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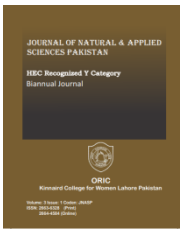

INCIDENCE AND PLANT EXTRACT MANAGEMENT OF FUNGAL PATHOGENS ASSOCIATED WITH SWEET PEPPER (*CAPSICUM ANNUUM L.)* FRUIT DISEASES IN SOKOTO STATE NIGERIA.

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Incidence, Sweet Pepper Fruit, Fungal Pathogens, Plant Extracts, Sokoto and Nigeria

Abstract

Disease infection is one of the major constraints to sweet pepper production in sub-Saharan Africa (Nsabiyera *et al.*, 2012). The major organisms causing diseases of most peppers in Sub- Sahara Africa are phyto-pathogenic fungi, bacteria, and viruses (Melanie *et al*., 2004). Attacks by fungal, bacterial or viral diseases, cause significant losses in pepper production (Ochoa-Alejo and Ramirez-Malagon, 2001). Three farms was selected in each of the four Agricultural zones of the State. A total of 14,868 Sweet Pepper fruits were studied, out of which 3504 (24%) were obseserved to be affected (damaged fruits with indication of fungal infection). The findings from this study revealed that fungal disease incidence is present in the study area with varying degree of percentage. Goronyo Local Government Area having the highest percentage disease incidence of 26%. The effects of three extracts (ethanol, N-hexane and water) of *A. nilotica* and *V. amygdalina* were evaluated *in-vitro* against the isolated fungi. The ethanolic extracts of *A. nilotica* and *V. amygdalina* show statistically significant ($P<0.05$) inhibitory activity against fungi isolated. The findings of this study recommended field trials and more in-depth investigations of plant extracts, along with development products with higher technological and health security for both famers and consumers.

1. Introduction

The reduction in disease incidence and severity of fungal growth and mycotoxin production by natural plant products has been reported. Cinnamon, clove, oregano, palmarosa and lemongrass oils (Marin *et al*., 2004), tea tree oil (Burgiel and Smaglowski, 2008), common thyme, cinnamon leaf and aniseed oils (Cosic *et al*., 2010), sweet basil, neem, eucalyptus, datura, garlic and oleander extracts (Nashwa & Abo-Elyousr, 2012). Thymol and carvacrol are definitely the most effective active constituents against most fungal species tested (Numpaque *et al*., 2011; Shin *et al*., 2014; Villanueva Bermejo *et al*., 2015; Gavaric *et al*., 2015). The mechanism of action of these compounds against fungi is not completely understood but it is supposed to be in relation to their general ability to dissolve or otherwise disrupt the integrity of fungal cell walls and cell membranes (Isman & Machial, 2006). Species of some plant families such as Solanaceae are known for their high alkaloid contents, Mimosaceae for their high tannins contents and Lamiaceae and Meliaceae for their wide diversity of terpenoids may be more feasible for investigations regarding their biofungicidal compounds. The concentration of a chemical in different parts of a plant such as roots, leaves, flowers and fruits may differ. It may even be absent in one or more parts, therefore it is convenient to collect integral samples (Montes-Belmont and Carvajal, 2008). Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity as a result of fungicide residues causes environmental pollution leading to health hazards of both human and animals (Ramamoorthy *et al*., 2002). Additionally, that *Pythium* spp. Develops resistance to fungicide and this further discourages the use of chemicals for the control of plant diseases (Punja and Yip, 2003). Also, global interest has been shifted towards the use of eco-friendly methods for protecting crops against pests and diseases (Paranthaman *et al*., 2009). Following the decline in yield and the high rate of post-harvest losses (Udegbe *et al*., 2012), which could be due to lack of adequate information on specific diseases affecting the plants by the farmers, and very limited information is available for growing sweet pepper through protected technology or green house and on the open field in Nigeria; hence, this study become imperative in order to identify the diseases incidence and ways of management of sweet pepper and information obtained would be made available on the farmers in order to improve production systems.

This study aimed at investigating the incidence and use of the plant extract in management of fungal diseases of sweet pepper (Capsicum annuum L.) fruit in Sokoto state, Nigeria.

2. Materials and methods

2.1 Study Area

Sokoto State is located in the Sudan Savanna ecological belt of Nigeria with longitude 11^{O}3’ to 13^{O} 50 E and Latitude 4^{O}to 6^{O}40’ N (Tsoho *et. al*., 2012). The wet season lasts from June to September (Ologe, 2002). Annual rainfall ranges between 300mm and 800mm while mean annual temperature is 34^{O}C with dry seasons temperatures often exceeding 40^{O}C. Agriculture is the mainstay of the people. The major upland crops include Millet, Sorghum, and Groundnut. Major dry season vegetable crops which are mainly grown under irrigation include Onion, Tomato, Sweet and Hot

pepper (Tsoho, 2004); others include Carrot, Rice, Wheat and garden egg. There are 23 local government councils in the state with Sokoto as the capital. The state is divided into four Agricultural zones (Figure 1), Isa, Tambuwal, Gwadabawa and Sokoto central zones.

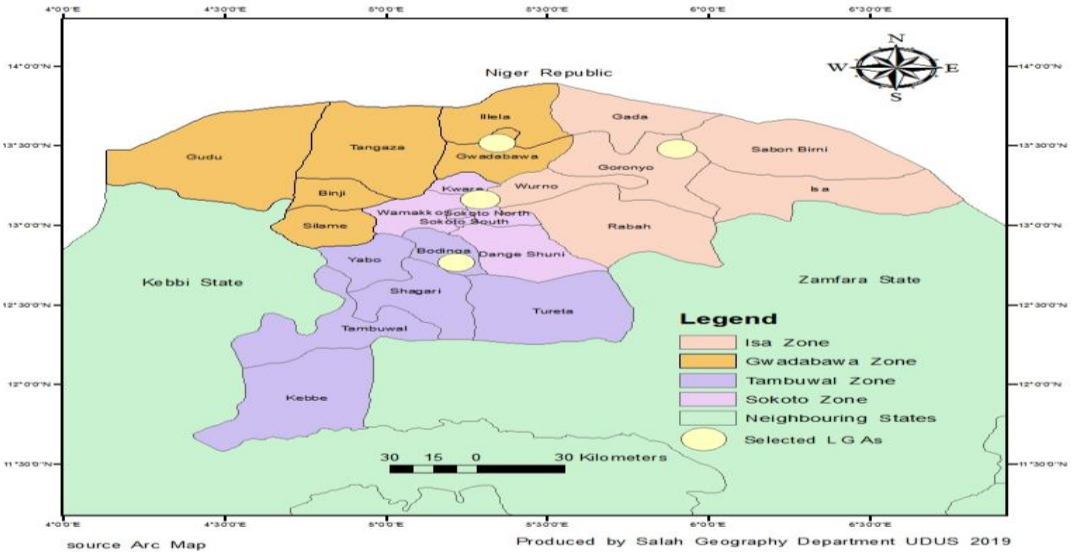

Figure 1: Map of Sokoto State Showing the Four Agriculture Zones and Purposive Selected Local Government Areas

2.2 Determination of Disease Incidence

The incidence of fungal diseases of Sweet Pepper fruit (*C. annuum*), was investigated in the four Agricultural Zones which was purposely selected (purposive sampling) to represent the zone, and three farms were selected from each Local Government to conduct the research. A total of 14,868 Sweet Pepper fruit were studied, out of which 3504 (24%) were obseserved to be affected (damaged fruits with indication of fungal infection). A total of 120 fruits (10 from each farm) were randomly selected for fungal identification and further analysis. Disease incidence was calculated based on Wokocha, (1990) which stated that

$$\text{Disease incidence (I)} = \frac{\text{Number of plant units affected}}{\text{Total number (healthy and infected of farms assessed)}} \times 100$$

2.3 Selection of Plants for Biological Assay

The antifungal effects of two plants extract *Vernonia amygdalina* and *Acacia nilotica* at different concentrations were evaluated in vitro against the test fungus using poisoned food techniques (Nene and Thapliyal, 1979). The plants were selected based on scientific reports concerning their potential antimicrobial activity against phytopathogens (Ijato *et. al.*, 2011), (Varahalarao, 2012).

2.4 Collection of Plant Samples

The leaves of *Vernonia amygdalina* and *Acacia nilotica* were collected from Arkilla and Gidan Salanke area of Arkilla Ward of Wamakko Local Government, Sokoto State by hand picking. The plant was identified at Botany Herbarium Department of biological Science Usman Danfodiyo University Sokoto. *Vernonia*

amygdalina with a vourcher number (UDUH/ANS/0409) and *Acacia nilotica* with a vourcher number (UDUH/ANS/0252).

2.5 Extraction of Plants for the Assay

In Preparation of Ethanol, n-hexane and Aqueous Extracts, the collected plant leaves samples were rinsed in clean water and dried at room temperature 25 to 30^{O}C for two weeks. The dried leaves sample was ground into powder using a mortar and pestle. An equal measurement of 100grams of *Acacia nilotica* and *Vernonia amygdalina* dried powdered leaves was soaked separately in 1000ml of ethanol, n-hexane and distilled water. The mixture of each extracts, were allowed to stand for 24 hours.The extract were then squeezed and then filtered with muslin cloth. The extracts were then placed into hot air oven to evaporate (Ijato, 2011). After evaporation, each extract was grinded to powder using mortar and pestle and then 100mg, 300mg and 500mg of each was dissolved in 5ml of their respective solvent (ethanol, n-hexane and distilled water) in a test tube, to produce 20, 60 and 100 mg/ml solution respectively.

2.6 Determination of Antifungal Activity of the Leaves Extracts

Agar well diffusion method as described by (Ahmad *et. al.*, 2012), was adopted for the evaluation of the plant extract. Spore suspension method was carried out according to Shoulkamy *et al*. (2006), in which 10ml of sterile distilled water was inoculated into Petri plates of each of the isolated fungi using sterile syringes. Sterile syringes were also used to inoculate 10ml of suspended fungal spores on sterile PDA, homogenised and poured into a sterile Petri plate where a uniform depth of 4mm was maintained. The Petriplates were then allowed to solidify. A sterile corn borer of 7mm in diameter was used to make a well at the centre of each Petriplate. A 100µl of each concentration (20mg/ml, 60mg/ml, and 100mg/ml) of leaves extract was loaded into wells for each of the isolated fungi. Sterile distilled water was loaded in the well of separate Petri plate as negative control while 100µl of standard antifungal Ketoconazol (100mg/ml) was loaded into the well of separate Petri plate as positive control. The plates were then incubated at 32°C for 24 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimetres (mm) with the help of transparent meter rule. Each treatment was repeated three times. Average zones of inhibition was calculated and presented in standard deviation.

3. Results

3.1 Incidence of Sweet Pepper Fruit Fungal Diseases

The results showed that, for the three farms visited in Goronyo Local Government (Isa zone, Sokoto East), farm 1, 2 and 3 had total percentage fungal disease incidence of 20%, 20% and 33% respectively as shown in Table 1, Gwadabawa Local Government (Gwadabawa Sokoto West zone), farm 1, 2 and 3 had total percentage fungal disease incidence of 25%, 25% and 25% respectively, as shown in Table 2, Bodinga Local Government (Tambuwal Sokoto South zone), had 16%, 33% and 17% as shown in Table 3, while Kware Local Government (Sokoto Central Zone), had in farm 1, 2 and 3 20%, 17% and 25% as shown in Table 4. The

percentage fungal disease incidence in the four zones was also calculated. Goronyo Local Government (Isa zone, Sokoto East) has 26%, followed by Gwadabawa Local Government (Gwadabawa Sokoto West Zone) 25%, Bodinga Local Government (Tambuwal Sokoto South zone) 24%, and the least was Kware Local Government (Sokoto Central Zone) 20%. This brought the total estimated fungal disease incidence of Sokoto State to stand at 24%, as shown in Table 5. The findings from this study on the incidence of fungal diseases of Sweet Pepper in Sokoto state, Nigeria shows that Goronyo Local Government Area had the highest percentage disease incidence of 26%, followed by Gwadabawa Local Government Area 25%, Bodinga Local Government Area 24% and the least was Kware Local Government Area with 20% respectively.

Table 1: Estimated Fungal Disease Incidence of Sweet Pepper Cultivated in Goronyo Local Government (Sokoto East) Agricultural Zones in Sokoto State, Nigeria, October 2019.

Sampling units	Estimated total number of fruits (A)	Estimated number of affected fruits (B)	% disease incidence B/A×100
farm 1	900	180	20%
farm 2	960	192	20%
farm 3	1344	448	33%
Total	3204	820	26%

Table 2: Estimated Fungal Disease Incidence of Sweet Pepper Cultivated in Gwadabawa Local Government (Sokoto West) Agricultural Zones in Sokoto State, Nigeria, October 2019

Sampling units	Estimated total number of fruits (A)	Estimated number of affected fruits (B)	% disease incidence B/A×100
farm 1	1120	280	25%
farm 2	1296	324	25%
farm 3	1456	364	25%
Total	3872	968	25%

Table 3: Estimated Fungal Disease Incidence of Sweet Pepper Cultivated in Bodinga Local Government (Sokoto South) Agricultural Zones in Sokoto State, Nigeria, October 2019

Sampling units	Estimated total number of fruits (A)	Estimated number of affected fruits (B)	% disease incidence B/A×100
farm 1	1836	306	16%
farm 2	1200	400	33%
farm 3	1176	196	17%
Total	4212	994	24%

Table 4: Estimated Fungal Disease Incidence of Sweet Pepper Cultivated in kware local Government (Sokoto Central) Agricultural Zones in Sokoto State, Nigeria, October 2019

Sampling units	Estimated total number of fruits (A)	Estimated number of affected fruits (B)	% disease incidence B/A×100
farm 1	1600	320	20%
farm 2	1080	180	17%
farm 3	900	225	25%
Total	3580	725	20%

Table 5: Estimated Fungal Disease Incidence of Sweet Pepper Cultivated in Sokoto State, Nigeria, October 2019

Local Government	Estimated total number of fruits (A)	Estimated number of affected fruits (B)	% disease incidence B/A×100
Goronyo (Sokoto East)	3204	820	26%
Bodinga (Sokoto South)	4212	994	24%
Gwadabawa (Sokoto West)	3872	968	25%
Kware (Sokoto Central)	3580	725	20%
Total	14868	3507	24%

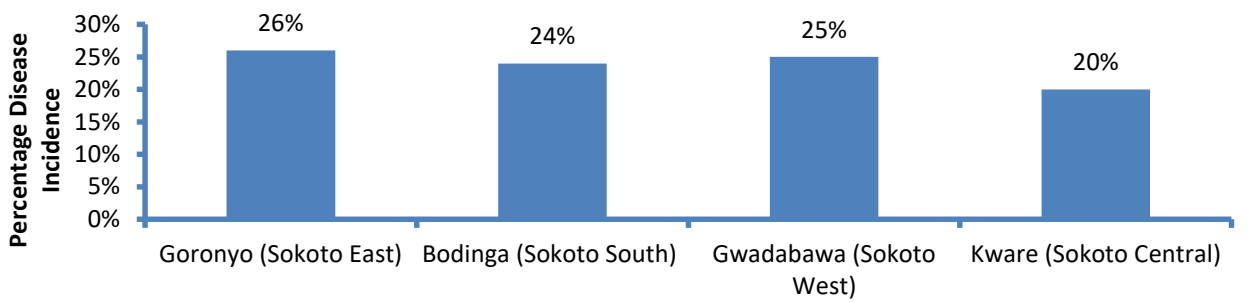

Figure 2: Sokoto State Agricultural Zones

The estimated total number of fruits for the four zones was 14868, number of affected was 3507 and percentage disease incidence was 24% with Goronyo local Government having the highest percentage of 26%.

3.2 Percentage sweet pepper fungal disease incidence in Sokoto state, Nigeria

3.2.1 Data Analysis of the Antifungal Activity of Plant Extract Against Isolated Fungi

The the result of antifungal activity of plant extract against isolated fungi indicated that there is significant effect of aqueous extrac of *A. nilotica* plant leaves in management of fungal diseases (F-test 18.200, P-value = 0.01). However, following the result of multiple comparison analyses of variance between the control group and treatment groups, there were no significant difference among the parameters (P>0.05) Table 6. The Table 7 indicate mean and standard deviation across all the parameters, SE = Standard of errors. The result indicated that there is significant effect of aqueous extraction on *V. amygdalina* plant leaves extract in management of fungal diseases considering the P-values (F-test 25.440, P = 0.01) hotelling`s trace. However following the result of multiple

comparison analyses of variance between the control group and treatment groups there were no significant difference among the parameters (P>0.05) The Table 8 indicate mean and standard deviation across all the parameters, SE = Standard of errors. The result indicated that there is significant effect of Ethanol extraction of *A. nilotica* plant leaves extract in management of fungal diseases considering the P-values (F-test 17.414, P = 0.01) hotelling`s trace. However following the result of multiple comparison analyses of variance between the control group subject and treatment groups there were significant difference among the variables carrying subscript in bold and the control group. (P<0.05) The Table 9 indicate mean and standard deviation across all the parameters, SE = Standard of errors. The result indicated that there is significant effect of Ethanol extraction of *V. amygdalina* plant leaves extract in management of fungal diseases considering the P-values (F-test 85.522, P = 0.01) hotelling`s trace. However following the result of multiple comparison analyses of variance between the control group subject and treatment groups there were significant difference among the variables carrying subscript in bold and the control group. (P<0.05) The Table 10 indicate mean and standard deviation across all the parameters, SE = Standard of errors. The result indicated that there is significant effect ofN-hexane extract on *A. nilotica* plant leaves extract in management of fungal diseases considering the P-values (F-test 137.543, P = 0.01) hotelling`s trace. However, following the result of multiple comparison analyses of variance between the control group subject and treatment groups there were significant difference among the variables carrying subscript in bold and the control group. (P<0.05) The Table 11 indicate mean and standard deviation across all the parameters, SE = Standard of errors. The result indicated that there is significant effect of aqueous extraction on *V. amygdalina* plant leaves extract in management of fungal diseases considering the P-values (F-teats 112.598, P = 0.01) hotelling`s trace. However following the result of multiple comparison analyses of variance between the control group subject and treatment groups there were no significant difference among the parameters (P>0.05).

Table 6: Effects of Aqueous Extract of *Acacia nilotica* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus Niger	*Aspergillus Fumigates*	*Fusarium oxysporum*	*Fusarium Equiseti*	*Fusarium culmorum*
100mg/ml	0.0±0.0	16.33±5.69	0.0±0.0	17.33±0.58	16.0±0.0
20mg/ml	0.0±0.0	10.67±1.16	0.0±0.0	15.33±0.58	10.67±1.16
60mg/ml	0.0±0.0	14.0±1.00	0.0±0.0	16.0±2.65	13.67±3.51
Positive Control Ketoconazole (100mg/ml)	10.0±17.32	9.33±16.17	6.67±11.55	9.67±16.74	10.33±17.90
Standard Error	2.50	12.58	1.66	2.44	2.63

P<0.05 Statistically significant difference

Table 7: Effects of Aqueous Extract of *Vernonia amygdalina* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus niger	*Aspergillus fumigates*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium culmorum*
100mg/ml	00±0.0	16.0±1.73	0.0±0.0	0.0±0.0	0.0±0.0
20mg/ml	00±0.0	14.33±2.52	0.0±0.0	0.0±0.0	0.0±0.0
60mg/ml	00±0.0	15.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0
Positive Control Ketoconazole (100mg/ml)	9.33±16.17	7.67±13.28	10.0±17.32	6.33±10.97	8.67±15.01
Standard Error	2.33	1.97	2.5	1.58	2.16

P<0.05 statistically significant difference

Table 8: Effects of Ethanol Extract of *Acacia nilotica* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus niger	*Aspergillus fumigates*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium culmorum*
100mg/ml	27.67±0.58	29.0±1.0*	29.33±1.15*	27.33±1.15*	29.0±1.0*
20mg/ml	20.33±0.58	22.33±0.58	20.33±0.58	17.67±0.58	19.0±1.0
60mg/ml	24.0±1.0	24.67±0.58	22.67±1.52	22.67±1.52	24.67±0.58
Positive Control Ketoconazole (100mg/ml)	10.67±18.47	9.67±16.74	9.33±16.16	10.33±17.89	10.6718.47
Standard Error	2.67	2.42	2.35	2.59	2.67

P<0.05 statistically significant difference

Table 9: Effects of Ethanol Extract of *Vernonia amygdalina* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus niger	*Aspergillus fumigates*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium culmorum*
100mg/ml	23.67±0.58	27.67±0.58*	28.0±1.0	29.0±1.0*	22.0±2.0
20mg/ml	17.33±0.58	18.67±0.58	15.0±0.0	13.33±1.53	12.67±0.58
60mg/ml	19.67±0.58	24.33±1.15*	20.0±1.0	23.33±0.58*	17.33±1.15
Positive Control Ketoconazole (100mg/ml)	10.0±17.32	6.67±11.54	10.67±18.47	3.0±10.39	10±17.32
Standard Error	2.5	1.67	2.67	1.52	2.52

P<0.05 statistically significant difference

Table 10: Effects of N-hexane Extract of *Acacia nilotica* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus niger	*Aspergillus fumigates*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium culmorum*
100mg/ml	0.0±0.0	17.0±1.0	17.0±1.0	0.0±0.0	20.0±1.00*
20mg/ml	0.0±0.0	10.67±0.58	0.0±0.0	0.0±0.0	14.33±1.16
60mg/ml	0.0±0.0	14.33±1.16	9.33±0.58	0.0±0.0	18.0±0.00*
Positive Control Ketoconazole (100mg/ml)	9.33±16.17	9.67±16.74	10.67±18.48	6.67±11.55	6.67±11.55
Standard Error	2.33	2.42	2.67	1.66	1.68

P<0.05 statistically significant difference

Table 11: Effects of N-hexane Extract of *Vernonia amygdalina* Plant Leaves Extract in Management of Fungal Diseases

Concentration of the plant extract (Mg/ml)	Average Zone of Inhibition (mm) ± Standard Deviation				
	Aspergillus niger	*Aspergillus fumigates*	*Fusarium oxysporum*	*Fusarium equiseti*	*Fusarium culmorum*
100mg/ml	19.0±1.0	16.67±0.58	0.0±0.0	0.0±0.0	13.33±0.58
20mg/ml	10.33±0.58	10.67±0.58	0.0±0.0	0.0±0.0	7.33±0.58
60mg/ml	16.67±0.58	14.67±0.58	0.0±0.0	0.0±0.0	10.67±0.58
Positive Control Ketoconazole (100mg/ml)	9.33±16.16	8.33±14.43	9.33±16.16	7.67±13.28	10.33±17.899
Standard Error	2.34	2.08	2.33	1.91	2.58

P<0.05 statistically significant difference

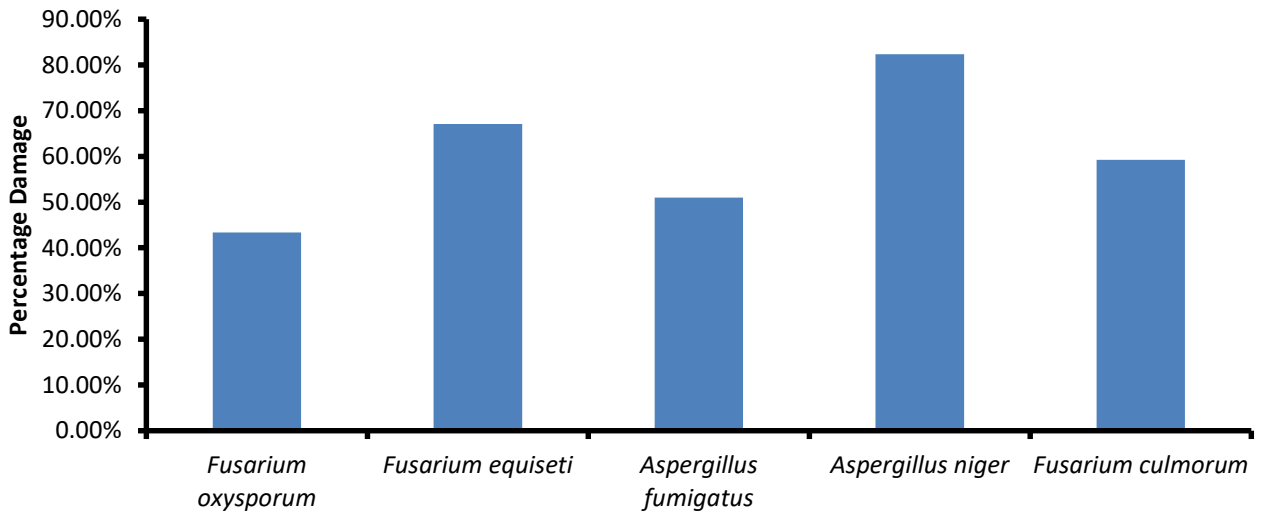

Figure 3: Fungal Species Isolate

4. Discussion

Determination of the fungal disease incidence in the study area revealed that fungal disease incidence is present in the study area with varying degree of percentage. This is lower than the percentage fungal disease incidence detected on Sweet Pepper from Ohimi and Gboko Local Government Area, in Benue State, even though the same type of media was used. Similarly, the percentage fungal disease incidence in this research is higher than the fungal disease incidence observed by El-Mougy *et al.* (2011) in cultivation station at Tookh, Kalubia Governorate, Egypt in their survey, of severe *Sclerotinia* foliage blight disease symptoms in sweet and hot peppers. The reason may be, they conducted the research in a controlled environment whereas this study was conducted in an open field where the sweet pepper fruits are prone to attack by plant's pathogens and different unfavorable environmental conditions like moisture which favor rapid development of fungal pathogens. Determination and comparison of the fungal antifungal efficacies *A. nilotica* and *V. amygdalina* revealed that, the ethanol extract of *Acacia nilotica* showed highest statistically significant inhibitory activity against sweet pepper pathogenic fungi at highest concentration i. e., 100mg/ml. In line with these findings, Nduagu *et al.,* (2008) demonstrated that crude extracts from stem bark and root bark of *Azadirachta indica*, *V. amygdalina* and *Cochlospermum planchonii* due to presence 23 of compounds like alkaloids, flavonoids, glycosides, saponins and tannins, has exhibited strong fungi toxicity against *Colletotrichum capcisi. Similarly,* Mahesh and Satish (2008) in their study used methanolic extract of *Acacia nilotica* bark and leaf and tested using disc diffusion assay. Khan and Nasreen, (2010) reported methanol extract of plants as it was also reporte for *Datura stramonium* root, stem and leaf; Choi *et al*., 2008) for *Myristica malabarica* fruit, Oh *et, al.,* 2008) for *Eucalyptus spp.*A study of Satish, (2009) and Satish, (2010) used aqueous, petroleum ether, benzene, chloroform, methanol and ethanol extracts of *Polyalthia longifolia* and *Acacia nilotica.*

5. Conclusion

Findings from the study indicated that Goronyo Local Government (Isa zone) has the highest percentage fungal disease incidence and Kware Local Government (Sokoto central) has the lowest percentage disease incidence of Sweet Pepper. Ethanol extract of both plant species used, had highest inhibitory activity against the isolated Fungus followed by n-hexane and the least was the aqueous extract. Pathogenesity tests on fresh and apparently healthy Sweet Pepper fruits, have established that the fungal isolates are pathogenic on the Sweet Pepper fruits with varying degree of Pathogenesity. The extracts showed the statistically significant highest inhibitory activity against *Aspergillus Fumigatus* and *Fusarium equiseti* followed by *Fusarium oxysporum* and *Fusarium culmorum* and the least was *Aspergillus niger.* Phytochemical, Saponins and Alkaloids may be responsible for the inhibition of the test Fungus because, they appear in high amount in both the plant extracts. From the result of this research, it can be concluded that the ethanolic extract of *V. amygdalina* and *A. nilotica* leaves extract show

statistically significant effect in the management of fungal diseases associated with sweet pepper fruit.

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