

EFFECT OF SAND PAPER (*Fiscus exasperata*) AND SODOM APPLE (*Calotropis procera*) LEAF EXTRACTS ON FUNGAL ORGANISMS ASSOCIATED WITH DISEASE OF MUSTARD IN ISHIAGU, EBONYI STATE

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SUMMARY

Effect of two aqueous plant extracts were tested on fungal organisms associated with diseased mustard at Central Laboratory, National Root Crops Research Institute (NRCRI), Umudike, Abia State. The diseased mustard seedlings were sourced from an existing experimental field during 2022 cropping season at Teaching and Research Farm, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria. Aqueous leaf extracts from *Fiscus exasperata* and *Calotropis procera* were used to inhibit the growth of the mycelial fungus at 0.5,1.0 and 1.5 w/v concentration levels in a Completely Randomised Design and replicated three times. Data collected were subjected to Analysis of Variance (ANOVA) using Minitab Version 17 and the means were separated using Tukey test at $P < 0.05$. The results revealed five fungi organisms belonging to four genera were isolated which includes *Trichoderma* species, *Fusarium oxysporum*, *Sclerotium* species, *Fusarium solani* and *Alternaria* species. The results showed that *Fusarium solani* had the highest percentage of occurrence (32.56 %), followed by *Alternaria* species (25.58 %) while *Trichoderma* species had the least (4.65 %). The inhibitory effect of *C. Procera* ranged from 3.73 to 93.39 % with *Fusarium solani* and *Trichoderma* species respectively across the three concentration levels. Similarly, the inhibitory effect of *F. exasperata* ranged from 2.33 to 89.59 % with *Fusarium solani* and *Trichoderma* species respectively across the three concentration levels. Therefore, the potentials of these two aqueous extracts on the inhibition of mycelial growth of fungal organisms associated with mustard diseases are worthy. This study showed the prospect of *Fiscus exeparata* and *Calotropis procera* in the control of fungal disease of mustard and a possible alternative to chemical pesticides.

Key words: Antifungal, *Fiscus exeparata*, *Calotropis procera*, Mustard diseases

Mustard (*Brassica juncea*, L.) belongs to the family *Brassicaceae* and is the third

most important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis*) (18). It is an annual

plant that originates from the Mediterranean region (21). Brown mustard has been reported to be grown as a leafy vegetable in Western and Southern Africa, known as 'laulau' in Nigeria, 'mpiru' in Malawi and 'tsunga' in Zimbabwe (4). It is a winter-spring plant that may be produced in short cycles, typically in rotation with other cereal crops, with the potential for second crop cultures (15). Mustard is an annual plant with broad leaves and yellow flowers that grows up to 100 cm tall and has a relatively short growing season of 85-95 days. The yellow blooms, which bloom from May through June, have four petals. Mustard crops include edible oilseeds (31), fast-growing salads (30), sauces, fodder, and green manure (24). Toxic heavy metals can be extracted from soil by the plant (23). Young seedling leaves, which are high in vitamins A, C, and E, are edible as fresh and flavourful salad leaves and have blood purifying properties (30). Due to its high protein and oil content and low starch content, mustard seed has substantial agronomic utility (6). The seeds are highly disinfectant and can be used to preserve food (30). Also, as a result of its powerful antibacterial activity, its essential oil can be utilized to preserve goods (29).

Sand paper tree (*Ficus exasperata*) is commonly known as "Ewe Ipin" in Yoruba and is widely spread in West Africa Forest re-growth. *Ficus* is a genus of about 800 species and 2000 varieties of *Ficus* of woody trees, shrubs and vines in the family *Moraceae* occurring in most tropical and subtropical forests worldwide (16). The potential antimicrobial activity of *Ficus* species has been extensively reported in many investigations (27). In Nigeria, young leaves of *F. exasperata* are prescribed as a common anti-ulcer action, as anti-diabetic, lipid lowering and antifungal activities (36). The activities of leaf extract of *F.*

exasperata against some pathogenic organisms have been extensively investigated (11; 28). Sirisha *et al.*, (34) reported the antifungal activities of the leaves of *F. exasperata*.

Sodom apple (Calotropis procera) belongs to the family *Apocynaceae* and is an invasive shrub, 2.5 m to 6.0 m tall adapted to poor soils (17). It is native to the arid regions of tropical Africa, India and Middle East (10). The species produces fruit all year round, and its seeds germinate with an enormous facility (7). The high potential of *C. procera* to invade pristine or economic important areas is of much concern, as it is also very difficult to eradicate (9;26). The antimicrobial activity of the leaf extracts of *C. procera* was evaluated and the inhibitory effect of extract of *C. procera* against *Candida albicans* was also observed (33). The antimicrobial activity of *Calotropis procera* was evaluated against pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and one pathogenic fungus, *Candida albicans* (22). The antifungal activities of aqueous extract of *Calotropis procera* was determined against *Epidermophyton floccosum* and *Tricophyton gypseum* using agar diffusion techniques (25). The antimicrobial effect of ethanol, aqueous and chloroform extracts of leaf and latex of *C. procera* were studied on *Aspergillus niger*, *Aspergillus flavus*, *Microsporium bouldarii* and one yeast *Candida albicans* using agar well diffusion and paper disk methods (20).

Infectious plant diseases are well known to account for a large share of health problems, particularly in underdeveloped nations. Many antimicrobials have developed resistance in microorganisms, posing a significant therapeutic challenge in the treatment of infectious disorders (14). This resistance has grown as a result of the indiscriminate use of commercial

antimicrobial medicines frequently used to treat infectious illnesses in plants. This circumstance forced scientists to look for new antimicrobial compounds in unexpected places, such as medicinal plants (19). Hence, the present study was carried out to investigate the effect of two aqueous plant extracts on mycelial growth of fungal organisms associated with mustard disease on the field.

MATERIALS AND METHODS

Research Location

These experiments were conducted at Central Laboratory, National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria.

Source of the two plants tested

The Sand paper (*Fiscus exeparata*) and Sodom apple (*Calotropis procera*) leaves were source from Amagu community, Ishiagu Ebonyi State

Source of diseased mustard seedlings

The diseased mustard seedlings were sourced from an existing experimental field during 2022 cropping season at Teaching and Research Farm, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria.

Preparation of Potato Dextrose Agar (PDA)

The Potato Dextrose Agar (PDA) was prepared by weighing 39.0 g of PDA powdered; this was poured into sterile Distilled water (SDW) in a conical flask and made up to 1.0 litre. The flask was swirled gently to allow dispersion of the PDA powder in the added water. The PDA medium contained in the conical flask were plugged with cotton wool and covered with aluminium foil paper this was later sterilized in an autoclave at 121 °C for 15 minutes. The sterilized medium was subsequently allowed to cool to 45 °C and streptomycin at the rate of 20ml/L (PDA) were added and poured aseptically into 9cm

diameter Petri dishes, covered and allowed to solidify.

Isolation and identification of fungi associated with mustard seedlings

Each sample of diseased mustard seedling showing symptoms were collected from an existing experimental plot were washed in clean water and sections of about 1cm in diameter were cut from the tissue, using sterilized scalpel, at the interface between healthy and infected portions of the sample. The pieces of the tissue were surface sterilized with 1% sodium hypochlorite for 2 minutes, and rinsed in 5 sections of the cleaned sample were plated out on potato dextrose agar (PDA). The inoculated Petri dishes were incubated at 28±2 °C for 5 days and observed daily for fungi development. The various fungi isolated from each of the samples were sub-cultured by transferring hypha tips from the colony edges to fresh PDA plates using a flame sterilized mounted needle to obtain a pure culture, and incubated at 28 °C. Cultures were identified using identification keys describe by Barnett and Hunter (8).

Determination of fungal frequency of occurrence

The most prevalent fungi in the study sample were identified by the frequency of occurrence of each of the isolated fungus from the infected tissue obtained from a particular treatment. This was determined by recoding the number of times each fungus was encountered. It was calculated using the formula below;

$$\text{Frequency (\%)} = \frac{\text{Number of isolates}}{\text{Total number of fungi occurrence}} \times 100$$

Preparation of plant extract

Fresh fully expanded leaves of *Fiscus exeparata* and *Calotropis procera* were washed thoroughly with water and soaked in a 1 % solution of sodium hypochlorite for 2 minutes, rinsed severally with sterile

distilled water. Three different aqueous extract concentrations were prepared by weighing 5, 10 and 15 g of the leaves on a Mettler-Toledo AG balance, AB104, Switzerland, and blended in a Eurosonic, ES210, Great Star, Asia, blender and 100 ml of sterile distilled water added for 12 hours. Extract concentrations of 0.5, 1.0 and 1.5 % were obtained. All the extracts were allowed for 12 hours to settle, sieved through four layers of sterile cheese-cloth and kept in the refrigerator until used.

Effect of aqueous plant extract on fungal mycelia growth

The method of Amadioha and Obi (3) was used to determine the effect of the aqueous extract on fungal growth using completely randomized design (CRD) with three replications. This involved creating four-equal sections on each petri-dish by drawing two perpendicular lines at the bottom of the plate, the point of intersection indicating the centre of the plate. This was done before dispensing PDA into each of the plates. One ml of each extract concentration was dispensed per petri dish and 9 ml of molten PDA were added to prepare a PDA-extract mixture with corresponding 0.5, 1.0 and 1.5 % w/v extract concentrations (32). The plates were gently rotated to ensure even dispersion of the extracts. The agar-extract mixtures were allowed to solidify and were used for the inhibition of mycelia growth of the tested fungi. A disc (4 mm diameter) of the pure culture of each isolate about 7 days old were placed on the extract just at the point of intersection of the two lines drawn at the bottom of the Petri dish. The negative controls were set up using blank agar plates (no extract) and the positive control consisted of the fungicide Mancozeb (ethylene bisdithiocarbamate) which were prepared according to manufacturer's directions by mixing 0.5 g in 100 ml of

sterile distilled water. Three replicates' plates of PDA-extract per isolate were incubated at 27 °C and radial growth were measured daily for 7 days. Colony diameter were taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates. Fungi toxicity of the extract were recorded in terms of percentage colony inhibition and calculated according to the formula by Egbonatan *et al.* (13):

$$M_p = \frac{M_1 - M_2}{M_1} \times 100$$

Where M_p is percentage reduction of mycelial growth, M_1 is mycelial growth in Petri dish and M_2 is mycelial growth in Petri dish containing extract.

Data analysis

Data collected were subjected to Analysis of Variance (ANOVA) using Minitab software version 17 and the significant means were separated using Tukey test at $P < 0.05$.

RESULTS

Fungi isolated, plant parts and percentage of occurrence

The results of the study revealed various fungi organisms isolated from different diseased mustard seed plant parts. *Trichoderma* species, *Fusarium oxysporum*, *Fusarium solani* and *Sclerotium* species were isolated from the diseased plant root. *Sclerotium* species, *Fusarium oxysporum* and *Alternaria* species were found recovered from the leaves while *Alternaria* species and *Fusarium solani* were isolated from the diseased mustard stem (Table 1). Five fungi organisms belonging to four genera were isolated which includes *Trichoderma*

species, *Fusarium oxysporum*, *Sclerotium species*, *Fusarium solani* and *Alternaria species*. The results showed that *Fusarium*

solani had the highest percentage of occurrence (32.56 %), followed by *Alternaria species* (25.58 %) while *Trichoderma species* had the least (4.65 %).

Table 1: Fungi isolated, plant parts and percentage of occurrence

S/N	Fungi isolates	Part isolated	Number of occurrence	Occurrence (%)
1	<i>Trichoderma species</i>	Roots	2	4.65
2	<i>Sclerotium species</i>	Roots, Leaves	6	13.95
3	<i>Fusarium oxysporum</i>	Roots, Leaves	10	23.26
4	<i>Alternaria species</i>	Leaves, Stem	11	25.58
5	<i>Fusarium solani</i>	Roots, Stem	14	32.56

Effect of aqueous plant extract of *Calotropis procera* on fungal mycelia growth

Table 2 revealed the inhibitory effect of aqueous plant extract of *C. procera* on fungal mycelia growth of fungal organisms associated with mustard on the field. The results showed a significant difference among the treatments and the levels of concentrations. At 5 % concentration level, *Fusarium oxysporum* had the highest mycelia inhibition (23.61%), followed by *Trichoderma species* (20.53 %) while

Fusarium solani had the least mycelia inhibition (3.73 %). At 10 % concentration level, *Trichoderma species* had the highest mycelia inhibition (45.24 %) while *Fusarium solani* had the least mycelia inhibition (6.13 %). Similarly, the mycelial inhibition at 15 % concentration levels followed the same patterns. However, the fungicide showed a high level of inhibition with different fungi organisms and at different levels except *Trichoderma species* at 15 % concentration that were not significantly different with the fungicide.

Table 2: Effect of aqueous plant extract of *Calotropis procera* on fungal mycelia growth

S/N	Treatments	5 %	10 %	15 %	Fungicide
1	<i>Trichoderma species</i>	20.53ab	45.24a	93.39a	97.92a
2	<i>Sclerotium species</i>	13.80b	27.89b	40.57c	91.45b
3	<i>Fusarium oxysporum</i>	23.61a	36.54ab	48.70b	91.95b
4	<i>Alternaria species</i>	14.23b	18.87c	23.58d	93.87ab
5	<i>Fusarium solani</i>	3.73c	6.13d	14.70e	94.58ab
	CV (%)	49.06	53.18	64.29	4.16

Means in the same column followed by different alphabets are significantly different ($P < 0.05$) using Tukey.

Effect of aqueous plant extract of *Fiscus exasperata* on fungal mycelia growth

Table 3 revealed the inhibitory effect of aqueous plant extract of *F. exasperata* on fungal mycelia growth of fungal organisms associated with mustard on the field. The results showed a significant difference

among the treatments and the levels of concentrations. At 5 % concentration level, *Sclerotium species* had the highest mycelia inhibition (27.27 %), followed by *Alternaria species* (15.67 %) while *Fusarium solani* had the least mycelia inhibition (2.33 %). At 10 % concentration level, *Alternaria species* had the highest

mycelia inhibition (45.10 %) while *Fusarium solani* had the least mycelia inhibition (9.43 %). The mycelial inhibition at 15 % concentration levels ranged from

Fusarium oxysporum to *Trichoderma* species with the values of 54.53–89.59 % respectively. However, the fungicide showed a high level of inhibition with different fungi organisms.

Table 3: Effect of aqueous plant extract of *Fiscus exasperata* on fungal mycelia growth

S/N	Treatments	5 %	10 %	15 %	Fungicide
1	<i>Trichoderma</i> species	14.39b	25.33b	89.59a	95.76a
2	<i>Sclerotium</i> species	27.27a	38.93a	89.53a	92.70a
3	<i>Fusarium oxysporum</i>	7.20c	37.40a	54.53b	84.80a
4	<i>Alternaria</i> species	15.67b	45.10a	59.70b	90.57a
5	<i>Fusarium solani</i>	2.33d	9.43c	30.00c	93.18a
	CV (%)	67.01	43.11	36.64	7.26

Means in the same column followed by different alphabets are significantly different ($P < 0.05$) using Tukey.

DISCUSSION

The isolation and identification of the fungal organisms from diseased mustard revealed five common fungi organisms. The fungal isolates were *Sclerotium* species, *Alternaria* species, *Fusarium oxysporum*, *Fusarium solani* and *Trichoderma* species. These fungi have been reported on several economic plants in Nigeria, such as *Fusarium oxysporum* reported on tomato (37), *Sclerotium* species on mustard and groundnut (38), *Fusarium solani* on maize (35) and *Trichoderma* species on tomato (5). Yekini *et al.* (39) reported that *A. niger*, *A. flavus*, *Fusarium oxysporum*, *Alternaria* species, *Penicillium* species, and *Rhizopus stolonifer* on mustard seed. Aqueous extract of *Fiscus exasperata* significantly inhibited the mycelia growth of the isolated fungus. This supports the findings of Durugbo *et al.* (12), who discovered that extracts of *Fiscus* species greatly inhibited the growth of *A. flavus*, *A. niger*, *Botryodiplodia theobromae*, *F. oxysporum*, *F. solani*, *Penicillium chrysogenum*, *P. oxalicum*, and *Rhizopus stolonifer* mycelial growth. Similarly, Ajala *et al.* (2) reported that antimicrobial properties of *F. exasperata* dependent on type of organism and extract concentration.

F. exasperata's antifungal properties may be attributed to chemical elements such as ficusamide, furanocoumarins, (S)-(_) oxypeucedanin hydrate, (R)-(_) oxypeucedanin hydrate, and bergapten (5-methoxypsoralen) found in the plant (1). **Similarly**, aqueous leaf extract of *C. procera* at 5.0, 1.0 and 1.5 % concentration levels significantly inhibited the mycelia growth of all the five fungal organisms isolated from diseased mustard plant. Kareem (20) reported the antimicrobial effect of ethanol, aqueous and chloroform leaf extracts of *C. procera* on *Aspergillus niger*, *Aspergillus flavus*, *Microsporium bouldardii* and one yeast *Candida albicans* using agar well diffusion and paper disk methods. The antimicrobial activity of the leaf extracts of *C. procera* was evaluated against *Candida albicans* and inhibitory effect was observed (38).

CONCLUSION

The use of *Fiscus exasperata* and *Calotropis procera* leaf extracts in the management of mustard diseases gave a relative value for all the organisms when compared with the systemic fungicide. The potentials of these two leaf extracts on the inhibition of mycelial growth of fungal

organisms associated with mustard diseases are worthy. It further buttresses the potential of natural plant products in the management of fungi disease of mustard on the field.

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