



## IN VITRO ANTIFUNGAL ACTIVITY OF SELECTED PLANT DIFFUSATES AGAINST POST HARVEST FRUIT ROT OF PEPPER (*Capsicum spp. L.*) IN YOLA

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### Abstract

Isolation and identification of the fungal pathogens of postharvest rots of pepper were carried out on rotted pepper fruits obtained from five markets (Jimeta Modern, Jambutu, Pallujah, Jimeta Shopping Complex and Lake Gariyo Markets) in Yola using Potato dextrose Agar (PDA). *Aspergillus niger* was frequently isolated with 34.7%, followed by *Aspergillus flavus*, *Botrytis cinerea*, *Colletotrichum capsici* and *Phytophthora capsici* with 21.3%, 20%, 10% and 10.7% respectively. Pathogenicity tests on fresh pepper (*Capsicum annum*, *Capsicum chinense* and *Capsicum frutescens*) fruits revealed that all the fungal isolates were pathogenic on the three pepper species. Among the five isolates, *Aspergillus niger* exhibited the highest level of virulence with 75% of rots covering the fruit surface while *Phytophthora capsici* had the least with 25% of the fruit rot surface. Effect of various concentrations of ethanolic extracts from the leaves of *Azadirachta indica*, *Tridax procumbens* and *Vernonia amygdalina* was carried out *in-vitro* under laboratory conditions. The efficacy of leaf extracts of the test plants against the five fungi isolates at different concentrations (20%, 40%, 60% and 80 %) revealed that ethanol extracts suppressed the mycelial growth of the five pathogens. The inhibition effect was proportional to concentration used. For ethanol extraction, *Azadirachta indica* was more effective on *Aspergillus niger* (86.87%), *Tridax procumbens* was also more effective on *Aspergillus niger* (88.03%), just as *Vernonia amygdalina* was also more effective on *Aspergillus niger* (87.21%). Statistically, mean diameter of mycelial growth differed significantly among concentrations and among plant extracts. Higher concentration of ethanol favoured higher mycelial growth reduction.

**Keywords:** *Aspergillus niger*, *Aspergillus flavus*, *Botrytis cinerea*, *Colletotrichum capsici*, *Phytophthora capsici*.

### 1. Introduction

Pepper is one of the most important agricultural crops, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colors and antioxidant compounds (Nevarro *et al.*, 2006). Pepper consumption in Nigeria accounts for 40 percent of the total vegetable consumed per day (Erinle, 1989). A total of 100-200ha is being assigned to pepper production annually in Nigeria (Ado, 1988). In 2003, FAO estimate of pepper production in Nigeria stood at 715,000 metric tons from total area of about 90,000 hectares. In many Nigerian diets, pepper accounts for a large source of vitamins A and C which is responsible for red colour in mature fruit, in Nigeria in particular, *Capsicum* is utilized in the dry state as spice, it content an alkaloid, a digestive stimulant that is used in ointment treatment of arthritic and neuropathic pains (Grubben and Tahir, 2004).

The major limiting diseases of most peppers in the world are by phytopathogenic fungi, bacteria, and viruses (Grubben and Tahir 2004; Melanie and Sally, 2004). Grubben and Tahir (2004) reported fungi as important disease agents of pepper. Anthracnose or fruit rot caused by *Colletotrichum gloeosporioides* (Jeffries *et al.*, 1990) and to a lesser degree *Colletotrichum capsici* (Isaac, 1992) which may cause yield losses of up to 50%. *Phytophthora* blight (crown rot or basal stem rot) caused by *Phytophthora capsici*, were reported (Bosland and Lindsey 1994; Ristaino and Johnson, 1999). *Botrytis* fruit rot (gray mold) is caused by the fungus *Botrytis cinerea* and is the most important disease of pepper worldwide (Vagelas *et al.*, 2009). Gray mold was also reported as rot agent of pepper (Elad *et al.*, 2004). Other fungi that are associated with fruit rot of pepper includes *Fusarium* Sally *et al.*, (1996), *Verticillium dahliae* Grubben and Tahir (2004). *Aspergillus niger*, *Aspergillus flavus*, *Penicillium digitatum* and *Verticillium* spp. (Balogun *et al.*, 2005).

The search for cultivars resistant to the major diseases and postharvest spoilage of pepper has been limited (Kiran *et al.*, 2006). Awareness about the risks involved in the use of synthetic fungicides is a measure concern to plant pathologist globally (Okigbo, 2009). Searching for harmless alternative methods of pathogen control is necessary (Ijato *et al.* 2011; Nsabiya *et al.*, 2012). Higher plants may contain secondary compounds that could effectively control plant diseases, but which are yet to be exploited and used as pesticides (Kurucheva *et al.*, 1997). Although there is a growing interest in the use of medicinal plants to control plant diseases, only about 2,400 plant species among more than 250,000 higher plants have been screened for phytoactivity (Oluwalana *et al.*, 1999; Khafagi & Dewedar, 2000). Extracts of leaves of *Azadirachta indica* were reported to have efficacy in control of *Candida* and *Aspergillus* species (Fabryl *et al.*, 1996). Ijato (2011) reported that an aqueous extracts of *Tridax procumbens* and *Vernonia amygdalina* at 80% were effective in control of *Geotrichum candidum* and *Aspergillus niger*. In view of that, this study is aimed to evaluate the antifungal effect of extracts of *Azadirachta indica*, *Tridax procumbens* and *Vernonia amygdalina* in *in-vitro* control of fungal isolates from pepper rots in Yola.

### 2. Materials and Methods

#### 2.1 Collection of infected and healthy tomato fruits

Pepper fruits showing the deterioration and rotting were collected from different markets/ shops of Yola, Adamawa

State, Nigeria. Fresh and healthy peppers were also collected and packed into sterilized polythene bags and were taken to the Plant Science laboratory at Modibbo Adama University of Technology (MAUTECH), Yola for isolation and other studies.

## 2.2 Isolation and identification of the fungal organisms

Diseased portion of the pepper fruits were cut under aseptic conditions into small bits of 5mm into a sterile dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit (Fawole and Oso,1988). The cut diseased bits sterilized with 70% ethanol were then placed centrally on Petri dishes containing solidified (potato dextrose agar) PDA. The solidified plates were incubated at room temperature ( $28 \pm 2^{\circ}\text{C}$ ) in the dark for 72hours. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure culture was obtained. Microscopic examination was used after examining the colony characteristics to establish identity of fungi. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide then stained with lactophenol cotton blue and examined under the microscope for fungal structures. The morphology and culture characteristics observed were compared with structures in (Snowdon, 1990). Forty grams (40g) Potato Dextrose Agar (PDA) powder was placed in five liters conical flask. 1000mls distilled water was added and boiled to completely dissolve the powder. To prevent bacterial growth, 0.2g of streptomycin was added to the potato dextrose broth. The supernatant was carefully transferred into sterile conical flasks and autoclaved at  $120^{\circ}\text{C}$  for 15 minutes at 101lbs pressure and poured into Petri-dishes for solidification.

## 2.3 Determination of the frequency of the isolates

The frequency of isolates of the different types of fungi associated with pepper fruit rot diseases was determined. The number of times each fungus was encountered was recorded. The percentage frequency of occurrence was calculated using the formula:

$$\text{Frequency} = \frac{\text{Number of times a fungus was encountered}}{\text{Total fungal isolations}} \times 100$$

## 2.4 Pathogenicity test

To ascertain the pathogenicity of the various fungi that were isolated, the approach of Balogun *et al.*, (2005) was employed. Apparently healthy matured pepper fruits, that is; Bell pepper '*Tattase*', Hot pepper '*Atarubu*' and Chilli pepper '*dogo-na-mara*' were surfaced - sterilized with 0.5% sodium hypochlorite for 30 seconds and then rinsed in three changes of sterile distilled water. With a 5mm diameter flame-sterilized cork borer, cylindrical cores were removed from each fruit which were then inoculated aseptically with 5mm diameter disc from the advancing edge of 7-day- old fungal culture of any one isolate. Vaseline jelly was smeared to completely seal the surface of each of the inoculated pepper fruit to prevent external infection before incubating for 10 days in three replicates. The controls were inoculated with disc of solidified potato dextrose agar medium. Fruits were inoculated in three replicates. Rot symptoms developed with different fungal isolates were compared to the natural original rot. The pathogens were re-isolated and identified using the same procedures described earlier.

## 2.5 Estimation of rot severity

Observation for level of fungal growth and fruit rot was made daily for 10 days and results were recorded, percentage rot severity was also determined adopting the method of (Balogun *et al.* 2005). In this study a fungus was considered pathogenic on the fruit if new mycelia emerged and extended radially and upwards from the originally inoculated disc and became visible outside the original wound hole on the fruit surface thereby causing fruit rot. On this basis, growth and pathogenicity were rated as follows; Low (rot covered less than 25% of the fruit surface); Medium (covered 25- 50% of the fruit surface); High (51- 75% covered) and Very high (covered 75% and above). Percentage rot severity was determined using the formula below;

$$\text{Percentage rot severity} = \frac{\text{Diameter of rot covered}}{\text{Diameter of fruit surface}} \times 100$$

## 2.6 Preparation of plant extracts

Thirty grams of the dried powdered plant were soaked separately in 150ml of ethanol. These mixtures were refluxed followed by agitation at 200 rpm (revolution per minute) for 1 hour. The ethanolic extracts were squeezed and then filtered by muslin cloth. The extracts were placed into a wide tray to evaporate ethanol and water added to make plant extracts (Ijato, 2011).

## 2.7 Evaluation of plant extracts against fungal growth

The approach of Amadioha and Obi (1999) and Ijato (2011) were used to evaluate the effect of the extract on fungal growth. This was done by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The centre of the plates indicated the point of intersection of the inoculum. This was done before dispensing the PDA into the plates. The extracts were poured into the flask plugged with cotton wool and heated for about 10 minutes to avoid contamination. Two (2ml) of the extract of various dilution percentages were separately introduced into the Petri-dish containing equal amount (10mls) of the PDA media (poisoned food method) Das *et al.* (2010); Nene and Thalpiyal (2000). Each plate was inoculated with 5mm plug of pure isolate taken from margins of actively growing culture of pathogen. The plates were incubated at  $25^{\circ} \pm 2^{\circ}\text{C}$ . The control plates were only added with equal quantity of ethanol and distilled water. Mycelial growth diameter of each isolates was measured and recorded and compared with the growth in

the control treatment. Each treatment was repeated three times. The set up was a completely randomized design. Mean radial mycelial growth of each isolate was recorded and data were transformed into inhibition percentage by using the following formula (Naz *et al.*, 2006).

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

Where DC - Average Diameter of fungal spore in control  
DT - Average diameter of fungal spore with treatment.

### 2.8 Statistical Analysis

Data was analyzed using the SAS computer program. Differences between means of inhibition of radial mycelial growth were determined using analysis of variance (ANOVA) and means that were significant were separated using Fisher's protected LSD test ( $P < 0.05$ ).

## 3. Results

### 3.1 Frequency of fungal isolates

Five fungal pathogens (*Aspergillus flavus*, *A. niger*, *Botrytis cinerea*, *Colletotrichum capsici*, and *Phytophthora capsici*) were found to be postharvest rot pathogens of pepper fruits sold in major markets in Yola, Adamawa State, North Eastern Nigeria. *Aspergillus niger* had the highest frequency of occurrence of fungal pathogen in all the pepper types with 34.70% frequency followed by *Aspergillus flavus* with 21.30% and *Phytophthora capsici* had the lowest frequency of 10.70% isolates (Table 1).

Table 1: Percentage Frequency of Occurrence of Fungi from Rotted Pepper Fruits

Fungi isolated	Percentage frequency according to markets					Total (%)
	LGM	JMM	JM	PM	JSC	
<i>A. niger</i>	30.0	43.3	36.7	30.0	33.3	34.7
<i>A. flavus</i>	33.3	23.3	6.7	23.3	20.0	21.3
<i>C. capsici</i>	10.0	6.7	13.3	6.7	33.3	14.0
<i>P. capsici</i>	6.7	10.0	13.3	16.7	6.7	10.7
<i>B. cinerea.</i>	20.0	16.7	20.0	23.3	6.7	19.3
Total	100	100	100	100	100	100

#### KEY:

JMM - Jimeta Modern Market  
JM- Jambutu Market  
PM- Pallujah Market  
JSC- Jimeta Shopping Complex  
LGM- Lake Gariyo Market

### 3.2 Pathogenicity of fungal isolates from pepper fruits

All the fungal isolates were pathogenic and exhibited significantly different degrees of pathogenicity ( $P > 0.05$ ) shown in Table 2. There was no significant difference in susceptibility among the pepper test varieties. Among the five isolates, *Phytophthora capsici* exhibited the least level of virulence. The pathogenic effect was rated as low. Mycelial growth and rots were observed only around the slit on the fruit on the 5<sup>th</sup> day of inoculation. This low level was maintained for the 10 days of inoculation. The effect was the same on all the three pepper types. On the other hand, the pathogenicity of *Aspergillus flavus* was rated as high level (i.e. mycelia and/or rods covering between 50% and 74% of the fruit surface). As with *Phytophthora capsici*, there seemed to be no considerable difference in the response of the three types of pepper fruits as they were all susceptible, manifesting fruit rots to about the same level. The virulence of *Aspergillus niger* on all types of pepper fruits was much higher than that observed for *Aspergillus flavus* (i.e. mycelial growth and/or rots covered at least 75% of the fruit surface at the 9<sup>th</sup> day of inoculation. Lastly, *Botrytis cinerea* and *Colletotrichum capsici* were rated as having only a medium pathogenic effect on the fruits. Growth and rots covered less than 50% of the fruit surface. Growth was first noticed on the 5<sup>th</sup> day after inoculation on the fruits of both pepper type and increase only minimally thereafter for the duration of the experiment.

Table 2: Pathogenicity of fungal isolates on the fruits of *C. annuum* (bell pepper type)

Fungi Isolated	No. of Fruit	Days after inoculation									
		1	2	3	4	5	6	7	8	9	10
<i>P. capsici</i>	1	–	–	–	–	+	+	+	+	+	+
	2	–	–	–	–	+	+	+	+	+	+
	3	–	–	–	–	+	+	+	+	+	+
<i>A. flavus</i>	1	–	–	–	+	++	+++	+++	+++	+++	+++
	2	–	–	–	+	++	+++	+++	+++	+++	+++
	3	–	–	–	+	++	+++	+++	+++	+++	+++
<i>A. niger</i>	1	–	–	–	+	++	+++	+++	+++	++++	++++
	2	–	–	–	+	++	+++	+++	+++	++++	++++
	3	–	–	–	+	++	+++	+++	+++	++++	++++
<i>C. capsici</i>	1	–	–	–	–	+	++	++	++	++	++
	2	–	–	–	–	+	++	++	++	++	++
	3	–	–	–	–	+	++	++	++	++	++
<i>B. cinerea</i>	1	–	–	–	+	+	+	++	++	++	++
	2	–	–	–	+	+	+	++	++	++	++
	3	–	–	–	+	+	+	++	++	++	++

**Key**

- No visible growth/fruit rot  
 + Low growth/fruit rot (less than 25% of fruit surface covered)  
 ++ Medium growth/fruit rot (26-50% of the surface covered)  
 +++ High growth/fruit rot (51-74% of the surface covered)  
 ++++ Very high growth/fruit rot (75% and above of fruit surface covered)

**3.3 Effect of ethanolic extract of *Azadirachta indica* on the mycelial growth of fungi isolates**

All concentrations of ethanolic *Azadirachta indica* leaf extracts suppressed the mycelial growth of the five tested pathogens. Statistically, there was significant difference in inhibition of radial mycelial growth in all the fungal isolates at all concentrations ( $P=0.05$ ) except in *P. capsici* where there was no significant difference in inhibition of radial mycelial growth observed between the concentrations of 20% and 40%. The effect was proportional to concentration and inhibition value at 80% concentration (highest concentration), and was higher for *Aspergillus niger* (86.87%), followed by *Aspergillus flavus* (85.27%) while the lowest inhibition value was observed for *Botrytis cinerea* (77.51%). There was no inhibition in radial mycelial growth exhibited in the control in all cases (Table 3).

Table 3: Percentage inhibition of mycelia growth of fungi isolates incorporated with ethanolic plant extracts of *Azadirachta indica*.

Concentration	Fungi isolates				
	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>	<i>C. capsici</i>	<i>B. cinerea</i>
20%	68.83	45.78	47.57	27.10	37.92
40%	80.71	53.17	48.20	46.54	53.26
60%	84.56	64.22	61.00	57.65	63.91
80%	86.87	85.27	81.72	80.56	77.51
Control	0.00	0.00	0.00	0.00	0.00
Mean	64.19	49.69	47.70	42.37	46.52
LSD( $P<0.05$ )	1.44	3.85	4.04	6.64	4.33

**3.4 Effect of ethanolic extract of *Tridax procumbens* on the mycelial growth of fungi isolates**

All concentrations of ethanolic *Tridax procumbens* leaf extracts suppressed the mycelial growth of the five tested pathogens. Statistically, there was significant difference in inhibition of radial mycelial growth for all the fungi isolates at all tested concentrations ( $P=0.05$ ). The effect was proportional to concentration and inhibition value at 80% concentration (highest concentration), was higher for *Aspergillus niger* (88.03%), followed by *Phytophthora capsici* (80.53%) while the lowest inhibition value was observed for *Botrytis cinerea* (69.57%). There was no inhibition in radial mycelial growth exhibited in the control (Table 4).

Table 4: Percentage inhibition of mycelia growth of fungi isolates incorporated with Ethanolic plant extracts of *Tridax procumbens*.

Concentration	Fungi isolates				
	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>	<i>C. capsici</i>	<i>B. cinerea</i>
20%	70.35	33.93	46.98	35.81	42.86
40%	81.87	46.01	57.37	49.33	50.32
60%	84.20	56.26	67.07	62.16	58.39
80%	88.03	73.74	80.53	80.40	69.57
Control	0.00	0.00	0.00	0.00	0.00
Mean	64.89	41.99	50.40	45.54	44.23
LSD( $P<0.05$ )	0.73	3.22	4.23	5.85	2.56

### 3.5 Effect of ethanolic extract of *Vernonia amygdalina* on the mycelial growth of fungi isolates.

All concentrations of ethanolic *Vernonia amygdalina* leaf extracts suppressed the mycelial growth of the five tested pathogens. Statistically, there was significant difference in inhibition of radial mycelial growth in all the fungi isolates at all concentrations ( $P=0.05$ ) except in *A. niger*, where no significant difference in inhibition of radial mycelial growth was observed between 40% and 60% concentration and between 60% and 80% concentration of the extract. The effect was proportional to concentration and inhibition value at 80% concentration (highest concentration), was higher for *Aspergillus niger* (87.21%), followed by *Aspergillus flavus* (83.95%) while the lowest inhibition value was observed for *Colletotrichum capsici* (64.10%). There no inhibition in radial mycelial growth exhibited in the control in all cases (Table 5).

Table 5: Percentage inhibition of mycelia growth of fungi isolates incorporated with ethanolic plant extracts of *Vernonia amygdalina*.

Concentration	Fungi isolates				
	<i>A. niger</i>	<i>A. flavus</i>	<i>P. capsici</i>	<i>C. capsici</i>	<i>B. cinerea</i>
20%	69.80	30.57	47.23	24.36	39.50
40%	80.00	57.52	56.44	32.69	50.62
60%	82.56	69.43	61.96	60.90	62.37
80%	87.21	83.95	71.16	64.10	68.52
Control	0.00	0.00	0.00	0.00	0.00
Mean	63.91	48.29	47.36	36.41	44.20
LSD( $P<0.05$ )	4.86	3.75	4.05	2.24	5.10

## Discussion

The findings of this study showed that *Aspergillus flavus*, *A. niger*, *Botrytis cinerea*, *Colletotrichum capsici*, and *Phytophthora capsici* were postharvest rot pathogens of pepper fruits sold in major markets in Yola, Adamawa State, North Eastern Nigeria. They have been previously reported as fruit rot pathogens of pepper fruits (Grubben and Tahir 2004; Nduagu *et al.*, 2008; Ekefan *et al.*, 2009 Balogun *et al.*, 2005). *Aspergillus niger* had the highest percentage frequency of occurrence in all the pepper types with 34.70% percentage frequency followed by *Aspergillus flavus* with 21.30% and *Phytophthora capsici* with lowest frequency of 10.70%. This agreed with the report of Akintobi (2011) who also found *A. niger* and *A. flavus* higher in the spoilage of pawpaw and tomato from three selected markets in Ibadan, Oyo State, South Western Nigeria.

The results from the pathogenicity tests of *Aspergillus niger*, *Aspergillus flavus*, *Botrytis cinerea*, *Colletotrichum capsici* and *Phytophthora capsici* on fresh partially ripe fruits of both bell pepper, hot pepper and chilli pepper types have established that the fungal isolates are pathogenic on the pepper types used in this study, with varying degrees of pathogenicity. They were able not only to grow on the fruits but also were able to induce some level of fruit rot indicating their virulence. Among the five isolates, *Aspergillus niger* exhibited the highest level of virulence (i.e. mycelia and/or rots covering more than 75% of the fruit surface), the pathogenicity of *Aspergillus flavus* was rated as high level (i.e. mycelia and/or rots covering between 50% and 74% of the fruit surface), *Botrytis cinerea* and *Colletotrichum capsici* were rated as medium levels having only a pathogenic effect on the fruits. Growth and rots covered less than 50% of the fruit surface thus *Phytophthora capsici* was rated as of the least virulence (i.e. mycelia and/or rots covering less than 25% of the fruit surface). Balogun *et al.* (2005) also reported the same result with *Aspergillus niger* with highest level of virulence in Ilorin, North central Nigeria. The effect was the same on all the three pepper types indicating that the test varieties were equally susceptible to the pathogens. *Botrytis cinerea* was found to be common but less severe on many plants such as bell pepper and hot pepper (Wilson *et al.*, 1997), so also *Colletotrichum capsici* on yam (Okigbo, 2009). The differences in the pathogenicity of the fungi isolates from the pepper fruits might be due to the fungi ability to overcome the natural defense mechanism of the pepper fruits or their ability to induce resistance in the fruits when infected. Jarret *et al.* (2007) found that pepper contain secondary metabolite capsaicin, the antimicrobial property of this capsaicin differs between individual organisms. Freeman and Beattie (2008) reported this compound to be important as it induced chemical defense mechanism against a broad range of fungal pathogens. Physiologically, spoilage fungi are considered toxigenic or pathogenic (Al-Hindi *et al.*, 2011). Toxigenic fungi have been isolated from spoilt fruits (Al-Hindi *et al.*, 2011). During refrigeration, some moulds may produce mycotoxins (Tournas and Stack, 2001). The fungi isolated in this study have been reported to produce secondary metabolites in plants tissues. These secondary metabolites are potentially harmful to humans and animals (Baiyewu *et al.*, 2007). A good example is Aflatoxin which has been associated in cancer of the liver (hepatoma), aflatoxicosis and also with acute hepatitis in humans, especially in the developing world (Muhammad *et al.*, 2004; Baiyewu *et al.*, 2007). Pathogenic fungi, on the other hand, could cause infections or allergies (Monso, 2004). Thus extra care should be taken during handling of these fruits, such as harvesting, cleaning, sorting, marketing, transport and storage (Al-Hindi *et al.*, 2011).

Ethanol extracts of the leaves of *Azadirachta indica*, *Tridax procumbens* and *Vernonia amygdalina* tested for antifungal activity against the five fungi isolated from pepper fruits (*Aspergillus niger*, *Aspergillus flavus*, *Botrytis cinerea*, *Colletotrichum capsici* and *Phytophthora capsici*) showed antifungal activity against all organisms tested. These plant extracts were previously reported to have antifungal activity against many fungal isolates. Wafaa *et al.*, (2007) and Winee *et al.*, (2013) separately used same concentrations of *Azadirachta indica* extract against *A. niger* in Egypt and India respectively. Nduagu *et al.* (2008) controlled *Colletotrichum capsici* using leaf extract of *Azadirachta indica* and *Vernonia amygdalina* in Makurdi, Nigeria. *Azadirachta indica* was also reported to significantly reduce mycelial growth of *Phytophthora capsici* *in vitro* (Sahu *et al.*, 2012). Aditi *et al.* (2011) controlled *Aspergillus niger* and *Phytophthora capsici* using extracts of the leaves of *Azadirachta indica* in India. Varahalarao (2012) used *Tridax procumbens* against *Aspergillus niger* and *Aspergillus flavus*. At 80% ethanol extract of *Vernonia amygdalina*, *Tridax procumbens* and *Azadirachta indica* inhibited *Aspergillus niger* up to 87.21%, 88.03% and 86.87% respectively. Ijato *et al.* (2011)

reported that ethanol extracts of 30% of both *Vernonia amygdalina* and *Tridax procumbens* had high inhibitory effect of 46.00% and 68.20% respectively against *A. niger*. At 20% concentration of *Vernonia amygdalina* and *Tridax procumbens* in this study an inhibitory effect of 70.35% and 69.80% respectively was recorded on *Aspergillus niger*. These differences could be due to the dilution method or difference in the medium used as reported by Ijato *et al.* (2011) and Sahu *et al.* (2012) as opposed to 30g of extract per 50mls of water in this study.

## Conclusion

It can be concluded that ethanolic leaf extracts of the test plants are effective against all the fungal isolates from these pepper fruits. Higher inhibition of fungal growth was observed at higher concentrations of the ethanolic extracts. The results also indicate the potential of plant extracts to control fungal pathogens. It is clear from the result that all the test plant extracts significantly reduced the radial growth of isolated fungi. These findings suggest an alternative control method to chemical control, because it has better results as they are biologically based and environmentally safe.

## References

- Aditi, G., Bhandari, B.S. and Nishant R. (2011). Antimicrobial Activity of medicinal plants *Azadirachta indica* A. Juss, *Allium cepa* L. and *Aloe vera* L. *International Journal of PharmTech Research*. **3** (2): 1059-1065 CODEN (USA): IJPRIF ISSN : 0974-4304.
- Ado, S. G (1988). Evaluation of capsicum in Nigeria. *PGRC/ E Newsletter*. **17**: 16-17.
- Amadioha, A. C. and Obi, V.I. (1999). Control of anthracnose disease of cowpea *Cymbopogon citratus* and *Ocimum gratissimum*. *Actaphytopathol. Entomol. Hungarica* **34** (92):85-89.
- Akintobi A.O, Okonko I.O, Agunbiade S.O, Akano O.R, Onianwa O. (2011) Isolation and identification of fungi associated with the spoilage of some selected fruits in Ibadan, South Western Nigeria. *Academia Arena*, 3(11):1-10] (ISSN 1553-992X). <http://www.sciencepub.net>
- Al-Hindi R.R, Al-Najada A.R, Mohamed S.A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research* **5**(4): 443-448.
- Baiyewu R.A, Amusa N.A, Ayoola O.A, Babalola O.O. (2007). Survey of the post harvest diseases and aflatoxin contamination of marketed pawpaw fruit (*Carica papaya* L) in South Western Nigeria. *African Journal of Agricultural Research*, **2**(4): 178-181.
- Balogun, O.S., Odeyemi, G.A. and Fawole O.B. (2005). Evaluation of the pathogenic effect of some isolates on fruits and seedlings of pepper (*Capsicum* spp). *Agric. Res & Dev*. **4** (2): 159-169
- Bosland, P.W., Lindsey, D.L. (1994). A seedling screen for phytophthora root rots of pepper, *Capsicum annum*. *Plant Disease*. **75**:1048.
- Das, K., Tiwari, R. K. S. and Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research* **4**(2): 104-111 Available online at <http://www.academicjournals.org/JMPR> DOI: 10.5897/JMPR09.030 ISSN 1996-0875.
- Ekefan, E.J., Jama A., and Gowen S. R. (1997). Potential of *Trichoderma harzianum* isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (*Capsicum* spp.). *Nigeria. Journal of Applied Biosciences* **20**: 1138 – 1145 ISSN 1997–5902
- Elad, Y., Williamson, B., Tudzynski, P., Delen N. (2004). *Botrytis*: Biology, Pathology and Control, Springer, Netherlands.
- Fabry, W.P., Okemo,.....and Ansorg, R. (1996). Fungistatic and fungicidal activity of East African medicinal plants. *Mycoses* **36**:67-70
- Erinle, I.D. (1989). Present status and prospects for increased production of tomato and pepper in Nigeria. In AVEDC Ed. Proc. Inter. I Symp. Integrated Management Practices 536-547.
- FAO. (2003). FAOST <http://apps.fao.org/page/collections?subset=agriculture>. Accessed 2013.
- Fawole, M.O and Oso, B.A. Laboratory Manual of Microbiology, Spectrum Books Ltd, Ibadan. 1988; pp. 14-18.
- Freeman, B.C. and G.A. Beattie. 2008. An Overview of Plant Defenses against Pathogens and insects. *Phytopathology*. **32**, 476
- Grubben, G.J.H. and Tahir, I.M., (2004). *Capsicum species*, In: Grubben, G.J.H. and Denton, O.A. (Editors). *Plant Resources of Tropical Africa 2. Vegetables* PROTA Foundation, Wageningen, Netherlands/Backhugs Publishers, Leiden, Netherlands/ICTA, Wageningen, Netherland, Pp 154–163.
- Ijato, J.Y., Otoide, J.E., Ijadunola, J.A. and Aladejimokun, A.O. (2011). Efficacy of antimicrobial effect of *Vernonia amygdalina* and *Tridax procumbens* in *in vitro* control of tomato (*Lycopersicon esculentum*) post harvest fruit rot. *Report and Opinion* **3**(1):120-123]. (ISSN: 1553-9873). <http://www.sciencepub.net>.
- Isaac, S. (1992). *Fungal Plant Interaction*. London: Chapman and Hall Press; p. 115.
- Jarret, R.L., Baldwin, E., Perkins, B., Bushway, R., Guthrie, K. (2007). Diversity of fruit quality characteristics in *Capsicum frutescens*. *HortScience* **42**: 16–19.
- Jeffries, P., Dodd, J.C., Jegerand, M.J., Plumbley, R.A. (1990). The biology and control of *Colletotrichum* species on tropical fruit crops. *Plant Pathology*. **39**(3):343–366.
- Khafagi I.K. and Dewedar. A. ( 2000). The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. *Journal of Ethnopharmacology* **71**: 365 – 376.
- Kiran, K.S., Linguratu and Adiver, S. (2006). Effect of plant extract on *Sclerotium rolfsii*, the incitant of stem rot of groundnut. *Journal of Mycology and Plant Pathology*, **36**:77-79.
- Kuruचेve V, Gerard, E.J. and Jayaraj, J. (1997). Screening of higher plants for fungitoxicity against *Rhizoctonia solani* in vitro. *Indian Phytopathology* **50** (2): 235-241.
- Melanie, L.L.I. and Sally A.M. (2004). Anthracnose Fruit Rot of Pepper [Internet]. Extension Fact sheet, Ohio State University [Online]. Available from: <http://ohioline.osu.edu> (Accessed: 2013).
- Monso E.M. (2004). Occupational asthma in greenhouse workers, *Curr. Opin. Pulm. Med.*, **10**: 147-150.
- Navarro, J.M., Flores, P., Garrido, C. and Martinez V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry* **96**:66–73.

- Naz, F., Rauf, C.A., Haque, I.U. and Ahmad, I. (2006). Management of *Rhizoctonia solani* with plant diffusates and chemicals. *Pakistan Journal of Phytopathology*, **18**(1):36-4.
- Nduagu, C., Ekefan E.J. and Nwankiti, A.O, (2008). Effect of some crude plant extracts on growth of *Colletotrichum capsici* (Synd) & Bisby, causal agent of pepper anthracnose. *Journal of Applied Biosciences* **6**(2): 184 –190.
- Nene, Y. and Thapilyal, L. (2000). *Poisoned food technique of fungicides in plant disease control*. 3rd Ed. Oxford and TBH Publishing Company, New Delhi.
- Nsabiyeera, V., Ochwo-ssemakula, M. and Sseruwagi, P. (2012). Hot pepper reaction to field diseases. *African Crop Science Journal* **20** (1):77 - 97 ISSN 1021-9730/2012
- Okigbo, R.N. (2009). Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi State, Nigeria. *American-Eurasian Journal of Sustainable Agriculture*, **3** (3):407-409.
- Oluwalana SA, Adetoro N.A, Adekunle M.F. and Momoh, S. (1999). The use of biopesticides in indigenous cropping systems in Ogun State Nigeria. *Bioprospector* **1**(2): 1-10.
- Ristaino, J.B., Johnson, S.A. (1999). Ecological based approaches to management of *Phytophthora* blight on bell pepper. *Plant Disease*, **83**:1080–1089.
- Sahu R. K. Mitali Madhusmita Pattnaik, Manoranjan Kar (2012) Bioefficacy of some plant extracts on growth parameters and control of diseases in *Lycopersicum esculentum*. *Asian Journal of Plant Science and Research*, **2** (2):129-142 ISSN : 2249-7412 Available online at [www.pelagiaresearchlibrary.com](http://www.pelagiaresearchlibrary.com)
- Sally, A.M., Randall, C.R. and Richard M.R. (1996). *Fusarium and Verticillium Wilts of Tomato, Potato, Pepper, and Eggplant*. Extension Factsheet. *Plant Pathology*, 2021 Coffey Road, Columbus, OH pp. **43**:210-1087.
- Snowdon, A.C. (1990). *A colour Atlas of post-harvest Diseases and Disorders of fruits and vegetables*. Cambridge University press. Pp. 128-139.
- Tournas VH, Stack ME. Production of alternariol and alternariol methyl ether by *Alternaria alternata* grown on fruits at various temperatures. *J. Food Prot.*, 2001; **64**: 528-532.
- Vagelas, A. Papachatzis, H. Kalorizou and E. Wogiatzi (2009). Biological control of *Botrytis* fruit rot (gray mold) on strawberry and red pepper fruits by olive oil mill wastewater. *Biotechnol. & Biotechnol.*1489-1491
- Varahalarao, V. (2012). Absence of anti fungicidal activity of few Indian medicinal plants methanolic extracts. *Asian Pacific Journal of Tropical Disease* S393-S395.
- Wafaa, A. H., Hassan, A. and Nefisa M.A. E. (2007). Biological and anti microbial activities of aqueous extracts from neem tree (*Azadirachta indica* A. Juss., Meliaceae). *Journal of Applied Sciences Research*, **3**(10): 1050-1055.
- Wilson, C.L., Solar, J.M., El Ghauth, A. and Wisniewski, M.E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against botrytis cinerea. *Plant Disease journal* **81**: 204 -210.
- Winee, S. L., Amit, A. C. and Akhilesh, B. (2011) Antimicrobial activity of methanolic and acetonetic extracts of *Azadirachta indica*, *Saraca asoca* and *Curcuma longa* *International Journal of PharmTech Research*. **3** (.2): 0974-4304