EVALUATION OF ANTIFUNGAL ACTIVITIES OF FIVE PLANT EXTRACTS AGAINST*Pseudoperenospora cubensis* (DOWNY MILDEW)IN MUSKMELON (*Cucumis melo* L).

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Abstract

Laboratory study was conducted to evaluate the effect of leaf extracts of five indigenous plant on conidia germination, growth and sporulation of*Pseudoperenosporacubensis* causing downy mildew disease of muskmelon. Extracts of five plant ; mexican sunflower (*Tithonia diversifolia),* bush banana *(Uvaria chamae),*salt and oil tree*(Cleistopholis patens),*goat weed*(Ageratum conyzoides)*and african eggplant*(Solanum macrocarpon)*at.fourconcentrations (15,30,45 and60% *)* were tested against the growth, conidial germination and sporulation of *Pseudoperenosporacubensis* in vitro.

Results show that all the plant extracts significantly inhibited conidia germination and radial growth compared to the control.The extracts had no significant ($p \leq 0.05$) effect on sporulation. The rate of inhibition of growth and conidia germination was concentration dependent being highest at 60% for the extracts. The extracts of *Solanum macrocarpon* was the most effective followed by *Ageratum conyzoides*, *Cleistopholis patens* and *Uvaria chamea* while*Tithonia diversifolia* caused the least inhibition of growth and conidia germination. At 15, 30, 45 and 60% concentrations growth of*Pseudoperenospora cubensis* on PDA modified with*Solanum macrocqrpon*were 3.79, 3.65, 3.33 and 2.87; and 4.25, 4.12, 3.92 and 3.89 for PDA modified with*Tithonia diversifolia*. Similarly, conidia germination percentages recorded at same concentration of extracts *S. macrocarpon*were 87, 85,70 and 62% while that of *T. diversifolia* were 91, 87, 84 and 72%. The study shows that the plant extracts has the potential for inhibition of the pathogen.

Keywords: Muskmelon,*Pseudoperenosporacubensis,* conidial germination, growth, sporulation.

1.0 INTRODUCTION

Muskmelon (*Cucumis melo L*) is a cucubitwidely grown in many tropical and subtropical regions of the world and consumed for its nutritional qualities(USDA, 2015).World output in 2013 was 29.4 million tons(t) (Ybi, 2007)with India being the largest producer producing 15.1 millon t. It contains 53kcal of energy, 13g of carbohydrates, 1.4g fibre, 12g of sugar, 1.3g of protein, 3126 IU vitamin A, 40.56mg vitamin C, 531.96mg potassium, 3,360mg of folate and 0.3g of fat (Entisar, 2014).The fruit when consumed help to suppress hypertension because of the richness in potassium, improvesvision due to high level of vitamin A that strengthens the eye muscle. It also helps to regulate the sugar level, thus controlling diabetes. Besides, the fruit helps to booster body immunity by stimulating the production of white blood cells (Entisar, 2014).

Downy mildew of muskmelon is an important fungal disease capable of causing 100% yield loss when not controlled (Savory, 2011). The pathogen affects all parts of the plant, reducing crop quality and quantity. It is an obligate parasite that needs living muskmelon plant to grow and survive. Symptoms of the disease are yellow to brown lesions on the upper leaf surfaces. The infection begins as small light green spots that are not water- soaked on the upper leaf surfaces but the spots enlarge and later turn to yellow or brown lesions (Colluci and Holmes, 2010). The disease is spread from plant to plant by air borne spores and infection is favoured by wet weather.

The disease can be controlled effectively by the use of fungicides and crop rotation (Mary,2014). The use of synthetic fungicides like benomyl had proven very effective but the increased awareness of environmental side effects of synthetic pesticides, development of resistant strains of pathogens and toxicity to non-target organisms have tilted attention on the development of alternative method of pathogen control.One of these is the use of plant extracts which are considered cheap and compatible with the farming practices of the farmers (Lowell, 2004).

The extracts of many plants have been reported to be toxic to many phytopathogenic fungi. The efficacy in plant disease management varies with the concentration of active ingredients in the plant extracts and the strain of the fungus (Mathukumal*et al.,* 2012). The

antifungal effects of goat weed (*Ageratum* conyzoides) (Eriyanto,2016), mexican sunflower (*Tithonia diversifolia*) (Mapa *et al.*,2016), bush banana (*Uvaria chamae*) (Chika *et al*.,2007)african garden egg (*S.macrocarpon*) (Yasnawan,2016) and salt and oil tree (*C. patens*) are well known but their use in the management of downy mildew disease of muskmelon has not been exploited. Based on this, it is imperative to evaluate the effectivenessof hot water extracts of these plants in the management of*P.cubensi,*the pathogen causingdowny mildew disease of muskmelon.

2.0 Materials and Method.

2.1 Collection of plant leaves and preparation of extracts.

Leaves of *T. diversifolia, A. conyzoides, U. chamae, C.patiens* and *S. macrocarpon* were collected from Ekiti State University Teaching and Research Farm,Ado-Ekiti and air-dried at ambient temperature (24±2°C) for 14 -28 days. The dried leaves were turned into powder using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C. Thereafter, 60, 45, 30 and 15 g of the powder of each plant were weighed into 250 ml standard flask and 100 mL of distilled water at 70°C was poured into each flask (Alves *et al.,* 2015). The flasks were maintained at this temperature in hot water bath-shaker for 30 minutes and thereafter the liquid extract was separated by vacuum filtration, poured into standard bottles and refrigerated at 4°C for subsequent use as the stock solution.

2.2 Isolation and morphological identification of *P. cubensis*.

Muskmelon plants showing distinct symptoms of downy mildew disease were collected from fields at Ekiti State University Teaching and Research farm, Ado –Ekiti, Nigeria. The leaves were cut into pieces of about 1-2 cm and surface sterilized by immersion in 0.2%NaOCl for two minutes. This was followed by two rinses in sterile distilled water and spraying with 70% isopropanol. The sterilized leaves were kept inside a laminar flow cabinet for 20-30 minutes to dry. Five sterilized leaf cuttings were appressed unto the surface of Potato Dextrose Agar (PDA) (Sigma-Aldrich) containing 0.05% chloramphenicol (company purchased) inside 9 cm sterile Petri dishes and removed. For the isolation of the downy mildew pathogen, three of the surface sterilized leaf cuttings were placed on PDAcontaining chloramphenicol to prevent growth of bacteria(Falade, 2017). The plates were sealed with parafilm and incubated separately at ambient

temperature for 5-6 days. There was no growth on the plates unto which leaves were appressed and this confirmed that the surface of the leaves was sterile. Single conidia from developing colonies in the isolation plate was transferred into prepared standard PDA media to obtain a pure culture. Agar plugs from single conidia cultures were used for morphological identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular) (Živković, *et al.*, 2010).

2.3 Effect of hot water extract on conidia germination

One mL of different concentrations (15, 30,45 and 60% w/v) of the extracts was added to 9 mL molten PDA. The plant extract-modified PDA was poured into 9cm Petri dishes and allowed for 1 hour to solidify. The media for the control treatment consisted of standard PDA media alone. The media were inoculated with 10 µL of *P. cubensis* conidia suspension containing 1.0 x 10^2 conidia ml^{-1} prepared from 21 days old culture and spread-plated using spatula. The Petri dishes were sealed with parafilm to prevent evaporation of moisture from the agar surface and incubated at ambient temperature for 12 hours. Thereafter, sterile coverslips were placed in three positions on the surface of the agar and viewed under x40 objective of compound microscope. A conidium with the germ tube length which waslonger than its diameter was considered as germinated. One hundred conidia were randomly counted in each of the coverslip field and the percentage germination was calculated as:

$$\% \text{ germination} = \frac{Germinated\ conidia}{Total\ counted\ conidia} X\ 100$$ (Borisade and Magan, 2014)

2.4 Effect of plant extract on growth

In order to evaluate the effect of the extracts on growth, standard PDA media (control) and plant extract-modified PDA based media were prepared as described previously. The plates were inoculated at the centre with 10 µL of conidia suspension containing 1 x 10^2 conidia ml^{-1} using micro-pipette (Eppendorf 1-10 µL).They were sealed with parafilm and incubated at 20°C for eight days. The treatments and the control were replicated three times. Daily measurement of the colony diameter along two orthogonal axes which were marked on the plates commenced at 24 hours after inoculation and this continued for 5-10 days. The values of the growth rates were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment and compared with the control (Amadioha,2003):

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

Where, R1= Radial extension of colony in the control plate and R2 =Radial extension of colony in sample plate.

2.5 Effect of plant extract on sporulation

Agar plugs were taken from three positions on 14 days old culture into a McCartney bottle using 1cm cork borer and 10 mL of sterile distilled water containing 0.05% Tween-80 (surfactant) was poured into each bottle. The bottle was vortexed for 1-2 minutes to dislodge conidia. The concentration of conidia in the suspension was estimated using a haemocytometer and the density of conidia (conidia cm^{-2} of the colony) was calculated (Borisade and Magan, 2014).

3.0 Results

3.1 Effect of Hot water Extracts on Conidia germination.

Table 1 shows the effect of different concentrations of the leaf extracts on germination rates of *P. cubensis.* All the extracts significantly ($p \leq 0.05$)inhibited conidia germination when compared with the control. There was 36-9% inhibition of conidia germination for all the extracts compared to the control that had no inhibition. Conidia germination with extracts of *S. macrocarpon* at 15, 30, 45 and 60% concentration was 87,85, 70 and 62% while that of *T. diversifolia* at same concentrationswere 91, 87, 84 and 72%.

Table 1: Effect of hot water extract of five plants on conidia germination

Concentration	T. diversifolia	U. chamae	C. patens	A. conyzoides	S. macrocarpon
15	91^{b}	89^{b}	89^{b}	92^{b}	87^{b}
30	87^{b}	86^{b}	84^{b}	80^{b}	85^{b}
45	84^{b}	81^{b}	73^{c}	73^{c}	70^{c}
60	72^{c}	70^{c}	72^{c}	64^{d}	62d
Control	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

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

3.2 Effects of Hot Water Extracts on growth rate.

Table 2 shows the effect of different concentrations of the five leaf extracts on growth rates of *P. cubensis.*The growth rate varied significantly in relation to plant extracts and their concentration, with values in the control significantly the highest. At 15,30, 45 and 60% concentration of extracts *S. macrocarpon* growth rates were 3.79, 3.65, 3.33 and 2.87 while that of *T. diversifolia* were 4.25, 4.12, 3.92 and 3.89 respectively. Lower growth rates were recorded at higher concentration of all the extracts used in the study.

Table 2: Effect of four concentrations of hot water extracts of five plants on growth rate of *Pseudoperenosporacubensis*

Concentration	*T. diversifolia*	*U. chame*	*C. patens*	*A. conyzoides*	*S. macrocarpon*
15	4.25^{b}	4.19^{b}	4.07^{b}	3.89^{b}	3.79^{b}
30	4.12^{b}	4.01^{b}	3.96^{b}	3.60^{c}	3.65^{b}
45	3.92^{c}	3.88^{c}	3.61^{c}	3.30^{c}	3.33^{c}
60	3.89^{c}	3.60^{c}	3.30^{d}	3.14^{d}	2.87^{d}
Control	4.34^{a}	4.34^{a}	4.34^{a}	4.34^{a}	4.34^{a}

3.3 Effects of hot water extracts on sporulation

Table 3 shows the effect of the five leaf extracts on sporulation of *P. cubensis.* There was no significant difference in conidia per colony area on all substrates containingthe different concentrations of the extracts. At 15, 30, 45 and 60% concentrations of *S. macrocarpon,*sporulation rates were 5.5, 5.4. 5.5 and 5.5 while at the same concentration that of *T. diversifolia* the rates were 5.6, 5.6, 5.4 and 5.5 respectively.

Table 3: Effect of extract on Sporulation density *P.cubensis*

Concentration	*T. diversifolia*	*U. chame*	*C. patens*	*A.*	*S. macrocarpon*

				conyzoides	
15	5.6[a]	5.6[a]	5.4[a]	5.6[a]	5.5[a]
30	5.6[a]	5.6[a]	5.5[a]	5.5[a]	5.4[a]
45	5.4[a]	5.5[a]	5.5[a]	5.6[a]	5.5[a]
60	5.4[a]	5.5[a]	5.6[a]	5.5[a]	5.5[a]
Control	5.9[a]	5.9[a]	5.9[a]	5.9[a]	5.9[a]

Discussion and Recommendation

In this study, all the leaves of the five indigenous plants were air dried and powdered to lower the surface area thus increasing the rate of reaction. It has been reported that air dried plant materials are less fragile and do not tend to deteriorate an advantage which it has over fresh samples (Falade, 2017). Bioactive constituents are present in varied form in tissues of plant species and can be used as natural protectants against diseases (Falade & Borisade, 2017). In this study, hot water was used for the extraction because it is considered as one of the best methods of extraction because it is capable of preserving the chemistry of the constituents (Vongsak *et al*., 2013).

In the study, all the extracts of the five plant: *T. diversifolia*, *U. chamae*, *C. patens*, *A. conyzoides* and *S. macrocarpon*reduced mycelia growth of *P.cubensis* and the rate of inhibition of growth was concentration dependent. Highest inhibition of growth occurred at relatively higher concentrations of the plant extracts. This was probably due to increased availability of anti-fungal chemicals in the medium that was responsible for suppressing growth.Mukrejee *et al.,*2011 evaluated the effects of the extracts of Mahogany, giant Indian milky weed, garlic and ginger at 30-70% concentrations on the growth and development of *C. gloeosporioides*. The study shows that garlic extract at 70% concentration was the most effective. Similarly, Falade (2017) evaluated the antifungal effects of six plant extracts: *Blighia sapida*, *Ricinus communis*, *Datura stramonium*, *Tridax procumbens*,*Jatropha gossypifolia* and *Sida acuta* on the mycelia growth of *C.lindemuthianum* the pathogen causing anthracnose disease of cowpea. The result shows that all the plant extracts inhibit the growth of the fungus and efficacy was concentration dependent which agree with the current study.

In this study, all the five plant extracts at the tested concentration did not have any effect on sporulation of *P.cubensis,* this result contradict the report of Obi and Bariuso-vurgas (2004) who reported that sporulation of C. lindemuthianum decreased as the concentration of the active ingredients increased.ln another study,Tegegne *et al.*,2008 reported crude extracts of *Agapanthus africana* plant which was screed against eight economically important plant pathogenic fungi, the result from the study shows that *Pythium ultimum* and to a lesser extent *Fusarium oxysporum* and *Alternaria alternata*showed high degree of tolerance to the extract, the report of which is similar to the current study.Susceptibility of phytopathogenic fungi to botanicals are controlled by anumber of factors which include mode of extraction of the plant active ingredients, age of the plant, mode of exposure to fungi toxic constituents all of which may be responsible for the result that is obtained in this study.

Conclusion

In this study, all the five extracts of the plant had significant effect on conidia germination when modified with PDA after 24 hours incubation at ambient temperature. This findings is in agreement with the work of Amadioha and Obi (2008) who reported that extracts of *Cymbopogon citratus* and *Ocimum gratissimum* inhibited the germination of *Colletotrichum lindemuthianum* the pathogen causing anthracnose disease of cowpea. Similarly, Anteneh *et al.*,(2011) evaluated the effect of 19 different botanicals on mycelia growth and conidia germination of *C. gloeosporioides*, the pathogen causing anthracnose of papaya, and the study shows that the plant extracts inhibited conidia germination.

The mechanism of some indigenous plants causing inhibition of mycelia growth and conidia germination without significant effect on sporulation is not fully understood. There may be a need for evaluating composite mixture of plant extracts in further studies. Thus, such mixtures that has inhibitory effect on growth and germination may produce a more promising result on sporulation if applied.The present study contribute to the list of researches that extracts of the indigenous plant are effective invitro in inhibiting growth of *P. cubensis*. However, further research must be carried out on the field to ascertain their effectiveness.

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