In-Vitro Evaluation of Selected Botanicals for the Control of *Ceratocystis Paradoxa* (Dade. Moreau.) The Causative Organism of Sugarcane Sett Rot Disease.

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Abstract

A laboratory study was carried out, using the food poison method, to evaluate the antifungal properties of crude extracts from four plant samples (Hyptis, Jatropha, Garlic and Ginger) against Ceratocystis paradoxa, the sugarcane sett rot pathogen. Filtered 100% conc. (w/v) of hot and cold crude extract of each plant sample was obtained using hot (70^o C) and cold (room temperature) water for soaking each crushed plant sample for 24hrs. Four concentrations, 40%, 60%, 80% and 100% of each crude extract was obtained through dilution of 100% stock solution with sterile distilled water as required. Exactly 2 ml of each concentration of the crude extracts was dispensed into 10 ml of hot Potato Dextrose Agar (PDA) in a Petri-dish to form PDA/crude extract complex. Exactly 5 mm agar disc from one week old culture of C. paradoxa was inoculated at the centre of each PDA/crude extract complex. Each crude extract concentration and control forms a treatment and each treatment was replicated four times. A total of 36 Petri-dishes were used and the experiment was laid out in a Completely Randomized Design. Incubation was done for 9 days at 27^o C ± 2^o C. Results from the study showed that significantly highest values of mycelia growth inhibition was recorded for garlic at all the levels of cold crude extracts evaluated, while the hot extract inhibited sporulation significantly at 40%, 80% and 100%. Garlic extract is therefore recommended for the control of C. paradoxa.

Key words: *Crude extract; food poison method; Ceratocystis paradoxa; sugarcane sett rot*

Introduction

Sugarcane is an annual crop belonging to the Poaceae family. The crop grows in parts of the world with warm climate with temperature playing an important role in sett germination. (Yang and Chen, 1980). The crop is believed to have originated from Polynesia, but its modern centre of diversity is Papua New Guinea. (Daniels and Rouch, 1987). It grows best in sandy loam soil rich in Calcium, Potassium, Phosphorus and Nitrogen. Nitrogen is particularly very important for cane germination and subsequent development. (Croft, 1998). Sugarcane plant usually grow erect with numerous repeating nodes and internodes. Each node gives rise to a single leaf and the base of each leaf forms a sheath around the node from which it is formed. The average height of the plant is usually around 2-3 m and maturity is attained 12 – 18 months after planting, (Bull, 2000). Harvesting is done by cutting matured cane a few centimetre above the soil surface.

Sugarcane is of immense importance worldwide because of the sucrose it contains. Sugar is the primary product obtained from sugarcane after processing. Other by-products includes bagasse, molasses and trash. (Mackintosh, 2000). It has been reported that about 70% - 80% of global sugar need is derived from sugarcane, while the remaining 30% - 20% is from sugar beet. (Vijaya, *et al*., 2007). Sugar is important as raw material in the production line of many industries like pharmaceutical, food and beverages. Ethanol and fertilizer can also be generated from molasses. (Mackintosh, 2000).

In Nigeria, only 3% of the sugar consumed is produced locally. The remaining 97% is imported. (Okwy, 2015). As a means of redressing this anomaly, the federal government recently lunched the National Sugar Master Plant. (Lubem, 2012). One of the aims of the plan is to put several hectare of land under sugarcane cultivation. This is a very ambitious plan, and the National Cereal Research Institute (NCRI) is currently carrying out field trials for several varieties of sugarcane. The aim is to find out the varieties of sugarcanes that are best adapted to the various agro-ecological zones of the country, such that the most promising varieties will eventually be released to peasant farmer for mass cultivation. It must be pointed out however that previous attempt at increasing sugarcane production in the country has not been very successful owing to a number of factors. Pest and diseases happens to be some of these factors. Common pest includes termites, (they eat up cane cuttings bringing about germination failure and reducing seedling vigour, wireworm and stem borer. (Agnew, 1997). Bacterial diseases includes ratoon stunting and leaf scald. (McLeod, *et al.,* 1999; Croft, *et al.*, 2000). Fungal diseases are particularly devastating. It includes red rot, whip smut, sett rot amongst others. (Courtney, 2002; Magarey and Bull, 2003). Sett rot has been reported to bring about complete germination failure, (Talukder, *et al.*, 2007) and yield reduction by as much as 31%- 35%. (Anon, 2000). The disease is caused by a fungus called *Ceratocystis paradoxa.* (Dade. Moreau.) The pathogen is sett and soil borne. (Vijaya, *et al*., 2007). Attempts at managing the disease has been through the use of synthetic chemicals like Benomyl, Carbendazim, Hexaconazoles and many others (Tiwari *et al*., 1988; Xiujian, *et al*., 2000) Chemical control, though highly effective in most cases, have associated problems. These problems includes a high level of toxicity to man and non-target but beneficial organisms, disruption of the natural ecosystem, development of resistance by pathogens after prolonged usage amongst others. (Rueeg and Siegfried, 1996). Consequently, a need has arisen to find alternative approaches in the control of the sett rot pathogen and by extension, pathogens of other crops.

Natural plant products is one of such possible alternatives. Literature abounds with research findings on the efficacy of plant extracts in the control of fungal diseases of crops. (Ajayi and Olufolaji, 2009; Olufolaji, *et al*., 2015 and Sesan, *et al*., 2015). It is for this reason that this

study was design to evaluate crude extracts from four plant samples for their efficacy in the control of *C. paradoxa*, the sugarcane sett rot pathogen. Previous research work has shown that the four plant samples evaluated in this study contains bioactive compounds with antimicrobial properties against some fungal pathogens of crops. (Depak, *et al.*, 2007; Ajayi and Olufolaji, 2009)

Methods

Study area.

The study was carried out at the pathology laboratory of the Department of Crop, Soil and Pest Management (CSP), Federal University of Technology, Akure. (FUTA). Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) were purchased from Akure main market (Oja-oba) Jatropha (*Jatropha curcas*) leaves was obtained from Jatropha farm established by CSP Department, while Hyptis (*Hyptis suaveolens*) leaves were obtained from students residential area off campus.

Preparation of hot and cold crude extracts of the four plant samples.

The four plant samples (Ginger rhizome, Garlic bulb, Jatropha and Hyptis leaves) were washed with sterile distilled water and air dried for 5 hrs. Exactly 100 g of each sample was weighed and crushed in a mortar with pestle. Each sample was soaked in 100 ml of hot water (70^o C) separately (hot crude extract) in conical flasks and allowed to stand for 24hrs. Thereafter, filtration was done aseptically with a double layered muslin cloth. The filtrate (100% concentration w/v stock solution) was then reconstituted to give four different concentrations of 40%, 60%, 80% and 100% by adding sterile distilled water as appropriate. The same procedure was adopted in the preparation of cold crude extracts, the only difference being that the crushed plant samples were soaked in sterile cold water (room temperature) and allowed to stand for 24 hrs. The different extract concentrations, hot and cold, were used immediately after preparation.

Preparation of culture medium:

The culture medium of choice was Potato Dextrose Agar (PDA). It was prepared by dissolving 3.9 g of PDA in 100 ml of distilled water in a conical flask. The mouth of the flak was plugged with a non-absorbent cotton wool and covered with aluminium foil. Sterilisation of the PDA along with all glass and metal wares used for the study was done in an autoclave at 121^o C for 15 minutes.

Isolation of Ceratocystis paradoxa from infected sugarcane stem.

Sugarcane sett infected with the sett rot pathogen was obtained from a sugarcane field at the Teaching and Research Farm. FUTA. Small segments (about 5mm x 8mm) from the advancing region were cut out with a scalpel and surface sterilized with 70% alcohol. The segments were rinsed in three changes of sterile distilled water and blotted dry with sterile blotting paper. Inoculation of the segments was done in three Petri-dishes at four cane segments per Petri-dish. Incubation was done at 27^o C ± 2^o C for 24 hrs. Sub-culturing was done after 24 hours of incubation. The pure culture obtained was confirmed to be *C. paradoxa* through the visual observation of the morphological characteristics displayed on the culture plate and by using standard texts on fungi identification by Barnet and Hunter, (1998) and Frank, (2005).

Evaluation of crude extracts for antifungal properties against *C. paradoxa*.

The food poison method was employed. Exactly 2 ml of each crude extract concentration was dispensed into 10 ml hot PDA in each Petri-dish. The PDA/crude extract complex was swerve gently to allow for proper mixing. Inoculation of 5 mm agar disc from one week old culture of *C. paradoxa* was done at the centre of each PDA/crude extract medium after cooling and gelling. The control had 2 ml sterile distilled water dispensed into 10 ml PDA. Each crude extract concentration and the control represented a treatment. A total of 9 treatments were evaluated and each treatment was replicated 4 times. The total number of Petri-dishes used for the study was 36. (4 Petri-dishes for the control and 16 Petri-dishes each for hot and cold crude extracts). The experiment was laid out in a Completely Randomized Design (CRD) and incubated at 27^{o} C ± 2^{o} C. for 9 days

Data collection and statistical analysis.

Data were collected on;

Mycelia growth of *C. paradoxa* in each culture plate. This was at 72 hours interval and the values obtained were converted to percentage inhibition of mycelia growth using equation 1.

$$Percentage\ inhibition\ of\ mycelia\ growth = \frac{dc - dt}{dc}\ x\ 100 \qquad equation\ 1$$

dc = average diameter of mycelia growth of fungal colony in control;

dt = average diameter of mycelia growth of fungal growth in treated Petri-dish

The number of spores in each culture plate was estimated with the aid of a haemocytometer slide 9 days after inoculation. The values obtained were converted to percentage inhibition of sporulation of *C. paradoxa* using equation 2.

$$Percentage\ inhibition\ of\ sporulatio = \frac{sc - st}{sc}\ x\ 100 \qquad equation \qquad 2$$

sc = average spores produced in control

st = average spores produced in treated Petri-dish

Data were transformed and subjected to analysis of variance (ANOVA) using Minitab version 17 software. Means were separated using Tukey test.

Results.

Effects of cold crude extracts of the four plant samples on the mycelia growth of *C. paradoxa.*

The highest percentage mycelia growth inhibitions of 57.40, 57.78, 76.15 and 100.00 at 40%, 60%, 80% and 100% respectively was recorded for Garlic cold crude extracts. These values were significantly higher than the others. The lowest value of percentage inhibition of mycelia growth inhibition at 100% concentration of cold crude extract (45.96) was recorded for Jatropha. This value was however not significantly different from the values recorded for Ginger (48.63) and Hyptis (46.27) at the same concentration. (Fig 1).

Effects of hot crude extract of the four plant samples on the mycelia growth of *C. paradoxa.*

Significantly highest values for percentage inhibition of mycelia growth, 67.67, 84.90 and 88.23 at 40%, 60% and 80% respectively was recorded for Ginger hot crude extract. At 100% extracts concentration, Garlic inhibited *C. paradoxa* mycelia growth significantly, giving a

value of 100.00%. Table 2. Jatropha gave significantly lowest values of mycelia growth inhibition at 60% and 80%. (Table 2).

Effects of cold crude extracts of the four plant samples on the sporulation of *C. paradoxa.*

Sporulation was inhibited by 61.62% and 71.57% by Garlic cold extract at 40% and 60% concentrations respectively. These values were significantly higher than those recorded for the other three plant samples. Fig 3. At 80% and 100% extract concentrations however, Ginger and garlic showed no significant difference in their percentage inhibition of sporulation. Significantly lowest value of percentage inhibition of sporulation (58.87) at 100% crude extract concentration was recorded for Hyptis. (Fig 3).

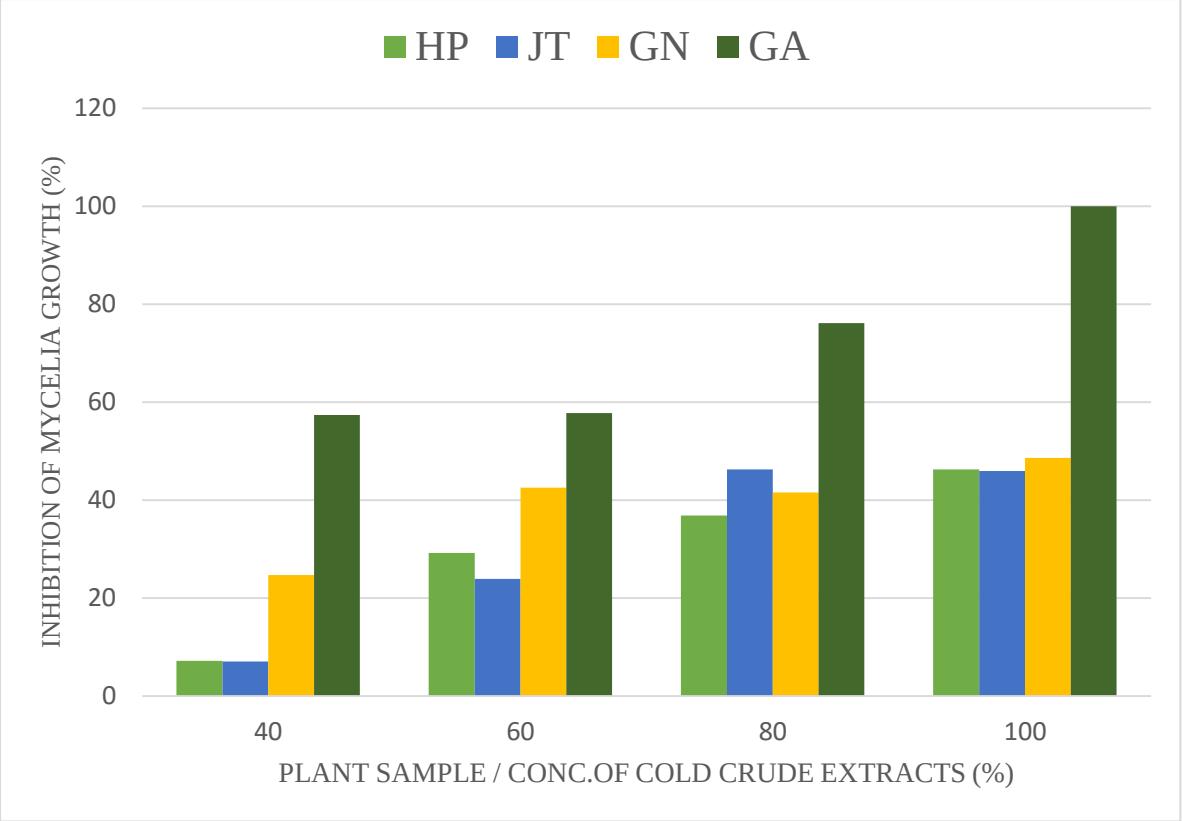

Fig 1. Percentage inhibition of mycelia growth of *C. paradoxa* by cold crude extracts of the four plant samples evaluated.

LEGEND

HP = Hyptis

JT = Jatropha

GN = Ginger

GA = Garlic

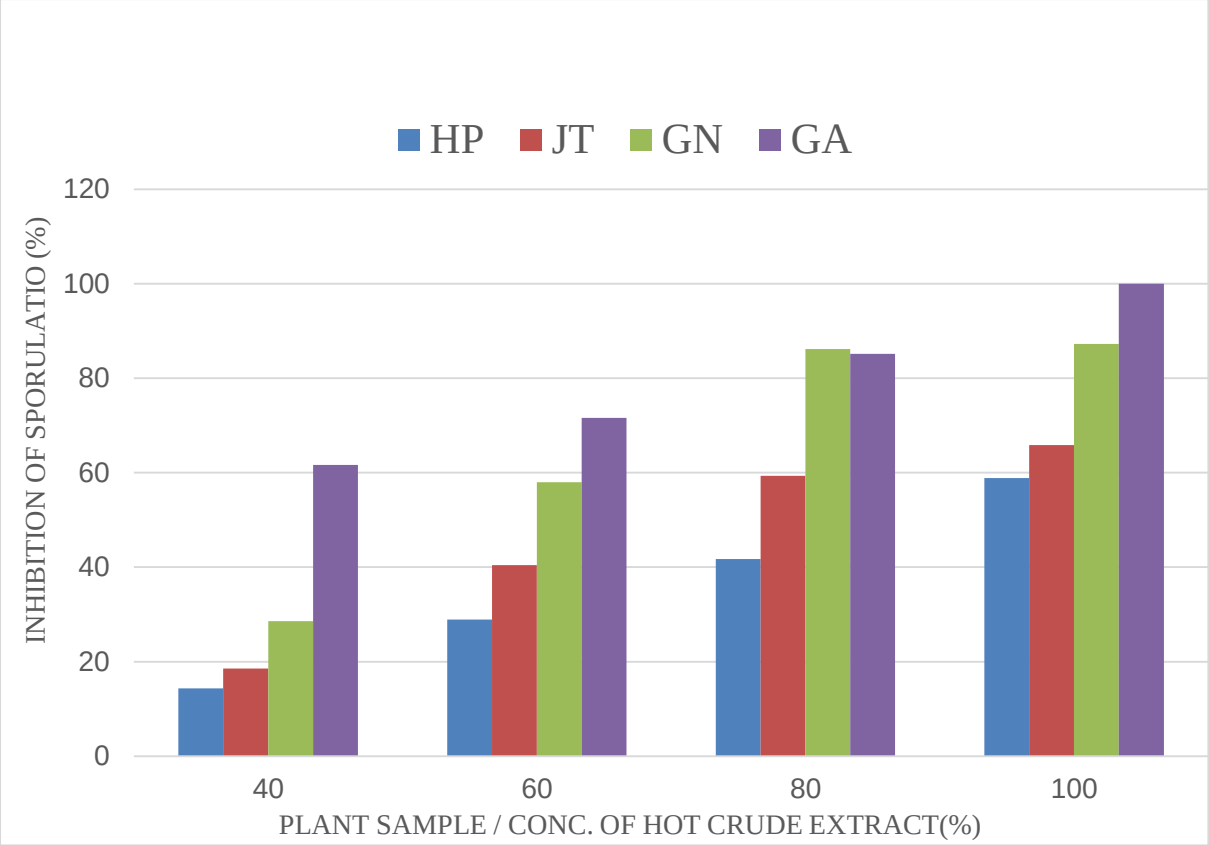

Fig 2. Percentage inhibition of mycelia growth by the hot crude extracts of the four plant samples evaluated

LEGEND

HP = Hyptis

JT = Jatropha

GN = Ginger

GA = Garlic

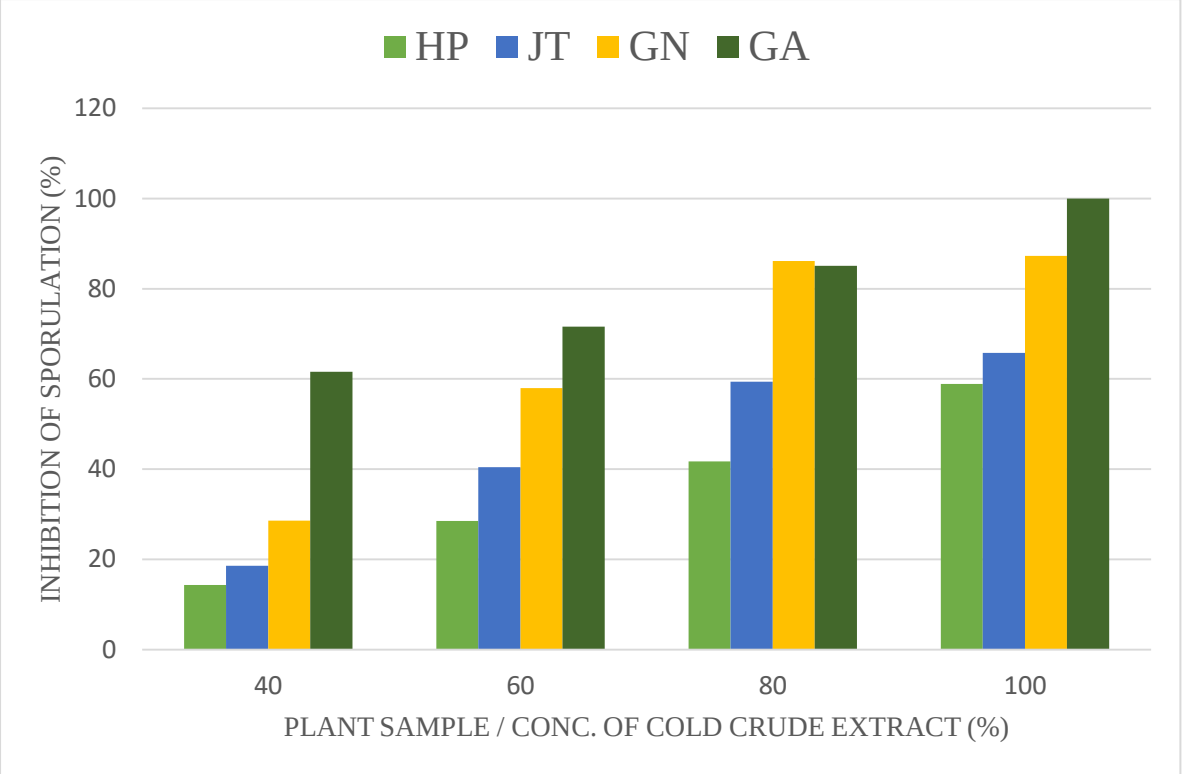

Fig 3. Percentage inhibition of sporulation by the cold crude extract concentrations of the four plant samples evaluated.

LEGEND

HP = Hyptis

JT = Jatropha

GN = Ginger

GA = Garlic

Effect of hot crude extracts of the four plant samples on the sporulation of C. paradoxa.

The highest values of percentage inhibition of sporulation with statistical significance was recorded for Garlic hot crude extract at 40% (69.24) and 100% (100.00). At 60% and 80% crude extract concentrations, the values for percentage inhibition of sporulation by Garlic and Ginger were not significantly different. Significantly lowest values of percentage inhibition of sporulation was recorded for Jatropha hot crude extract at 40%, 80% and 100% extract concentrations. The values were 28.56, 61.62 and 71.17 respectively. Fig 4.

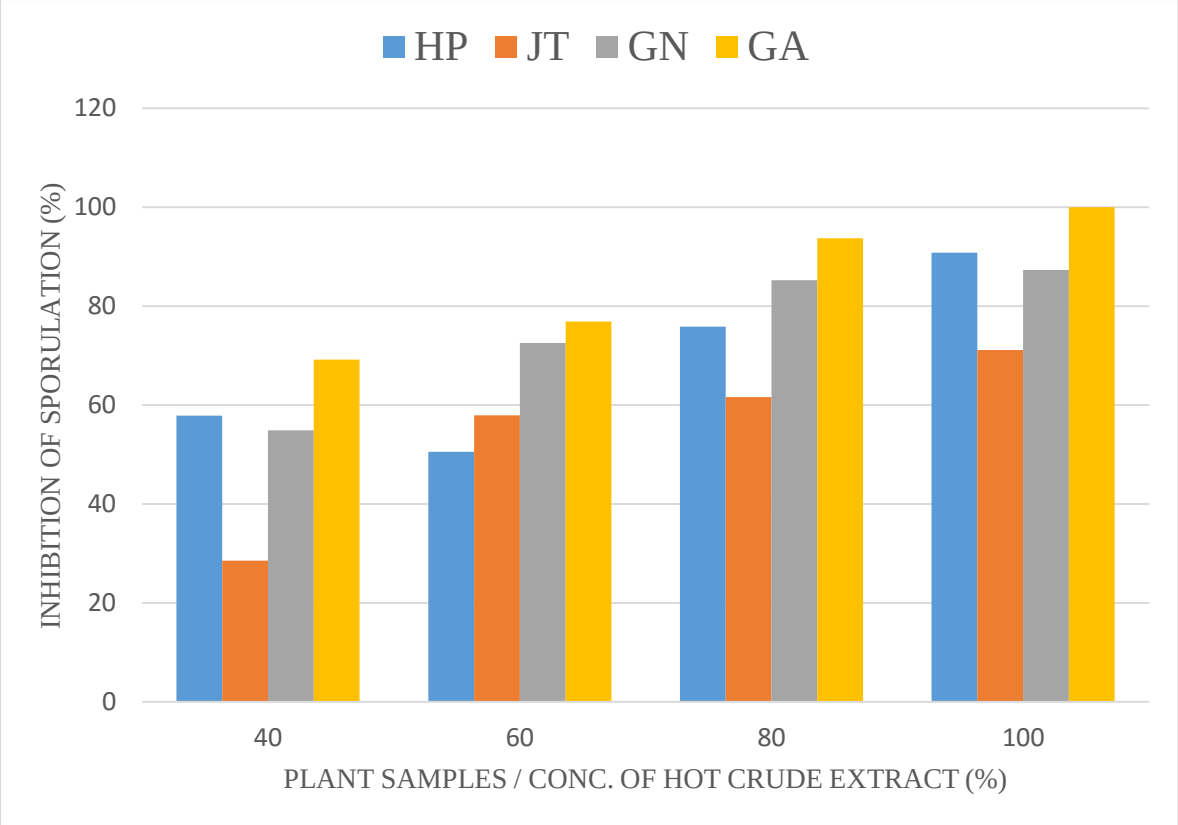

Fig 4. Percentage inhibition of sporulation by the hot crude extracts of the four plant samples evaluated

LEGEND

HP = Hyptis

JT = Jatropha

GN = Ginger

GA = Garlic

Discussion.

The result obtained for percentage inhibition of mycelia growth by cold crude extracts showed that increasing concentration of crude extracts brought about increasing inhibition of mycelia growth for the four plant samples evaluated. The percentage inhibition was however significantly highest in all the concentrations of garlic cold crude extracts. Ginger extract was second best at 40%, and 60% concentrations. Hyptis and Jatropha cold crude extracts did not do too well at all the concentrations evaluated. The values recorded at 40% and 60% concentrations were significantly lower than those of Garlic and Ginger. There was a noticeable increase in the percentage inhibition of mycelia growth across all the concentrations of hot crude extracts in the four plant samples evaluated when compared to the performance of the cold extract. Ginger was particularly interesting as it gave significantly highest values of mycelia growth inhibition at 40% and 60% concentrations, which was better than the performance of Garlic. The inhibition of mycelia growth by plants extracts is due to the different bioactive compounds present in them. The increased inhibition recorded for hot crude extracts of Ginger may be due to the fact that hot water extracts more of the bioactive compounds present in it. Allicin has been reported to be the key compound with anti-

microbial activity in Garlic (Lawal, *et al.*, 2010). Saponin and glycosides have been isolated from Hyptis leaves (Ladan, *et al.*, 2009). Gingerol is fingered as one of the many bioactive compounds in Ginger (Wohlmuth, *et al*., 2005), while phenols and flavonoids are some of the bioactive compounds in Jatropha. (Namuli, *et al.*, 2011)These compounds have fungistatic, fungitoxic or fugicidal properties. Results from previous research works showed that extracts from these samples exhibited different percentages of inhibition of mycelia growth on different fungal pathogens of crops. The method of extraction also plays an important role in determining their efficacy. (Deepak, *et al.*, 2007; Ajayi and Olufolaji, 2009; Sesan, *et al*., 2015).

Spores are to fungi what seeds are to higher plants. The inhibition of sporulation therefore has the effect of preventing fungal dissemination and consequently the spread of plant diseases. Sporulation is initiated in fungi asexually after a given period of active cell division or sexually in matured fungal thallus. Any factor that affects normal mycelia growth usually produces a corresponding effect on sporulation. It was as expected therefore that increasing concentrations of both the cold and hot crude extracts of the four plant samples brought about increasing inhibition of sporulation of *C. paradoxa*. Garlic extract was particularly effective with significantly highest values for sporulation inhibition at almost all the concentrations evaluated for both hot and cold crude extracts. Ginger crude extracts were next to Garlic in effectiveness at sporulation inhibition. Saligrama, *et al*., (2005) recorded significant inhibition of sporulation in *Sclerospora graminicola* with increasing concentration of Ginger and some other plants samples. Deepak, et al., (2007) reported a similar finding on the same organism using Garlic and ginger. Other extraction methods can be evaluated by other researchers to determine their level of efficacy.

Conclusion

Results from this study showed that Garlic cold crude extract at 40% concentration and above will inhibit mycelia growth and sporulation of *C. paradoxa* significantly. Hot crude extracts of Ginger was also highly effective at inhibiting sporulation of *C. paradoxa*. Consequently, Garlic and Ginger are both recommended for the control of *C. paradoxa*. Further research work can be carried out *in-vivo* to determine the efficacy of Garlic and Ginger crude extracts on sugarcane setts as both therapeutic and prophylactic agents in the control of sett rot disease.

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