EVALUATION OF ANTIFUNGAL ACTIVITY OF DIFFERENT LEGUME EXTRACTS ON SELECTED PATHOGENS OF SORGHUM

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ABSTRACT

Plants consist of various secondary metabolite compounds on their barks, flowers, leaves and fruits. Plant extracts have been reported to exhibit insecticidal, antifungal and antibacterial properties. Secondary metabolites (saponins, flavonoids, tanins, triterponoid and glucosides) and plant based pesticides have got minimal effect to the environment compared to synthetic pesticides. The inhibitory activity of dolichos and soybean extracts (leaf, root, stem) were evaluated against two test fungi; *Fusarium moniliforme* and *Colletotrichum sublineolum* that causes stem rot and foliar diseases respectively resulting in low yields in sorghum. An aqueous extract was prepared by blending fresh legume extracts and the homogenate centrifuged at 14000 rpm for 10 minutes to obtain a supernatant and sterilized at 121 ^{0}C for 15minutes. Culturing of sorghum infected tissues was done using poisoned food technique and incubated under alternating 12- hour light and 12- hour darkness to induce sporulation. Data on Inhibitory index, mycelia diameter and percent inhibition were recorded. Data analysis was done using descriptive statistics and results presented on tables, trellis plot and graphs. Extracts of dolichos leaves had the highest inhibitory (92.34%) effect on mycelia growth of *Colletotrichum sublineolum* followed by Soybean leaves (91.3%) while soybean stem extracts had the highest inhibition (62.87%) of mycelia growth on *Fusarium moniliforme*. This study suggests the use of legume plant extracts as natural fungicides.

Key: words. Inhibitory, Extract, Mycelia, Metabolites

INTRODUCTION

Sorghum (*Sorghum bicolor* L., Moench) is a nutritive food for millions of people across the globe (Fetene, 2014) livestock feed and an industrial crop (edible oil, flour and alcoholic beverages) (Parthasarathy, 2010). The crop is ranked fifth as one of the most important cereal grown worldwide for food security after maize, wheat, rice (FAOSTAT, 2014) and barley but second after maize in Sub-Saharan Africa (FAOSTAT, 2015). Globally, sorghum is grown in 46 million hectares accounting for an annual production of 60.46 million tons (USDA, 2020). Inherent challenges to increased low production are anthropogenic, edaphic, abiotic and biotic factors. Biotic stresses remain the most limiting factors comprising of diseases (viral, fungal, bacterial) (Ramathani, 2011), insect pests (shoot fly, midge, fall armyworm, stem borer, African ball-worm, head bugs, sorghum aphids and webworm) (CABI, 2005) and weeds (striga). Severe yield loses have been reported in susceptible cultivars as high as 50% due to anthracnose (Erpelding, 2007), 50% at flowering for turcicum leaf blight (Ogliaril, 2007) and 85% zonate leaf spot (Ngugi, 2002).

Lack of sustainable management of anthracnose and Fusarium stem rot diseases in sorghum production have been of great concern. Successful and continuous production of sorghum is key to global food security especially in the semi-arid tropics. However, the gap between achievable and actual yields in farming systems is quite large because of various foliar disease damage among others constraints (Mohan, 2012). Foliar diseases have become a challenge for the peasant farmer to manage in Kenya as majority of farmers own small tracts of land hence do not practice crop rotation, intercropping and field sanitation (Ngugi, 2000). Less time is therefore given for the disease to break its cycle. Furthermore, continuous use of fungicides has led to moderate resistance of the pathogens as there are different strains of anthracnose, zonate leaf spot, Fusarium stem rot and turcicum leaf blight (Ramathani, 2011) which have evolved. Thus the study was conducted to ascertain the percent *in vitro* inhibitory index of legumes (dolichos, soybean) leaf, stem and root extracts on selected isolates of fungal pathogens obtained from experimental sites in Western Kenya.

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MATERIALS AND METHOD

Collection Sites

Plant legume extracts (roots, leaf, stem) and infected sorghum leaf, stem, panicle (anthracnose, Fusarium stem rot) tissues were collected from the experimental sites in Kisumu (Kibos) and Siaya (Sega) counties and transported to the pathology laboratory at Kenya Agricultural and Livestock Research Organization, Sugar Research Institute Kibos.

Preparation of the aqueous crude plant extracts

An aqueous extract was prepared by blending legume extracts with distilled water in a blender for 2 minutes. The homogenate was filtered through a layer of cheese cloth and centrifuged at 14000 rpm for 10mins to obtain a supernatant. The supernatant was sterilized at 121°C for 15minutes and stored in +4 °C until use for experiment as outlined by (Rodriguez- Garcia, 2019).

PDA preparation from Commercial Medium Powder

Thirty-nine grams of commercial PDA powder mixed with 40mg of chloramphenicol was suspended in 1Litre of distilled water. This was followed by boiling the mixture to dissolve the powder completely and autoclaving at 121 °C for 15 minutes to sterilize the media.

Isolation and incubation

Infected leaf samples by anthracnose and Fusarium stem rot collected from Kisumu and Siaya Counties, were taken to the laboratory for isolation of fungi as described by Salano (2015). The samples were surface sterilized in 1% hypochlorite, rinsed in distilled water then plated on sterile potato dextrose agar (PDA). Five to ten leaf pieces were placed and incubated at (28 ± 2 °C) under continuous white fluorescent light. After a period of 7 days, a single conidia representing each collection was transferred on freshly prepared PDA and incubated at similar conditions. Sub-cultures were made from the emerging colonies and pure cultures obtained for subsequent studies.

Efficacy of legume (soybean, dolichos) extract on the mycelial growth

The antifungal efficacy of extracts was determined by poisoned food technique. Legume (dolichos and soybean) extracts and two checks (sterile media and fungicide) were tested for spore germination. Aqueous leaf, root, stem extracts were mixed with PDA and the mixture poured into sterile labeled petri-plates. A mycelia plug obtained from 10-day culture of actively growing colonies of *Colletotrichum sublineolum* and *Fusarium moniliforme* was inoculated at the center of the petri-plates. The petri-plates were incubated at room temperature (28 ± 2 °C) and mycelia growth of the test fungi recorded for 4 days. Three replications were maintained for each legume extract (leaf, stem and root). Antifungal activities were recorded in terms of inhibition of mycelial growth (%) and calculated as suggested by (Gupta, 2011).

Per cent inhibition of mycelial growth (%) = (C- T/C) × 100

Where C is the average diameter of fungal colony in control plate and T is the average diameter of fungal colony in poisoned plates.

RESULTS

Effect of soybean and dolichos extracts (roots, stem and leaves) on the mycelia growth of *Fusarium moniliforme* and *Colletotrichum sublineolum*

Results of the analysis indicate legume extracts had an effect on mycelial growth of the two pathogenic fungi, *Colletotrichum sublineolum* and *Fusarium moniliforme.* However, the inhibition varied significantly ($P<0.05$) among the legume extracts used (roots, stems and leaves) and also among the two pathogenic fungi tested (Table I).

Among the legume extracts used, soybean and dolichos leaves were the most effective against *C. sublineolum.* On *C. sublineolum,* dolichos leaves had the highest inhibition of 93% followed by soybean 91.3% respectively. However, the leaves were significantly least effective in the inhibition of mycelial growth of *F. moniliforme* where the soybean and dolichos leaves extracts recorded 22.9% and 3.1% mycelial inhibition respectively. The study also showed that legume stem extracts of soybean and dolichos were the least effective inhibitors of mycelial growth of *C. sublineolum.* However, stem extracts of soybean were effective in the inhibition of *F. moniliforme* (62.9%) compared with 4.9% in *C. sublineolum.*

Below are mean percent inhibition of the fungal pathogen mycelial growth of *Fusarium moliniforme* and *Colletotrichum sublineolum* (Table I).

TABLE I-EFFECT OF DIFFERENT LEGUME EXTRACTS ON MYCELIAL GROWTH OF TWO FUNGAL PATHOGENS, *FUSARIUM MONIFORME AND COLLETOTRICHUM SUBLINEOLUM*

Micro-organism	**Zone of inhibition (%)**					
Pathogenic fungi	**Soybean**			**Dolichos**		
	Root	**Stem**	**Leaves**	**Root**	**Stem**	**Leaves**
Colletotrichum sublineolum	35.0a	3.9b	91.3a	39.9b	13.0a	93.0a
Fusarium moniliforme	33.1b	62.9 a	22.9b	61.6a	4.9b	3.1b
$LSD_{(0.05)}$	2.5	9.9	5.3	3.3	3.8	3.4
CV%	2.1	8.4	2.6	1.9	12.0	2.0

Mean % marked with the same letter within a column are not significantly different P<0.05

Among the legume extracts (dolichos, soybean), two extracts showed significant inhibition against *Fusarium moniliforme.* Mycelial growth of *Fusarium moniliforme* was inhibited by soybean stem and dolichos roots extracts. Soybean extracts showed the highest inhibitory effect from day 5 and 6 while on the other hand, dolichos root extracts had moderate inhibition of mycelial growth from day 4, 5 and 6. However, extracts from dolichos leaves, soybean leaves, soybean roots, dolichos stem were not effective against the tested pathogen and recorded lowest inhibition of mycelial growth (Figure 1).

The inhibitory effect of legume extracts (dolichos and soybean leaves) exhibited marked antifungal activity against *Colletotrichum sublineolum* by inhibiting mycelial growth from day 3, 4, 5 and 6. Whereas moderate inhibition activity of dolichos stem was observed against the fungal pathogen. The lowest mycelial growth inhibition was recorded by soybean stem, soybean roots and dolichos roots (Figure 2).

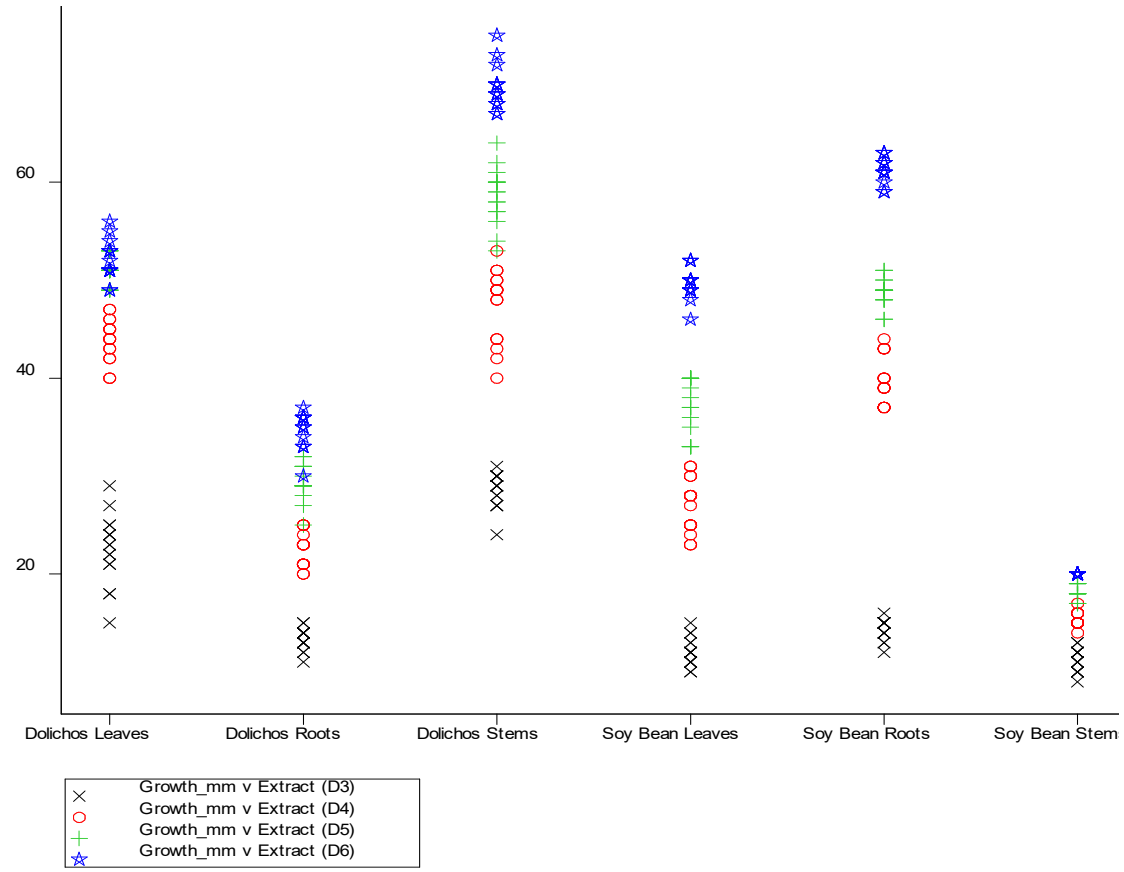

Figure 1. Percent inhibition effect of soybean and dolichos extract on mycelia growth of *Fusarium moniliforme*

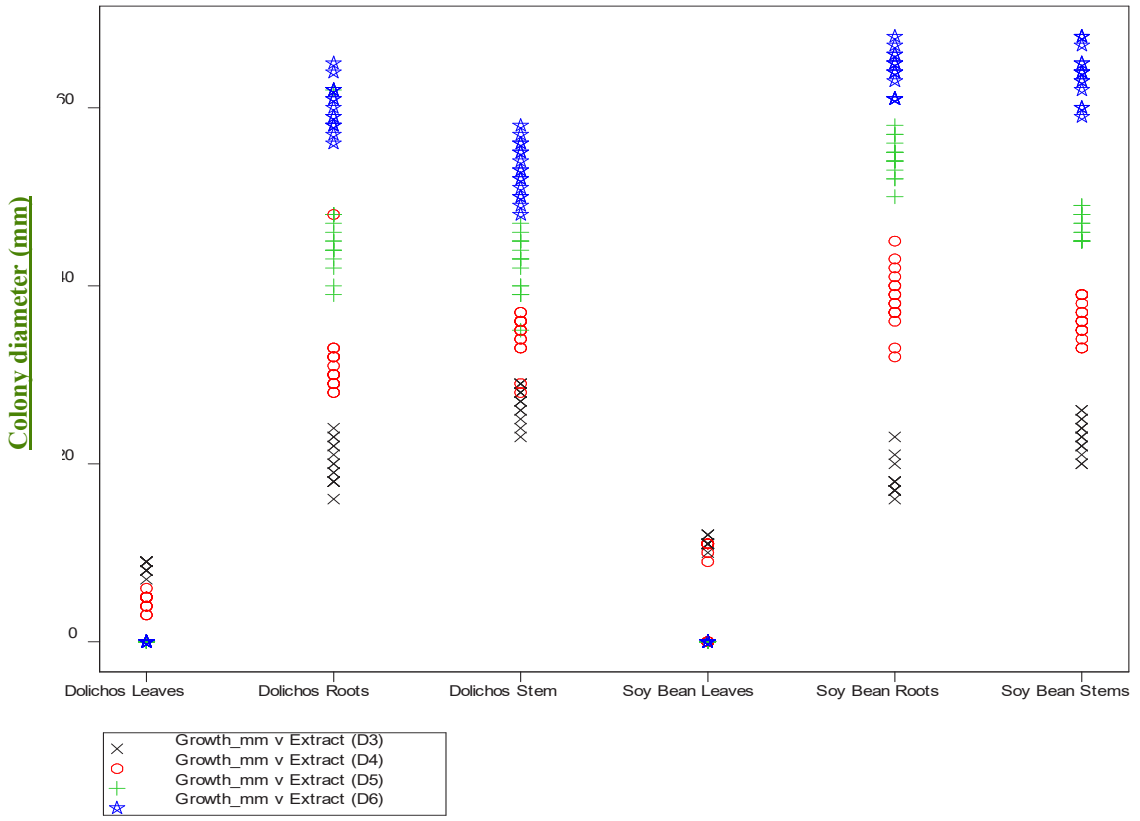

Figure 2. Percent inhibition effect of soybean and dolichos extract on mycelia growth of *Colletotrichum sublineolum*

The results of antifungal activity of the legumes (dolichos, soybean) extracts on *Colletotrichum sublineolum* showed that soybean and dolichos stem extracts had no effect on the test microorganism. However, dolichos and soybean leaves extracts showed the highest significant percent inhibition growth on the tested fungi. Dolichos and soybean roots showed moderate inhibition. On the other hand, dolichos root, soybean stem extracts were more efficient and had a shown in Figure 3.

Dolichos and soybean leaf extracts expressed highest inhibitory index on *Colletotrichum sublineolum.* However, dolichos root and soybean extracts showed selective inhibition on *Fusarium moniliforme* as shown in Figure 4.

DISCUSSION

The current study showed that all the aqueous extracts obtained from legumes (soybean, dolichos) revealed antifungal activity but differed significantly among the different plant parts of the legumes (roots, stems, leaves) in their effectiveness in controlling the *in vitro* growth of the two tested pathogens. This study was in agreement with Sakshi (2019) findings that antifungal activity vary with different plant species and plant material. In addition, their effect was linear to the percent inhibition of mycelial growth of *Colletotrichum sublineolum* and (*Fusarium moniliforme)*. This study concurs with Sakshi (2017) who reported that the use of aqueous extraction method on various plant extracts has been shown to have antifungal activities against some tested fungal pathogens such as *Colletotrichum musae*, *Fusarium proliferatum, Fusarium graminearum*, *Fusarium sporotrichioides* among others

Inhibition in growth of *Colletotrichum sublineolum* observed in this study from dolichos leaves, was similar to previous findings by Chadalavada (2015) who demonstrated antifungal potency of three fungi using the extract. The results of this current study using dolichos leaf extract show that plants possess antimicrobial activity. In addition, dolichos root and flower powdered extracts have been used to manage diseases such as sorghum covered kernel and loose kernel smuts screened under field conditions and the results were positive (Senthilkumar, 2009). These studies concur with the current study that focused on the use of (soybean, dolichos) stem, roots

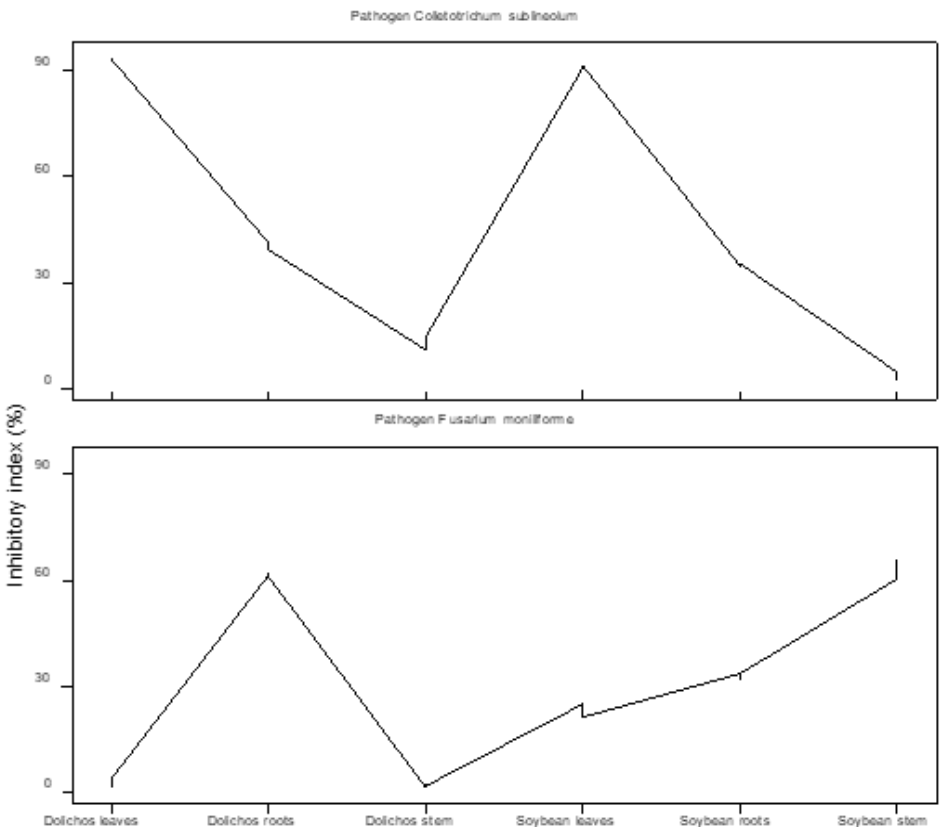

Figure 3. Percent inhibitory index of *Fusarium moniliforme* and *Colletotrichum sublineolum*

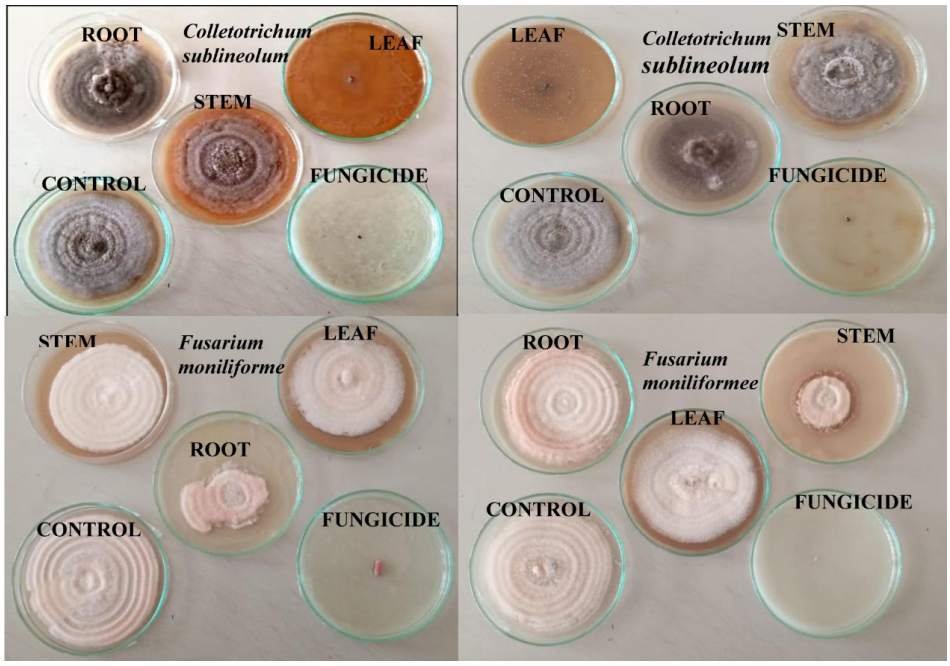

Figure 4. *In vitro* expression of dolichos (a), soybean (b), dolichos (c) soybean (d) leaf, stem, root extracts in growth media

and leaf extracts for antifungal activity of *Fusarium moniliforme* and *Colletotrichum sublineolum.*

The antifungal activity of the legumes is due to the presence of natural compounds which has got both antibacterial and antifungal properties. Dolichos and soybean leaves contain secondary metabolites (flavonoids, alkalloids) and poly-phenolic compounds (isoflavons) respectively. Thus the inhibition of fungi observed from this study using aqueous dolichos leaf, soybean stem extracts may be related to flavonoids and isoflavons respectively which curbs the performance of some enzymes that are important to fungi. In addition, phytoalexins found in soybean has got antiviral, antibacterial and antifungal effects as isoflavons produces daidzein metabolized to produce glyceollins which plays a major role in plant protection from attack by fungal pathogen (Liu, 2017).

CONCLUSION AND RECOMMENDATIONS

The findings of the present study revealed that legume (dolichos leaf, soybean leaf, soybean stem, dolichos root) extracts are safe and should be considered as safe alternatives to replace chemical fungicides since they are eco- friendly. Therefore, further studies are required to be undertaken to increase the efficacy of these legume extracts in the field and understand the mechanisms of observed activities. There is also need to isolate, purify, characterize active phytochemicals responsible for these bioactive ingredients. Lastly, further work should be done on dolichos leaf, soybean leaf and soybean stem, dolichos root to investigate their durability as natural fungicides in the plants for increased efficiency against *Colletotrichum sublineolum* and *Fusarium moniliforme*.

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