

**EFFICACY OF THREE SELECTED BOTANICALS IN THE CONTROL OF
BOTRYTIS CINEREA ASSOCIATED WITH DAMPING OFF IN *CITRULLUS
LANATUS* (THUNB.) MATSUM AND NAKAI**

***ADEBOLA, M. O., BELLO, T. S., YUSUF, H. AND EGUBAGI, J. M.**

Federal University of Technology Minna, School of Life Sciences, Department of
Plant Biology, P.M.B. 65, Minna Niger State, Nigeria

*Corresponding author: adebolamo@gmail.com

ABSTRACT

The use of plant extracts in controlling plant diseases has witnessed appreciable success; such was exploited in this study when efficacy of extracts from *Azadirachta indica*, *Calotropis procera* and *Anacardium occidentale*, each at different concentrations; 30 mg ml⁻¹, 60 mg ml⁻¹, 90 mg ml⁻¹ and 0 mg ml⁻¹ (Control), were examined *in vitro* to control *Botrytis cinerea* that causes damping off disease of *Citrullus lanatus* var. 'lanatus'. Result showed that the mean inhibition in colony diameter at 90 mg ml⁻¹ ranged from 0.26-2.26 (*A. indica*), 0.43-3.30 (*C. procera*) and 0.13-2.50 (*A. occidentale*) compared respectively to 0.5-4.36, 0.57-4.33 and 0.43-4.10 in their 0 mg ml⁻¹ concentration. The 90 mg ml⁻¹ concentration that has the least fungal growth evident at different days in all extract used was significantly different ($p \leq 0.05$) from the 0 mg ml⁻¹. Furthermore, percentage inhibition in *A. occidentale* (37.50%) and *A. indica* (43.33%) were slightly significant ($p \leq 0.05$) at 90 mg ml⁻¹ concentration but was significant ($p \leq 0.05$) in *C. procera* (17.50%). From this study, it can therefore be recommended that field trial of these medicinal plants on the control of damping off disease caused by *B. cinerea* can be achieved as the concentration of the extracts used here increases. Also, the use of these extracts should be inculcated as part of cultural control measure, since they are easy, simple and cost-effective in making.

Keywords: Damping off, Disease control, Fungicidal, Phytochemicals, Methanolic extract, Soil-borne fungi

INTRODUCTION

Watermelon (*Citrullus lanatus* var. 'lanatus') is a tender, warm season vegetable belonging to the family Cucurbitaceae (Renner and Chomicki, 2017). It is enjoyed by many people across the world as fresh fruit. It is highly nutritious and thirst-quenching that contains vitamins A and C in the form of disease-fighting beta-carotene (Adesanya *et*

al., 2011). Watermelon is rich in carotenoids, some of the carotenoids of which include lycopene, phytofluene, phytoene, beta-carotene, lutein and neurosporene (Pinto *et al.*, 2011). Potassium is also available in it, which is believed to help, in the control of blood pressure, and possibly prevention of stroke (Darin, 2003). Citrulline is a non-essential amino acid first identified from the juice of

watermelon. Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles (Adesanya *et al.*, 2011). Watermelon production has been low in spite of its values (FAOSTAT, 2018).

Plants and their metabolites are beneficial in fungal biomass production (Adebola *et al.*, 2018). Fungi in turn causes diseases such as *Fusarium* wilt, powdery mildew, downy mildew, gummy stem blight, damping-off, etc., that can result in significant loss in plant yield, including watermelon (Michael *et al.*, 2017). Mary (2011) found that damping-off is caused by several soil-borne fungi or fungus-like organisms commonly found in soils that may or may not have been previously cultivated. It is most severe when temperatures, light and other environmental conditions are unfavourable for seedling growth (Mary, 2011). It is commonly caused by several soil borne pathogens like: *Rhizoctonia solani*, *Thielaviopsis basicola*, *Pythium* spp., *Sclerotinia minor*, *Verticillium dahlia*, *Botrytis cinerea*, etc. (Mary, 2011). Damping-off caused by *Botrytis cinerea* is a filamentous, heterothallic ascomycete soil borne fungi (Ratna *et al.*, 2015). Seedlings are attacked either before they emerge (called pre-emergence damping off) or after (called post-emergence damping off).

The use of botanicals instead of chemical fungicides is one of the recent approaches for plant disease control. These plants contain some organic compounds which produce definite physiological action. Victor and Chidi (2009) listed these compounds to include; tannins, alkaloids, terpenoids, steroids and flavonoids. They also included alkaloids, coumarin, flavonoids, saponins and volatile constituents of the essential oils as being allelopathic agents.

Efforts have been made in controlling wide range of seed borne pathogens by different botanicals (Adebola *et al.*, 2018). A few researches have been geared to evaluate the efficacy of botanicals against soil borne pathogens (Monaim *et al.*, 2011; Mahmood and Muhammad, 2013).

In view of the above, this study was embarked on to evaluate the efficacy of extracts from *Azadirachta indica*, *Calotropis procera* and *Anacardium occidentale* in controlling mycelial growth of *Botrytis cinerea*; causal agent of damping off disease in watermelon.

MATERIALS AND METHODS

Experimental Site and Design

This experiment was carried out at the Botanical Garden and at the Department of Plant Biology laboratory, Federal University of Technology, Minna. The experiment was laid in a Completely Randomized Design with three replicates.

Collection of Materials

At the Botanical Garden, the healthy and matured leaves of *Calotropis procera* (Sodom apple), *Azadirachta indica* (Neem plant) and *Anacardium occidentale* (Cashew plant) were aseptically collected. Rhizosphere sample of watermelon showing symptoms of damping off were collected aseptically.

Plant Preparation and Extraction Procedures

Fresh leaves of *Calotropis procera*, *Anacardium occidentale* and *Azadirachta indica* were washed with sodium hypochloride and rinsed with clean water. They were dried at room temperature for 10-12 days (Cashew and Neem) and for 14-16 days (Sodom

apple). The dried materials were homogenized into powdered form. The powder of *C. procera*, *A. occidentale* and *A. indica* (50g each) were heated in 250ml methanol (in a ratio of 1:4) using a Soxhlet extractor for 4 hrs at temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper (No. 1), concentrated in vacuum, dried at 45°C for ethanol removal, and the extracts were kept in sterile bottles under refrigerated conditions until use (Jensen, 2007).

Isolation of Fungi

From the collected rhizosphere of *C. lanatus* showing symptoms of damping off, soil sample of it, about 1.0g, was obtained to make sample suspension when aseptically mixed with 10ml of distilled water. This was mixed well for 15 minutes and was serially diluted to 10⁻⁶. Five (5) ml was pipetted into plates with PDA medium, swerved slightly and incubated at 27±2°C for 3-7 days (Nazir *et al.*, 2007). After the incubation period, subculturing was done so as to have pure culture of *B. cinerea* (Jonathan *et al.*, 2017).

Identification of the Fungi

Identification of *B. cinerea* was based on morphological examination (length, width colour and texture of the colony, the presence or absence of aerial mycelium) and microscopic examination was carried out using compound microscope (Ratna *et al.*, 2015).

Pathogenicity Test

Pathogenicity of the isolated fungi was established by testing for their

ability to induce damping off in healthy watermelon. Pathogenicity test was performed using a cork borer of 5mm to place *Botrytis cinerea* culture onto the surface of the soil around a healthy potted watermelon plant. Inoculated plants were incubated in humid sterile plastic pots (Adebola and Amadi, 2012).

Phytochemical Analysis on the Extracts

A preliminary phytochemical screening of the plant extracts were carried out for the presence of phytochemical compounds using methods described by Hassan (2006) and Prashanth *et al.* (2011).

Antifungal Activity of the Plant Extracts

This was done using direct plate method. The leaf extract of each plant was incorporated into replicated plates after dilution in 100ml of water at the following concentrations: 30 mg ml⁻¹, 60 mg ml⁻¹, 90 mg ml⁻¹ and 0 mg ml⁻¹ (Control). The plates were allowed to solidify. Isolates of *B. cinerea* were inoculated into the solidified medium using mycelia disc of 5mm disc per plate. The antifungal activity of the extracts was determined by measuring the mycelia growth of *B. cinerea* on each of the plate by drawing two perpendicular lines which meets at a right angle at the centre of the plate. The plates are then incubated at 27±2°C for 7 days. After each day, the diameter of the growth was measured using a meter rule. Antifungal action of the extracts was calculated using the formula below;

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter of (Control-Treatment)}}{\text{Colony Diameter of Control}} \times 100$$

Data Analysis

Statistical analyses of inhibition of radial growth was subjected to one way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 17.0 and means was separated according to Duncan's Multiple Range Test (DMRT) at 5% probability level.

RESULTS

Plate 1a shows the appearance of *B. cinerea* cultured for 7 days on a PDA plate. The fungus appears as pale brown in colour. Plate 1b shows the photomicroscopy of the fungus when

examined under light microscope. The mycelium was observed to have a set of loculated hyphae that are septate and hyaline. Conidiophore (Cd) of *B. cinerea* that branched at the apical region appears like a tree-like structure. At the terminal end of the branched conidiophore were observed globose vesicles where conidia (C) are grouped.

Symptoms of post emergence damping off were observed after six days of inoculation on healthy watermelon during pathogenicity test conducted *in vitro*. Symptoms observed include leaf blight and stem rot as shown in Plate 1c.

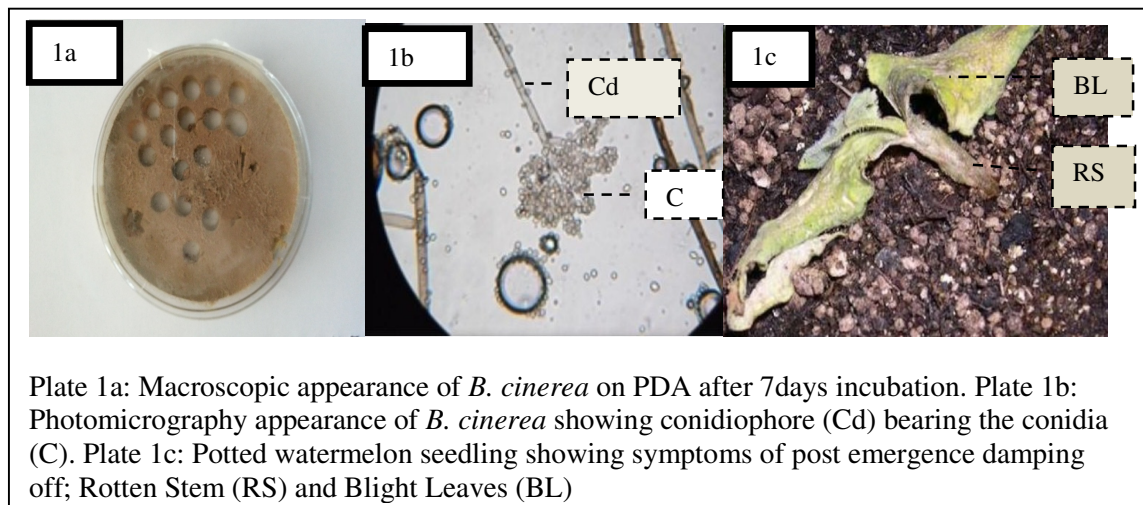


Plate 1a: Macroscopic appearance of *B. cinerea* on PDA after 7 days incubation. Plate 1b: Photomicrography appearance of *B. cinerea* showing conidiophore (Cd) bearing the conidia (C). Plate 1c: Potted watermelon seedling showing symptoms of post emergence damping off; Rotten Stem (RS) and Blight Leaves (BL)

Phytochemical Screening

Phytochemical analysis (Table 1) revealed the presence of the alkaloid, saponin, tannin, anthocyanin, phenol and flavonoid in the four plant extracts screened. Alkaloids, saponin, tannins

and flavonoids were found in all the plant extracts while *C. papaya* and *A. indica* did not contain steroid and phenols; and only steroid was not found in *A. occidentale*.

Table 1: Phytochemical constituent of the methanolic leaf extract of *Azadirachta indica*, *Anacardium occidentale* and *Calotropis procera*

Phytochemical constituents	<i>A. indica</i>	<i>A. occidentale</i>	<i>C. procera</i>
Tannin	+	+	+
Flavonoid	+	+	+
Saponin	+	+	+
Steroid	-	-	-
Alkaloid	+	+	+
Terpenes	+	+	+
Phenols	-	+	-

Note: + = Present, - = Absent

Table 2: Effects of methanolic leaf extract of *Azadirachta indica* at varying concentrations on mycelial growth of *Botrytis cinerea*

mgml ⁻¹	Day 1(mm)	Day 2(mm)	Day 3(mm)	Day 4(mm)	Day 5(mm)	Day 6(mm)	Day 7(mm)
30	0.00 ± 0.00	0.23±0.03 ^b	0.96±0.06 ^c	1.70±0.10 ^c	2.46±0.20 ^c	3.46±0.29 ^c	3.76±0.14 ^c
60	0.00 ± 0.00	0.16±0.03 ^b	0.50±0.00 ^b	1.00±0.05 ^b	1.86±0.06 ^b	2.66±0.12 ^b	3.16±0.12 ^b
90	0.00 ± 0.00	0.00±0.00 ^a	0.26±0.03 ^a	0.50±0.00 ^a	1.10±0.10 ^a	1.43±0.09 ^a	2.26±0.14 ^a
0	0.00 ± 0.00	0.50±0.01 ^c	1.60±0.02 ^d	2.58±0.02 ^d	3.23±0.11 ^d	4.05±0.03 ^d	4.36±0.27 ^d

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p \leq 0.05$).

Effects of methanolic leaf extract of Azadirachta indica on mycelial growth of Botrytis cinerea

The effect of the methanolic leaf extract of *A. indica* against *B. cinerea* was presented in Table 2. It shows that at day one (1), the different concentration used have 0.00% effect on the fungal mycelia growth. At day two (2), there was no significant difference ($p \leq 0.05$) between the fungal growth of the 30 mg ml⁻¹ (0.23±0.03) and 60 mg ml⁻¹ (0.16±0.03) although, there was significant difference ($p \leq 0.05$) between the treatment 90 mg ml⁻¹ (0.00±0.00) that was observed to have no fungal growth. The control treatment (0.50±0.01) however, has the highest fungal growth. At day three (3), there was much significant difference ($p \leq 0.05$) between the concentrations of 90 mg ml⁻¹ (0.26±0.03) and the control (1.60±0.02), compared to the

concentrations of 30 mg ml⁻¹ (0.96±0.06) and 60 mg ml⁻¹ (0.50±0.00) that has slight significant differences ($p \leq 0.05$).

At day four (4), there was significant difference ($p \leq 0.05$) between concentration of 90 mg ml⁻¹ (0.50±0.00) and the control (2.58±0.02), while there was no significant difference ($p \leq 0.05$) between the concentrations of 30 mg ml⁻¹ (1.70±0.10) and 60 mg ml⁻¹ (1.00±0.05). At day five (5), the concentrations of 30 mg ml⁻¹ (2.46±0.20) and 60 mg ml⁻¹ (1.86±0.06) shows little significant difference ($p \leq 0.05$), as the concentration of 90 mg ml⁻¹ (1.10±0.10) is significantly different ($p \leq 0.05$) from the control (3.23±0.11). At day six (6), there was great significant difference ($p \leq 0.05$) between 90 mg ml⁻¹ (1.43±0.09) and the control (4.05±0.03) concentrations, and there was a slight significant difference

($p \leq 0.05$) between the concentrations of 30 mg ml⁻¹ (3.46±0.29) and 60 mg ml⁻¹ (2.66±0.12). At day seven (7), there was significant difference ($p \leq 0.05$) between the concentrations of 30 mg ml⁻¹ (3.76±0.14) and 60 mg ml⁻¹

(3.16±0.12), the concentration 90 mg ml⁻¹ (2.26±0.14) that has the least fungal growth was significantly different ($p \leq 0.05$) from the control (4.36±0.27) that was observed to have the highest growth.

Table3: Effects of methanolic leaf extract of *Calotropis procera* at varying concentrations on mycelial growth of *Botrytis cinerea*

mg ml ⁻¹	Day 1(mm)	Day 2(mm)	Day 3(mm)	Day 4(mm)	Day 5(mm)	Day 6(mm)	Day 7(mm)
30	0.00 ± 0.00	0.83±0.12 ^b	1.83±0.03 ^c	3.03±0.26 ^c	3.53±0.06 ^c	3.93±0.03 ^b	4.00±0.00 ^b
60	0.00 ± 0.00	0.80±0.10 ^b	1.16±0.03 ^{ab}	2.73±0.08 ^{bc}	3.23±0.08 ^{ab}	3.73±0.08 ^b	3.90±0.05 ^b
90	0.00 ± 0.00	0.43±0.06 ^a	0.90±0.20 ^a	1.80±0.15 ^a	2.50±0.05 ^a	3.00±0.05 ^a	3.30±0.11 ^a
0	0.00 ± 0.00	0.57±0.064 ^{ab}	1.43±0.16 ^{bc}	2.24±0.32 ^{ab}	3.13±0.18 ^b	3.78±0.29 ^b	4.33±0.29 ^b

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p \leq 0.05$).

Effects of methanolic leaf extract of *Calotropis procera* on mycelial growth of *Botrytis cinerea*

The result in Table 3 shows effect of the methanolic leaf extract of *Calotropis procera* against *Botrytis cinerea*. At day one (1), record shows that the different concentration used have 0.00% effect on the fungal mycelia growth. Day two (2), there was no significant difference ($p \leq 0.05$) in fungal growth between the concentrations of the 30 mg ml⁻¹ (0.83±0.12) and 60 mg ml⁻¹ (0.80±0.10). Also there was no significant difference ($p \leq 0.05$) between the concentration of 90 mg ml⁻¹ (0.43±0.06) and the control (0.57±0.064) treatment. Day three (3), there was significant difference ($p \leq 0.05$) between the concentrations of 90 mg ml⁻¹ (0.90±0.20) and the control (1.43±0.16), however, the concentrations of 30 mg ml⁻¹ (1.83±0.03) and 60 mg ml⁻¹ (1.16±0.03) shows slight significant differences ($p \leq 0.05$).

At day four (4), there was no significant difference ($p \leq 0.05$) between the concentrations of 30 mg ml⁻¹ (3.03±0.26) and 60 mg ml⁻¹ (2.73±0.08), and there was little significant difference ($p \leq 0.05$) between the concentration of 90 mg ml⁻¹ (1.80±0.15) and the control (2.24±0.32). Day five (5), the concentrations of 30 mg ml⁻¹ (3.53±0.06) and 60 mg ml⁻¹ (3.23±0.08) shows significant difference ($p \leq 0.05$), as the concentration of 90 mg ml⁻¹ (2.50±0.05) shows high significant difference ($p \leq 0.05$) from the control group (3.13±0.18). Day six (6), there was slight significant difference ($p \leq 0.05$) between concentration 90 mg ml⁻¹ (3.00±0.05) and the control group (3.78±0.29), and there was no significant difference ($p \leq 0.05$) between the concentrations of 30 mg ml⁻¹ (3.93±0.03) and 60 mg ml⁻¹ (3.73±0.08). Day seven (7), there was no significant difference ($p \leq 0.05$) at all between the concentrations of 30 mg ml⁻¹ (4.00±0.00) and 60 mg ml⁻¹

(3.90±0.05), the concentration 90 mg ml⁻¹ (3.30±0.11) was significantly different (p≤0.05) from the control

(4.33±0.29) which has the highest growth.

Table 4: Effects of methanolic leaf extract of *Anacardium occidentale* at varying concentrations on mycelial growth of *Botrytis cinerea*

mg ml ⁻¹	Day 1(mm)	Day 2(mm)	Day 3(mm)	Day 4(mm)	Day 5(mm)	Day 6(mm)	Day 7(mm)
30	0.00 ± 0.00	1.00±0.05 ^c	1.80±0.05 ^d	2.10±0.05 ^d	2.60±0.15 ^b	3.10±0.10 ^c	3.80±0.11 ^c
60	0.00 ± 0.00	0.50±0.00 ^b	0.56±0.03 ^b	0.90±0.05 ^b	1.26±0.08 ^a	2.46±0.06 ^b	3.23±0.03 ^b
90	0.00 ± 0.00	0.13±0.03 ^a	0.30±0.10 ^a	0.53±0.03 ^a	0.76±0.12 ^a	1.53±0.12 ^a	2.50±0.15 ^a
0	0.00 ± 0.00	0.43±0.02 ^b	1.42±0.01 ^c	1.91±0.01 ^c	2.72±0.01 ^b	3.36±0.12 ^c	4.10±0.05 ^c

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different (p ≤ 0.05).

Effects of methanolic leaf extract of *Anacardium occidentale* on mycelial growth of *Botrytis cinerea*

The result in Table 4 shows the inhibitory effect of the aqueous leaf extract of *Anacardium occidentale* against *Botrytis cinerea*. At day one (1), record shows that the different concentration used have 0.00% effect on the fungal mycelia growth. Day two (2), there was little significant difference (p≤0.05) in fungal growth between the concentrations of the 30 mg ml⁻¹ (1.00±0.05) and 60 mg ml⁻¹ (0.50±0.00). Also little significant difference (p≤0.05) was observed between the concentration of 90 mg ml⁻¹ (0.13±0.03) and the control treatment (0.43±0.02). Day three (3), there was significant difference (p≤0.05) between the concentrations of 30 mg ml⁻¹ (1.80±0.05) and that of 60 mg ml⁻¹ (0.56±0.03). The concentration of 90 mg ml⁻¹ (0.30±0.10) was significantly different (p≤0.05) from the control (1.42±0.01).

At day four (4), there was significant difference (p≤0.05) between

the concentrations of 30 mg ml⁻¹ (2.10±0.05) and 60 mg ml⁻¹ (0.90±0.05). Also, there was significant difference (p≤0.05) between the concentration of 90 mg ml⁻¹ (0.53±0.03) and the control (1.91±0.01). Day five (5), the concentrations of 30 mg ml⁻¹ (2.60±0.15) and 60 mg ml⁻¹ (1.26±0.08) shows much significant difference (p≤0.05); just as the concentration of 90 mg ml⁻¹ (0.76±0.12) shows significant difference (p≤0.05) with the control (2.72±0.01). Day six (6), the concentration of 30 mg ml⁻¹ (3.10±0.10) was significantly different (p≤0.05) from that of 60 mg ml⁻¹ (2.46±0.06). Also, the concentration of 90 mg ml⁻¹ (1.53±0.12) shows significant difference (p≤0.05) with the control (3.36±0.12). Day seven (7), there was little significant difference (p≤0.05) between the concentrations of 30 mg ml⁻¹ (3.80±0.11) and 60 mg ml⁻¹ (3.23±0.03). The concentration 90 mg ml⁻¹ (2.50±0.15) having the least fungal growth was significantly different (p≤0.05) from the control (4.10±0.05) which has the highest growth.

Table 5: Effects of tested plant extracts on percentage mycelia growth inhibition of *B. cinerea*

mg ml ⁻¹	<i>A. indica</i> (%)	<i>C. procera</i> (%)	<i>A. occidentale</i> (%)
30	5.88±1.34 ^b	0.00±0.00 ^a	5.00±0.03 ^b
60	20.83±2.45 ^c	2.50±2.34 ^b	19.16±2.75 ^c
90	43.33±4.56 ^d	17.50±2.56 ^c	37.50±4.56 ^d
0	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Values along the same column with different superscripts are significantly different ($p \leq 0.05$).

Percentage mycelial growth inhibition of *Botrytis cinerea* by *Anarcadium occidentale*, *Azadirachta indica* and *Calotropis procera* leaf extracts

The result in Table 5 present the percentage mycelial growth inhibition of *Botrytis cinerea* by *Anarcadium occidentale*, *Azadirachta indica* and *Calotropis procera* leaf extracts. The methanolic leaf extract of *A. indica* at 90 mg ml⁻¹ concentration significantly ($p \leq 0.05$) prevents the fungal mycelial growth by 43.33% when compared with the 30 mg ml⁻¹ concentration of the leaf extract that inhibited the mycelial growth by 5.88%. The fungal growth in the methanolic leaf extract of *C. procera* showed no inhibition at all at the concentration of 30 mg ml⁻¹ compared to the concentration of 90 mg ml⁻¹ which showed about 17.50% inhibition. The methanolic extract of *A. occidentale* showed potential of inhibition at the concentration of 90 mg ml⁻¹ as it inhibited the mycelial growth by 37.50% but showed low inhibition to be only 5% at the concentration of 30 mg ml⁻¹.

DISCUSSIONS

The descriptive characteristics of *B. cinerea* observed in this study were in agreement with those described by Ratna et al. (2015). These features could

be indicative of a typical ascomycete fungus.

Symptoms observed in this finding were in accordance with those reported by Michael (2014). These which were observed few days after inoculation with a healthy watermelon seedling may be indications that post emergence damping off do exist in this plant.

The presence of methanolic compounds in the extracts used conforms to previous findings (Qian and Nihorimbere, 2004; Adebola et al. (2018). Presence of these phytochemicals could be an indication of their fungicidal activity.

Records on daily growth of *Botrytis cinerea* showed that the methanolic plant extract acted as an inhibitory constituent to the amended media. The inhibitory effect of the methanolic leaf extracts of the plants may be due to the presence of some phytochemicals like tannins, saponin, flavonoid, and alkaloids etc., that have antimicrobial properties. This can be supported by painstaking studies that have been carried out on different phytochemicals of plants (Tasleem et al., 2009; Adebola et al., 2018).

The *in vitro* control of *B. cinerea* using plant extracts obtained from leaves of *A. indica*, *C. procera* and *A. occidentale* showed that they all significantly reduced the pathogenic

activities of the fungus from the second day. This agrees with previously reported findings (Goyal and Mathur, 2011; El-Hawary *et al.*, 2013; Omojate *et al.*, 2014; Aderiye *et al.*, 2015; Manoorkar *et al.*, 2015). Perhaps it could be that these extracts were unable to arrest the physiology of *B. cinerea* not until the second day when there are noticeable vegetative growths by this fungus.

The use of low-cost, readily available and phytotoxic approaches are needed when exclusion of pathogen and disease management are issue of concern. Independent researchers have studied the phytochemical properties of leaves and stem of these plants. Their result showed that these plants are potentially fungicidal (El-Hawary *et al.*, 2013, Manoorkar *et al.*, 2015; Adebola *et al.*, 2018).

CONCLUSIONS

This study has shown the antifungal activities of the methanolic leaf extract of three medicinal plants. The efficacy of the methanolic leaf extract of these plants increased with increase in concentration; 90 mg ml⁻¹ concentration showed the highest level of inhibition. The methanolic leaf extract of *Azadirachta indica*, *Anacardium occidentale*, *Calotropis procera* in that order, proved to be effective in the inhibition of *Botrytis cinerea* at the three concentrations. Since the plants are easy to find and prepare, they can be used as substitute to chemical fungicide in the control of damping off disease in watermelon.

REFERENCES

- Adebola, M. O. and Amadi, J. E. (2012). Studies on *Penicillium digitatum*, *Botryodiplodia theobromae*, *Alternaria tenuis* and *Trichoderma harzianum* for Bicontrol of *Phytophthora palmivora* Cocoa Black Pod Disease Pathogen. *American-Eurasian Journal of Agronomy*, 5(2):30-34.
- Adebola, M. O., Ayeni, O. B., Aremu, M. B. and Kalesanwo, A. O. (2018). Evaluation of Leaf Extracts of Four Plant Species against Rice Blast Pathogen (*Magnoportha oryzae* (T.T. Hebert) M.E. Barr.). *Nigerian Journal of Mycology*, 10: 88-101.
- Adebola, M. O., Yusuf, S., Bello, T. S. and Kalesanwo, A. O. (2018). Effects of Different Plant Sawdust substrates on the cultivation of *Pleurotus ostreatus*. *Nigerian Journal of Mycology*, 10:116-128.
- Aderiye, B. I., David, O. M. and Atere, V. A. (2015). Administration of cashew extracts in the treatment of some infections and diseases. *Advancement in Medicinal Plant Research*, 3(3): 75-86.
- Adesanya, A. O., Olaseinde, O. O., Oguntayo, O. D., Otulana, J. O. and Adefule, A. K. (2011). Effects of Methanolic Extract of *Citrullus lanatus* Seed on Experimentally Induced Prostate Hyperplasia. *European. Journal of Medicinal Plants*, 1(4): 171-179.
- Darin, I. (2003). Watermelon Juice a Rich Source of Lycopene and Beta-Carotene. *Journal of Nutrition*, 13(3): 1043–5032.

- El-Hawary, S. S., El-Tantawy, M. E., Rabeh, M. A. and Badr. W.K. (2013). Chemical composition and biological activities of essential oils of *Azadirachta indica* A. Juss. *International Journal of Applied Research in Natural Products*, 2(6): 33- 42.
- Food and Agriculture Organization of the United Nations, Statistics Division (2018). Watermelon production in 2016. Crops/ Regions (World list)/ Production quantity. Retrieved 10 February 2018.
- Goyal, M. and Mathur, R. (2011). Antimicrobial potential and Phytochemical analysis of plant Extracts of *Calotropis procera*. *International Journal of Drug Discovery and Herbal Research*, 1(3): 138-143.
- Islam, M. T., Islam, M. R., Aminuzzaman, F. M. and Yesmin, S. (2006). Management of extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, 28(1): 99
- Jensen, W. B. (2007). The origin of Soxhlet extractor. *Journal of Chemistry Education*, 84(12):1913-1914.
- Jonathan, S. G., Bello, T. S. and Asemoloye, M. D. (2017). Food Values, Spoilage Moulds and Aflatoxin Detection in 'Attieke' (A Cassava Fermented Product). *Journal of Microbial and Biochemical Technology*, 9: 244-248.
- Mahmood, A. and Muhammad, W. (2013). Plants as potential source of antimicrobial agents. *Journal of Pharmacy and Alternative Medicine*, 4(7): 222-566
- Manoorkar, V. B., Mandge, S. V. and Gachande, B. D. (2015). Antifungal Activity of Leaf and Latex Extracts of *Calotropis procera* against Dominant Seed-Borne Storage Fungi of Some Oil Seeds. *Bioscience Discovery*, 6(1): 22-26
- Mary, W. O. (2011). Damping off. The University of Arizona College of Agriculture and Life Sciences Tucson, Arizona 8(5): 7-21.
- Michael, D. O., William, J. L., Lynn, F. K., Steven, M. B. and Jayson, K. H. (2017). Watermelon Production. *Penn State Extension, Northumberland*, 8(1): 7857-9723.
- Monaim, A. M. F., Elyousr, K. A. and Morsy, K. M. (2011). Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (*Lupinus termis* Forsik). *Crop Protection*, 1(30): 185-195.
- Nazir, N., Mirza, J.H., Akhtar, N., Bajwa, R. and Nasin, G. (2007). Some studies of thermophilic and thermotolerant fungi from Lahore. *Pakistan. Mycopathology*, 5: 95-100.
- Omojate, C. G., Felix, O. E., Clement, O. A., Oghenejobo, M. and Akpotu, M. (2014). A Review on the Phytochemical and Anti-Hyperglycaemic Properties of the Fractionated *Anacardium occidentale* Leaves, Seeds and Stem Barks Extracts. *IOSR Journal of Pharmacy*, 2(25): 3-13.
- Pinto, M. P., dos Santos, C. N., Henriquesa, C., Lima, G. and

- Quedas, F. (2011). Lycopene content and antioxidant capacity of Portuguese watermelon fruits. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 10(4): 2090–2097.
- Prashanth, T., Bimlesh, K., Mandeep, K., Gurpreet, K. and Harleen, K. (2011). Phytochemical Screening and extraction. A review. *International Pharmaceutical Science*, 1(2): 34-60.
- Qian, H. and Nihorimbere, V. (2004). Antioxidant power of phytochemicals from *Psidium guajava* leaf. *Journal of Zhejiang Universal Science*, 5: 676-683.
- Ratna, K. H., Shiny, N. P. and Samuel, K.K. (2015). Isolation and identification of soil mycoflora in agricultural fields at tekkalimandal in srikakulam district. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 2(1): 2277-4688.
- Renner, S. S. and Chomicki, G. (2017). Watermelon origin solved with molecular phylogenetics including Linnaean material: Another example of museomics. *New Phytologist*, 205: 526-532.
- Tasleem, A., Bhosale, J. D., Naresh, K., Mandal, T.K., Bendre, R.S., Lavekar, G. S. and Rajesh, D. (2009). Natural products – antifungal agents derived from plants. *Journal of Asian Natural Products Research*, 11(7): 621–638.
- Victor, N. O. and Chidi, O. (2009). Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry*, 3(11): 228-233.