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Full Length Research Paper

Efficacy Of Plant Extracts in the Management Of Cucumber Anthracnose Caused By *Colletotrichum Lagenarium*

M. J. Falade.

Department of Crop, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti, Nigeria

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Cucumber anthracnose caused by *Colletotrichum lagenarium*is responsible for about 60% yield loss of cucumber in Nigeria. Chemical control using fungicides usually cause accumulation of toxic residues, development of fungicide resistance in pathogens and adverse effect on soil and beneficial micro-organism. This study investigated the effect of hot water leaf extracts of three plant: *Ricinus communis, Datura stramonium and chromoleana odoratum* at three concentrations (65,50 and 30%) in the management of anthracnose disease of cucumber. In the field experiment, cucumber variety (cu999) was intercropped with maize (SUWAN-ESR) and inoculated with conidia suspension of *Colletotrichum lagenarium*two weeks after planting.After 48hours, inoculated plants were sprayed with three different concentration of *D. stramonium, R. communis and C. odoratum* in a randomized complete block experiment. Data collected were subjected to Analysis of Variance and means were separated using least significant difference at ($p<0.05$).Result from this study shows that all extracts significantly reduce($p<0.05$) disease spread and the greatest control occur at 65% concentration. With the application of extract of *D.stramonium*, disease incidence in percentage for both sole and intercropped cucumber were 22.6% and 14.10% compared to the control (100%).Cucumber yield in the cucumber maize intercrop was significantly higher than the sole cucumber at all the tested concentration. At 65,50 and 30% concentration with extracts of *R. communis*, yield of cucumber in the sole crop were 262,257 and 230kg while that of intercropped were 300, 285 and 250kg respectively. Seed germination and fungi infection were generally low in the intercrop and sole plant at all tested concentrations. At 50% concentration with application of extracts*C. Odoratum,*seed germination percentage was 21.12% while that of intercrop was 23.52%. The study concluded that the extracts has the potential for the control of anthracnose disease in cucumber and intercropping maize with cucumber can reduce the incidence and severity of anthracnose with increased yield.

Keywords: Plant Extracts, Cucumber Anthracnose, Colletotrichum Lagenarium

INTRODUCTION

Cucumber (*Cucumissativus*) is one of the most important exotic vegetables in the country. It is the fourth most cultivated vegetable in the world and known to be one of the best foods for body's overall health (Natural News, 2014). It is one of the most popular members of the cucurbitaceae family.Cucumber is native to tropical regions of southern Asia, having been cultivated in India for over 3,000 years. From there it extended to Greece and then to Rome and subsequently was introduced into China. The cucumber crop was introduced by the Romans in other parts of Europe; records of this crop appeared in France in the ninth century, in England in the fourteenth century and in North America in the mid-sixteenth century (Avi and Torey, 2014), as Columbus took cucumber seeds to America (Infoagro 2011). Cucumber is a valuable source of conventional

antioxidant nutrients including vitamin C, beta-carotene, and manganese.

The estimated total world production for cucumbers in 2017 was 83,753,861 metric ones, which has increased by about 3.9% compared to 80,616,692 tonnes produced in 2016. China is the largest producer, accounting for over 77% of global production (64,824,643 tonnes) (FAOSTAT 2017). West Africa especially Nigeria, Mali, Ghana, Burkina Faso, Senegal and Niger are the key cucumber producing countries. Mali is the largest producer of cucumber in Africa and Nigeria is ranked second (FAOSTAT 2017).

In Nigeria, cucumber production has not been ranked; it is grown mainly in Jos, Imo, Ekiti and in some other states of the federation. Cucumber does well on well-drained fertile soils with pH 6.0 -7.0 and ample richness in organic matter. It is often planted on raised beds and thrives in sandy loam soils. The crop requires a good amount of sunshine, warmth and is mostly grown in green houses. In spite of the increasing relevance of cucumber, low yields are obtained in farmers farms and its production in southeastern Nigeria is constrained by scarcity of planting seed, lack of capital, climatic factors, pests and diseases, high fruit perishability and lack of production experience (Jeffery, 2001).

Cucumber plants are susceptible to a variety of insect, bacterial, fungal, and nematode infections. Early Identification of such infections or infestations is key to appropriate and rapid control methods. Disease prevention strategies include use of crop rotation, careful selection of field, farm sanitation, soil treatments, appropriate seed selections and control using chemicals

Cucumber anthracnose is caused by the fungus, *Colletotrichum lagenarium*. The disease shows up from the start of cultivation of cucumbers, however, it was only officially reported in 1979. Although anthracnose is known worldwide to infect all parts of the plant above ground, on cucumber it mainly infects the leaves. Infected leaves first appear as yellowish spots that enlarge and develop brown centers of dead tissue. A single leaf may have anywhere from a few spots to a hundred. The spots may remain small or enlarge to half an inch in size. The disease has the potential to spread several feet in a mild rain event, thereby having the potential to infect an entire planting in a matter of days, thus, reducing the harvesting period of cucumber (Wehner and Gunner, 2004).

This disease can be controlled effectively by the use of resistance varieties where they exist and culturally by removing sources of inocula. The use of synthetic fungicides like (Benzimidazole) and Mancozeb (Dithiocarbamate) had proved very effective (Alves *et al.*, 2015). However, due to the side effects of these synthetic pesticides, much attention is being focused on the alternative methods of pathogen control like the use of plant extracts. Extracts obtained from plants are cheap, readily available and have no effect on soil or environment. Extracts of plants such as garlic (*Allium sativum*), black pepper (*Piper nigrum*), ginger (*Zingiberofficinale*), lemon-grass (*Cymbopogon citratus*), cinnamon (*Cinnamomumzeylanicum*), lemon-balm (*Lippia alba*), mint (*Menthapiperita*), eucalyptus (*Eucalyptus globulus*), have been tested against fungal infections in cucumber with varying degrees of success (Venturoso*et al*., 2011). The potentials of plants extracts for disease control have been recognized such that there is the need for research into the use of extracts of some other indigenous plants.

1.1 JUSTIFICATION

Anthracnose, caused by the fungus *Colletotrichum lagenarium*, is probably the most destructive disease of cucurbits or vine crops in warm seasons with frequent rains (IPM, 1996). Plant diseases are mostly controlled by agricultural chemicals, which results in high costs, environmental and toxicological risks to non-target pest and favors plant pathogen resistance to agricultural chemicals. Society's concern with dependence on toxic agricultural chemicals, that contaminate the environment, has led to the search for alternative control methods that are safe, viable and efficient in plant pathogen fungus control (Silva *et al*., 2010). At some point in time, the use of chemical pesticides are been prohibited from those which can be used in the management of pests of horticultural crops, considering their environmental health effects and toxicity of their residues in food (Melloto *et al*., 2000).

The antifungal effects of castor oil (*Ricinus communis*) (Naz and Bano, 2012), jimson weed (*Datura stramonium*) (Usha*et al*., 2009) and (*Chromolaena odoratum*) are well known but their use for the management of anthracnose in cucumber has not been studied. Therefore, this study will be conducted to evaluate the effect of extracts of some indigenous plants on growth and sporulation of *C. lagenarium* using in-vitro bioassays and to select the best botanicals for the control of anthracnose disease of cucumber in the field.

1.2 Objectives of the Study

The objectives of the study are to:

1. Determine the effect of foliar spray of extracts of the leaves from three plant species on anthracnose disease incidence, severity and yield of cucumber in the field.
2. Determine the effects of intercropping maize with cucumber on anthracnose disease incidence, severity and crop yield.

3.0 MATERIALS AND METHODS

3.1 Location of experiments

Laboratory studies and field experiments were conducted at Ekiti State University Teaching and Research farm (7.7129^0 N, 5.2532^0 E), Ado Ekiti, Nigeria.

3.2 Preparation of laboratory apparatus

Glasswares used in the studies were washed with detergent containing 0.05% sodium hydrochlorite for 30 minutes, rinsed with tap water and dried inside an oven (Gallenkamp 300) at 50^0C for 2 hrs. Thereafter, Erlenmeyer flasks, beakers, glass pipettes and petri-dishes were wrapped in aluminum foil and sterilized in oven for 35 minutes. Inoculating loop, cork borers and scalpels were sterilized by dipping in 70% ethanol and flaming to red hot before and after use. Laminar flow cabinet and all other working surfaces were disinfected by swabbing with 70% ethanol. Sterile distilled water was used in the study.

3.3 Preparation of media

Potato Dexose Agar (PDA) was prepared by dissolving 39g dehydrated PDA (E. Merck, Darmstadt Germany) in 1L of sterile distilled water in Erlenmeyer flask. The flask was stoppered using cotton wool plug, wrapped with aluminium foil and autoclave at 121^0C for 15 minutes at pressure of 100 kpa. Agar was allowed to cool to room temperature (45^0C) and amended with 300 mg/L streptomycin sulphate and 20 ml of cooled media were poured into 9cm diameter sterile Petri-dishes (Sterilin Product, UK) inside a laminar flow cabinet and allowed to solidify.

3.4 Collection and preparation of plant materials (leaves)

Leaves of three plants namely; *Daturastramonium*Linn, *Ricinuscommunis* Linn and *Chromolaenaodoratum*Linn (Burkill, 2000) were collected at Ekiti State University, Ado Ekiti and air-dried at $28^0 \pm 2^0$C for 2-3 weeks to constant weight. Dried leaves were milled using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4^0C used within two weeks.

3.5 Preparation of plant extracts

Extracts were prepared by mixing equivalent grams of prepared plant powder (65, 50 and 30) with 100 ml of distilled hot at 70^0C water in 500 ml bottles and kept in hot water bath-shaker for 30 minutes. Thereafter, the liquid extract was separated by using two folds cheese cloth and poured inside standard bottles which were refrigerated ta 4^0C. These extracts were used as the stock solution from which 65%, 50% and 30% (w/v) of each extract were prepared.

3.6 Isolation and identification of *C. lagenarium*

Cucumber plants showing distinct symptoms of the disease were collected of the fields at Ekiti State University Teaching and Research farm, Ado Ekiti. Leaves were cut into pieces (1-2 cm^2) and surface sterilized by immersion in 0.2% NaOCl for 2 minutes and rinsed in two changes of sterile distilled water. Three leaf cuttings (1cm^2) per plate were placed on PDA. The plates were sealed with Parafilm tape and incubated at 28^0C for 5-6 days. Single spore of developing colonies was isolated and sub-cultured to obtain pure cultures. Samples from single spore cultures were for morphological identification of agar at x400 magnification of a compound microscope (OLMPUS Binocular) (Zivkovic*et al.,* 2010)

Plate 2: Jimson weed, *Daturastramonium*Linn.

Plate 3: Castor oil plant, *Ricinuscommunis* Linn

Plate 4: Siam weed*, Chromolaenaodoratum* Linn

3.7 Maintenance and storage of culture

Pure culture of *C. lagenarium* were either maintained on PDA agar slants in McCartney bottles secured with a screw cap for short-term storage (1-2 weeks) at 4^0C or stored in 20% aqueous glycerol and refrigerated for long term preservation (1-3 months) at 4^0C.

3.8 Effect of plant extracts on anthracnose incidence and yield of cucumber

Cucumber was intercropped with maize (Variety: SUWAN-ESR). Hot water extracts of *D. stramonium*, *R. communis* and *C. odoratum* were appliedas a foliar spray. The 2 x 3 x 3 factorial experiment was a split plot design and two cropping systems were used: (a) sole cucumber (which was the control) and (b) cucumber intercropped with maize. The plot size was 2m x 2m, separated by boarder row 1m and the total area of the plot was $180m^2$. The population of cucumber in each cropping system was 324 and population of maize in the cucumber-maize intercrop was 162. Cucumber was planted at 3 seeds $hole^{-1}$ at a spacing of 60cm x 30cm and the intercropped maize was planted at a spacing of 60cm x 90cm. Three surface sterilized seeds of each variety of cucumber and maize were sown at spacing of 60cm x 30cm. Seedlings were thinned to two per stand after one week.

Two weeks after plant establishment, the cucumbers were sprayed with *C. lagenarium* conidia suspension containing 10^4 conidia ml^{-1} while the control was sprayed with sterile distilled water. Incidence and severity of anthracnose disease was monitored and recorded.

3.9 Effect of plant extract on severity of infection

After 5 days after inoculation of the disease on the field experiment, the plant extracts demonstrated 95% control of *C. lagenarium* at a concentration of 16 mg mL and the MIC was 2.596 mg mL. It was observed that the extracts had a significant impact on the morphology of the fungus, leading to shorter hypae that were not branched. This observation was associated with the inhibition of mycelial growth. The extracts were relatively stable at 80^0C, under acidic conditions and when exposed to light and short periods of UV radiation.

3.10 Effect of plant extracts on yield of cucumber

A field experiment was carried out to observe the effects of the plant extract i.e. siam weed at rate of 0.5,1 and 1.5 g/l, castor plant at rate of 50, 100 and 150 g/l and jimson weed at rate of 2.5, 5 and 7.5 g/l on growth, yield and it components and chemical constituents of cucumber. The results obtained revealed that, all foliar treatments improved growth, yield parameters and chlorophyll content compared with control treatment. The highest significant increments in plant length, weight, average of leave area, fruit weight, and number of fruit/plant, yield and chemical content were recorded with foliar sprays using siam weed extract at the rate of 1.5 gm/l, castor plant at the rate of 150 gm/l and jimson weed at the rate of 7.5 gm/l. respectively, in both growing seasons.

Linear correlation and regession of cucumber traits on each other were carried out. It may be worth to mention that for each increased of one square centimeter of leaves area/plant, fresh weight of a real part and total yield correspondingly increased by (5.195

and 3.64 gm/plant) and 0.050 and 0.055 ton/field) in the first and second season respectively.

4.0 RESULTS AND DISCUSSION

4.1.1 Field experiments

The climate of the experimental area is tropical with distinct wet and dry seasons. The range of annual rainfall is between 1,200 and 1,600 mm, average annual temperature is 25.1^{o}C; 2000 annual total sunshine hours while annual range of radiation is between 110 and 140 kcalcm^{2} per year. The vegetation is forest mixed with various types of bush re-growth, grasses and creepers (Oso, 2014). The physical properties of the sampled soil at the location of the experiment are shown in Table 1.

4.1.2 Effect of Plant extracts and concentrations on Incidence of cucumber anthracnose disease caused by *Collectotrichum lagenarium*

Table 2 shows the effect of plant extracts and concentrations on incidence of cucumber anthracnose disease. Disease incidence varies with the concentrations of the extracts for both the sole and intercropped plants. At higher concentrations, disease incidence was low and at lower concentrations, disease incidence was high. At 65% concentration with extracts of *D. stramonium*, disease incidence for both the sole and intercropped plant were 24.50% and 15.20% whereas at 30% concentration with the same extracts, disease incidence were 47.10% and 21.32% respectively.

Extracts of *Datura stramonium* was the best in reducing the incidence of the disease for both the sole and intercropped plants. At all the tested concentration, disease incidence in the intercropped plant was generally lower than that of sole crop. Significant variations were observed at all the tested concentrations for the extracts used in the study

Table 1: Physical properties of soil of the experimental site

Soil property	Content
Sand	64%
Silt	11%
Clay	25%
PH (1:2, 5, H_2O)	3.87%
Organic matter	3.74%
Total nitrogen	0.28%
Available P	34.84 (mg/kg)
Na	0.07(c mol/kg)
Mg	0.30(c mol/kg)

Table 2: Effect of plant extracts and concentrations on Incidence of cucumber anthracnose disease caused by *C. lagenarium* in Ado-Ekiti

Disease Incidence %

Plant extracts	Extract Conc. % (w|v)	Sole cucumbe	Intercropped
D. stramonium	65	24.50^{d}	15.20^{c}
	50	35.00^{c}	19.45^{c}
	30	47.10^{b}	21.32^{b}
	0	100^{a}	100^{a}
R. communis	65	26.40^{d}	16.43^{c}
	50	37.21^{c}	20.10^{b}
	30	48.75^{b}	22.75^{b}
	0	100^{a}	100^{a}
C. odoratum	65	28.65^{d}	18.55^{c}
	50	38.94^{c}	21.90^{b}
	30	49.50^{b}	24.50^{b}
	0	100^{a}	100^{a}

0 Means with the same letter in each column are not significantly different (P<0.05) (Turkey's HSD)

4.1.3 Effect of plant extracts and concentrations on Incidence of normal and abnormal seeds of cucumber.

The effect of the plant extracts and concentration on incidence of normal and abnormal seeds of cucumber anthracnose are shown in Table 3. Incidence of normal seeds were generally higher than the abnormal seeds for both sole and intercropped plant. At 50% concentration with extracts of *R. communis*, incidence of normal seeds for both sole and intercropped plant were 67.52% and 73.35% whereas abnormal seeds were 32.48% and 26.65%.

4.1.4 Effect of Plant extracts and Concentration on seed germination and fungal infection of anthracnose of cucumber caused by *Collectotrichumlagenarium*

The effect of extracts on seed germination and fungal infection is shown in Table 4. Seed germination were generally low in both the sole and intercropped cucumber. Seed germination for sole plant at 50% concentration with application of extracts of *C. odoratum*

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Table 3: Effect of plant extracts and concentrations on Incidence of normal and abnormal seeds of cucumber anthracnose caused by *C. lagenarium* in Ado-Ekiti

		Incidence of normal and abnormal seeds (%)			
		Sole cucumber		cucumber intercropped	
Plant extract	Conc.	Normal	Abnormal	Normal	Abnormal
D. stramonium	65	71.56[a]	28.44[c]	86.50[a]	13.50[d]
	50	69.55[b]	30.45[c]	75.43[b]	24.57[c]
	30	67.40[b]	32.60[b]	74.73[b]	25.27[c]
	0	53.44[c]	46.56[a]	58.50[c]	41.50[a]
R. communis	65	70.48[a]	29.52[c]	85.20[a]	14.80[d]
	50	67.52[b]	32.48[b]	73.35[b]	26.65[b]
	30	66.90[b]	33.10[b]	72.48[b]	27.52[b]
	0	54.10[c]	45.90[a]	53.70[c]	46.30[a]
C. odoratum	65	68.90[b]	31.10[c]	83.30[a]	16.70[d]
	50	66.65[b]	33.35[b]	71.45[b]	28.55[b]
	30	65.40[b]	34.60[b]	70.35[b]	29.65[b]
	0	53.21[c]	46.79[a]	52.80[c]	47.20[a]

Means with the same letter in each column are not significantly different (P<0.05) (Turkey's HSD)

was 21.12% while that of intercropped plant was 23.52%. Fungi infection on the other hand were generally low in both the sole and intercropped cucumber. At 65% concentration with the extracts of D. stramonium, fungi infection for both sole and intercropped plant were 26.30% and 15.64%. Significant variations was observed at all the tested concentration 65% respectively.

Abnormal seeds were generally low at all the tested concentrations and significant (P<0.05) variations were observed in the result obtained in this study

Table 4: Effect of Plant extracts and concentration on seed germination and fungal infection of anthracnose of cucumber caused by Collectotrichumlagenarium in Ado-EkitiSeed germination/Fungal infection (%)

Sole cucumberCucumber intercropped

Plant extracts	Conc.	S.G	F.I	S.G	F.I
(Treatments)	(% w/v)				
D. stramonium	65	19.44^{b}	26.30^{b}	20.65^{b}	15.64^{c}
	50	17.52^{c}	25.42^{c}	21.42^{a}	15.32^{c}
	30	18.32^{b}	23.75^{c}	20.43^{b}	17.84^{b}
R. communis	65	21.53^{a}	27.42^{b}	21.75^{a}	16.45^{c}
	50	20.84^{a}	28.34^{b}	20.45^{b}	17.75^{b}
	30	19.52^{b}	30.11^{a}	21.55^{a}	18.40^{b}
C. odoratum	65	20.11^{a}	29.42^{b}	21.56^{a}	16.10^{c}
	50	21.12^{a}	30.10^{a}	23.52^{a}	17.71^{b}
	30	18.94^{b}	31.65^{a}	22.65^{a}	18.53^{b}
	0	19.30^{b}	38.47^{a}	19.40^{c}	41.34^{a}

Means with the same letter in each column are not significantly different (P<0.05) (Turkey's HSD)

4.1.5 Effect of plant extracts and concentration on the yield of cucumber infected with *Collectotrichum lagenarium*

The effect of plant extracts on yield of cucumber anthracnose is shown in Table 5. Yield of cucumber varies with the concentration of the extracts for both the sole and intercropped plants. At lower concentrations, yield is low and at higher concentrations, yield is generally high. At 50 and 30% concentrations with extracts of *D. stramonium*, yield for sole crop were 241 and 220kg while that of intercropped plant were 301 and 270kg respectively. Similar results were obtained with other extracts used in the study.Extracts of *D. stramonium* was the best in increasing the yield of cucumber for both sole and intercropped plant.

Table 5: Effect of Plant extracts and concentrations on Yield of cucumber infected with *C. lagenarium* in Ado-Ekiti

Plant extracts	Extract Conc. % (w|v)	Yield (kg/ha) Sole	Yield (kg/ha) cucumber Intercropped
D. stramonium	65	270[a]	324[a]
	50	241[b]	301[a]
	30	220[b]	270[b]
	0	143[c]	145[c]
R. communis	65	262[a]	300[a]
	50	257[a]	285[b]
	30	230[b]	250[b]
	0	132[c]	127[c]
C. odoratum	65	268[a]	294[b]
	50	251[a]	270[b]
	30	230[b]	245[b]
	0	145[c]	130[c]

Means with the same letter in each column are not significantly different (P<0.05) (Turkey's HSD)

4.2 DISCUSSION

In this study, leaves of *Datura stramonium, Ricinus communis and chomoleana odoratum* were powdered and tested against *C. lagenarium,* the pathogen causing anthracnose disease of cucumber. The leave extracts were collected and powdered so as to offer higher surface area for the reaction. It has been observed that air dried leaf extracts perform better than the sundried ones because the chemistry of the constituents are well preserved (Falade, 2018). Apart from this, air-dried and powdered leaf offer a higher surface area for the reaction which is in agreement with this study. All the plant extracts reduced the growth of the pathogen and higher imbibition of growth occurred at relatively higher concentrations. The imbibition may be due to higher fungitoxic substances released by the extracts (Falade and Borisade, 2017).

The effect of intercropping maize with cowpea in this study showed that the incidence and severity of anthracnose disease of cucumber were less in the intercropped than in sole cucumber. Intercropping has been reported to alter the response of host plant and pathogens to changes in the environment and this generates diverse alterations in the ecosystem which may be responsible for reduced incidence of infection. This is in line with the work of Enikuomehin *et al., (*2008) who reported that the incidence and severity of foliar disease of sesame *(Sesanum indicum)* was reduced when intercropped with maize and sprayed with the extracts of *Ocimum gratissimum*

In this study, the plant extracts were tested at various concentrations on the incidence of the disease, result from this study shows that incidence of disease were relatively lower where higher concentration of plant extract were used and significant variations exist among all the plant extracts used . In this study, Incidence of normal seeds were generally higher than the abnormal seeds for both sole and intercropped plant at all the tested concentrations of plant extracts used in the study. Abnormal seeds were generally low at all the tested concentrations and significant ($P<0.05$) variations were observed in all the treatments used. This study agrees with the work of Falade*et al*., (2017) who reported that Incidence of normal seeds were relatively higher than abnormal seeds when extracts of *R. communis* and *D. stramonium* were used in the control of *Colletotrichum lindemuthianum* causing anthracnose disease of cowpea.

In this study, seed germination was generally low in both cucumbers intercropped with maize and the sole plant. Seed germination for sole plant at 50% concentration with extracts of *R. communis* was 20.84% while that of intercropped was 20.45%. The result from the study possibly suggest that the extracts were inhibitory to the germination which contradict the report of Falade*et al*., (2017) that seed germination of cowpea inoculated with *C. lindemuthianum* and sprayed with extracts of *D. stramonium* were high at all tested concentrations. Fungi infections on the other hand were generally low. At 30% concentration with the extracts of *C. odoratum*, fungi infection for both sole and intercrop were 18.94% and 22.65% respectively.

In this study, yield of cucumber intercropped with maize were higher than that of the sole crop at all the tested concentrations. Intercropping has been reported to alter the response of host plant to changes in the environment which may be responsible for the result of this study (Mundth, 2002). This however generates a diverse alterations in the ecosystem which may be responsible for reduced incidence of the disease. Apart from this, intercropping tends to reduce the speed of wind which will invariably reduce air circulation and hence reduction in the rate of inoculum transfer (Enikuomehin, 2007).

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