Direct Research Journal of Agriculture and Food Science
Vol. 9, Pp. 212-217, 2021
ISSN 2354-4147
DOI: https://doi.org/10.26765/DRJAFS7092572913
Article Number: DRJAFS7092572913
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Full-Length Research Paper

Assessment of potency of different plant extracts on growth of fungal rot pathogens of Ghana and Cameroon pepper (*Capsicum* species) seeds in storage

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Received 7 May 2021; Accepted 18 June 2021 Published 2 July 2021

ABSTRACT: Potency of *Cucuma longa, Moringa oleifera* and *Occimun grattisimum* were tested in-vitro to control post harvest seed rot of two types of pepper seeds (Cameroon and Ghana) from four different markets; Orie Ugba, Urbani, Ndoro and the University general markets in Umuahia, Abia State. The study was carried out in the Department of Plant Health Management, Michael Okpara University of Agriculture Umudike, Abia state, Nigeria. Rotted pepper seeds were collected from four (4) different markets in Umuahia, Abia State. The experiment was laid out in a Completely Randomized Design (CRD) with three treatments replicated three times while the analysis was carried out using SAS package (Institute Inc, 2001). Fungal pathogens isolated from the two types of pepper seeds (Cameroon and Ghana) 7 days after inoculation in a PDA media at 28±2ºC were; *Aspergillus niger, Pennicilium sp, Fusarium sp, Rhizopus stolonifer. Aspergillus niger* (80%) had the highest percentage occurrence when compared with other isolates and the pathogenicity test conducted also showed that *A. niger* incited rot more than the other fungal isolates from the four markets studied. The result further revealed that 50, 100 and 200 mls concentrations of *Occimum grattisimum* were the most effective though the other plant extracts (*C. Longa* and *M. Oleifera*) had comparative effect in reducing the radial mycelial growth of the test organisms at varied levels of concentrations. It is therefore recommended that aqueous leaf extracts of these plants which are readily available, easy to prepare and affordable could be used to control post harvest seed rot of the two types of pepper seeds.

Keywords: Pepper seeds, mycelial growth, fungal rot, plant extracts, *Capsicum* spp.

INTRODUCTION

Peppers (*Capsicum solanum)* are part of the Solanaceae family and are considered the first spice to have been used by human beings (Hill *et al.*, 2013). Nigeria is the largest producer of pepper in Africa and cultivated mainly with irrigation in the north (Olaniyi and Ojetayo, 2010; Abdulmalik *et al.*, 2012). A high value crop rich in vitamins A and C also contains appreciable quantities of proteins and minerals (Temu and Temu, 2005), and the second most important vegetable in the world after tomato, used mainly as spices in various cuisines (Olaniyi and Ojetayo, 2010). The common types of dry pepper found in Umuahia markets in Abia state, include: Cameroon and Ghana peppers e.t.c. These peppers are currently the object of much attention due to the possible links to the prevention of certain types of cardiovascular diseases, atherosclerosis, haemorrhage, delaying of the ageing process, improving physical resistance and improving appetite (Marin *et al., 2008)*. Although the use

of plant based insecticides (PBI) to control pathogens prior to and after harvest has been practiced for many centuries, it was rather limited in potential and ignored (Oparaeke *et al*., 2005). The toxicity of these plant materials on pathogens is thought to be the effects of secondary metabolites including alkaloids, saponins and flavonoid compounds of this plant (Bouchelta et al., 2005). The efficacy of extracts from many tropical plants has been studied by previous workers e.g *Cucuma longa, Ocimum grattissimum, Moringa oleifera. Piper guinense* extracts e.t.c with extracts from other plants such as cashew nutshell and garlic bulb which create a synergistic effect between pathogen and their respective toxic compounds (Oparaeke *et al.,* 2005). The objectives of this study therefore were to isolate and identify fungal rot pathogens associated with two pepper types (Cameroon and Ghana pepper) seeds; and to evaluate the potency of different aqueous plant extracts on the growth of the fungal rot isolates *in vitro.*

MATERIALS AND METHODS

The experiment was conducted at the Department of Plant Health Management, Michael Okpara University of Agriculture Umudike, and a Plant Pathology Laboratory attached to National Root Crop Research Institute (NRCRI), Umudike in Abia State, South Eastern Nigeria. The survey was carried out in four markets which were Ndoro, Ubani, Orie ugba, and the University markets all in Abia state in other to collect the samples .In each of the selected markets, two (2) shops were randomly selected and samples of dry Cameroon and Ghana seeds were collected from each shop making it a total of four (4) samples from each market .The samples (dry Cameroon and Ghana pepper seed) collected per market were separately placed in clean sample bags and properly labelled and taken to the Laboratory for analysis and identification of the fungal pathogen. The Potato Dextrose Agar (PDA) was measured using a weighing balance and diluted with 1000mls of distilled water in a conical flask and heated in an autoclave for 60 minutes at 110ºC. After autoclaving, the media was allowed to cool for 15minutes. 1g of Streptomycin in 10mls of sterile water was added to prevent bacterial growth and the medium was poured into sterilized Petri dishes and left to solidify under aseptic conditions. The isolation technique used was similar to that of Chiejila (2008). Thin sections (1mm diameter) were cut from the periphery of diseased pepper seeds and sterilized in 0.1% ethanol for 2 minutes. The diseased pepper seeds were rinsed in 3 changes of sterile distilled water and plated in Potato Dextrose Agar (PDA) plates. The (Plates 1 and 2) were incubated at room temperature (28±2ºC) for 3 days (72 hours). Pure cultures were obtained by several transfers

Plate 1: Pepper seeds (Var. Ghana pepper).

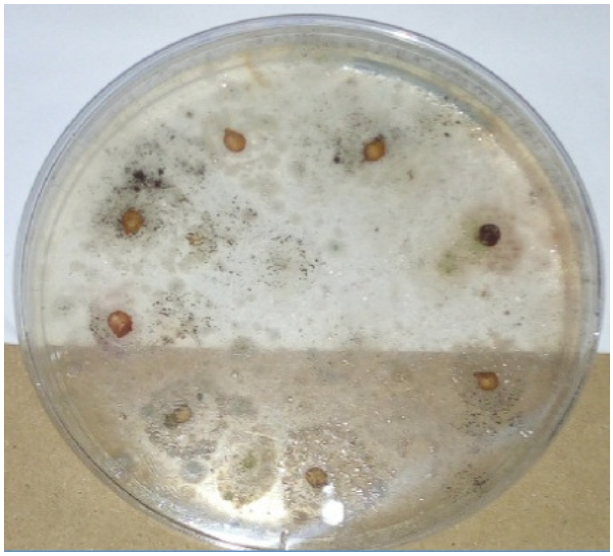

Plate 2: Pathogenicity test on the pepper seeds.

of the colony growth from PDA plates to clean PDA plates aseptically. To determine the frequency of occurrence of the fungal isolate. The number of occurrences for each of the isolates in the two different dry pepper seeds was recorded, calculated, and expressed as a percentage.

Percentage of occurrence= $\frac{X}{N} \times 100/1$

X = Total number of each organism in all the dry pepper seeds.
N = total number of the entire organism in all the dry pepper seeds screened.

The pathogenicity test was carried out using the technique of Okigbo (2009). Healthy pepper seeds were rinsed in sterile distilled water and surface sterilized with 0.1% ethanol solution, a sterilized inoculation needle was used to insert wounds on the seeds and then suspension of the pure culture was spread on the seeds and observed (Ebele, 2010). These were kept for seven (7) days, on the establishment of disease symptoms, inoculums from the infected seeds were taken and cultured, pure cultures were obtained according to (Barnett and Hunter 1999; Aziaba et al., 2015). Plant materials used, *Curcuma longa Moringa oleifera*, and *Ocimum grattissimum* were thoroughly washed and rinsed in three changes of clean tap water, air dried and milled into powder using a milling machine. Cold water extraction was used, and 5g, 10g 15g and 20g of the various plant materials were weighed and dissolved into 50mls, 100mls, 150mls and 200mls of sterile distilled water and filtered differently using a clean four folded muslin cloth. These filtrates 50%, 100%, 150% and 200% which constituted the various concentrations were evaluated. All the extracts used were applied using the food poison technique by pouring aseptically 1ml of the plant extracts onto the sterile Petri plates containing the molten PDA media and gently swirled. The 5mm sterile cup borer was used to pick aseptically 5mm disc of the pure isolates which were placed onto the centre of the sterile Petri plates and incubated at a temperature of ±27 ºC in the incubation chamber and examined for radial growth of the pathogen for 7 days. The experiments were laid out in a Completely Randomized Design (CRD) while the treatments were replicated three (3) times,

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS (Institute Inc., 2001) while mean differences were separated at 5% probability.

RESULTS

The pathogenicity test shown in (Table 1) established the susceptibility of the healthy seeds of two types of pepper (Cameroon and Ghana) and invasion by the rot-causing organism (*Aspergillus niger)* after inoculation. The result showed that in all the four (4) markets studied, there was a highly significant ($P \leq 0.05$) difference in susceptibility among the two types of pepper seeds tested. In the Ndoro market, Cameroon seeds (62.50) were significantly ($P \leq 0.05$) more susceptible to the test pathogen than in Ubani (47.50) and Orie Ugba (37.50) markets respectively. Similarly, the trend was the same for Ghana pepper in the Ndoro market (60.00) which showed a high level of susceptibility to the pathogen followed by Ubani (50.00) and Orie Ugba (35.00) market respectively. However, the fungal isolate caused the same level of rot diseases on the two pepper seed types Cameroon (45.00) and Ghana (45.00) in the University general market. The result in (Table 2) showed that aqueous extracts of *Occimum gratissimum* (2.33) (3.85) at 50 and 100mls concentrations inhibited significantly ($P \leq 0.05$) the radial growth of the pathogen in culture followed by *Cucuma longa* (2.72) (3.91), while on the contrary, the control (5.84) (8.81) showed significantly low anti fungal activity against the test organism. Furthermore, at 150mls concentration *Cucuma longa* (4.32) and *Occimum grattissinum* (4.76) were the most effective in reducing the mycelial growth of the test fungi. Also, at 200mls concentrations, *Occimum gratissimum* (5.50) and *Moringa oleifera* (5.53) were most active and suppressive to the mycelia growth of the pathogen in culture. This result, therefore, showed that aqueous extracts of *Occimum grattissimum* at 50, 100, and 200mls concentrations were the most potent extract effective against the radial growth of the pathogen which causes rot in Cameroon pepper seeds. The result in (Table 3) showed that aqueous leaf extracts of *Occimum gratissimum* at 50 mls (2.31) concentrations inhibited significantly ($P \leq 0.05$) the radial mycelial growth of the pathogen (*A. niger*) followed by *Cucuma longa* (2.44) and *Moringa oleifera* (2.74). The result was the same at 100mls concentration; *Occimum gratissimum* (3.84) and *Cucuma longa* (3.86) effectively reduced the radial mycelial growth of the pathogen when compared with *Moringa oleifera* (4.54). On the other hand at 150 and 200 mls concentrations, *Cucuma longa* (4.20) and *Occimum gratissimum* (5. 67) were highly potent against the radial mycelial growth of the pathogen, while the control (9.00) which had no suppressive elements significantly ($P<0.05$) increased the radial growth of the pathogen in culture. The result, therefore, revealed that the different plant extracts tested were more virulent against the pathogen inciting pepper rot (Ghana pepper) at 50 mls concentration as shown from (Table 3). Different percentages (%) of fungi were isolated from the two types of pepper seeds (Cameroon and Ghana) from the study areas as indicated in (Table 4). The result showed that for Cameroon pepper, 90% of *A. niger* was isolated from the Ubani market followed by Ndoro (89%) and University general markets (85%). In the Ndoro market, *Penicillum sp* was more predominant by 65% followed by Orie Ugba (61%) and Ubani (60%) markets. Furthermore, Orie-Ugba recorded the highest percentage (%) of *Fusarium sp* (50%) and University general market (41%) while the lowest percentage (%) of the pathogen was observed in the Ndoro market. *Rhizopus stolonifer* was the least occurring fungal isolate on the Cameroon pepper seeds and the highest percentage (%) was

Table 1: *In-vitro* pathogencity test of the fungal isolates on two types of pepper seeds Ghana and Cameroon) from four (4) markets in Umuahia, Abia State.

Fungal Isolate Pepper Types Markets	*Aspergillus niger*	
	Cameroon	Ghana
Ndoro	62.50	60.00
Orie ugba	37.50	35.00
Ubani	47.50	50.00
University general market	45.00	35.00
LSD ($P \leq 0.05$)	24.86	27.42

Table 2: Effect of selected extract, their concentrations on the radial mycelia growth of *Aspergillus niger* inciting rot in (Cameroon) pepper seed type cultured in PDA media at 28±2ºC.

Treatments	50 mls	100 mls	150 mls	200 mls
Curcuma longa	2.72	3.91	4.32	6.23
Moringa oleifera	2.75	4.55	5.87	5.53
Ocimum gratissimum	2.33	3.85	4.76	5.50
Control	5.84	8.81	7.93	8.41
LSD($P > 0.05$)	0.66	0.33	0.12	0.10

Table 3: Effect of selected aqueous plant extracts, their concentrations on radial mycelia growth of *Aspergillus niger* inciting rot on (Ghana Pepper Seeds) in Umudike.

Treatments	50 mls	100 mls	150 mls	200 mls
Curcuma longa	2.44	3.86	4.20	6.44
Moringa oleifera	2.74	4.54	5.85	6.67
Occimum gratissimum	2.31	3.84	4.72	5.67
Control	6.25	3.97	9.00	9.00
LSD ($P > 0.05$)	0.58	0.23	0.60	0.45

recorded in the University general market (24%), however, Ndoro (20%) and Ubani (20%) markets had similar % fungal occurrence. The result also showed a similar trend for Ghana pepper seeds. The highest % occurrence *A. niger* was also recorded in Ubani (89%) and Orie Ugba (81%) markets respectively. However, *Penicillium sp* was more predominant on pepper seeds from Orie Ugba (79%) and Ubani (72%) markets. *Fusarum sp* occurred most in Ubani (49%) and Orieugba (42%) markets while the same % of *Rhizopus sp* was isolated from pepper seeds gotten from Orie Ugba (32%) and the University general markets (32%) respectively. The result from the (Table 4**)** therefore, revealed that *A. niger* and *Penicillum sp.* had the highest % occurrence in all the four markets studied for the two types of pepper seed.

DISCUSSION

The results from this study showed that the predominant fungi *A. niger*, isolated from the seeds of the two types of pepper seeds (Cameroon and Ghana) sold in different markets in Umuahia, Abia State was pathogenic and therefore caused rot at varying degrees. The rot of pepper is associated with various fungal pathogens both in fresh fruits and seeds. The pathogen *A. niger* has been previously reported as a fruit rot pathogen of pepper fruits (Grubben and Tahir (2004), Balogun *et al.,* (2005), Nduagu *et al.,* (2008), Ekefan *et al*., (2009) and seeds as well as fruits and seeds of other tropical crops. The differences in the result from the pathogenicity test of the fungi isolated from the two varieties of pepper seeds might be due to the fungi ability to overcome the natural defence mechanism of the seeds or the ability to induce resistance in the seeds when infected (Jarret et al., 2007), this author, also found out that pepper contains secondary metabolite capsaicin, and the antimicrobial property which differs in activity on individual organism. Freeman and Beathie (2008) also reported this compound to be important as it induced a chemical defence mechanism against a broad range of fungal pathogens. Physiologically, spoilage fungi are considered

toxigenic or pathogenic (Al-Hindi et al., 2011). More so, the secondary metabolites isolated from these types of pepper seeds are potentially harmful to humans and animals (Baiyewu et al., 2007). The result also revealed that the aqueous extracts of *Occimum gratissinum* at 50, 100, and 200mls concentrations were the most effective against the radial mycelial growth of the pathogen inciting rot in Cameroon pepper seeds. This could be as a result of the amount of bioactive principle present in the plant extract, differences in the dilution methods, and the type of media used. Furthermore, at 150 and 200mls concentrations, the aqueous *Cucuma longa* (4.20) and *Occimum grattissimum* (5.67) also proved to be most superior to Moringa oleifera in reducing the radial mycelial growth of the pathogen in Ghana pepper seeds.

This could be due to the secondary metabolites of plants which contain an array of secondary metabolites that are cost-effective and are eco-friendly alternatives to chemical compounds. Plant extracts degrade easily and are systemic in function and due to the presence of various compounds; it becomes difficult for the pathogen to acquire resistance (Zhao *et al.,* 2011; Basim *et al.,* 2012).

Results from the study also showed that *A. niger* and *Pennicillum expansum* had the highest (%) occurrence in all the four markets studied for both Cameroon and Ghana pepper. This result indicates that the two pathogens *A. niger* and *P. expansum* were more virulent than the other fungal pathogens isolated thereby causing rot on the seeds of the two types of pepper tested. This is in agreement with the findings of (Al-Hindi et al., 2011) who reported that the second most serious problem in pepper production and consumption is the post-harvest losses due to rot deterioration by fungal pathogens such as carcinogenic *Aspergillus spp.* etc. Balogun *et al*. (2005) further opined that Penicillium *digitatum, Aspergillus flavus and Aspergillus niger* were isolated from pepper fruits and seeds and were *demonstrably* pathogenic on pepper fruits. This report collaborates the isolation of *A. niger*, *A. flavus*, *Penicillium corylophilum* and *Penicillium janthinellum* from pepper seeds (Goldberg, 2011). Seeds attacked by *Rhizoctonia solani, Fusarium* spp, and *Pythium* spp. usually fails to germinate resulting in poor stand development (Goldberg, 2011). Plants are vulnerable to infection by *P. aphanidermatum* during germination and juvenile stages resulting in pre-emergence damping off. Many of the isolated fungi have been reported to be pathogenic to the seeds of different crops causing diseases such as seed rot, damping-off, root rot, fruit rot, wilt, and foliar diseases (Al- kassam and Monawar, 2000). Some of the seed-borne fungi are also known to cause seed rot, decrease seed germination and cause pre and post-damping-off and seedling death (Al-kassam and Monawar, 2000). Anjorin and Mohammed (2009) reported that factors such as moisture content of the seed, temperature, and the degree of invasion of seed by pathogens influenced the development of seed-borne fungi.

Conclusion and Recommendation

In conclusion, the study showed that the predominant fungi *A. niger*, isolated from the seeds of the two types of pepper seeds (Cameroon and Ghana) sold in different markets in Umuahia, Abia State was pathogenic. Furthermore, the effect of different concentrations of the aqueous plant extracts tested on the radial growth of *A. niger* which incites rot on two types of pepper seeds (Cameroon and Ghana) in storage proved that the aqueous extracts of *Occimum grattissium* and *Cucuma longa* were the most effective against the growth of the fungal isolate which incites rot in dry pepper seeds in culture. Higher inhibition of fungal growth was observed at lower to higher concentrations of the test material and this suggests an alternative control method to chemical control. Based on the results obtained from this study, therefore, the use of plant extracts namely: *Moringa oleifera, Ocimum gratissimum, Curcuma longa* in the control of post-harvest fungal rot of two pepper seed types is highly recommended and considering its efficacy in controlling the fungal pathogen of pepper seeds during storage. The majority of the pathogens persist on seeds which possibly explain why isolated fungi were mainly seed-borne. Diagnoses of seed-borne pathogens will allow for the development of control strategies and prevention of disease spread to other areas of the state. The need to further research, on the effect of active principles of these test materials against the pathogen in the field is necessary.

ACKNOWLEDGMENTS

We sincerely appreciate the permission given by the Executive Director of the National Root Crops Research Institute, (NRCRI) Umudike, Abia-state to use their facility and the support by the staff Plant Pathology Unit of the Institute.

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