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Full Length Research Paper

Antifungal Activity of *Gueira Senegalensis* and *Mangifera Indica* On loose Smut Pathogen (Sporisorium Cruentum)

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Methanolic extracts from two different plant species were tested at concentrations 250, 125, 62.5 and 31.2mg/ml as potential sources of antifungal agents for the fungi *Sporisoriumcruentum.* The plant species were *Guierasenegalensis* (leaves) and *Mangiferaindica* (leaves). The objective of the research was to determine the antifungal activities of leave extract with methanol as solvents on the most frequently occurring sorghum pathogens of the loose smut *(Sporisoriumcruentum)* in Nigeria. All extracts exhibited moderate to good activities on the tested fungi with minimum inhibitory concentrations (MICs). The test organism was sentitive to the *G. senegalensis* extracts, with 14mm and 10mm in diameter at 250mg/ml and 125mg/ml, respectively. The test organism was sensitive to *M. indica* leaf extract with 12mm in diameter at 250mg/ml. The result showed that all the plants exhibited minimum inhibitory concentration against the test organism. Therefore, it is concluded that extracts of *G. senegalensis* and *M. indica* could be used in controlling the fungal pathogen of loose smut *(Sporisoriumcruentum).*

Keywords: Methanolic extracts, antifungal activities, *Mangiferaindica, Guierasenegalensis, Sporisoriumcruentum*

INTRODUCTION

Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties, like analgesics, anti-inflammatory, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). For example, a large proportion of the South American population use plant extracts obtained from traditional medicinal plants as medicine for many infectious diseases. Plants from the genus Pterocaulon, known as “quitoco”, are commonly used in veterinary medicine in southern Brazil to treat animal problems popularly diagnosed as “mycoses” (Demo and Oliva, 2008). Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers.

Loose kernel smut, caused by fungus *sporisoriumcruentum* (synonym *Sphacelothecacruenta*) is less widespread than covered kernel smut. Loose kernel smut attacks all group of sorghums, including Johnsongrass, is usually not infected. Normally, all kernels

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in an infected panicle are smutted. Partial destruction is rare. Some kernels may be transformed in to leafy structures or escape infection completely. Individual kernels are replaced by small smut galls (or sori) that are 2.5cm or longer, poited and surrounded by a thin gray membrane. This membrane usually ruptures when or soon after the panicle emerges from the root. Smutted panicles appear earlier than the remainder of the crop and are more open than healthy panicles (Chilean, 2011). The powdery, dark brown to black spores (teliospores) are soon blown away, leaving a long, black, pointed, conical, often curved structure (culumella) in the centre of what was the gall. Some smut spores (6 to 10 microns in diameter) adhere to the surface of healthy kernels on neighboring plants in the same field or ones nearby before and during harvest. When such infected kernels are planted, the teliospores germinate along with the seed by first forming a thick, usually 4-celled promycellum bearing lateral sporidia (Doshimov 1982) The sporidia germinate and infect the developing sorghum seeding. Most infections, however, result from the teliospores producing hypae which penetrate young seedings before emergence. Seeding infection occurs over a wide range of soil moisture andPH at a temperature of 68 to 77f (20 to 25). The fungus continues to grow systemically within the plant unobserved until heading, when the long, black.

Importance

If uncontrolled, loose smut can wipe out entire crops, since it replaces the grain. In areas where people depend on their grain crops for survival and don't have the money or technology resources to control it, the disease can be devastating. Not only does it wipe out the crop, growers cannot even try again next year since any seeds they were able to harvest will be infected and will not produce seed the following season. Loose smut has recently become a more serious problem in many areas in Nigeria. These areas have been particularly affected by global climate change and have experienced many new crop pests and diseases because of it. In places like the United State where there are ample resources and technologies such as fungicide seed treatment, loose smut is not an especially important disease (Martin *et al.,* 1980).

The loose Smut of Sorghum is caused by the fungus (*Sphacelothecacruenta)* which forms teliospores(hibernating stage of the pathogen) and sporidia in its life cycle. Spherical or ellipsoidal, tinted, smooth teliospores are 4.8 x 10.8 mm, rarely to 12 mm in diameter. At sprouting they give a four-celled basidium with laterally and apically growing sporidia. The sporidia are spindle-shaped or oblong, 2.8 x 12.7 mm in size. Mycelium is multicellular. The disease external symptoms become apparent immediately after panicle emergence from flag leaf sheath. All ovaries of inflorescences are affected, and smut sori can develop on peduncles, glumes, stamens as well. Their size can reach 1.5-2.0 cm in length and 0.4-05 cm in width. After rupture of sous coat, fungal teliospores disperse, leaving clearly visible central column (columella). A panicle spangled with a lot of teliospores looks like a burnt fire-brand, sharply contrasting with healthy plants. The infection takes its origin from infected seeds. The infection occurs during only seeding stage of plant development (Silaev, 2005)

Distribution

The loose smut of Sorghum was for the first time recorded in Russia in 1917. Within the area of sorghum growing in the former USSR territories, the disease is recorded in states of Central Asia and Transcaucasia, in Ukaraine and Moldova (Zaprometov, 1917)

Economic significance

The pathogen affects plant growth and development adversely. Grain yield losses correlate well with percentage of the disease distribution in fields. Control measures include cultivation of resistant varieties and hybrids, seed treatment before sowing (Akpa, 1991)

Justification

Today, loose smut is among the most prevalent disease infecting sorghum and it causes a great loss of significant economic value, as such it has to be prevented. this problem lead to some incision like:-

1. The modern chemicals used to control loose smut is much cost which are hard for most people to afford it especially the less privilege ones.
2. This disease can be treated using traditional plants extract. And we have this plant plenty or are abundant around us.
3. As a result of the abundant nature and the effect of this plant, it is important to verify the fact on them. If this plant extracts were verified and identified their inhibitory effect of this fungus, it will be recommended in treatment of loose smut.

The objectives of this study are;

1. To determine the antifungal activity of the two plants leaf extracts on the test organism *(S.cruentument)* at different concentration of the extract.
2. To determine the appropriate concentration of the various leaves extracts that can inhibit the growth of *S.cruentum.*

MATERIAL AND METHODS

Study Area

The research was conducted at Biology Laboratory, Kano University of Science and Technology Wudil at latitude $11^{0}48^{1}$N, longitude 8^{0} 51^{1}E and altitude 403M above the sea level.

Collection of Samples

Guierasenegalensis and *Mangiferaindica* leaves were collected according from the environment of Kano University of Science and Technology Wudil, close to the river sites. The plants were first identified at the field using standard keys and descriptions (Dalziel, 1956; Keay, 1989). Its botanical identity was further confirmed and authenticated at the herbarium section of the botany unit of Biological science, Kano University, Wudil, Kano State.

Sample Preparation

The preparation of samples was carried out in accordance with the method of (Kareem *et al,* 2003). The leaves samples were separately collected, sterilized by immersing in 3% sodium chloride solution for three minutes, and rinsed in three changes of running of tap water and kept in shade for air-drying for one (1) weeks. The leaves were separately grinded in to powder in a clean mortar and pestle and packaged for use (Kareem *et al,* 2003).

Test Organism

The test organism used was loose smut of sorghum which was collected from the stalk of the infected plant. It was obtained from international crop research institute for the semi arid tropics (ICRI SAT). The organism was confirmed by the ICRI SAT officials. The isolate was maintained in a wrapped paper until required for use.

Extractions

The extraction was conducted according to the method of (Fatope *et al,* 1993). Soxlet extraction technique was used. A quantity (50g) of the fine powder of the extract was weighed and suspended in to a conical flask and percolated with 250ml of methanol. The process was carried out for both of the extracts and later each was allowed to stand for one (1) week with a constant shaking at interval of days under a room temperature. The percolates were then filtered and solvent (methanol) was evaporated and obtained methanol and aqueous extracts and these served as solutions. The exracts were then put in a small sample bottles for future use.

Preparation of Stock Concentrations

This was carried out using standard method (Cheesbrough, 2002). Stock solution of the methanolic extract was prepared by weighing 0.25mg of it and dissolved in 0.75ml of dimethylsulphur dioxide (DMSO) in small sample bottle. This gave an extract concentration of 250mg/ml (stock solution). Three varied extract concentrations (125mg/ml, 62.5mg/ml and 31.25mg/ml) were prepared from the stock solution.

Preparation of (PDA) Culture Media

The medium used was potato dextrose agar (PDA) which is the faster and best media for fungal growth. Exactly 19.5 grams of Mueller-Hinton agar was weighed and mixed with 250ml of sterile distilled water. The mixture was the sterilized by autoclaving at 121^{0}C for 20minutes. Under aseptic conditions in the laminar flow hood, 20ml of agar media was uniformly dispensed in to sterilized Petri dishes. They were then covered and allowed to cool at room temperature until the culture media hardened. The inoculation of the fungi was cultured on the agar surface by spread plating technique (Prescott, et al, 2002).

Sensitivity Testing

Sensitivity was carried out using agar diffusion method (Kirby,*et al,* 1966). The freshly prepared potato dextrose agar (PDA) plates were dried in a dryer for 10minutes to remove the surface moisture. The plates were aseptically inoculated uniformly with the test organism by spread plating technique. A sterile cork borer (6mm) in diameter was used to bore 4 wells and one (1) at the center for control for each sample numbered 1-5, respectively then a 0.1ml of the extract was transferred in to respective agar plates and the ethanol was placed at the center to serve as control (Meyer, and Dillika; 1996) the plates were incubated aerobically at $37^{0}{}_{c}$ for 72hours. Diameter of zone of inhibition was been measured using millimeter rule. The result was interpreted and recorded in accordance with Chessbrough (2002).

Preparation of Potato Dextrose Broth (PDB)

The potato dextrose broth was used to determine the minimum inhibitory concentration of the extracts which is in

Table 1: Physical characteristic of the leaf extracts of *G. senegalensis and M.indca*

Plant part	Solvent	Colour	Odour	Texture
G. senegalensis Leaf	Methanol	Dark brown	Slightly repulsive	Oily
M. indica Leaf	Methanol	Brown	Pleasant fruity	Soft

Table 2: Antifungal effects of Guierasenegalensis against loose smutpathogen at different concentrations

Concentration in (mg/ml)	250	125	62.5	31.2	25 ethanol (control)
Zone of inhibition in (mm)	14	10	0	0	14

Table 3: Antifungal effect of Mangiferaindica against loose smut pathogen at different concentrations

Concentration in (mg/ml)	250	125	62.5	31.2	28 ethanol (control)
Zone inhibition in (mm)	12	0	0	0	16

liquid form. Exactly 6.25g of (PDB) was weighed and mixed with 125ml of sterile distilled water. The mixture was then sterilized by autoclaving at 121^0C for 20minutes. Under aseptic conditions, the media was allowed to cool at room temperature until required for use (Prescott, *et al,* 2002).

Determination of Minimum Inhibitory Concentrations of Theextracts

The (MIC) was carried out according to the method of Prescott et al, (2002). The MIC was determined using tube dilution method. 8ml of potato dextrose broth was pipette in to a set of 10 tubes for each sample extracts that show sensitivity to the test organism. Serial dilution was carried out using 2ml of stock solution in the first test tube contained 8ml potato dextrose broth give a dilution of 250mg/ml. 2ml of this later concentration was then pipetted into the next test tube containing 8ml potato dextrose broth to give a dilution of 125mg/ml. This was continued until 10 serial dilutions equivalent to 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.6mg/ml, 7.81mg/ml, 3.9mg/ml and 1.9mg/ml all in 100% w/v were obtained. The first tube contained the potato dextrose broth and the extract which served as positive control. The remaining 8 tubes were then inoculated with 0.1ml of the test organism and incubated at 37^0C for 72 hours. The MIC inhibitory concentration was then recorded i.e. turbid tubes indicated growth while clear tubes showed inhibition. The lowest concentration without visible signs of growth was taken as the minimum inhibitory concentration.

RESULTS

The present study tested the antifungal activity of leaf extracts and their respective inhibition against loose smut pathogen. These plants extract were chosen based on either traditional usage, suggestive of antimicrobial activity, or previous studies that have demonstrated antifungal properties using different kinds of extracts (*Guo et al,* 1997; Wilson *et al,* 1997) each of the extract shows inhibitory activity to the test organism but not all of them show inhibition to the different concentrations used.

DISCUSSION

Many naturally occurring compounds found in plants have been shown to possess antifungal functions and thus serve as a source of both traditional and orthodox medicine (Kim *et al,* 1995; Abhay *et al,* 1997; Adoum *et al,* 1997; Awune, 2002; Musa *et al,* 2002; Kalamba and Kuneika, 2003; Akinyemi *et al.,* 2007; Yusha'u *et al.,* 2008). For example, flavonoids are known to inhibit fungal growth (Lutterodt *et al.,* 1994; Mbuh *et al.,* 2007) and thus responsible for the antifungal activity exhibited in this study. The results of the present study further support Bibitha *et al.,* (2002) who reported variation in the antifungal activities of different plants extracts. On the other hand, the variations observed in this result of the plants parts screened to inhibit fungal growth are in conformity with the reports of Yusha'u et al., (2008) that antifungal activity may vary from one plant to another. The results in Table 2 and 3 showed that *G.senegalensis* shows inhibition to two different

Table 4: Minimum inhibitory concentration of G. senegalensis at 125mg/ml

Concentrations (mg/ml)	Inhibition
250	-
125	-
62.5	-
31.2	+
15.6	+
7.81	+
3.90	+
1.90	+

Table 5: Minimum inhibitory concentration of M. Indica at 250mg/ml

Concentration (mg/ml)	Inhibition
250	-
125	-
62.5	-
31.2	-
15.6	+
7.81	+
3.90	+
1.90	+

Key: + Growth (Turbid)

_ No growth (Clear)

MIC = Minimum inhibitory concentration

concentrations 250mg/ml and 125mg/ml at 14mm and 10mm respectively and therefore, is the appropriate concentration while *M. indica* shows inhibition on only one concentration 125mg/ml and it is also the appropriate concentration. The other Tables i.e. 4 and 5 showed the minimum inhibitory concentrations (MIC). The (MIC) of *G. senegalensis* was 62.5mg/ml while the (MIC) of M*. indica* was 31.2mg/ml.

CONCLUSIONS

The study has shown that two (2) locally available indigenous plants namely; *Guierasenegalensis* and *Mangiferaindica* are very effective in inhibiting the fungal growth of loose smut. The fungitoxic effects of the extracts indicate the potential of some plant species as a natural source of fungicidal material. Antifungal activity was confirmed in all the plant species tested, although the

results showed that different plant extracts varied in their effectiveness in inhibiting the fungal growth of the pathogen tested. *Guierasenegalensis* has the higher inhibitory activity against the test organism. Therefore, it may be concluded that plant extract can successfully be used for controlling loose smut fungal pathogen of sorghum instead environmental hazardous chemicals for treating looses Smut of sorghum in Nigeria.

RECOMMENDATION

To broaden the scope of this study, is to achieve the goal for ideal medicinal plant of acceptable standard, further work is therefore recommended on the other extraction methods, and on the chemical basis of the active compounds of the plants and their toxicology.

Further research is also suggested on the study of the antifungal activities of these plants.

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