Original Research Article

In-vitro Evaluation of the Efficacy of Two Plant Extracts *Allium sativum* (Garlic) and *Azadirachta indica* (Neem) in the Control of Powdery Mildew Caused by *Erypside cichoracearum* of *Abelmoschus esculentus* (Okra)

ABSTRACT

Aim: The aim of this study is to determine the efficacy of extracts of some selected plant materials in the control of Powdery Mildew of Okra plant.

Study design: Completely randomized design.

Place of Study: This study was carried out in the biology laboratory of the department of Plant science and Biotechnology.

Methodology: Three Okra farms showing symptoms of powdery mildew disease were surveyed, disease occurrence was recorded, and disease incidence was calculated. Leaves of okra (*Abelmoschus esculentus*) showing symptoms of powdery mildew were collected from the farms. The disease incidence was recorded and the samples of infected leaves were collected from the farms and taken to the laboratory for microscopic examination. The fungus associated with the disease was isolated and cultured to obtain a pure ed. Pathogenicity test was carried out on a healthy okra plant. Extraction of the two plants were done using methanol and it was then diluted to different concentrations. The antifungal susceptibility test on *Erysiphe cichoracearum* was carried out using the agar diffusion method and recorded at various concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml under in-vitro conditions. The minimum inhibitory concentrations and antifungal activity were recorded at these concentrations.

Results: Results of phytochemical analysis showed that Alkaloids, reducing sugars, carbohydrates, Flavanoids, Tannins and Resins were present in both *Allium sativum* and *Azadirachta indica*. These phytochemicals present are suspected to be responsible for the antifungal activity of the plants. Out of the two plants, Neem leaf extract was found to be best in the inhibition of the fungus at 50mg/ml with a significantly high inhibition of 26.77+-0.50a after the treatment. At P<= 0.05 there was a significant difference in the antifungal activity of the extract of *A. indica* on *Erysiphe cichoracearum*.

Conclusion: The extracts of both *A. sativum and A. indica* possess antifungal properties, and may have the potential for the production of biopesticides, that can be used for the control of fungi.

Keywords: Antifungal; Azadirachta indica; Allium sativum, Fungi, Abelmoschus esculentus

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L.) locally known as Kubewa is an annual commercial fruit vegetable for small scale rural farmers in Nigeria, grown on a limited or large area. However, Disease management remain the most considerable factor in it production system, this is because it is affected by a number of diseases caused by fungi, such as wet rot, powdery mildew and wilt, causing significant losses in yield and quality. Powdery mildew occurs on leaves, stems and fruits. Due to powdery mildew infection, all aerial parts of the plants are infected at all stages of their growth causing premature defoliation and reducing the yields [1]. The disease initiates as white minute patches first on the upper surface of lower older leaves and then spread to younger ones, while greyish powdery coating visible on severely

affected leaves. Leaves finally show necrosis resulting in withering, drying and defoliation [1].

Control methods currently available under commercial conditions include the use of repeated applications of elemental sulphur and other fungicides [2]. However, as okra is known to be harvested every two days, the dependence on chemical fungicides will result in several ecological problems and resistance development against these fungicides [3]. In recent years, as a result of growing concerns for health hazards and environmental pollution, agricultural programmes have been developed to aim at high productivity while ensuring conversion and utilisation of natural resources on a sustainable basis. Therefore, in addition to existing disease management tools, new and interesting approaches are being explored to suppress diseases through natural and eco-friendlier means to reduce the use of synthetic fungicides [4]. Also, the reduction of total fungicides application rates is one of the dominant trends in agricultural production. This can be achieved in different ways, either by the introduction of new and more effective chemicals, or by the purification of the chemicals, or by the combination of the formulation with an adjuvant or with plant extracts or culture filtrates of biocontrol agents (BCAs). The use of adjuvants, culture filtrates of BCAs and plant extracts alone or in combination is a practice that has gained more and more acceptance as the cost of the development of new active ingredients is still much higher than the cost of the development of new adjuvants [5,6]. Recently in some studies, volatile compounds produced by antagonistic fungi and bacteria have been shown to have potential antifungal activities [7, 8, 9, 10, 11], and the biological control of plant diseases by antifungal volatiles from fungal strains had been carried out under the greenhouse conditions [12, 13].

Abundant use of chemicals for the control of plant disease is neither economical nor beneficial for environment. Moreover, the continuous use of these chemicals continues to exert serious health hazard as well as it resulted to the evolution of new races of plant pathogens that inhibit the healthy growth of plants. There is dire need to search out alternate methods of controlling plant pathogens. Some plant extracts which consist of numerous anti-microbial compounds are effective in controlling pathogens and also safe for the environment. A number of plants possess antifungal agents which were used by different researchers in the control of powdery mildew disease of okra [14]. Similarly, turmeric, garlic and pepper extracts expressed significant results against powdery mildew disease [15]. Therefore, this study is focused on determining the efficacy of extracts of some selected plant materials in the control of Powdery Mildew of Okra plant.

The specific objectives are;

1. To examine the Okra farms for symptoms of the disease and isolate the fungal pathogen.
2. To carry out pathogenicity test on the isolated pathogen.
3. To test and analyze the fungicidal effects of the two plants extracts on powdery mildew disease in vitro.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh cloves of garlic and leaves of neem plants were bought from the Farin gada market of Jos, Jos North, Plateau State Nigeria and identification was done in the herbarium of the Department of Plant Science and Biotechnology, University of Jos.

2.1.1 Preparation of Samples

Infected leaf parts of okra plant were collected at two different botanical gardens located at the Permanent Site farm of the University of Jos and also at a farm located at the Farin gada area of Jos north Plateau state, the 3 farms were labelled as farm A, B and C. Infected okra leaves showing characteristic symptoms of the disease were examined using hand held lens and were collected in a sterile transparent bag and brought to the laboratory,The tissues showing symptoms of the disease (that is, between healthy and affected tissue) were cut with a sterilized scissor and examined under a dissecting microscope for the presence of powdery mildew pathogen. Surface contamination was avoided using a solution of 10% bleach in a petri dish for 30seconds to 5minutes in order to remove secondary or opportunistic fungi and bacteria that could cause contamination in the isolation plate. After surface sterilization, it was then rinsed with three changes of sterile distilled water. Smaller pieces of infected tissues were cut with sterile razor blades and placed in a prepared agar (PDA) poured in the sterilized petri dishes. The petri dishes were labelled and incubated under favourable condition (23^0C) and were checked 3-4 days for signs of growth. After five days, sub culturing from the margin was done in order to obtain a pure culture.

2.1.2 Disease Incidence

From all the three farms surveyed, Farm A having a population of 25 okra plant; Farm B having 20 while at farm C, a total number of 30 plants were observed. The percentage of disease incidence was calculated using the formula below;

$$Disease\ incidence\ (\%) = \frac{\text{No.of diseased plant}}{\text{Total No.of plants}} \times 100$$

2.2 Preparation of Spore Suspension

15ml of distilled water was poured into the petri dish containing pure culture of the organism, the mycelium was scraped with a clean microscope glass slide, and liquid was then poured into the mesh which was over a clean cup. The plate was rinsed with an additional 15ml of distilled water and it was filtered through the mesh into the cup. The filtrate formed the spore suspension [16].

2.3 Pathogenicity Test

The initial disease was observed on the leaf of okra plant and was labelled as inoculated. A spore suspension already prepared was carefully inoculated on a wounded and unwounded surface of the okra leaf. Wounding was achieved by cutting a slit into the tissue. Transfer of the spores was done using a sterile fine brush on the leaf surface. For negative control, part of the leaf of another okra plant was wounded but was not inoculated. A small piece of damp cotton wool was placed on to the infected area for 24-48 hours, to maintain humidity at the beginning of the test; the plant was then placed in a larger damp chamber.

2.4 Preparation of Methanolic Plant Extract

Locally available plant materials of Neem and Garlic obtained were washed thoroughly with distilled water, neem leaves and garlic cloves were oven dried at 50°C and then were prepared by blending 50g of the dried leaf and cloves with 100ml of methanol using an electric blender, and the mixture was kept for 24hours, it was then taken to the laboratory for Phytochemical analysis. Each extract was filtered through a muslin cloth and was diluted to obtain different concentrations.

2.4.1 Phytochemical Screening of Extracts

Following the method described by [17], the phytochemical analysis of the Methanolic extract of *Allium sativum* and *Azadirachta indica* was carried

out to determine the presence of their chemical constituent.

2.5 Sterility Test of Plant Extracts

Each of the above extracts were tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on PDA agar and incubated at 37˚C for 24 hours. The plates were observed for growth. All glassware used were washed with detergent, rinsed properly with tap water and dried. They were then sterilized in the oven at 160˚C for 2 hours. Cork borer was sterilized by dipping into 70% alcohol followed by flaming over Bunsen burner. Inoculating loop was heated to redness in an open flame. Potato dextrose agar, distilled water bottles were sterilized in the autoclave at 121˚C for 15 minutes. Finally, the laboratory bench was swabbed with 70% alcohol before and after each round of experiment.

2.6 Minimum Inhibitory Concentration (MIC) of the Extracts

This is the lowest concentration that the antimicrobial agent will inhibit the growth of the test organism.

The minimum inhibition concentration (MIC) of the extract was determined using broth dilution method as described by Wiegand et al. [18]. 2ml of each of the plant extracts were prepared at different concentration of 50%, 25%, 12.5%, and 6.25% respectively; they were then aseptically pipetted into a set of test tubes each containing 10ml of sterile broth. A set of 4 test tubes were used for each test organism. The test tubes were carefully agitated and then incubated at 37^{o}c for 3 days, after which they were examined for turbidity. The lowest concentration that prevented visible growth of the test organism were noted and taken as the minimum inhibitory concentration of the extract.

2.7 Antifungal Susceptibility Test

Sensitivity testing of the plant extracts was done using the agar diffusion method [19]. 10ml of the broth culture was introduced into a sterile PDA medium in a test tube and agitated to obtain a homogenized mixture. The inoculated agar was poured into sterile Petri-dishes and allowed to solidify. Four wells of 4mm in diameter were made in each plate using a sterile cork-borer. Appropriate volume of each of the extract's concentration was aseptically introduced into each of the four wells. The plates were appropriately labelled and were then incubated at 37^{o}c for 3-4 days. Inhibition zone formed on the medium were measured in millimetre(mm) using a sterile transparent ruler. All tests were carried out in duplicate and their mean values were recorded respectively. b

3. RESULTS AND DISCUSSION

3.1 Disease Incidence

To record the occurrence and percentage incidence of the disease, surveys of the okra growing farms at Botanical garden at Permanent site and Farin Gada farm were conducted for one crop season in August 2019. The data for the plant population and the diseases occurrence and percentage incidence were recorded and presented in Table 1.

3.2 Symptoms of Powdery Mildew on Okra Plant

All the infected okra plant surveyed in the field showed milled to sever symptoms of powdery mildew infections. The fungus symptoms on Individual samples of infected Okra plants were clearly observed on both sides of the leaves (both upper and lower surface), infected leaf parts showed symptoms of irregular patches of white powdery appearance which extended to whole leaves and other parts of the plants. The leaves were seen to be more infected than the other plant parts (Plate 1). There were no symptoms observed

on the fruits. Severely infected leaves were seen to be yellowish and dried.

3.3 Microscopic Identification of Fungi from Samples

The fungus isolated from the infected okra leaves from the Botanical garden was found to be *E. circhoracearum*. its characters were studied from the infected leaves by preparing semi-permanent slides and observing the fungal structures under microscope, this is illustrated in Plate. 3.

3.4 Pathogenicity Test

Pathogenicity indicates the ability of the fungus to produce disease in the test organism or host. In order to prove the pathogenicity of *E. cichoracearum*, okra plants grown in plastic pots in greenhouse of Federal College of Forestry, Jos, were used. The plant were brought to the laboratory, inoculated with *E. cichoracearum* tissue already grown in the lab (Plate. 2) and incubated under moist conditions. Symptoms were observed, the symptoms observed were similar to that of the originally infected plants. This shows that Koch's postulate has been established (Plate.4).

Plate. 1 Whitish powdery mildew symptoms observed in the field

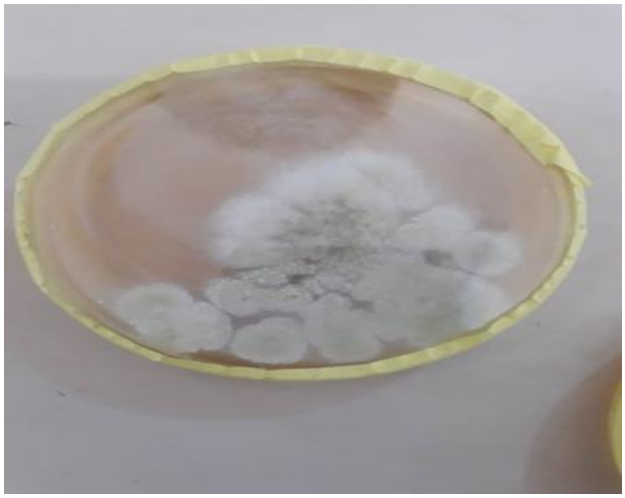

Plate 2 Pure Culture of E. cichoracearum Grown in the Lab

Plate 3 Pictomicrograph of *E. circhoracearum*

Plate 4 Confirmation of Koch's Postulate

Table 1: Population of Plant and Incidence of Powdery mildew on Farms surveyed (%)

Location	Total Population	Occurrence	Disease Incidence	% incidence
Farm A	25	15	0.60	60%
Farm B	20	13	0.65	65%
Farm C	30	14	0.46	46%
Total	75	42	1.71	171.1%

3.5 Phytochemicals Analysis of the Methanolic Extracts

The result for the phytochemical analysis of the methanolic extracts of *A. Sativum* and *A.* is summarized in Table 2 and Table 3.

3.6 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined, and there were no growth of the pathogen at 50% and 25% concentrations of *A. indica* methanolic extract, and growth were observed at 12.5% and 6.25% concentrations of the extract. For the methanolic extract of *A. sativum*, it was observed that there were no growth of pathogen at 50%, while at 25%, 12.5% and 6.25% concentrations, growth were observed (Table 4).

3.7 Antifungal Analysis of the Phytoextracts

In Figure 1, the result for the determination of the antifungal activity of the methanolic plant extracts revealed that *A. indica* at 50% showed highest antifungal activity with 26.77mm zone of inhibition while *A. sativum* at 50% showed 23.83mm zone of inhibition. At 25%, *A. indica* had a zone of inhibition of 22.86mm and *A. sativum* 15.33mm. At 12.5%, *A. indica* showed 14.88mm zone of inhibition while *A. sativum* showed 5.73mm. At 6.25%, 4.60mm zone of inhibition was recorded for *A. indica* and 0.00mm was recorded for *A. sativum*.

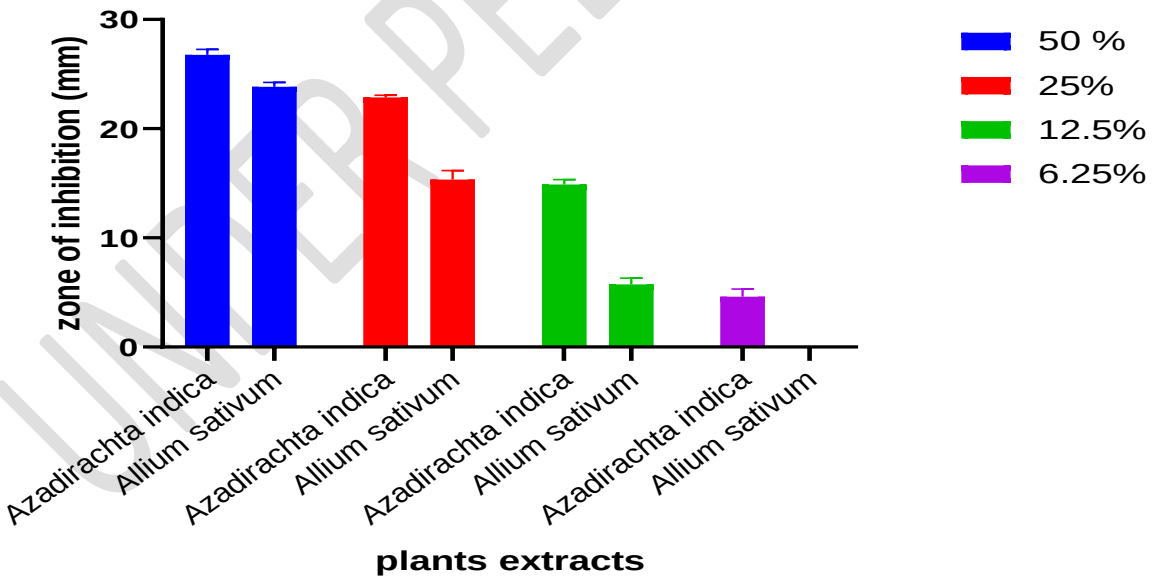

Figure 1: Showing antifungal activity of *A. indica* and *A. sativum extracts* on *E. cichoracearum* (%)

Table 2: Phytochemical analysis of methanolic extract of *A. sativum*

Phytochemical	Result
Alkaloids	+ +
Flavonoids	+
Glycosides	+ + +
Reducing Sugar	+ +
Saponins	+ +
Steroids	+ +
Terpenoids	_
Oils	+ +
Taninns	+
Resins	-
Carbohydrate	-

Key: + = Present, - = Absent, +++ = Highly Present

Table 3: Phytochemical constituent of methanolic extract of *A. indica*

Phytochemicals	Methanol Extract
Flavonoids	+
Saponins	+ +
Glycosides	+ +
Tannins	+
Alkaloids	+
Anthraquinones	-
Terpernoid	+
Coumarin	+
Resin	-
Carbohydrate	-

Key: + = Present, - = Absent, +++ = Highly Present

Table 4: Effects of methanolic extracts on *E. cichoracearum* (%)

Plant Extracts	50%	25%	12.5%	6.25%
A. Indica	-	-	+	+
A. Sativum	-	+	+	+

Key:
+ = presence of growth
- = absence of growth

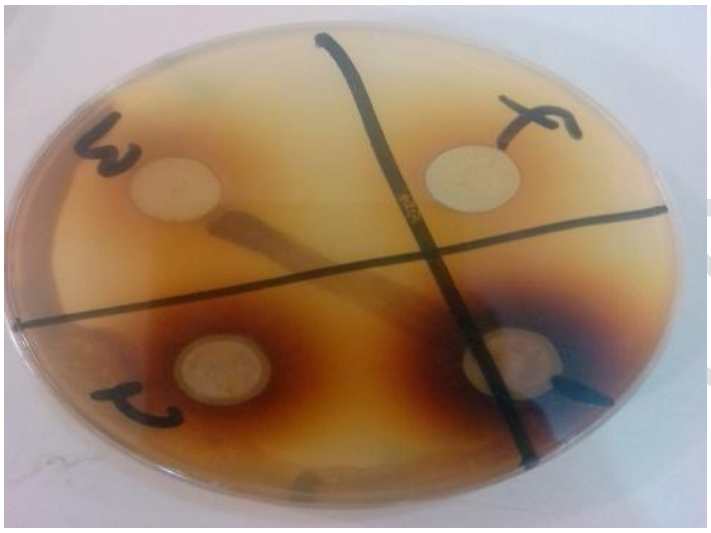

Plate 5 Fungi inhibitory germination zones for Neem

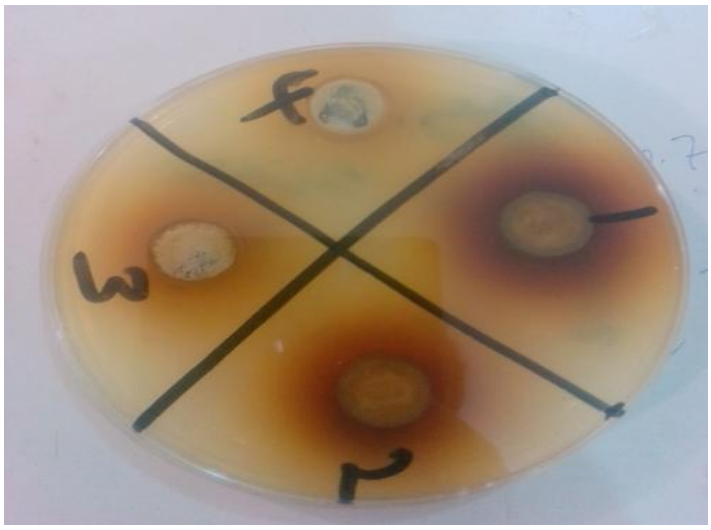

Plate 6 Fungi inhibitory germination zones for Garlic

4.0 Discussion

The results for the field observation and identification of the powdery mildew infection on okra plant showed the characteristics features of white powdery mass appearance on both the lower and upper sides of the leaves (Plate 1); this is similar to the report of Haidar et al., [20]. The isolated fungus is an obligate parasite; its characters were studied from the isolated fungus under microscope. The conidia were seen to be formed singly or in short and long chains, which were barrel or cylindrical shape, hyaline and septate. This result is also in congruent to that of Moyer and Grove [21].

The phytochemical analysis of the two plants extract revealed the presence of large number of secondary metabolites such as Alkaloids, Tarpenoids, Glycosides, Reducing sugar, Coumarins, Tannins, Phenols, Saponins, and Flavanoids compounds are present in *A. Indica extract* except Carbohydrate and Anthraquinones. It showed from the result that the extract exhibited similar number of phytochemicals as in the report of Madaki et al., [22] and consistently with that of Keta et al., [23]. Similarly, despite the absence of Carbohydrate in the extracts of *A. sativum*, Tannins and Saponins were present, this is contrary to the result of Fadiji [24] who reported Tannins and saponins to be absent in the methanol extract of *A. sativum*. The phytochemical constituents present in both Neem and Garlic extracts are suspected to be responsible for the antifungal activities of the plants. This agrees with the report of Keta et al., [23]. Also, the use of these extracts as antifungal agents can serve as a cost effective and environmentally-friendly measure. A good and cheap control measure will go a long way to increase yield since economic factor is a very important consideration in any control measure and since fungi can reduce crop yield of up to 10-18% [25].

It is evident that out of two plant extracts, the maximum fungal inhibition in this study was observed with neem extract at 50% concentration (26.77mm), followed by Garlic extract at 50% concentration (23.83mm). Garlic extract concentration at 6.25% was found to be least effective in fungal inhibition. Fungal inhibition was slow in agar when treated with 6.25% of both plant extracts, as compared to 50% concentration (fig. 1). The results conform with the report of Jadav and Kadvani [2019] that With increasing the concentration of phytoextract, fungal growth inhibition was found to be increased. The effectiveness of neem and garlic extract against *E. cichoracearum* is also reported in the studies of Dinesh et al., [27] and Dhaliwal *et al*., [28]. The findings of this study are in agreement with that of Gangwar *et al*., [29] who evaluated the effect of phytoextracts against powdery mildew disease, and showed *A. indica* to be more effective compared *to Chromolaena odorata, A. sativum, Adhatoda zelanica and Mirabilis jalapa*. Similarly, Ahmed and Din [19] tested antifungal potential of Tumeric, Garlic, Neem and Pepper and observed that neem extracts showed more action against powdery mildew disease.

5.0 CONCLUSION

The *In vitro* evaluation of the efficacy of *A. indica and A. sativum* phytoextracts in the control of powdery mildew diseases of okra cuased by *E. cichoracearum* revealed that, both extracts can be effective in the control of powdery mildew disease, with neem leaf extract having the highest inhibition effect at 50% concentration and Garlic clove extract having the least inhibition effect at 6.25% concentration. Moreover, these botanicals can be used as a source of cheap and effective fungicides for the control of *E. cichoracearum*. There is increasing hope for a bright future in identifying

botanicals as bio-pesticides which will reduce or replace the dependence on chemical pesticides used presently which are usually expensive and non-environmentally friendly.However, further study on identification of bioactive compounds of these plant extracts is needed to classify their antifungal efficacy, especially on *E. cichoracearum.*

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