Effect of Water Activity and Plant Extracts on the Growth of Phytophthora *palmivora* (Black Pod of Cocoa) in South Western Nigeria

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Abstract

Laboratory studies was conducted to evaluate the effect of water activity and hot water extracts of African eggplant (*Solanum macrocarpon)*, salt and oil tree (*Cleistopholis patens)* and the composite mixture on growth, conidia germination and sporulation of *Phytophthora Palmivora* invitro. The extracts and the composite mixture were applied in situ at three concentrations (15, 45 and 60%) by mixing 1 ml of the different concentrations with 10 ml of molten PDA. Unmodified potato Dextrose Agar (PDA) media with water activity (a_w = 0.995) and PDA modified with non-ionic solute glycerol (a_w = 0.97, 0.964 and 0.91) were poured into 9 cm petri- dishes and inoculated at the center with one microliter of standardized conidial suspension for evaluation of growth and conidial density of the fungus. Result from the study shows that the growth rate and conidiation varied significantly at different water activity levels being highest (4.34mm day^{-1}) at (a_w = 0.995) when water was freely available and decreased with water stress (a_w = 0.97 – 0.91). The conidial density was highest under the greatest water stress condition (a_w= 0.91). The extracts applied at all the concentrations reduced mycelia growth and conidial germination. The composite mixture of the extracts was more effective in reducing mycelia growth (3.15mm day^{-1}) compared to the control (4.34mm day^{-1}). Similarly, conidial germination were reduced significantly by the composite mixture values being (38, 25 and 16%) at 60, 45 and 15% concentration respectively. There was no significant effect of the extracts on sporulation at the tested concentrations. The study therefore shows that extracts of the plants and water activity significantly affect the growth and conidiation of *Phytophthora palmivora* and the information obtained can be used in the management of the disease on the field.

Keywords: Cocoa, *P. palmivora*, water activity, plant extract, growth rate.

Introduction

Cocoa (*Theobromo cacao*) originated from Amazon Region of South and Central America, the fruit contains about 20-40 seeds and it is grown by small holder farmers in Nigeria (Vanegtern, *et al.,* 2015). The beans are consumed for the good nutritional qualities being useful in production of beverages, it also play role in reducing blood pressure, cholesterol, diabetes, cancer and various neurodegenerative diseases (FAOSTAT, 2015). Apart from this, it is useful in wound healing, skin care, and cocoa butter production and in improving brain health. World output in 2015 was 730 Million Metric Tonnes (MMT), Ivory Coast is the largest producer followed by Ghana and Nigeria (Afolayan, 2017). Production in the last few decades has decreased in Nigeria due to the problem of climate, fire outbreak, soil fertility, plantation age, management strategies, government policies and diseases (Konam, 1999).

Phytophthora palmivora is the pathogen causing the black pod disease of cocoa. The

pathogen belong to the Phylum Oomycota, order Peronosporales, family Pythiaceae and genus Phytophthora (Opoku *et al*, 1999). The disease is capable of causing about 75% yield loss when not controlled [Widmer, 2016]. Infection starts from the pods that are close to the ground level and the symptoms of the disease are production of brown to black coloration on the pods and seeds as well as necrotic lesions, this invariably reduce the quality of cocoa in world market (Akrofi *et al*., 2003).

The disease is spread by water and wind. Water from rainfall liberates the sporangia from the surface of infected fruits to the atmosphere and wind is required for the dispersal of the inoculum and disease epidermics (Appiah, 2001). Temperature of 18-20^{oc} along with high humidity activate the pathogen. Management of the disease can be achieved by pruning to increase airflow and reduce relative humidity, mulching to reduce the number of spores released through rain splash, removal of infected pods, use of companion crops, host resistance and chemical control using fungicides (Vanegtern *et al*., 2015).

However, due to the rising cost of fungicides and toxicity on non-target organisms, attention is being focused on alternative methods of disease control. One of these is the use of plant extracts that are cheap, readily available and compatible with the farming practices of the farmers (Falade *et al.*, 2017). Extracts of many plants have shown toxicity to many phytopathogenic fungi by inhibiting their growth and the efficacy varies with the concentration of active ingredients in the plant extracts and the strain of the fungus (Mathukumal *et al*., 2012).

Phytophthora palmivora, like any other fungi, is affected by nitrogen, p^{H}, temperature and water availability (water activity) (Gideon and Anita, 2013). Water activity significantly affects the growth, infectivity and sporulation of phytopathogenic fungal species such that the management of irrigation regimes in fields sown to cocoa can play important roles in modulating water activity (Falade, 2016). Based on this, experiment was conducted to evaluate the effect of different water activity on growth and conidiation of *P. palmivora* and to test the efficacy of hot water extracts of *Solanum macrocarpon* and *Cleistopholis patens* in the management of the disease

Materials and Method

The laboratory experiments was conducted at the Crop Protection Laboratory, Ekiti State University, Ado Ekiti, Nigeria. Leaves of the two plants namely African garden egg (*S. macrocarpon)* and sait and oil tree (*C. patens)* were collected at Odo-Oro in Ikole Local Government Area Ekiti State and air-dried at 28°C for 6-8 weeks until each plant had a constant weight. The dried leaves were milled using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C for about 2 weeks until they were required for bioassay Extracts were prepared by mixing equivalent grams of prepared plant powder (15, 45 and 60%) with 100 ml of distilled water at 70°C in 500 ml bottles and kept in hot water bath-shaker for 30 minutes. Thereafter, the liquid extract was separated by vacuum filtration and poured inside standard bottles for refrigeration at 4°C. These extracts were used as the stock solution from which 60%, 45% and 15% of each extract were prepared

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Isolation and identification of *P. palmivora*

Cocoa pods showing distinct symptoms of the pathogen were collected from a cocoa plantation at Odo- Oro in Ikole Local Government Area of Ekiti State. The pods were cut into small pieces of about 1-2 cm^2 and surface sterilized by immersion in 0.2 % NaOCl for two minutes and followed by two

rinses in sterile distilled water in a laminar flow cabinet. Three pod cuttings per plate were placed on Potato Dextrose Agar (PDA). The plates were sealed with parafilm and incubated at 28°C for 5-6 days. Single spore of developing colonies was isolated and sub-cultured to obtain pure cultures. Samples from single spore cultures were used for identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular) (Zivkovic *et al*, 2010).

Evaluation of growth *P. palmivora*

Ten mls of molten PDA were mixed with 1 ml of the different concentrations (60%, 45% and 15%) of the plant extract and the composite mixture and allowed to solidify. The composite mixture was obtained by adding 30.0, 22.5 and 7.5% each of the extract concentration to get 60,45 and 15% concentrations respectively. The control treatment consisted of plates containing 1 ml of sterile distilled water. Thereafter, the plates were inoculated with the conidia of the fungus at the center inside a laminar flow cabinet sealed with Parafilm (PM-992®) (Borisade and Magan, 2014) and incubated at 25°C for eight days. The colony diameter after 24 hours was measured along two orthogonal axes which were marked on the plates. The rates of growth (radial extension) were measured using a ruler and growth inhibition (RGI) was calculated for each treatment and compared with the control (Anteneh *et al.,* 2013), as stated in the equation:

$$RGI = \frac{(R1-R2)\ 100}{R1}$$

where R1= mycelial growth of colony in control plate, R2 = mycelial growth of colony in sample plate, RGI= Rate of Growth Inhibition. The treatments were replicated three times.

Evaluation of Sporulation Density of *P. palmivora*

Spore suspension was prepared from 10-day old culture by flooding the surface of the growing colonies in each Petri-dish with 10 ml sterile distilled water containing 0.05% Tween 80 (Polyethylene glycol sorbitan monolaurate) and Drigalsky spatula was used to dislodge the spores carefully. The suspensions were serially diluted and spores counted using Improved Neaubaur Haemocytometer. Sporulation density, which was the estimated number of spores per colony area was calculated as:

$$\text{Sporulation density (spores cm}^{-2}\text{ colony area)} = \frac{Estimated\ number\ of\ spores}{colony\ area}$$

(Borisade and Magan, 2015).

Evaluation of Conidia Germination

Sterile PDA in 9 cm Petri-dishes were inoculated with 10 µl of *P. palmivora* conidia suspension measured with a micropipette and spread-plated using Drigalsky spatula. The lids were replaced and sealed with Parafilm. Incubation was done at ambient temperature (25 ± 2ºC) for 24 hours. Thereafter, sterile coverslip was placed on the spread-plated area and percentage germinated conidia was estimated for 100 conidia in the coverslip area under a compound microscope using X40 objective. Conidium with germ tube length longer than its diameter was considered as germinated. Percentage conidia germination (PCG) was calculated as:

$$PCG = \frac{No\ of\ Germinated\ conidia}{Total\ counted\ conidia\ within\ field\ of\ view} \text{x}100$$

(Borisade and Magan, 2015)

Effect of water activity on growth of *P. palmivora*

Conidia from 10-day old culture were harvested by flooding the surface of the agar plate with distilled water containing 0.02%

Tween 80. Conidia suspension was poured into universal bottles and centrifuged at 1500 rpm for 30 min. The supernatant was discarded and the conidia suspension was made to 1 ml. Serial dilutions were made and the conidia were counted with Improved Neubauer Haemocytometer microscope. Thereafter, the conidia suspension was standardized to 10^4 conidia ml^{-1}. Standard Potato Dextrose Agar (PDA, a_w=0.995) and modified PDA media containing calculated amounts of non-ionic solute glycerol at three water activity (a_w) levels; 0.97, 0.964 and 0.91 were prepared and poured into 9 cm Petri-dishes. Three replicate plates at each a_w were inoculated at the center with one micro-litre of the standardized conidial suspension. The plates were sealed with Parafilm and incubated at ambient temperature (25±2°C) for 10-12 days or until ¾ of the surface of the agar in the 9 cm Petri-dish was covered with the growing colony. Measurement of radial extension of the colony along two pre-marked orthogonal axes was done daily for the entire incubation period. Growth rate was calculated by plotting the graph of radial extension against the period of growth. The slope of the log phase of growth (growth rate) was estimated using the regression equation of the linear model (Borisade and Magan, 2014).

Effect of water activity on sporulation of *P. palmivora*

One centimetre agar disks from the culture used to estimate growth were taken randomly from three portions on the PDA plate into 10 ml disposable universal bottles. One ml sterile distilled water containing 0.02% Tween 80 was added into each bottle and vortexed for 1-2 minutes to dislodge the spores. The conidial suspension was thereafter made to 10 ml and spore count was done at X40 objective of light microscope using Neaubaur Haemocytometer. Sporulation density was calculated as the number of conidia cm^{-2} of fungal colony

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Results

Table 1 shows the effect of different concentrations of *S. macrocarpon, C. patens* extracts and composite mixture of the two plants on germination rates of *P. palmivora.* All the extracts significantly ($p \leq 0.05$) inhibited conidia germination at all the tested concentration compared to the control. There was 15-33, 10-27 and 16-38% inhibition of conidia germination for S. macrocarpon, C. patens and the composite mixtures respectively compared to the control that had no inhibition. Conidia germination with extracts of *S. macrocarpon* at 15, 45 and 60% concentration was 85, 74, and 67% while that of *C. patens* were 90, 81, and 73%. Similarly at the same concentrations of extracts, the values of the composite mixtures were 84, 75 and 62%.

Table 1: Effect of three concentrations hot water extract of two plants on conidia germination of *P. palmivora*

Concentration	S. macrocarpon	C. patens	Composite Mixture
15	85^b	90^b	84^b
45	74^c	81^c	75^c
60	67^d	73^d	62^d
Control	100^a	100^a	100^a

Means with the same letter are not significantly different according to Turkeys test

Table 2 shows the effect of different concentrations of hot water extracts of *S. macrocarpon, C. patens* and composite mixture of the two plants on growth rates of *P. palmivora.* The growth rate varied significantly in relation to plant extracts and

their concentration, with values in the control significantly the highest. At 15, 45 and 60% concentration of extracts *S. macrocarpon* growth rates were 4.35, 3.97, and 3.69 mm day^{-1} while that of *C. patens* were 4.10, 3.78 and 3.30 mm day^{-1} respectively. Similarly, at the same concentrations, growth rates with that of composite mixtures were 3.95, 3.41 and 3.15 mm day^{-1}. The least growth rates were recorded at higher concentrations of all the extracts.

Table 2: Effect of three concentrations of hot water extracts of two plants on growth rate (mmday^{-1}) of *P. palmivora*

Concentration	*S. macrocarpon*	*C. patens*	*Composite Mixture*
15	4.35^{b}	4.10^{b}	3.95^{b}
45	3.95^{c}	3.78^{c}	3.41^{c}
60	3.69^{d}	3.30^{d}	3.150^{d}
Control	4.34^{a}	4.34^{a}	4.34^{a}

Means with the same letter in each column are not significantly different according to tukeys test

Table 3 shows the effect of the hot water extracts of two plants on sporulation of *P. palmivora.* There was no significant difference in conidia per colony area on all substrates containing the different concentrations of the extracts at all the tested concentrations. At 15, 45 and 60% concentrations of *S. macrocarpon,* sporulation rates were 5.6, 5.4 and 5.2 while that of *C. patens* were 5.6, 5.8, and 5.7 respectively. Similarly, at this same concentrations the sporulation rates for the composite mixtures were 5.4, 5.6, and 5.5 respectively.

Table 3: Effect of three concentrations of hot water extract two plants on Sporulation *P. palmivora*

Concentration	*S. macrocarpon*	*C. patens*	Composite mixture
15	5.6^{a}	5.6^{a}	5.4^{a}
45	5.4^{a}	5.8^{a}	5.6^{a}
60	5.2^{a}	5.7^{a}	5.5^{a}
Control	5.9^{a}	5.9^{a}	5.9^{a}

Means with same letter in each column are not significantly different according to tukey's test

The rates of growth *P. palmivora* on glycerol modified PDA at different a_w levels (0.995, 0.97, 0.964 and 0.91) are shown in Table 4. The highest radial growth rate was observed at 0.995 a_w, where water was freely available and the growth rate reduced significantly as the a_w decreased from 0.995 – 0.964. Growth rate was 4.34 mm day^{-1} at 0.995 a_w and this reduced to 3.77 mm day^{-1} at a_w 0.97. The reduction of a_w to 0.91 from 0.96 did not decrease growth. At 0.97 a_w, growth inhibition rate was 13 % whereas at 0.96 and 0.91 a_w levels, percentage inhibition were 23.0%.

Table 4: Growth rate (mm day^{-1}) of *P. palmivora at* different level of water activities.

Water activity levels	Growth rate (mm day^{-1})	%inhibition
0.995 (control)	4.34 [a]	-
0.97	3.77 [b]	13
0.96	3.36[c]	23
0.91	3.36[c]	23

Means with the same letter in each column are not significantly different (P<0.05) (Tukey's HSD)

Fig 1 shows the effect of a_w on *P. palmivora* conidia density. At 0.91 a_w, *conidia* density was significantly higher compared to (0.995 a_w) when water was freely available. The conidia density of the culture under water stress condition a_w 0.97 and the control ($a_{w=}$ 0.995) was comparable. The water activity that supported highest sporulation was a_w 0.91.

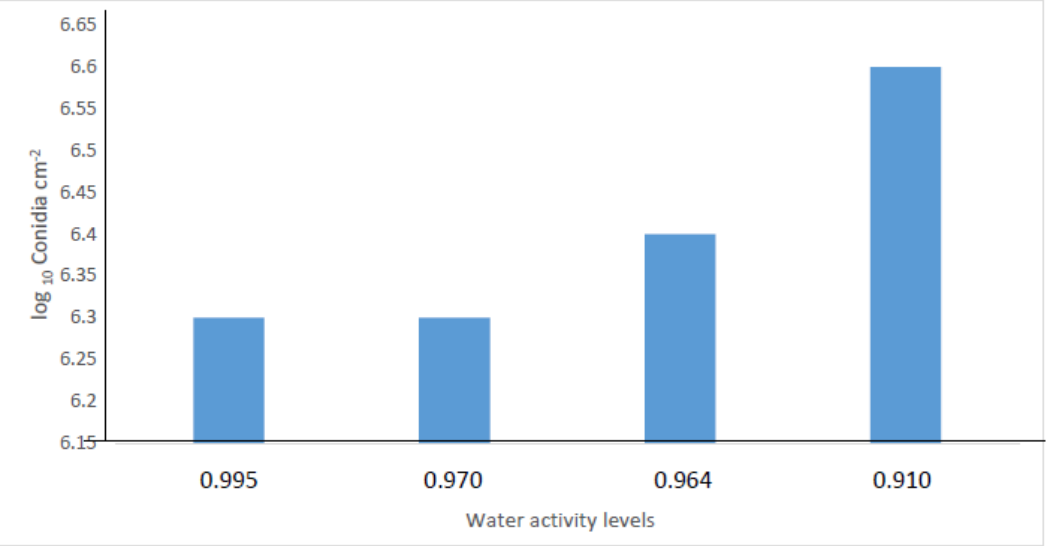

Fig. 1: Effect of water activities on conidial densiy of *P. palmivora*

Discussion

In this study, radial growth of *P. Palmivora* was recorded over the period of 10 days and the rates of growth on glycerol modified PDA was measured at different a_w levels (0.995, 0.97, 0.964 and 0.91). The growth of *P. Palmivora* was rapid when water was freely available ($a_{w=}$ 0.995) and decreased with water stress. However, higher conidia density was observed under water stress condition (a_w = 0.91). The optimum condition that supports conidial formation (a_w = 0.91) was different from the one that supports mycelia growth (a_w=0.995).

Water activity affects the growth and conidiation of phyto- and entomopathogenic fungi in different studies (Abadias *et al*., 2003; Falade, 2016. Borisade and Magan, 2014. Peromingo, *et al.*, 2016). The effect of water activity and temperature on the growth and sporulation of *Aspergillus niger* was reported by Roberto *et al.*, (2004). The result of the study shows that growth of the fungus was faster under moderate water activity level.

Similarly, sporulation was higher when the organism was in a water stress condition (a_w = 0.97 and 0.93) which is in agreement with the current study.

Borisade and Magan (2014) reported that the growth of entomopathogenic *Beauveria bassiana, Metarhizium anisopliae, Isaria farinose* and *Isaria fumosororea* strains were faster on glycerol modified PDA when water was freely available and decreased with water stress. The result also shows that sporulation of the pathogen was higher under water stress condition which is in agreement with this study.

Phytopathogenic fungi are known to respond to water stress by production of large numbers of conidia that are tolerant to abiotic stress factors like temperature and water as an adaptation to survival in marginal environments (Ysilos and Magan, 2004, Amani *et al*., 2016). In this study, the conidial density of P. palmivora under water stress condition (a^w 0.91) was higher than those obtained when water was freely available. This agrees with Mousa *et al.,* (2016) that higher conidial density was obtained when *Aspergillus flavus* was isolated from paddy at low water activity level, because more aflatoxins were produced. However, the effect of temperature on the growth and conidial density of the fungus was not observed in the current study.

The two extracts used in the study (*S. macrocarpon* and *C. Patens*) as well as the composite mixture reduced the mycelia growth of *P. Palmivora* at all the tested concentrations and the efficacy was concentration dependent. Higher imbibition of growth occurred as the concentration of the plant extracts increased. The composite mixture of the extracts at all the tested concentration were more effective than single application of the extracts. This may be due to increased availability of fungitoxic chemicals released by the mixture like saponin, flavonoids, tannins ascorbic acid, sabiene, linalool and caryophyllene all of which exert their effect on the composite mixture. Enyiukwu and Awurum (2013) extracts of *Carica papaya* roots and seeds as well as seeds of *Piper guineense* controlled the growth of *Colletotrichum destructivum* O Gara, the study shows that all the extracts reduced mycelia growth of the fungus in-vitro. Similarly, Shovan *et al*., (2008) controlled the growth of *Collectotrichum dematium* causing anthracnose of soyabean with extracts of ginger, garlic, onion and neem at three different concentrations (5, 10 and 20%) the result shows that all the extracts inhibited mycelia growth of the fungus and efficacy was concentration dependent. In the study, all the two extracts at the tested concentrations as well as the composite mixture did not affect sporulation. The mechanism of plant extracts in inhibiting mycelia growth and conidial germination without any significant effect on sporulation is not fully understood. It may be suggested that different concentrations of the extracts in the substrate may present a dissimilar osmotic environment in addition to its phytotoxicity which may be responsible for the result of this study.

There is need for molecular studies to understand the mechanism of bioactive phytochemicals against growth, sporulation and germination of plant pathogenic spores and further screening is necessary to identify plants with active ingredients that can inhibit sporulation. It may be interesting to study the temperature relations for inhibition of growth, conidial density and sporulation in further studies.

Conclusion

This research provided information that *P. palmivora* disease can be controlled with extracts of *S. macrocarpon* and *C. patens* invitro, in addition water activity play significant role in growth and conidiation of the pathogen which may be a useful

information in the management of the disease on the field

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References

Abadias, M., Usall, J., Teixidó, N. & Vinas, I. (2003). Liquid formulation of the postharvest biocontrol agent Candida sake CPA-1 in isotonic solutions. *Phytopathology*, *93*(4): 436-442.

Akrofi, A. Y., Appiah, A. A. & Opoku, I. Y. (2003). Management of *Phytophthora palmivora* Rot Disease on Cocoa Farms in Ghana. *Crop Protection*, 22(3): 469 – 477.

Amani, L. & Vicente, S. (2016). Effect of Temperature, Water Activities and Incubation Time on Fungal Growth and *Aflatoxin B.* Production in toxinogenic *Aspergillus flavus* Isolate on Sorghum Seeds. *Elsevier Revista Argentia Demicrobiologia*, 48(1): 78 – 85.

Anteneh, A., Amare, A. & Kebede, W. (2013). Evaluation of Antifungal Activity of Plant Extracts against Papaya Anthracnose (*Colletotrichum gloeosporiordes*). *Journal of Plant Pathology and Microbiology*, 4: 273 – 277.

Appiah, A. A. (2001). Variability of *Phytophthora species* Causing Black Pod Disease of Cocoa (*Theobroma cacao L.*) and Implications for Assessment of Host Resistance. Imperial College London (University of London).

Borisade, O. A. & Magan, N. (2014). Growth and sporulation of entomo pathogenic *Beauveria bassiana, Metarhizium anisopliae. Isaria farinosa* and *Isaria fumosorosea* strains in relation to water activity and temperature interaction**.** *Biocontrol Science and Technology*: 24: 999 – 1011.

Borisade, O. A. & Magan, N. (2015). Resilience and relative virulence of strains of entomopathogenic fungi under interactions of abiotic stress. *African Journal of Microbiology Research*, *9*(14): 988-1000.

Enyiukwu, D. N. & Awurum, A. N. (2013). Fungitoxic Principles and Antifungal Activities of Extracts from *Carica papaya* and *Piper quineense* on *Colletotrichum desctructiuum* O Gara. *Continental Journal of Agricultural Sciences.* 7: 23-28.

Falade, M. J. (2016). Growth and Conidiation of *Colletorichum lindemuthianum* Under Conditions of Different Water Activity. *International Journal of Bacteriology, Virology and Immunology,* 2(1): 38 – 42.

Falade, M. J., Enikuomehin, O. A., Borisade, O. A. & Aluko, M. (2017). Control of Cowpea (*Vigna linguiculara L. walp*) Diseases with Intercropping of Maize (*Zea mays L*) and Spray of Plant Extracts. *Journal of Advances in Microbiology:* 7(4), 1 – 10.

FAOSTAT, (2015). Food and Agriculture Organization of the United Nations. Site internet: http//www.fao.org/statistics

Gideon, K. O. & Anita (2013) *In vitro* control of Anthracnose disease of cowpea *Vigna unguiculata* L. Walp caused by *C destructivum* with *Cyathula prostrata* L and *Diodia scadens* S. W. *Journal of Intercultural Ethnopharmacology* 2 (1):29-36.

Konam, J. K. (1999). Integrated Management of *Phytophthora palmivora* Diseases in Papua New Guinea. School of

Botany, University of Melbourne (255 pg).

Mathukumar, A., Eswaran, A., Nakkeeran, S. & Sangeetha, G. (2010). Efficacy of plant extracts and biocontrol agents against *Pythum aphanidermatum* Inciting Chilli dam ping-off. *Journal of Crop Protection* 29: 1483 – 1488.

Mousa, W., Ghazall, F. M., Jinap, S., Ghazali, H. M. & Radu, S. (2011). Modelling the Effect of Water Activity and Temperature on Growth Rate and *Aflatoxin* Production by two Isolates of *Aspergillus flavus* on Paddy. *Applied Microbiology,* 3: 1262 – 1274.

Opoku, J. Y., Akrofi, A. Y. & Appiah, A. A. (1999). The Spread of *Phytophthora megakarya* on Cocoa in Ghana. *Journal of Ghana Science Association,* 2(3): 110 – 116.

Peromingo, B., Rodriguez, A., bernaldez, V. Delaado, J. & Rodriguez, M. (2016). Effect of Temperature and Water Activity on Growth and *Aflatoxin* Production by *Aspergillus flavus* and *Aspergillus parasiticus* on Cured Meat Model System. *Journal of Meat Science,* 122: 76 – 83.

Roberto, P., David, A., David, B. & Naresh, M. (2004). Water Activity Solute and Temperature Modify Growth and Spore Production of Wild Type and Genetically Engineered *Aspergillus niger strains. Journal of Enzyme and Microbial Technology,* 35: 232 – 237.

Shovan, L. R., Bhuniyan, M. K., Begun, J. A. & Pervez, Z. (2008). Invitro control of *Colletotrichum dermatium* causing anthracnose of soyabean by fungicides, plant extracts and *Trichoderma hazianium*. *International Journal Sustainable Crop Production* 3(3): 10 – 17.

Vanegtern, B., Rogers, M. & Nelson, S. (2015). Black Pod Rot of Cacao Caused by *Phytophthora palmivora.* College of Tropical Agriculture and Human Resources, University of Hawai.

Ypsilos, I. K., & Magan, N. (2004). Impact of water-stress and washing treatments on production, synthesis and retention of endogenous sugar alcohols and germinability of Metarhizium anisopliae blastospores. *Mycological research*, *108*(11): 1337-1345.

Zivkovic, S., Stojanovic, S., Ivanović, Z, Trkulja, N., Dolovac, N., Aleksic, G. & Balaz, J. (2010). Morphological and molecular identification of *Colletotrichum trucutatum* from tomato fruit. *Pesticidi fitomedicina*, *25*(3): 2 – 8.