E-ISSN: 2789-3073
P-ISSN: 2789-3065
IJPPM 2021; 1(2): 19-23
Received: 19-03-2021
Accepted: 22-04-2021

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Timing of fungal infection of water melon (*Citrullus lanatus* var *lanatus*) inoculated with *Colletotrichum orbiculare* in south western Nigeria

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Abstract
Anthracnose of water melon is an important fungal disease reducing yield by 20-100% when not controlled. Field experiment was carried out to evaluate the effect of time and sequence of application of infective conidia and extracts of three plants: *Physalis angulata*, *Nicotiana tabacum* and *Hyptis suaveolens* on disease incidence, severity and fungal infection of water melon inoculated with *Colletotrichum orbiculare*. Three concentrations of the extracts (20, 40 and 60%) were used in the study. Total area of the farm (625m^2) was divided into three sub plots in a factorial experiment. The first plot was sprayed with extracts of the three plants at the tested concentrations and after 48 hours inoculated with the conidia of the fungus. The second plot was sprayed with the conidia of the fungus followed by application of the different extracts after 48 hours. The third plot was treated simultaneously with the different concentrations of the extracts and the inoculum of the fungus. Control plot in each case was sprayed with distilled water. The result showed that all the extracts were effective in reducing the incidence and severity of the disease and values obtained were significantly ($p \leq 0.05$) lower than the control. At 20, 40 and 60% concentration of *P. angulate* extract, disease incidence in percentages were 23.5, 22.7 and 18.3% compared to the control (75.4%) when extracts were applied two days before inoculation. Similarly, percentage germination of seeds was 81.9, 84 and 92.7% when extracts of *P. angulata* were applied at 20, 40 and 60% concentrations respectively compared to the control (53.7%). In terms of disease severity, trace infections of symptoms were noticeable on treated plots compared to the control (severe infection). Extracts of *P. angulata* were the most effective followed by *N. tabacum* and *H. suaveolens*. The study demonstrated that the extract of the plants can be used in the management of anthracnose disease of water melon.

Keywords: *C. orbiculare*, disease incidence, fungal infection, watermelon

1. Introduction
Water melon (*Citrullus lanatus varlanatus*) is a Curcubit family cultivated both in the tropical and temperate zones as a means of staple food. The crop originated from North Eastern Africa but it is now naturalized in all agro ecological zones (Toluwase and Owoeye, 2017) [12]. In terms of chemical composition, water melon contains about 92% water, 30Kcal energy, 7.6g carbohydrate, 0.6g protein, 0.15g fat, 0.4g fiber, 3ug folates, 0.178mg niacin, 0.221mg Pantothenic acid, 0.045mg pyridoxine, 0.033mg thiamin, 56gIu vitamin A, 8.1mg vitamin C, 0.05mg vitamin E and varying amounts of electrolyte, minerals and other phytonutrients (FAOSTAT, 2015) [7]. The fruit when consumed helps to increase nitric oxide level in the body thus helping the blood vessels to expand which invariably lower blood pressure, the fruit has nine vitamins and minerals useful to the body, it also contains antioxidant that help to prevent cell damage. Apart from this, the fruit helps to improve digestion and conditions of the heart thus lowering oxidative stress (USDA, 2015) [13].
World production of water melon in 2012 was 88 million metric tonnes, half of which is produced annually in Asia (Kerri- Ann Jennings, 2016) [8]. China is recorded as the leading producer with over 67% of the total world production, within the last few decades, output is on the increase in south western Nigeria (Ajewole, 2015) [1]. Anthracnose of water melon is an important fungal disease that causes significant yield loss of between 20-100% when not controlled (Savory *et al.*, 2011) [11]. The disease occurs during warm and moist season and infection is favoured by high humidity. The disease affects all the above ground parts of the crop, symptoms of the disease are brown to black spots which are found on the plants after the production of vines, on fruits round sunken Salmon-coloured lesions are observed (Colluci and Holmes, 2010) [3].

The disease can be controlled by good farm sanitation and the use of fungicides like benomyl and phosphonate antibiotics. However, no single fungicide or tank mixture has been able to achieve excellent result across all trials (Mary, 2014) [9]. Successful control therefore requires preventive rather than a curative means. Apart from the fungicides that are costly, the environmental hazards like pollution is of great concern all over the world. There is therefore the need to consider alternative method of control like the use of plant extracts which do not have residual effect on soils (Falade, 2017) [5, 6]. Various studies have shown that extracts of many plants are toxic to many phytopathogenic fungi with promising results. Efficacy of which vary with concentrations of active ingredients, strains of the fungus, method of extraction of bioactive components and mode of application of phytochemicals. Based on the above, this study was carried out to examine the effect of time and sequence of application of infective conidia of *C. orbiculare* on watermelon and management of the disease with extracts of *Physalis angulata*, *Nicotiana tabacum* and *Hyptis suavecolens*.

2. Materials and Methods

2.1. Location of experiment

The experiment was carried out at Ekiti State University Teaching and Research Farm, (7.7129° N, 5.2523° E), Ado Ekiti, Nigeria.

2.2. Collection and preparation of plant leaves

Leaves of the three plants used in this study (*P. angulata*, *N. tabacum* and *H. suaveolens*) were collected at Ekiti State University Teaching and Research Farm, Ado Ekiti and air-dried at 28°±2^{0}C for 6-8weeks to constant weight. After drying, the leaves were powdered with blender and stored for use inside a refrigerator at 4°C for 2 weeks until they were required for bioassay.

2.3. Plant extracts preparation

The extracts used in this study were prepared by mixing exact quantity (grams) of the plant powder (60, 40 and 20) with 100 ml of distilled hot water maintained at 70°C in 500 ml bottles and left in hot water bath-shaker for thirty minutes. After that, the liquid extract was separated using vacuum filtration and transferred inside standard bottles which were stored at 4°C inside a refrigerator. These extracts were used as the stock solution from which 60%, 40% and 20% (W/v) of each extract were prepared.

2.4. Isolation and identification *C. orbiculare*

Watermelon plants indicating the symptoms of the disease were obtained from Ekiti State University Teaching and Research Farm Ado Ekiti. The leaves were sliced into small pieces (1-2 cm^2) and surface sterilized by dipping it in 0.2% NaOCl for 2 minutes and rinsed in two changes of sterile distilled water. The leaf cuttings (size approximately 1 cm^2) per plate were placed on 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 morphological identification on Malt Extract Agar (MEA) at x400 magnification of a compound microscope (OLYMPUS Binocular) (Zivkovic *et al*, 2010).

2.5. Effect of extracts on anthracnose disease of water melon

The variety of water melon used for the study was the yellow/orange type otherwise called crimson variety. Three plant extracts (a) *Physalis angulate (b) Nicotiana tabacum* and (c) *Hyptis suaveolens* were sprayed at three different concentrations (20, 40 and 60%). The total farm area (625m^2) was laid in a factorial experiment in which variety, concentrations and botanicals represent the main plot, sub-plot and sub-sub-plot factors respectively. Three seeds of yellow/orange variety of water melon susceptible to anthracnose were planted on 15th September 2015 at a spacing of 1.5m × 1.5m. Seedlings after germination on 12th day were reduced to two per stand. Prior to planting, seeds of the water melon were sterilized by rinsing in 0.5% Sodium hypochlorite solution for 30 minutes and later rinsed in sterile distilled water three times before sowing. The total area of the farm was partitioned into three sub plots, making the water melon in the first sub plot to be sprayed with the different concentrations of the plant extracts and after 48 hours sprayed with the conidia suspension of the fungus.

The conidia suspension of the fungus was first sprayed in the second sub plot and after 48 hours sprayed with different concentrations of the extracts. Plants in the third sub plot were sprayed with the inoculum suspension followed by immediate application of the plant extracts after twenty minutes. In this experiment, a conventional knapsack sprayer was used for spraying the plant extracts and inoculum, conidia suspension of 10^4 conidia was used throughout the experiment. Percentage disease incidence was measured and severity of disease was recorded as shown in table 1. The experiment was repeated in 2016 planting season.

2.6. Disease Assessment

This was done using five randomly tagged plants per plot. The diseased leaves were counted and expressed as a percentage relative to the total number of tagged leaves per plant. The commencement of disease assessment started at four weeks after planting (WAP) and continued till 12 WAP. Thereafter, severity of disease for watermelon was examined at 12 WAP through the count of lesions number and rating of symptom expressed with the aid of a visual scale (Shamim and Najmun, 2015). In each plot, five plants were selected from each plot and lesion number was counted. Severity of the disease was assessed through measurement of the size of five randomly selected lesions per plot. After maturity, fruits were harvested and subjected to seed test.

2.7. Assessment of Seed Health

Four hundred seeds harvested from each treatment were divided into four replicate samples of 100 seeds each and examined with the aid of a stereomicroscope for normal and abnormal seeds which was done virtually. Abnormal seeds were those with malformed shape, wrinkled seed coat or those showing evidence of abortion with fungal propagules and data was analysed using ANOVA and treatment means were separated using Turkey significant honest test. Severity of disease was recorded on a scale of 0 – 80 as shown in Table 1.

Table 1: Severity Rating for Watermelon

Scale	Rating	Symptoms of Anthracnose on watermelon
0	No disease	No trace of infection
1-10	Hypersensitivity	Tiny spots on lower leaves only
11-20	Sensitivity	Tiny spots on lower and upper leaves
21-30	Trace infection	Small lesions on leaves and stem of lower leaves
31-40	Slight infection	Lesions on upper and lower leaves
41-50	Moderate infection	Advanced lesions on upper and lower leaves and slight infection of flowers
51-60	High infection	Advanced lesions on upper and lower leaves, flowers, buds and stems
61-70	Very high infection	Advanced lesions on upper and lower leaves, flowers, buds, stems and fruit
71-80	Severe infection	All features above with heavy infection of fruit
>80	Very severe infection	All features above with severe infection of fruit, defoliation and death of leaves

Source: Shamim and Najmun (2015)

2.8. Effect of crude extracts on seed germination and fungal infection

Seeds after harvesting from each of treatment were grouped into 4 samples of 100 seeds each, 30 seeds out of which was selected randomly and planted on bloater paper using a petri dish. The seeds were incubated in Gallenkamp growth chamber for fifteen days at 28±2 ºC under alternating cycles of 12 hours light and darkness. Seeds were observed with the aid of a microscope for evidence of fungal infection and identification of the fungus was based on hyphal and conidial characteristics. Germinated seeds were expressed as a percentage of the total number of seeds. Data obtained were analyzed using ANOVA and treatment means separated using Turkey Significant Honest Test.

3. Results

Effect of time and sequence of application of inoculum and plant extracts on disease incidence in water melon inoculated with *C. orbiculare*.

Result from Table 2 revealed the effect of time lag and sequence of application of plant extracts and infective conidia of *C. orbiculare* on incidence of the disease. The result further showed that incidence of disease at all concentrations were significantly (P≤0.05) lower when extracts were applied 48 hours before inoculation of the fungus compared to application of the extracts two days after inoculation or simultaneous application of extract followed by inoculation. Incidence of disease were 23.5, 22.7 and 18.3% at 20, 40 and 60% with extracts of *P. angulata* compared to the control (75.4) when applied two days before inoculation. Noticeable variations were observed among the different concentrations used in the study.

Table 2: Effect of time and sequence of application of inoculum and plant extracts on disease incidence in water melon in Ado-Ekiti.

Treatment (Leaf extract)	Conc.	Anthracnose Disease Incidence (%).		
		2DB1	2DAI	CAEI
P. angulate	60	18.3^{c}	21.5^{d}	23.1^{d}
N. tabacum		20.8^{d}	23.1^{d}	25.2^{c}
H. suaveolens		22.5^{d}	24.8^{d}	25.3^{d}
P. angulate	40	22.7^{d}	23.6^{c}	25.5^{c}
N. tabacum		22.3^{c}	25.7^{c}	28.7^{b}
H. suaveolens		25.1^{c}	27.6^{b}	29.1^{b}
P. angulate	20	23.5^{c}	25.2^{c}	27.4^{d}
N. tabacum		24.8^{c}	26.6^{c}	29..4^{b}
H. suaveolens		28.3^{b}	30.4^{b}	31.2^{b}
Control	0	75.4^{a}	74.8^{a}	76.9^{a}

DBI, Days before Inoculation, DAI, Days after Inoculation CAEI, Concurrent Application of Extract and Inoculum. Means with the same letter in each row are not significantly different ($P< 0.05$) (Tukey's HSD). Values are average for 2015/2016 planting season

The effect of extracts *P. angulata*, *N. tabacum*, and *H. suaveolens* on severity of anthracnose disease in watermelon, is shown in Table 3. Result from the study shows that where relatively high concentrations (60%) of the extracts were used, trace infections of the symptoms were noticed on the water melon compared to 40% and 20% concentrations that resulted in slight and moderate infections. On the other hand, symptoms observed on the control plants were severe. The result obtained showed that there were variations observed in disease severity based on the different concentrations used in the study.

Table 3: Effect of *P. angulata*, *N. tabacum*, and *H. suaveolens* leaf extracts on anthracnose disease severity of water melon in Ado-Ekiti.

Leaf extract	Concentration	Severity	Symptom rating
Physalis angulate	20	4	Moderate infection
	40	3	Slight infection
	60	2	Trace infection
Nicotiana tabacum	20	4	Moderate infection
	40	3	Slight infection
	60	2	Trace infection
Hyptis saveolens	20	4	Moderate infection
	40	3	Slight infection
	60	2	Trace infection
Control		6	Severe infection

Values are average for 2015/2016 planting season. Symptom rating as contained in table 1.

The effect of application of sprayed leaf extracts *P. angulata, N. tabacum* and *H. suaveolens* on the percentage of normal and abnormal seeds of watermelon in 2015 and 2016 planting seasons is shown in Table 4. The result from the study shows that the percentage of normal seeds was significantly higher ($P \leq 0.05$) than the abnormal seeds for the two planting seasons. The percentage of anthracnose infection on watermelon seeds was relatively lower where higher concentration of the extracts was used. At 60, 40 and 20% concentrations of *P. angulata* leaf extracts, the percentage of normal seeds were 88.5, 85.95 and 78.05% respectively on pooled mean basis compared to the control (59.90%). Similarly, incidence of abnormal seeds were low values being 11.50 14.05 and 21.95 on pooled mean basis when extracts of *P. angulata* were applied at 60, 40 and 20% concentrations compared to the control (40.10%).

Table 4: Comparative effect of foliar spray extracts *P. angulata, N. tabacum* and *H. suaveolens* on incidence of normal and abnormal seeds watermelon in Ado-Ekiti.

Incidence of Normal and Abnormal seeds (%)							
2015		2016		Pooled Mean			
Leaf extract	Conc.	NS	AB	NS	AB	NS	AB
P. angulata	60	89.20^{a}	10.80^{d}	87.80^{a}	12.20^{d}	88.50^{a}	11.50^{d}
	40	85.50^{b}	14.50^{c}	86.40^{b}	13.60^{c}	85.95^{b}	14.05^{c}
	20	77.40^{b}	22.60^{b}	78.70^{b}	21.30^{b}	78.05^{b}	21.95^{b}
N. tabacum	60	85.20^{b}	14.80^{c}	84.60^{b}	15.40^{c}	84.90^{b}	15.10^{c}
	40	83.30^{b}	16.70^{c}	81.90^{b}	18.10^{c}	82.60^{b}	17.40^{c}
	20	72.80^{c}	27.20^{b}	73.20^{c}	26.80^{b}	73.00^{c}	27.00^{b}
H. suaveolens	60	82.60^{b}	17.40^{c}	81.70^{b}	18.30^{c}	82.15^{b}	17.85^{c}
	40	79.90^{b}	20.10^{b}	78.60^{b}	21.40^{b}	79.25^{b}	20.75^{b}
	20	71.40^{c}	28.60^{b}	72.30^{c}	27.70^{b}	71.85^{c}	28.15^{b}
Control		59.60^{d}	40.40^{a}	60.20^{d}	39.80^{a}	59.90^{d}	40.10^{a}

Means with the same letter in each column are not significantly different ($P < 0.05$) (Tukey's HSD).
NS=Normal seed. AB=Abnormal seed

The effect of extracts of *P. angulata, N. tabacum* and *H. suaveolens* at three different concentrations (20, 40 and 60%) on the germination and fungal infection of water melon is shown in Table 5. Seed germination was high at all the concentrations used in this study and was significantly ($P \leq 0.05$) higher than the control. At 20, 40 and 60% concentration of *P. angulata* leaf extracts, seed germination was 81.9, 84 and 92.7% on pooled mean basis compared to the control (53.7%). Similarly, fungal infection on the seeds at all the tested concentrations were low. At 20, 40 and 60% concentration of *P. angulata* fungal infection on seeds were 10.18, 6.42 and 3.57% respectively on pooled mean basis compared to the control (36.4%). There were significant differences on fungal infection of watermelon at all the three concentrations.

Table 5: Effect of *P. angulata, N. tabacum,* and *H. suaveolens* leaf extracts on seed germination and fungal infection of water melon in Ado-Ekiti.

Treatment (Plant extract)	Conc. (%w/v)	Seed germination /Fungal infection (%)					
		2015		2016		Pooled mean	
		SG	FI	SG	FI	SG	FI
P. angulata	60	90.40^{a}	04.14^{c}	95.00^{a}	03.00^{c}	92.70^{a}	03.57^{c}
	40	82.10^{a}	06.94^{b}	85.90^{a}	05.90^{b}	84.00^{a}	06.42^{b}
	20	80.00^{a}	10.56^{b}	83.80^{a}	09.80^{b}	81.90^{a}	10.18^{b}
N. tabacum	60	88.10^{a}	05.72^{c}	92.10^{a}	03.72^{c}	90.10^{a}	04.72^{c}
	40	78.30^{a}	09.10^{b}	81.20^{a}	06.70^{b}	80.70^{a}	07.90^{b}
	20	77.60^{a}	12.90^{b}	80.00^{a}	11.40^{b}	78.80^{a}	12.15^{b}
H. suaveolens	60	85.20^{a}	06.95^{b}	90.40^{a}	04.97^{b}	87.80^{a}	05.96^{b}
	40	76.80^{a}	09.50^{b}	80.40^{a}	07.84^{b}	78.60^{a}	08.67^{b}
	20	74.70^{a}	14.02^{b}	77.50^{a}	12.00^{b}	76.10^{a}	13.01^{b}
Control		68.80^{b}	37.40^{a}	68.60^{b}	35.40^{a}	53.70^{b}	36.40^{a}

S.G=seed germination. F.I=fungal infection. Means with same letter in each row are not significantly different according to Tukey's test.

4. Discussions

In this experiment, *Colletotrichum orbiculare,* the pathogen causing anthracnose disease of water melon was controlled with leaf extracts of *P. angulata, N. tabacum* and *H. suaveolens.* The leaves were powdered, extracted and tested against the pathogen. All the leaves of the three plant extracts reduced incidence of the disease and it was concentration dependent. Disease incidence was significantly ($P \leq 0.05$) lower where higher concentrations of the extracts were used compared to the control. At 20, 40 and 60% concentrations with extracts *P. angulata* prior to inoculation, disease incidence were 23.5, 22.7 and 18.3% compared to the control that gave 75.4%. Extracts were more effective in reducing incidence of disease when applied 48 hours before inoculation of the pathogen compared to application of extracts after inoculation or simultaneous application of extracts followed by inoculation of pathogen. The result from this study suggest that the plant extracts were more effective as preventive rather than curative means at the tested concentrations. The result obtained from this study agrees with Amadioha (1999) [2] who reported the control of rice blast caused by the fungus *Pyricularia oryzae in-vitro* and *in-vivo* with extracts of *Azadirachta indica,* the study showed that the extracts reduced the spread of the disease and incidence of disease were lower when extracts were applied two days before inoculation. Similarly, Falade *et al*., (2017)

[5, 6] reported that extracts of *Datura stramonium, Ricinus communis* and *Jatropha gossypifoli*a reduced incidence and severity of infection cowpea seeds inoculated with *Colletotrichum lindemuthianum*, the pathogen causing anthracnose disease in cowpea, the study also shows that extracts were more effective, when applied two days before inoculation of the pathogen compared to post inoculation which is in agreement with the current study.

In this study, the seeds of the water melon were removed after harvesting and tested for seed germination and fungal infection, the result of which shows that germination were high at all the tested concentration and fungal infection on the seeds were considerably low. Percentage germination of seeds were 76.1, 78.6 and 87.8% when extracts of *H. suaveolens* were applied at 20, 40 and 60% concentration compared to the control (53.7%). Similarly, fungal infection were low values being 13.01, 8.67 and 5.96% respectively at 20, 40 and 60% concentration compared to the control (36.4%). This result suggests the fact that the extracts prevented the growth of the fungus. Jimoh *et al.*, (2016) [10] reported that incidence of the foliar diseases of sesame were reduced when treated with extracts of *Tithonia diversifolia* and *Ocimum gratissimum.* The study further shows that better outcome in terms of seed yield, seed health, quality and germinability were evident and fungal infection was considerably low, which is in consonance with the current study.

In this study, all the plant extracts reduced severity of the disease of water melon and it was concentration dependent where higher concentrations (60%) of the plant extracts were used, trace infections of the symptoms were observed compared to 40% and 20% concentrations that gave slight and moderate infections. The extract sprayed plots were characterized with small lesions on leaves while the control plots were characterized with advance lesions. This may possibly account for the reason why lower incidence of abnormal seeds were recorded on treated plots than the control. This is pertinent if the seeds are to be used as planting materials since it will affect the viability and germination percentage. The result of this study agrees with the work of Enikuomehin and Peters (2002) [4] who used extracts of *A. indica* to control field diseases of sesame, the study shows that the extracts reduced severity of the disease and efficacy was concentration dependent.

In this study, the fruits after harvesting were cut and the seeds removed and sorted into normal and abnormal seeds. Abnormal seeds were those having fungal propagules or rough seed coat. The result of which shows that the percentage of normal seeds were high at all the tested concentration. Percentage of normal seeds were 78.05, 85.95 and 88.50% when extracts of *P. angulata* were applied at 20, 40 and 60% concentration compared to the control (59.90%). Similarly, abnormal seeds were low values being 11.05, 14.05 and 21.95% when extracts were applied at 20, 40 and 60% concentration compared to the control (40.10%). This result probably suggest that all the plant extracts have some chemical substances that was inhibitory to the growth of *C. orbiculare*, resulting in better quality seeds. However, further studies must be carried out to ascertain the active ingredients in the plants that is responsible for their activity, in addition, composite mixture of the extracts can be formulated to determine their efficacy in the management of anthracnose disease of watermelon. The present study therefore contributes to scientific research of disease control using methods that are not having deleterious effect on the environment.

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