SADI International Journal of Science, Engineering and Technology
ISSN: 2837-1941 | Impact Factor : 8.27
Volume. 12, Number 4; October - December, 2025;
Published By: Scientific and Academic Development Institute (SADI)
8933 Willis Ave Los Angeles, California
https://sadijournals.org/index.php/sijset|editorial@sadijournals.org

DETERMINATION OF PLANT EXTRACTS FOR THE CONTROL OF PEPPER (*Capsicum SPP*) FUNGAL DISEASES IN NORTHEAST NIGERIA

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ACKNOWLEDGEMENT: I acknowledged Tertiary Education Trust Fund (Tetfund) for Sporing this Research Work

DOI: https://doi.org/ 10.5281/zenodo.17454774

Abstract: Pepper (Capsicum spp.) is cultivated in almost every part of Northern Nigeria which faces significant challenges due to the prevalence of fungal diseases. This identified plant parts that inhibit the growth of fungal pathogens reduces the quality of pepper in the study area especially, stem rot, vascular wilt and anthracnose, the study also examine the incidence and severity of the disease caused by the fungi. The research was conducted in two study areas: Adamawa and Taraba states. The laboratory experimental design was a CRD with three replications. To obtain a concentrated solution of the plant extracts, three lots (20, 40, and 60g) of the powdered form of the extracts were separately dissolved in 1 L of distilled water, vigorously agitated, and left for 24 h to stand before filtration. All treatments were found to be superior to the control, and the effects were directly proportional to the concentration. Casia occidentalis was the most effective plant extract in inhibiting the mycelia growth of the test fungi in the following order: Colletotrichum capsici (69.23%), Fusarium solani (55.20%), and Phoma species (42.73%), followed by Vitex doniana on Colletotrichum capsici (54.03%) and Fusarium oxysporum (42.49%). Anogeissus leiocarpus, Detarium microcarpum, and Nauclea latifolia were the least effective plant extracts in the reduction of test fungi mycelial growth, with less than 30% inhibition in all cases. Mancozeb was found to be statistically significantly ($p \leq 0.05$) superior to all plant extracts. All data were subjected to analysis of variance (ANOVA) using the Paleontological Statistics (PAST) package version 4.07, and means were separated using Fisher's least significant difference (FLSD) at the 5% probability level.

Keywords: Pepper cultivation, plant extracts, fungal pathogens, vascular wilt, and Anthracnose disease.

INTRODUCTION

The morphological characteristics of pepper plants have also been reported. Joshi *et al*. (2015) described *Capsicum* as a highly heterogeneous plant which exhibits considerable morphological variation, especially in fruit shape, color, and size. Leaf and stem pubescence ranges from glabrous to very pubescent. Akilu *et al*. (2018) described bisexual pepper flowers that are borne at the intersection between the stem and leaves at points where the stem splits into a fork. The inflorescences may vary from solitary to seven flowers at one node. The calyx may range from long, green sepals to truncate sepals and spine-like projections. The pedicel length varies among

cultivars, ranging from 3 to 8 cm. In *C. annuum*, the petals are usually white with five to seven individual stamens, which vary in color from pale blue to purple anthers. He maintained that greenish-white corolla was observed in *C. frutescens* and added that corolla color is one of the most consistent features of distinguishing *Capsicum* species (Joshi *et al*., 2015).

Chigoziri and Ekefan (2013) described *Capsicum* species as tender annual or perennial plants with straight woody stems and single, star-shaped white flowers in the axils of their leaves. Their flowers are followed by pods and vary in shape and size; they are green at first and change to yellow when ripe. They contain many flat, kidney-shaped fruits, with a very hot taste; it is a shrub perennial plant 30 cm to 180 cm high. Branches angular, usually enlarge and slightly purple at the nodes; petioles medium; peduncles slender, often in pairs and longer than fruit; calyx cup shaped, clasping base of fruit which is red, ovate and from 1 to 4 cm long; seeds small and flat, taste very pungent.

Chigoziri and Ekefan (2013) further reported that the pepper pistil is made up of an ovary, which contains two to four carpels or locules, and a stigma borne at the tip of a slender style. The length of the style and relative position of the stigma and the anthers vary among genotypes, and it is an important factor determining the level of natural cross-pollination of the flowers (*Lema et al*., 2018). The flower color, shape, length, and relative positions of the styles also vary with different species and cultivars. The fruits are, botanically, classified as berries with different shapes, colors, and sizes that vary among cultivars. The seeds are cream colored, except for *C. pubescens* which has black seeds.

The largest producers in West Africa are Nigeria and Ghana, ranked eighth and thirteenth in the world, respectively (FAOSTAT, 2022). They also estimated the world production of pepper at an average yield of 13.4 t/ha, 7.9 t/ha in Africa, and 8.4 t/ha in Nigeria. This implies that Nigeria has fertile soils, good weather, and land that can readily support the growth and production of pepper. The estimated yield of 8.4 t/ha reported on farmers' fields in Nigeria was still very low compared with the estimated yield of 15 t/ha reported in Western Europe. The low yields in Nigeria were once attributed to production constraints, including diseases, pests, and poor weed management. Zakari et *al*. (2016) reported that the northern region between latitude 10°N and 12°301N is the major area for pepper production in Nigeria. A high percentage of peppers grown in Nigeria come from the Savanna zones of Guinea and Sudan (Kaduna, Kano, Katsina, Kogi, Kwara, Yobe, and Zamfara states) in northern Nigeria.

Many higher plants and their constituents have been successfully used in plant disease control and have proven to be safe and non-phytotoxic. Some studies have been conducted to control a wide range of seed-borne pathogens using different botanicals (Abubakar *et al*., 2017). Plant extracts used to control phytopathogens have been obtained from tree plant species such as *Eucalyptus* and neem (Alanwood, 2013). These plants control pathogens that cause disease in the plant canopy, such as the genera *Alternaria*, *Bipolaris*, *Crinipellis*, *Corynespora* and *Colletotrichum* (Asare-Bediako *et al*., 2015). Oil from the leaves of *Cymbopogon flexuosus* was found to be effective against *Aspergillus flavus, Penicillium italicum, and Alternaria alternata* (David *et al*., 2017).

Chukwunonye *et al*. (2017) investigated the in vitro antimicrobial activity of leaves and stem bark of *Vitex doniana* on clinical isolates of *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*

Statement of the problem

The largest producers of pepper in West Africa are Nigeria and Ghana, ranked eighth and thirteenth in the world, respectively (FAOSTAT, 2022). They also estimated the world production of pepper at an average yield of 13.4

t/ha, 7.9 t/ha in Africa, and 8.4 t/ha in Nigeria. This implies that Nigeria has fertile soils, good weather, and land that can readily support the growth and production of pepper. The estimated yield of 8.4 t/ha reported on farmers' fields in Nigeria was still very low compared with the estimated yie ld of 15 t/ha reported in Western Europe. The low yields in Nigeria were once attributed to production constraints, including diseases, pests, and poor weed management. A high percentage of peppers grown in Nigeria came from the Savanna zones of Guinea and Sudan (Kaduna, Kano, Katsina, Kogi, Kwara, Yobe, and Zamfara states) in northern Nigeria Mohammed *et al*., 2016). Despite the global efforts toward archiving this eco-friendly plant-based fungicide and the high potential of tropical plants to provide varieties of these phytochemicals, there is no published information about the use of local medicinal plants in many parts of the tropics, especially Nigeria, on the specific plant fungicide to control specific plant diseases. The documentation of the medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of their natural habitat d ue to anthropogenic activities (Sofowora *et al*., 2013). This study identified botanicals that can reduce the infection rate caused by fungi on pepper plants.

The objectives of the study were as follows:

i. To isolate and identify fungi responsible for the diseases of pepper in the study areas.
ii. To determine the in vitro efficacy of plant extracts against fungi isolated from pepper diseases
iii. To determine the quality and quantity of phytochemicals associated with the medicinal plants used in the study.

MATERIALS AND METHODS

Study area:

The study was conducted in Jalingo, Taraba State and Yola, Adamawa State. The two areas lie under the Northern Guinea Savannah ecological zone of Nigeria between latitudes 8º47' to 9°19'N and longitudes 11º09' to 12°30''E (Shawulu *et al*., 2008). Both locations have a tropical continental climate characterized by well-marked wet and dry seasons.

Experimental design:

The laboratory experimental design was a CRD with three replications.

Sterilization of the glassware and media

The Petri dishes were sterilized in an oven at 160℃ for 2 hours. Potato dextrose agar (PDA) medium, water agar medium, and water were used in the experiment and sterilized at 121.6 º C for 15 minutes in an autoclave. The inoculating needle and other metallic instruments were sterilized by immersion in alcohol and heated over a flame. Surface sterilization of plant parts and diseased materials was performed by dipping them in 0.1 % mercuric chloride for 30 s, followed by three washings in sterilized water. The bench top was sterilized using 70 % alcohol for 30 min.

Preparation of the culture media

Potato dextrose agar (PDA) is the growth media used. The media was prepared according to the manufacturer's instructions. To 1 L of distilled water in a conical flask, 39 g of commercial PDA powder was added and mixed to dissolve. The conical flask was covered with cotton, wrapped with aluminum foil, and autoclaved at 121^0 C for 15 min at 10 psi pressure. After cooling, 500 mg of chloramphenicol was added to discourage bacterial contamination.

Isolation of fungal pathogens from the plant

Diseased parts of pepper plants, such as fruits, leaves, roots, and stems of the three cultivars (Tattase, Atarugu, and Shambo), were collected from the field in the study areas and were surface sterilized by dipping them in 90

% ethyl alcohol for 1 min and then rinsed in three sterile distilled water. Small pieces of infected tissue (2-3 mm in length) were cut at a point between the diseased and healthy portions using a disinfected knife. These bits were surface sterilized in 0.1 % mercuric chloride solution ($HgCl_2$) for 30 s and then washed three times with sterilized distilled water in Petri dishes under aseptic conditions. The bits were then dried by placing them on sterilized blotting paper. Three bits were transferred aseptically to Petri dishes containing sterile potato dextrose agar (El Karkouri *et al*., 2010).

In vitro assessment of the effects of plant extracts on fungal pathogens

In a laboratory experiment, medicinal plants (*Anogeissus leiocarpus Boswellia dalzielii* and *Cassia occidentalis*) were collected and evaluated for antifungal properties against *Colletotrichum capsici* and*, Fusarium oxysporum* using the poisoned food technique. The extracts were poured into a flask, plugged with cotton, and sterilized in an autoclave for 10 min to avoid contamination (Madari and Singh, 2005). Twenty (20), forty (40), and sixty (60) ml of the extract of various plant materials were separately introduced into a Petri dish containing the media (Endriyas and Tola, 2020). A 5-mm mycelium disc of a 7-day-old culture of fungal pathogens was placed on the medium, and the plates were kept upside down to maintain the growth of the pure culture. The control was not supplemented with plant extracts. The Petri dishes were marked and arranged in a completely randomized design with three replications. A total of 36 Petri dishes were used in this experiment. The radial growth of the colony was measured after 7 days of growth. The inhibition percentage was calculated using the following formula:

$$\text{Inhibition percentage (\%)} = \frac{DC - DT}{DC} \times 100$$

Where, DC is the average diameter of fungal spores in the control,

DT; average diameter of fungal spores with treatment

Qualitative determination of plant extract chemical constituents

Qualitative phytochemical analysis of the plant extracts used in this study was conducted according to the procedure described by Sofowora (2013).

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using the Paleontological Statistics (PAST) package version 4.07, and means were separated using Fisher's least significant difference (FLSD) at the 5% probability level.

Frequency of Fungal Pathogen Isolation

A total of five fungi were isolated from different parts of diseased pepper plants collected from different pepper fields in the study sites in Jalingo and Yola. Fungal isolates were identified according to their characteristics and appearance on culture media and under a light microscope as *follows: A. niger*, *C. capsici*, *F. oxysporum, F. solani,* and *Phoma* species. The frequency of fungi isolation is presented in Table 1. Among the isolates, *F. oxysporum* was the most frequent (91.7 %) fungus isolated from all the diseased samples collected in the study areas. It appeared in all four plant parts examined at the seedling and maturity stages and was only absent in the fruits of the plants examined at the flowering stage, appearing 11 times out of 12 possible times. Colletotrichum spp. appeared second (58.3 %) most frequent isolates in the study areas with a total of 7 out of 12 isolates, followed by *F. solani* (50.0 %) with 6 out of 12 isolates. *Phoma* spp. (25.0 %) and *A. niger* (16.7 %) appeared as the least frequent isolates, with 3 and 2 of 12 isolates, respectively.

Table 1: Percentage Frequency of Fungi Isolated from Pepper Plants at Different Growth Stages in Jalingo and Yola during the 2024 Rainy Season

Growth stage / Isolates	Seedling stage				Flowering stage				Maturity stage				Occurrence	
	PR	PS	PL	RS	PR	PS	PL	RS	PR	PS	PL	PF	Frequency	Percentage
A. niger	×	×	×	√	×	×	×	√	×	×	×	×	2	16.7
Colletotrichum spp.	×	×	√	√	×	×	√	√	×	√	√	√	7	58.3
F. oxysporum	√	√	√	√	√	√	√	×	√	√	√	√	11	91.7
F. solani	√	√	×	√	√	×	×	×	√	√	×	×	6	50.0
Phoma spp.	√	×	×	×	×	×	×	×	√	×	×	√	3	25.0

Key: PR-Plant root, PS-Plant stem, PL-Plant leaf, PF-Plant fruit, RS-Root soil, √ = Detected, × = not detected.

Screening Plant Extracts for Disease Control

Eight crude plant extracts, viz., Anogeissus *leiocarpus, Cassia occidentalis, Detarium microcarpum, Boswellia dalzielii, Guiera senegalensis, Metracarpus scaber, Nauclea latifolia, and Vitex doniana, and a conventional* chemical (mancozeb) were evaluated against *Aspergillus niger*, *Colletotrichum capsici*, *Fusarium oxysporum, Fusarium solani,* and *Poma* species under *in vitro* conditions at three different concentrations of 20%, 40%, and 60% v/v compared with the control.

They were evaluated against the fungi using the poisoned food technique. Fungal growth was recorded at 7th day after inoculation, and the data are summarized in Table 2. All treatments were found to be superior to the control, and the effects were directly proportional to the concentration. *Casia occidentalis* was the most effective plant extract in inhibiting the mycelia growth of the test fungi in the following order: *Colletotrichum capsici* (69.23%), *Fusarium solani* (55.20%), and *Phoma* species (42.73%), followed by *Vitex doniana* on *Colletotrichum capsici* (54.03%) and *Fusarium oxysporum* (42.49%). *Anogeissus leiocarpus, Detarium microcarpum*, and *Nauclea latifolia,* were the least effective plant extracts in the reduction of test fungi mycelial growth, with less than 30% inhibition in all cases. Mancozeb was found to be statistically significantly ($p \leq 0.05$) superior to all plant extracts.

Table 2: *In vitro* Effect of Plant Extracts at Different Concentrations on Radial Mycelial Growth and Inhibition of Isolated Fungal 7 Days after Inoculation

Treatments	Mycelial growth inhibition (%)											
	**C. capsici*			**F. oxysporum*			*F. solani*			*Phoma* spp.		
Conc. (%)	20	40	60	20	40	60	20	40	60	20	40	60
A. leiocarpus	11.30	20.51	28.97	3.97	5.55	15.83	5.93	5.19	17.43	15.93	9.01	18.07
B. dalzielii	8.00	7.81	30.70	1.94	1.89	24.03	20.24	14.73	19.83	16.23	13.13	32.80
C. occidentalis	1.29	2.23	69.23 ++	6.07	9.11	46.33	8.37	3.98	55.20 ++	17.28	8.21	42.73
D. microcarpum	14.61	20.75	24.10	7.56	14.00	27.97	13.73	11.10	24.17	27.59	13.21	19.57
G. senegalensis	10.93	14.16	20.90	12.05	15.44	35.60	19.69	18.11	21.20	20.91	13.00	24.23

M. scaber	9.46	16.79	34.00	8.15	15.27	25.70	17.90	16.70	18.50	8.74	5.78	11.47
N. latifolia	12.86	21.98	16.93	11.96	9.40	10.67	12.65	8.56	20.27	11.35	3.81	27.17
V. doniana	38.94	54.03 ++	29.57	42.49	32.53	27.87	34.30	31.41	22.90	37.61	33.66	22.17
Mancozeb	78.48 +++	79.01 +++	92.00 +++	80.94 +++	79.83 +++	87.57 +++	80.34 +++	79.08 +++	92.73 +++	80.65 +++	79.25 +++	91.80 +++
Distilled water	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	18.59	23.73	34.64	17.51	18.30	30.16	21.32	18.89	29.22	23.63	17.91	29.00
p-value	0.99	0.89	0.73	0.92	0.88	0.93	0.96	0.95	0.94	0.99	0.99	0.97
LSD	22.00	22.91	25.89	23.41	21.79	25.45	21.08	21.19	22.52	21.02	21.59	23.93

0% () = not effective, 6- 49 % (+) = Effective, 50-69% (++) = Very Effective, 70-99% (+++) = highly effective.

CONCLUSION

It may be concluded from this study that all eight medicinal plants screened were effective against the fungal pathogens tested under controlled and field conditions. *Cassia occidentalis* was the most effective plant extract for controlling fungal diseases evaluated in this study. It has high tannin content, which could be responsible for the high antifungal activity displayed in this study.

i. More research should be conducted to further test the effects of these tested plant extracts, especially *Cassia occidentalis* and other more effective extracts, on other known fungal pathogens, such as *Cercospora capsici*, *Phytophthora capsici*, *Verticillium* species, and *Phythium* species. Further research should be conducted to examine other indigenous medicinal plants for antimicrobial properties and their potential incorporation into modern pesticides.

ii. Research work should be conducted to fractionate the extracts to identify, isolate, purify, and then characterize the principle(s) responsible for their reported control activities.

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