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ASSESSING THE EFFICACY OF SELECTED CRUDE PLANT EXTRACTS IN INHIBITING FUNGAL PATHOGENS OF *Amaranthus hybridus* (L.)

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ABSTRACT

Fungal pathogens pose a significant threat to horticultural crops, including *Amaranthus hybridus* (L.) a commonly cultivated leafy vegetable. This research was undertaken to assess the effectiveness of unrefined extracts of some plants in inhibiting some fungal pathogen of *A. hybridus*. Symptomatic leaves were collected from a garden in Okwuta Ibeku and the suspected pathogens were isolated following standard procedures. The pathogens were subcultured to obtain pure cultures and thereafter Pathogenicity test was conducted. Antimicrobial test was done with *Tagetes erecta* (marigold) and *Allium sativum* (garlic) at four different concentrations (0% control, 25%, 50% and 50% garlic + 50% marigold) for garlic and marigold respectively. The test was laid out in Completely Random Design (CRD) of 2 x 4 x 3 factorial. Radial growth and percentage inhibition of the extracts against the isolates after 2 days of incubation were determined. Analysis of Variance (ANOVA) on scientifically generated data was obtained using SPSS package. Means were differentiated at 95% confident intervals. Results showed the presence of *Rhizoctonia solani*, *Pythium sp.*, *Rhizopus stolonifer*, and *Fusarium solani* in the diseased vegetable. Pathogenicity test of the isolates showed that only *R. solani* and *Pythium* species produced vivid symptoms of damping off on the succulent *Amaranthus* twig. The antimicrobial test on *R. solani* and *Pythium* species revealed that both garlic and marigold crude extracts were highly potent in inhibiting the growth of the test pathogens. Garlic extract fractions produced maximum (100%) inhibition and were consistently more effective of the two extracts. Overall, this experiment offers important awareness into the potential use of the selected crude plant extracts as natural alternatives for controlling fungal pathogens in *Amaranthus* and other horticultural vegetables. The findings contribute to the development of sustainable and environmentally friendly strategies for crop protection in agriculture.

Keywords: Efficacy, Crude, Plant Extracts, Isolates, Pathogens, Inhibition, Control.

INTRODUCTION

The genus *Amaranthus* belongs to Amaranthaceae family and 298 kinds in this group have been identified. Species in the *Amaranthus* genus generally consist of annual and herbaceous plants. *Amaranthus* is a genus composed of about 74 annual species with a wide morphological diversity distinctly characterized by monoecy and dioecy (Waselkov *et al.*, 2018). The name *Amaranthus* stems from the Greek term ‘amarantos’ which denotes ‘undying’, ‘immortal’, ‘everlasting’ or unfading, due to the ability of its flower to persist for a long period. The most popular leafy species are *Amaranthus hypochoeriacus*, *Amaranthus tricolour*, *Amaranthus hybridus* and *Amaranthus blitum* (Jimoh *et al.*, 2019). They are among the first leafy vegetables that are universally known for various features as grains, leafy vegetables, ornamentals, colorants and weeds in tropical, subtropical and temperate climates (Jimoh *et al.*, 2018). *Amaranthus*, a dense nutrient vegetable, contains a number vital nutrients including plant based proteins, dietary fibre, unsaturated fatty acids, essential minerals like iron, manganese, magnesium and phosphorus and other vital organic materials from leaves, seeds and roots (Venskietonis *et al.*, 2013; Jimoh *et al.*, 2020). Rastogi and Shukla (2013) reported that *Amaranthus* acclimatizes simply to unfavourable environmental conditions because they evolved through the C4 photosynthetic/ anabolic pathway of manufacturing food. The authors noted that *Amaranthus* have advanced unique physiological features that make them easily planted and allow them to subsist attacks from disease-causing agents and improve their phenotypic flexibility and inherent diversity (Jimoh *et al.*, 2019).

Due to the versatility of its production in most tropical countries as indigenous vegetable coupled with its nutritional value, resistance to drought, heat and low production cost, *Amaranthus* has been described as a poor man’s vegetable and rediscovered as a promising food crop (Dada *et al.*, 2017). Seeds as well as leaves of the crop are known for their exceptional essential micronutrients such as calcium, iron, beta carotene, vitamin C and folic acid (Caselato-Sousa and Amaya-Farfan, 2012; Mensah *et al.*, 2008). Assad *et al.*, (2017) reported that the total dietary worth of *Amaranthus* is considered as considerably higher than several protein foods such as milk and soybean and is therefore used as an important dietary supplement for HIV/AIDS patients in some African countries. In addition, *Amaranthus* species has medicinal properties against constipation, fevers, haemorrhage, anaemia, kidney ailments and worms (Jimoh *et al.*, 2018). Osawaru *et al.*, (2013) included *Amaranthus* in a list of regular plants used in Nigeria. Thirteen species of *Amaranthus*: *A. blitum* L. (syn. *A. lividus* L.), *A. caudatus* L., *A. dubius* Mart. Ex Thell., *A. deflexus* L., *A. graecizans* L. (Basionym *A. silvestris* Vill.), *A. cruentus* L., *A. hybridus* L., *A.*

hypochondriacus L., *A. spinosus* L., *A. thunbergii* Moq., *A. retroflexus* L., *A. tricolor* L., and *A. viridis* L. have been documented as commonly found in Nigeria under cultivation, as weeds or ornamental. However, none of the thirteen species is native to Nigeria but most have since acclimatized to the climate and are valued for their highly nutritious leaves, herbaceous stem, inflorescence, seeds and chemical by-products Osawaru and Ogwu (2014).

America is in the centre of origin of the majority of amaranth species where the Aztec and Inca scripts show early utilization, however, the exact date of introduction to Nigeria remains uncertain. Nevertheless, Nigerians have been cultivating and consuming *Amaranthus* species for many decades resulting in different traditional practices and values that have locally maintained the germplasm and made it appealing to the people. Some of the diverse ethnic groups in Nigeria call almost all the varieties with the same local name like “shokoyokoto”, “efo tete”, “arowo jeja” (Yoruba), “akwukwo inene” (Igbo), “boroboro” (Fulani), and “alaiyaho” (Hausa). Indigenous people consider most of the amaranth species available in their vicinity as the same (Burkill, 1985). Burkill (1985) reported the many ethno-dimensional roles of amaranths as human food, animal feed, medicines, dyes, home decorators, and superstitious practices to local gods. The plant is used in medicines in the treatment of eye, ear, and stomach troubles as well as for dysentery, diarrhoea, diuretics, lactation boost, haemorrhoids, menstrual cycle, venereal diseases, paralysis, epilepsy, convulsion, and spasm (Burkill, 1985).

Alegbejo (2013) reported several pharmacological benefits of boiled leaves and roots of *Amaranthus* species including its diuretic, laxative, anti-diabetic, antipyretic, anti-snake venom and antileprotic effects which are associated with their anti-inflammatory, immuno-modulatory activity, anti-androgenic activity and anthelmintic properties. Other medicinal benefits as anti-gonorrhoeal, expectorant to relieve breathing in acute bronchitis have also been reported. There is a belief system in the south eastern parts of Nigeria that the consumption of *Amaranthus* leaves and stem in the soup will improve blood count and revitalize the body system. Consequently, hot amaranth soup with fish is served to nursing mother to help boost their immunity soon after child delivery. In the South Western region of Nigeria, fresh but mature amaranth leaves are consumed raw for the treatment of mouth and stomach ulcers whereas in certain parts of Northern Nigeria, the red inflorescence serves as raw material for making dyes as well as a special traditional drink for soothing stomach pains. In the North Central parts of Nigeria, raw amaranth extract is used for treatment of inflammation caused by boils. The liquid extract is smeared on boils until the abscess is dispelled and the wet plant also helps to dry up Whitlow faster (Mowobi *et al.*, 2016).

The presence of fungal diseases in *Amaranthus* poses a significant threat to crop yield and food quality. Food remains the most vital item in the hierarchy of needs because of its centrality to human existence.

These fungal diseases have posed a serious threat to production of important food and vegetable crops, a serious challenge to nutritional and economic welfare of both farmers and consumers alike. Traditional chemical fungicides have been widely employed to mitigate these diseases but concerns about their environmental impact and human health risks necessitate the exploration of alternative, sustainable solutions. During the past years it has been observed that the severity of fungal attacks is on the rise among *Amaranthus*. The disease has brought significant reduction and loss among *Amaranthus* farmers in Nigeria. Botanical extracts have shown promise as potential sources of natural antimicrobial agents. However, there is a critical knowledge gap regarding the specific botanicals and their efficacy against fungal pathogens affecting *Amaranthus*, necessitating need to address the gap. This research aims to investigate the antimicrobial activities of selected crude plant extracts against prevalent fungal diseases in *Amaranthus hybridus*, with the goal of providing environmental friendly and effective disease management strategies for sustainable agricultural practices.

MATERIALS AND METHODS

Location of Study: The study was conducted in the Department of Plant Science and Biotechnology Laboratory, Michael Okpara University of Agriculture Umudike, Abia State. Umudike is located approximately at latitude 05029N, longitude 07032E. Umudike lies within the tropical rain forest zone of South Eastern Nigeria with altitude of 123metres. Daily mean temperature varies from 23°C to 32°C. The Laboratory work was done between July and October, 2024.

Collection of samples: *Amaranthus hybridus* plant with external visible symptoms of fungi infection was collected from Lois mother's garden in Okwuta Ibeku, Umuahia, The garlic plant materials used for the antimicrobial test were obtained from Oriugba Market while the marigold was collected from Michael Okpara University of Agriculture school premises.

Isolation of pathogen: The diseased *Amaranthus* plant was brought into the Laboratory and 2 mm portion of the diseased leaf was incised from the peripheral of the lesion and was surface sterilized with 10% ethanol for 2 minutes and washed with 3 changes of sterile distilled water. This was done in order to remove surface contaminants. The surface sterilized explants were aseptically inoculated unto a Water Agar (WA) in Petri dishes and incubated for 3 days. After 3 days of incubation, fungal colonies emerging from the explants were sub-cultured individually on new Potato Dextrose Agar (PDA) medium amended with antibiotics such as streptomycin in order to eliminate bacterial and fungal contaminants. This exercise was repeated 3 times until clean cultures were attained. The Petri dishes of all the test fungi were sealed with masking tape to prevent contamination.

Identification of pathogens: Observation of the cultural characteristics on colony, colour, and growth rate was recorded. Characteristics like sporulation and colour of spores; white, creamy or milky colour and appearance of the colony on plate: rosette form, velvet or fluffy was observed and recorded after incubation. The mycelium of fungal isolates were studied in 7 days old culture of each isolates grown on PDA stained with 0.1 percent lactophenol on cotton blue and secured with a cover slip then viewed under the light microscope using x40 and x10 lens. The size and shape of hyphae and mycelium were examined microscopically and the observation compared with those in standard atlas.

Pathogenicity test: Pathogenicity test was conducted on a fresh *Amaranthus hybridus* plant which had no trace of disease. It was brought to the Laboratory and surface washed under running tap water. A sterilized cork borer was used to take an equal size of mycelial plug of each organism and the samples were placed in the punched hole of the healthy *Amaranthus hybridus* plant using sterilized forceps. It was then sealed with Vaseline and maintained inside water contained in a bottle to retain turgidity then incubated for four days. The inoculated plants were monitored for symptoms development and those which had symptoms were re-isolated using 5g of water Agar in 250ml of distilled water.

Preparation of crude extracts: The plant materials used for the antimicrobial activity were washed under a running tap and rinsed in three changes of distilled water. 20grams of each of the plant material (Garlic and Marigold) were measured out and ground using 20ml of distilled water with a manual grinding machine to obtain the juice, sieved through a four layered cheese cloth to remove unwanted particles then squeezed into a beaker. The crude plant extract of both garlic and marigold were thus derived.

Antimicrobial test: The poisoned food technique described by Ufoegunne *et al.*, (2015) was used to evaluate the antifungal effect and determine the percentage inhibition of mycelia growth of the crude plant extracts on the fungal pathogen. Four concentrations were used in this research work to study the efficacy of each extract. The concentrations include: 0% (control), 25% garlic, 50% garlic, 25% marigold, 50% marigold and 50% garlic and marigold). The extracts were integrated into the liquefied agar at the chosen final concentration, thoroughly mixed and dispensed into Petri dishes. Inoculation on to the solidified medium was done using a sterile cork borer of about 5mm diameter which was used to puncture the fungi culture under aseptic conditions. After that, three replications per concentration of fungi discs were placed on the gelled agar plate. Following incubation of the test fungi under appropriate condition ($25\pm 2^{\circ}\text{C}$), the radial growth diameter in test and control plates were determined and the antimicrobial effect was quantified using the following formula adopted from Ugwuja *et al.*, (2023).

$$\% \text{ inhibition of radial growth} = (DC-DT)/DC \times 100$$

Where DC = Diameter of fungal colony growth in the control plate

DT = Diameter of fungal colony growth in the test plate.

Statistical Analysis: The experimental design consisted of 2 x 4 x3 factorial in Completely Randomized Design; two plant extracts in four concentrations and three replications. Data obtained from the experiment was statistically analysed using Analysis of Variance (ANOVA) and IBM SPSS 23 statistical package. Means were separated at 95% confident intervals.

RESULTS

Effect of garlic and marigold extracts on radial

Growth and percentage inhibition of *Rhizoctonia solani*:

The effect of garlic and marigold extracts revealed that the inhibition of the growth of the pathogen was higher than that of the control (0%) as shown in (Table 1). This reflects the effectiveness of both extracts (Garlic and Marigold) in its various concentrations. For the isolate containing *Rhizoctonia solani* inoculum, the lowest inhibition of growth of pathogen was observed in the control (0%) concentration while the garlic and marigold 25%, 50% each and combined as 50% was observed to have the highest inhibition of fungal pathogen. From the result, it was observed that there was significant difference ($P \geq 0.05$) between the treatments (0.00 ± 0.00), which had no radial growth and the control treatment (5.35 ± 1.70) that showed the highest radial growth.

Table 1: Effect of garlic and marigold extracts on

Radial growth and percentage inhibition of <i>Rhizoctonia solani</i>		
Treatment	Radial growth (mm)	% Inhibition
Control 0%	$5.35 \pm 1.70a$	$0.00 \pm 0.00a$
Garlic 25%	$0.00 \pm 0.00b$	$100.00 \pm 0.00b$
Garlic 50%	$0.00 \pm 0.00b$	$100.00 \pm 0.00b$
Marigold 25%	$0.00 \pm 0.00b$	$100.00 \pm 0.00b$
Marigold 50%	$0.00 \pm 0.00b$	$100.00 \pm 0.00b$
Garlic + Marigold	$0.00 \pm 0.00b$	$100.00 \pm 0.00b$

Key = mean \pm standard error with the same superscript indicate insignificance ($P \leq 0.05$)

Effect of garlic and marigold extracts on the radial growth and percentage inhibition of *Pythium sp* in *Amaranthus hybridus*:

The results obtained from the study as relayed in Table 2 for isolate *Pythium sp* revealed that garlic at 25 and 50% concentrations, as well as marigold at 50% and the combination of the two extracts at 50% respectively, had maximum inhibition (100.00 ± 0.00 %) of radial growth of the fungus compared to the other treatments. The difference in percentage inhibition for these treatments were highly significant (P ≥ 0.05) compared to the treatment with marigold 25% which showed a lower inhibition of 76.59 ± 2.40%. However, the control which induced 3.70 ± 0.34 mm mycelial growth of the fungus, had a corresponding zero inhibition. From the results the various fractions of garlic and marigold treatments showed a highly significant inhibition (P ≥ 0.05) of the pathogen compared to control.

Table 2: Effect of garlic and marigold extracts on the radial growth and percentage inhibition of *Pythium sp* in *Amaranthus hybridus*

Treatment	Radial growth (mm)	% Inhibition
Control 0%	3.70 ± 0.34a	0.00 ± 0.00a
Garlic 25%	0.00 ± 0.00b	100.00 ± 0.00b
Garlic 50%	0.00 ± 0.00b	100.00 ± 0.00b
Marigold 25%	0.80 ± 0.00c	76.59 ± 2.40c
Marigold 50%	0.00 ± 0.00b	100.00 ± 0.00b
Garlic + Marigold 50%	0.00 ± 0.00b	100.00 ± 0.00b

Key = mean ± standard error with the same superscript indicate insignificance (P ≤ 0.05)

DISCUSSION

The experiment was conducted to investigate the antimicrobial activities of selected crude plant extracts against prevalent fungi pathogens causing rot/damping off symptoms in *Amaranthus hybridus*, with the goal of providing environmental friendly and effective disease management strategies for sustainable agricultural practices. The results of this present study has revealed the efficacy of selected crude plant extracts (Garlic and Marigold) in inhibiting fungal pathogens of *A. hybridus*. The results of this study agrees with the findings of Daniel *et al.*, (2015); Jimoh *et al.*, (2020) who reported on the inhibitory effects of the crude extracts on fungal pathogens. According to previous researchers, the inhibition may be attributed to a sulphur compound in garlic, chemically identified as di-allyl thiosulfinate (allicin), an active component responsible for inhibition of the growth of fungi and bacteria. Moreover, the essential oil from leaves and thiophene rich extracts from marigold roots have high antifungal activity against a number of soil borne and foliar pathogens.

The study of Slusarenko *et al.*, (2008) also revealed the effectiveness of garlic juice against a series of plant pathogenic bacteria, fungi and oomycetes *in vitro*. They reported that the active compound allicin effectively controlled seed borne *Alternaria sp.* The antifungal properties of marigold extracts in the present study corroborates the findings of Garzim *et al.*, (2008), who stated that the essential oil from the flowers of *Calendula officinalis* had displayed significant antifungal activities against common pathogenic fungal strains. Since all the pathogens were inhibited with respect to growth, sporulation, and these parameters are vital in fungal virulence, it is a strong indication that the extracts of garlic and marigold have a wide spectrum of disease inhibition. All the different concentrations of these plant extracts under study exhibited promising control effects against fungal pathogens on *Amaranthus*. The result obtained from the antimicrobial test showed that both crude plant extracts (Garlic and Marigold) were very effective in inhibiting the growth of the test pathogens. Garlic (*Allium sativum*) extracts were persistently the most active in reducing the radial growth of the pathogens.

CONCLUSION AND RECOMMENDATION

In conclusion, this study confirmed that Garlic (*Allium sativum*) and Marigold (*Tagetes erecta*) possess potential inhibitory effects on fungal pathogens. The study suggests the possible use of extracts of garlic and marigold as an alternative means of fungal disease management especially against the pathogens *Rhizoctonia solani* and *Pythium sp.* Adoption of these botanicals in disease control and management will reduce the risk of pesticide residues build up and pathogens resistance to fungicides. Farmers could either spray the extracts directly onto the *Amaranthus* crops or they could use the extract to create a natural fungicide that could be applied to the farm because of its safety and availability.

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