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1 ***Original Research Article***

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

6

7 Abstract

8 Laboratory study was conducted to evaluate the effect of leaf extracts of five indigenous plant on
9 conidia germination, growth and sporulation of *Pseudoperenospora cubensis* causing downy
10 mildew disease of muskmelon. Extracts of five plant ; mexican sunflower (*Tithonia*
11 *diversifolia),* bush banana *(Uvaria chamae),*salt and oil tree *(Cleistopholis patens),*goat weed
12 *(Ageratum conyzoides)* and african eggplant *(Solanum macrocarpon)* at.four concentrations
13 (15,30,45 and 60% *)* were tested against the growth, conidial germination and sporulation of
14 *Pseudoperenospora cubensis* in vitro.

15 Results show that all the plant extracts significantly inhibited conidia germination and radial
16 growth compared to the control. The extracts had no significant ($p \leq 0.05$) effect on sporulation.
17 The rate of inhibition of growth and conidia germination was concentration dependent being
18 highest at 60% for the extracts. The extracts of *Solanum macrocarpon* was the most effective
19 followed by *Ageratum conyzoides*, *Cleistopholis patens* and *Uvaria chamea* while *Tithonia*
20 *diversifolia* caused the least inhibition of growth and conidia germination. At 15, 30, 45 and 60%
21 concentrations growth of *Pseudoperenospora cubensis* on PDA modified with *Solanum*
22 *macrocqrpon* were 3.79, 3.65, 3.33 and 2.87; and 4.25, 4.12, 3.92 and 3.89 for PDA modified
23 with *Tithonia diversifolia*. Similarly, conidia germination percentages recorded at same
24 concentration of extracts *S. macrocarpon* were 87, 88, 70 and 62% while that of *T. diversifolia*
25 were 91, 87, 84 and 72%. The study shows that the plant extracts has the potential for inhibition
26 of the pathogen.

27 **Keywords:** Muskmelon, *Pseudoperenospora cubensis,* conidial germination, growth,
28 sporulation.

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30 INTRODUCTION

31 Muskmelon (*Cucumis melo L*) is a cucubit widely grown in many tropical and subtropical
32 regions of the world and consumed for its nutritional qualities (USDA, 2015).World output in
33 2013 was 29.4 million tons (t) (Ybi, 2007) with India being the largest producer producing 15.1
34 millon t. It contains 53 kcal of energy, 13 g of carbohydrates, 1.4 g fibre, 12 g of sugar, 1.3 g of
35 protein, 3126 IU vitamin A, 40.56 mg vitamin C, 531.96 mg potassium, 3,360 mg of folate and
36 0.3g of fat (Entisar, 2014). The fruit when consumed help to suppress hypertension because of
37 the richness in potassium, improves vision due to high level of vitamin A that strengthens the eye
38 muscle. It also helps to regulate the sugar level, thus controlling diabetes. Besides, the fruit helps
39 to booster body immunity by stimulating the production of white blood cells (Entisar, 2014).

40 Downy mildew of muskmelon is an important fungal disease that can cause up to 100%
41 yield loss when not controlled (Savory, 2011). The pathogen is an obligate parasite that needs
42 living muskmelon plant to grow and survive. Symptoms of the disease are yellow to brown
43 lesions on the upper leaf surfaces. The infection begins as small light green spots that are not
44 water- soaked on the upper leaf surfaces but the spots enlarge and later turn to yellow or brown
45 lesions (Colluci and Holmes, 2010). The disease is spread from plant to plant by air borne spores
46 and infection is favoured by wet weather.

47 The disease can be controlled effectively by the use of fungicides and crop rotation
48 (Mary, 2014). The use of synthetic fungicides like benomyl had proven very effective but the
49 increased awareness of environmental side effects of synthetic pesticides, development of
50 resistant strains of pathogens and toxicity to non-target organisms have tilted attention on the
51 development of alternative method of pathogen control. One of these is the use of plant extracts
52 which are considered cheap and compatible with the farming practices of the farmers (Lowell,
53 2004).

54 The extracts of many plants have been reported to be toxic to many phytopathogenic
55 fungi. The efficacy in plant disease management varies with the concentration of active
56 ingredients in the plant extracts and the strain of the fungus (Mathukumal *et al.,* 2012). The
57 antifungal effects of goat weed (*Ageratum* conyzoides) (Eriyanto, 2016), mexican sunflower
58 (*Tithonia diversifolia*) (Mapa *et al*., 2016), bush banana (*Uvaria chamae*) (Chika *et al*., 2007)
59 african garden egg (*Solanum macrocarpon*) (Yasnawan, 2016) and salt and oil tree (*Cleitopholis*

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60 *patens*) are well known but their use in the management of downy mildew disease of muskmelon
61 has not been exploited. Based on this, it is imperative to evaluate the effectiveness of hot water
62 extracts of these plants in the management of *Pseudoperenospora cubensi,* the pathogen causing
63 downy mildew disease of muskmelon.

64 **2.0 Materials and Method.**

65 **2.1 Collection of plant leaves and preparation of extracts.**

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

75 **2.2 Isolation and morphological identification of *Pseudoperenospora cubensis*.**

76 Muskmelon plants showing distinct symptoms of downy mildew disease were collected from
77 fields at Ekiti State University Teaching and Research farm, Ado –Ekiti, Nigeria. The leaves
78 were cut into pieces of about 1-2 cm and surface sterilized by immersion in 0.2% NaOCl for two
79 minutes. This was followed by two rinses in sterile distilled water and spraying with 70%
80 isopropanol. The sterilized leaves were kept inside a laminar flow cabinet for 20-30 minutes to
81 dry. Five sterilized leaf cuttings were appressed unto the surface of Potato Dextrose Agar (PDA)
82 (Sigma-Aldrich) containing 0.05% chloramphenicol (company purchased) inside 9 cm sterile
83 Petri dishes and removed. For the isolation of the downy mildew pathogen, three of the surface
84 sterilized leaf cuttings were placed on PDA containing chloramphenicol to prevent growth of
85 bacteria. The plates were sealed with parafilm and incubated separately at ambient temperature
86 for 5-6 days. There was no growth on the plates unto which leaves were appressed and this
87 confirmed that the surface of the leaves was sterile. Single conidia from developing colonies in
88 the isolation plate was transferred into prepared standard PDA media to obtain a pure culture.

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89 Agar plugs from single conidia cultures were used for morphological identification on Malt
90 Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular)
91 (Živković, *et al.*, 2010).

92 **2.3 Effect of hot water extract on conidia germination**

93 One mL of different concentrations (15, 30, 45 and 60% w/v) of the extracts was added to 9 mL
94 molten PDA. The plant extract-modified PDA was poured into 9 cm Petri dishes and allowed for
95 1 hour to solidify. The media for the control treatment consisted of standard PDA media alone.
96 The media were inoculated with 10 μL of *P. cubensis* conidia suspension containing 1.0 x 10^2
97 conidia ml^{-1} prepared from 21 days old culture and spread-plated using spatula. The Petri dishes
98 were sealed with parafilm to prevent evaporation of moisture from the agar surface and
99 incubated at ambient temperature for 12 hours. Thereafter, sterile coverslips were placed in three
100 positions on the surface of the agar and viewed under x40 objective of compound microscope. A
101 conidium with the germ tube length which was longer than its diameter was considered as
102 germinated. One hundred conidia were randomly counted in each of the coverslip field and the
103 percentage germination was calculated as:

104 $$\% \text{ germination} = \frac{Germinated\ conidia}{Total\ counted\ conidia} X\ 100$$

105 **2.4 Effect of plant extract on growth**

106 In order to evaluate the effect of the extracts on growth, standard PDA media (control) and plant
107 extract-modified PDA based media were prepared as described previously. The plates were
108 inoculated at the centre with 10 μL of conidia suspension containing 1 x 10^2 conidia ml^{-1} using
109 micro-pipette (Eppendorf 1-10 μL).They were sealed with parafilm and incubated at 20°C for
110 eight days. The treatments and the control were replicated three times. Daily measurement of the
111 colony diameter along two orthogonal axes which were marked on the plates was commenced at
112 24 hours after inoculation and this continued for 5-10 days. The values of the growth rates were
113 averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each
114 treatment and compared with the control (Amadioha, 2003):

115 $$\text{PIMG} = \frac{(R1-R2)\ 100}{R1},$$

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

118 2.5 Effect of plant extract on sporulation

119 Agar plugs were taken from three positions on 14 days old culture into a McCartney bottle using
120 1 cm cork borer and 10 mL of sterile distilled water containing 0.05% Tween-80 (surfactant) was
121 poured into each bottle. The bottle was vortexed for 1-2 minutes to dislodge conidia. The
122 concentration of conidia in the suspension was estimated using a haemocytometer and the
123 density of conidia (conidia cm^{-2} of the colony) was calculated (Borisade and Magan, 2014).

124 3.0 Results

125 3.1 Effect of Hot water Extracts on Conidia germination.

126 Table 1 shows the effect of different concentrations of the leaf extracts on germination rates of
127 *Pseudoperenospora cubensis.* All the extracts significantly ($p \leq 0.05$) inhibited conidia
128 germination when compared with the control. There was 36-9% inhibition of conidia
129 germination for all the extracts compared to the control that had no inhibition. Conidia
130 germination with extracts of *Solanum macrocarpon* at 15, 30, 45 and 60% concentration was 87,
131 85, 70 and 62% while that of *Tithonia diversifolia* at same concentrations were 91, 89, 89 and
132 92%.

133 **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

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

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137 **3.2 Effects of Hot Water Extracts on growth rate.**

138 Table 2 shows the effect of different concentrations of the five leaf extracts on growth rates
139 of *Pseudoperenospora cubensis.* The growth rate varied significantly in relation to plant
140 extracts and their concentration, with values in the control significantly the highest. At 15,
141 30, 45 and 60% concentration of extracts *Solanum macrocarpon* growth rates were 3.79,
142 3.65, 3.33 and 2.87 while that of *Tithonia diversifolia* were 4.25, 4.12, 3.92 and 3.89
143 respectively. Lower growth rates were recorded at higher concentration of all the extracts
144 used in the study.

145 **Table 2: Effect of four concentrations of hot water extracts of five plants on growth rate**
146 **of *Pseudoperenospora cubensis***

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}

147

148 **3.3 Effects of hot water extracts on sporulation**

149 Table 3 shows the effect of the five leaf extracts on sporulation of *Pseudoperenospora*
150 *cubensis.* There was no significant difference in conidia per colony area on all substrates
151 containing the different concentrations of the extracts. At 15, 30, 45 and 60% concentrations
152 of *Solanum macrocarpon,* sporulation rates were 5.5, 5.4. 5.5 and 5.5 while at the same
153 concentration that of *Tithonia diversifolia* the rates were 5.6, 5.6, 5.4 and 5.5 respectively.

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157 Table 3: Effect of extract on Sporulation density *Pseudoperenospora cubensis*

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

158

159 **Discussion**

160 In this study, all the leaves of the five indigenous plants were air dried and powdered to
161 lower the surface area thus increasing the rate of reaction. It has been reported that air dried
162 plant materials are less fragile and do not tend to deteriorate an advantage which it has over
163 fresh samples (Falade, 2017). Bioactive constituents are present in varied form in tissues of
164 plant species and can be used as natural protectants against diseases (Falade & Borisade,
165 2017). In this study, hot water was used for the extraction because it is considered as one of
166 the best methods of extraction because it is capable of preserving the chemistry of the
167 constituents (Vongsak *et al*., 2013).

168 In the study, all the extracts of the five plant: *Tithonia diversifolia*, *Uvaria chamae*,
169 *Cleitopholis patens*, *Ageratum conyzoides* and *Solanum macrocarpon* reduced mycelia
170 growth of *Pseudoperenospora cubensis* and the rate of inhibition of growth was
171 concentration dependent. Highest inhibition of growth occurred at relatively higher
172 concentrations of the plant extracts. This was probably due to increased availability of anti-
173 fungal chemicals in the medium that was responsible for suppressing growth. Mukrejee *et al.,*
174 2011 evaluated the effects of the extracts of Mahogany, giant Indian milky weed, garlic and
175 ginger at 30-70% concentrations on the growth and development of *C. gloeosporioides*. The
176 study shows that garlic extract at 70% concentration was the most effective. Similarly,
177 Falade (2017) evaluated the antifungal effects of six plant extracts: *Blighia sapida*, *Ricinus*
178 *communis*, *Datura stramonium*, *Tridax procumbens*, *Jatropha gossypifolia* and *Sida acuta* on

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179 the mycelia growth of *C .lindemuthianum* the pathogen causing anthracnose disease of
180 cowpea. The result shows that all the plant extracts inhibit the growth of the fungus and
181 efficacy was concentration dependent which agree with the current study.

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

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

202 The mechanism of some indigenous plants causing inhibition of mycelia growth and conidia
203 germination without significant effect on sporulation is not fully understood. There may be a
204 need for evaluating composite mixture of plant extracts in further studies. Thus, such
205 mixtures that has inhibitory effect on growth and germination may produce a more promising
206 result on sporulation if applied. The present study contribute to the list of researches that
207 extracts of the indigenous plant are effective invitro in inhibiting growth of

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208 *Pseudoperenospora cubensis*. However, further research must be carried out on the field to
209 ascertain their effectiveness.

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