth inhibition after five days.

| Conc. of extract (µL/ml) | *Alternaria* sp. | *Aspergillus* sp. | *Fusarium* sp. |
|---|---|---|---|
| 1 | 75.20 ± 1.16 | 88.60 ± 1.06 | 72.22 ± 0.96 |
| 2 | 79.51 ± 0.70 | 92.20 ± 0.96 | 76.16 ± 1.12 |
| 3 | 85.44 ± 1.67 | 100 | 80.22 ± 1.06 |
| 4 | 95.23 ± 1.12 | 100 | 90.01 ± 0.88 |
| 5 | 100 | 100 | 94.10 ± 0.90 |
| 6 | 100 | 100 | 100 |
| 7 | 100 | 100 | 100 |
| 8 | 100 | 100 | 100 |
| 9 | 100 | 100 | 100 |
| 10 | 100 | 100 | 100 |

**Discussion**

This study reveals a high percentage occurrence of phytopathogenic fungi in farmlands soil samples, in Ethiope east Local government area of Delta State. A number of food crop disease, as well as mycotoxicosis have been associated with the fungi isolated in this study (Agrios, 2004).

Hence, it is possible to experience a high degree of losses of crops and possible infection of individuals who consumes food contaminated with their mycotoxins. These values are considerably higher as compared with results obtained from previous studies (Gakuubi *et al.,* 2017; Siramon *et al*., 2103).

It is quite intriguing that the various phytochemicals observed in this study, were prevalent in *Heteropogon contortus* (L.) Buauv. There is paucity of information regards to spear grass phytochemical screening but this study has revealed a higher yield of these active phytochemicals using aqueous extraction. The Alkaloids however, had a higher yield using methanol extraction. Yields obtained may probably be as a result of the various solubilities of the phytochemicals in the respective solvent (Bibhuti *et al*., 2013). Mycelia growth inhibition of the tested fungi by the aqueous extract of spear grass increase in the order, *Fusarium* sp. ≤ *Alternaria* sp. ≤ *Aspergillus* sp. In other words, *Aspergillus* sp. was best inhibited while *Fusarium* sp. was least. Also growth inhibition increased with increasing concentration of extracts. The inhibition of *Fusarium* has been reported in previous study (Nefzi *et al*., 2016; Gakuubi *et al*., 2017). These values of inhibition are quite comparable with the present study, though studies were not based on the use of spear grass extract. Also worthy of note, is the enhanced activity of the plant extract with increasing period of exposure.

**Conclusion**

Further studies into the specific active phytochemical present in this weed are recommended. The aqueous extract of spear grass may serve as a suitable replacement for chemical herbicide against some phytopathogenic fungi in the future.

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