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Water Activity Profiles and Efficacy of Some Plant Extracts on the Growth of *Podosphaeraxanthii* (Powdery Mildew of Muskmelon) in Ado Ekiti, South Western Nigeria

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Abstract **– Laboratory studies was conducted to evaluate the effect of water activity and hot water extracts of Drum stick tree (Moringa oleifera), Jimson weed (Datura stramonium) and the composite mixture on growth, conidia germination and sporulation of *Podosphaeraxanthii* the pathogen causing powdery mildew of muskmelon. The extracts and the composite mixture were applied in situ at three concentrations (20, 40 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 (aw = 0.995) and PDA modified with non-ionic solute glycerol (aw = 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 (aw = 0.995) when water was freely available and decreased with water stress (aw = 0.97 – 0.91). The conidial density was highest under the greatest water stress condition (aw = 0.91). The extracts applied at all the concentrations reduced mycelia growth and conidial germination. The composite mixture of the extracts at 60% concentration was more effective in reducing mycelia growth (3.14mm day-^{1}) compared to the control (4.34mm day-^{1}). Similarly, conidial germination was reduced significantly by the composite mixture values being (40, 27 and 18%) at 60, 40 and 20concentrations 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 *Podosphaeraxanthiiand* the information obtained can be used in the management of the disease on the field.**

Keywords **– Muskmelon, *Podosphaeraxanthii*, Water Activity, Plant Extract, Growth Rate.**

I. INTRODUCTION

Muskmelon (Cucumis melo L) originated from Persia (Iran) and is a cucubit widely grown in many tropical and subtropical regions of the world and consumed for its nutritional qualities (Falade, 2019). World output in 2020 was 28.47 million metric tons (MMT) with China being the largest producer (12.96 MMT), this is closely followed by Turkey (1.779 MMT) and Iran (1.680 MMT). Musklemon is rich in vitamin C being an antioxidant, it helps to boost immunity and contains phytochemicals like beta carotene which helps to keep the gut healthy (Soumya *et. al.,* 2012). In addition, it contains 53 kcal of energy, 13 g of carbohydrates, 1.4 g fibre, 12 g of sugar, 1.3 g of protein, 3126 IU vitamin A, 40.56 mg vitamin C, 531.96 mg potassium, 3,360 mg of folate and 0.3 g of fat (Gene, 1997). The fruit when consumed helps to suppress hypertension because of the richness in potassium, it improves vision 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).

Powdery mildew of muskmelon is an important fungal disease capable of causing between 40-60% yield loss

when not controlled (Savoury *et. al*, 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 small circular yellow lesions observed on the leaves and petioles, the lesions quickly become covered with white spots and may appear on the other side of the leave (Krarup*et. al.*, 2009). White powdery spots can also be formed on both the upper and lower surfaces which can expand into large blotches. The blotches can cover entire leave petioles and stem surfaces (Mary 2014). 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. 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 (Mathu kumar *et. al.*, 2010). The antifungal effects of Jimson weed (*Datura stramonium*) (Usha *et. al.*, 2009 and Falade, 2021), drum stick tree (*Moringa oleifera*) (Ahmadu *et. al.*, 2020 and Falade, 2022) are well known but their use in the management of powdery mildew disease of muskmelon has not been exploited. Based on this, it is imperative to evaluate the effectiveness of hot water extracts of these plants in the management of *P. xanthii*and also determine the effect of varying levels of water activities on the pathogen.

II. MATERIALS AND METHODS

2.1. *Collection of Plant Leaves and Preparation of Extracts*

Leaves of *Moringa oleifera* and *Datura stramonium* were collected from Ekiti State University Teaching and Research Farm, Ado-Ekiti and air-dried at ambient temperature (24 ± 2°C) for 5-6weeks. The dried leaves were turned into powder using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C. Thereafter, 60, 40 and 20g 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. The flasks were maintained at this temperature in hot water bathshaker 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. Xanthii*

Muskmelon plants showing distinct symptoms of powdery 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 powdery mildew pathogen, three of the surface sterilized leaf cuttings were placed on PDA containing chloramphenicol to prevent growth of bacteria. 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 were 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).

2.3. *Effect of Hot Water Extract on Conidia Germination*

One mL of different concentrations (20, 40 and 60% w/v) of the hot water extracts was added to 9 mL molten PDA. The plant extract modified PDA was poured into 9 cm 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. xanthii* 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 was longer 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:

$$\% \text{ germination} = \frac{Germinated\ \ conidia}{Total\ \ counted\ \ conidia} x\ 100 \quad (1)$$

2.4. *Effect of Hot Water Extract on Growth*

In order to evaluate the effect of the hot water 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.

$$\text{PIMG} = \frac{R1-R2}{R1} X\ 100 \quad (2)$$

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

2.5. *Effect of Hot Water Extract on Sporulation*

Agar plugs were taken from three positions on 14 days old culture into a McCartney bottle using 1 cm 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.

2.6. *Effect of Water Activity on Growth of P. Xanthii*

Conidia from 10-day old culture were harvested by flooding the surface of the agar plate with distilled water containing 0.02% Tween 80. The conidia suspension was poured into universal bottles and centrifuged at 1500rpm for 30 minutes. The supernatant was discarded and the conidia suspension was made to 1ml. Serial

dilutions were made and conidia were counted with Improved Neubauer Haemocytometer under x400 objective of Microscope. Thereafter, the conidia suspension was standardized to 10^4 conidia ml^{-1}. Standard Potato Dextrose Agar (PDA, aw = 0.995) and modified PDA media containing calculated amounts of non-ionic solute glycerol at three water activity (aw) levels; 0.97, 0.964 and 0.91were prepared and poured into 9cm Petri-dishes. Three replicate plates at each aw were inoculated at the center with one micro-litre of the standardized conidia suspension. The plates were sealed with parafilm and incubated at ambient temperature 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 axis 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 (Falade, 2016).

2.7. *Effect of Water Activity on Conidiation of P. Xanthii*

One centimeter 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. 1 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 conidia suspension was there after made to 10 ml and spore count was done using x40 objective of light microscope and Haemocytometer. Sporulation density was calculated as the number of conidia cm-2 of fungal colony.

2.6. *Statistical Analysis*

Data were subjected to Analysis of Variance (ANOVA) where significant difference exists a Post-Hoc Turkeys Honesty significant difference was used to separate mean values (IBM SPSS 23).

III. RESULTS

Results Table 1 shows the effect of different concentrations of D. stramonium, M. oleifera extracts and composite mixture of the two plants on germination rates of P. xanthii. All the extracts significantly ($p \leq 0.05$) inhibited conidia germination at all the tested concentration compared to the control. There was 17-35, 12-29 and 27-40% inhibition of conidia germination for D. stramonium, M. oleifera and the composite mixtures respectively compared to the control that had no inhibition. Conidia germination with extracts of D. stramonium at 20, 40 and 60% concentration was 83, 72, and 65% while that of M. oleifera were 88, 79, and 71%. Similarly, at the same concentrations of extracts, the values of the composite mixtures were 82, 73 and 60%.

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

Conc.	D. Stramonium	M. Oleifera	Composite Mixture
20	83*(17)	88*(12)	82*(28)
40	72*(28)	79*(21)	73*(27)
60	65*(35)	71*(29)	60*(40)
Control	100	100100	

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

- Values in parenthesis are % inhibition conidia germination.

Table 2 shows the effect of different concentrations of hot water extracts of *D. stramonium, M. oleifera* and composite mixture of the two plants on growth rates of *P. xanthii.* The growth rate varied significantly in relation to plant extracts and their concentration, with values in the control significantly the highest. At 20, 40 and 60% concentration of extracts *D. stramonium* growth rates were 4.35, 3.94, and 3.68-mm day^{-1} while that of M. oleifera were 4.10, 3.77 and 3.29-mm day^{-1} respectively. Similarly, at the same concentrations, growth rates with that of composite mixtures were 3.94, 3.40 and 3.14 mm day^{-1}. The least growth rates were recorded at the highest 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. xanthii.*

Conc.	D. Stramonium	M. Oleifera	Composite Mixture
20	4.35	4.10	3.94
40	3.94	3.77	3.40
60	3.68	3.29	3.14
Control	4.34	4.34	4.34

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

- Values in parenthesis are % growth radii

Table 3 shows the effect of the hot water extracts of two plants on sporulation of *P. xanthii.* 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 20, 40 and 60% concentrations of D. stramonium, sporulation rates were 5.6, 5.4 and 5.2 while that of M. oleifera were 5.6, 5.8, and 5.7 respectively. Similarly, at this same concentration, 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. xanthii.*

Conc.	D. Stramonium	M. Oleifera	Composite Mixture
20	5.6	5.6	5.4
40	5.4	5.8	5.6
60	5.2	5.7	5.5
Control	5.9	5.9	5.9

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

The rates of growth *P. xanthii* on glycerol modified PDA at different aw 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 aw, where water was freely available and the growth rate reduced significantly as the aw decreased from 0.995 – 0.964. Growth rate was 4.34 mm day^{-1} at 0.995 aw and this reduced to 3.77 mm day^{-1} at aw 0.97. The reduction of aw to 0.91 from 0.96 did not decrease growth. At 0.97 aw, growth inhibition rate was 13 % whereas at 0.96 and 0.91 aw levels, percentage inhibition was 23.0%.

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

Water Activity Levels	Growth Rate (mmday^{-1})	% Inhibition
0.995 (Control)	4.34^{a}	-

Water Activity Levels	Growth Rate (mmday^{-1})	% Inhibition
0.97	3.77^{b}	13
0.96	3.36^{c}	23
0.91	3.36^{c}	23

Means with the same letter are not significantly different according to Turkey's test.

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

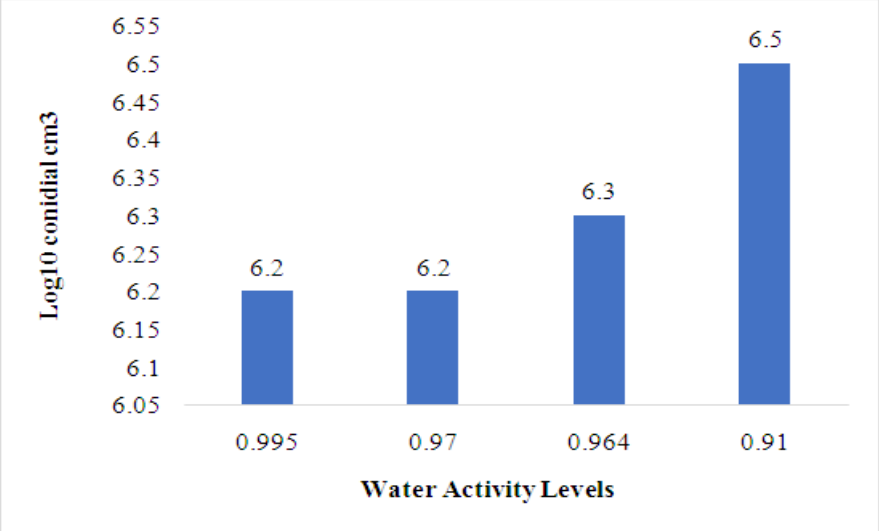

Fig. 1. Effect of water activities on conidial density of *P. xanthii*.

IV. Discussion

Radial growth of *P. xanthii* was monitored over the period of 8 days and the rates of growth on glycerol modified PDA was measured at different levels of water activities (0.995, 0.97, 0.964 and 0.91). Growth of *P. xanthii* was faster when water was freely available (aw = 0.995) and decreased with water stress. However, higher conidia density was noticed under water stress condition (aw = 0.91). The optimum condition that supports conidial formation (aw = 0.91) was different from the one that supports mycelia growth (aw=0.995). Water activity affects the growth and conidiation of phyto- and entomopathogenic fungi in different studies (Abadias *et al.*, 2003 and Falade, 2016. 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 (aw = 0.910 and 0.93) which is in agreement with the current 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. xanthii* under water stress condition (aw 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.

In the study, all the extracts of the two plants (*Moringa oleifera* and *Datura stramonium*) with the composite mixture reduced mycelia growth of *P. xanthii* and the rate of inhibition of growth was concentration dependent. Highest inhibition of growth occurred at relatively higher concentrations of the plant extracts. In the study, the composite mixture of the extracts was more effective in inhibiting growth of the fungus, this was probably due to increased availability of anti-fungal chemicals in the medium that was responsible for suppressing growth (Mukherjee *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)examined the antifungal effects of six plant extracts: *Blighiasapida, Ricinus communis, Datura stramonium, Tridaxprocumbens, Jatropha gossypifolia* and *Sidaacuta* 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, the two plant extracts and the composite mixture at the tested concentration did not have any effect on sporulation of *P. xanthii*, this result negates the study of Obi and Barriuso-Vurgas, 2004 who concluded that sporulation of *C. lindemuthianum* decreased as the concentration of the active ingredients increased. Susceptibility of phytopathogenic fungi to botanicals are influenced by many factors such as 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 the current study.

V. CONCLUSION

The research provided information that water activity significantly affects the growth and reproduction *P. xanthii* causing powdery mildew muskmelon. Management of the disease can be successfully controlled with the use of *Moringa oleifera* and *Datura stramonium* with the aim of increasing crop yield, thereby replacing the conventional fungicide that are costly alongside the attendant side effects.

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