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Control of *Colletotrichum capsici* (Pathogen of Brown Blotch of Cowpea in the Savanna) Using Garlic Oil

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ABSTRACT

Colletotrichum capsici was isolated from an infected cowpea pod collected on the outskirts of Yola in the month of December, 2012. The *in vitro* control was carried out on potato dextrose agar (PDAS) poisoned with garlic oil in a completely randomized design with replicates of 4; and inhibited mycelial growth of the pathogen by 65% as against 0% inhibition by control. All growth characters (seedling emergence, stem height, stem girth, leaf number and leaf size significantly improved compared to the control values at p=0.01.There was a significant reduction in the incidence and severity of infection on cowpea seedlings (2.50% for garlic treated and 10.80% for control, 4.50mm for treated and 17.5omm for control respectively). There was a corresponding improvement in performance of garlic oil as the quantity increased (0.2ml-1.0ml/20ml of PDA *in vitro* and 2.0ml-10.0ml/1kg of soil for the *in vivo* tests).

Keywords: *Colletotrichum capsici*, garlic oil control

INTRODUCTION

Cowpe [*Vigna unguiculata (*L) Walp] is an important crop for the livelihood of the poor in the undeveloped countries, and is used for various purposes such as food crop, cash crop and animal feed (Singh *et al*., 1997). It is considered the most important legume crop in Africa (Langyintuo *et al*., 2003).

Emechebe (1986) reported that **c**owpea highly rated as an important plant to mankind, was susceptible to a wide range of diseases. Among which brown blotch caused by *Colletotrichum capsici* (which brings about seedling damping-off, stem or branch girdling, flower abortion, immature pod mummifying, thereby reducing yield, taste and market value) is rated as one of the most devastating diseases (Summerfield and Robert, 1985).The crop is susceptible to *Colletotrichum capsici*, at all stages (Summerfield and Robert 1985). A collaborative report (Amusa and Adegbite, 2006) rated brown blotch disease of cowpea as one of the most destructive diseases of cowpea. A survey in the south Western Nigeria by these authors covering 74 cowpea lines revealed that 64% were susceptible to the pathogen.

A reduction in stand establishment from 88% (for healthy seeds) to 24% (for seeds infected by *Colletotrichum capsici)* has been reported (Emechebe, 1981). Emechebe and Soyinka, (1985) reported that in the forest ecological zone yield loss could be as high as 85%. According to Singh and Rachie (1985), there is low yield especially in rural areas in developing countries where farmers are unable to control the disease because of financial constraints leading to great losses in production, due to seed decay and damping-off.

In the Northern Guinea Savanna zone, result of surveys conducted in Adamawa State shows that brown blotch caused by *Colletotrichum capsici* is spread across the whole state (Channya, 2011). Since the disease is seed- borne (Emechebe, 1981) and most farmers obtain their seeds from markets other than reliable sources and hardly treat them, it may be reason for its wide spread within the state.

Various control measures against brown blotch diseases of cowpea have been reported, these include use of resistant varieties (Singh, 1994), folia fungicides (Sohi and Rawal, (1994), seed treatment with fungicides (Emechebe, 1994) as well as application of phosphorus fertilizer (Owolade *et al.*, 2008),

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use of cultural method consisting of spacing and cropping pattern (Adebitan *et al.*, (1996) and the use of *Aspergillus niger* (Channya, 2011).

Lee *et al.*, (2003) reported that synthetic fungicide might pollute the atmosphere while accumulation of the chemical in the soil may encourage the development of chemical resistance. A cheaper and safer alternative control of this menace within easy reach of farmers, majority of whom are poor and ignorant, is the target of this research. It has been reported that the use of synthetic chemicals is dangerous to the environment, whereas the use of plant oils or plant extract is safer and cheaper to be used and easier to handle by farmers (Olowe *et al.*, 2003). There is therefore need to employ the use of plant oils and plant extracts in controlling the brown blotch pathogen. Atia (2011) reported a successful fungal control using garlic extracts.

There is no report indicating that control attempt *of Colletotrichum capsici* has been carried using garlic oil. Hence the objective of the present study is to investigate the effect of garlic oil, *in vitro* and *in vivo* as a means of controlling the brown blotch pathogen.

MATERIALS AND METHODS

Study Area

The study was conducted in the Department of Plant Science laboratory and Biological garden of Modibbo Adama University of Technology Yola in Adamawa state in 2013. Adamawa state is located at the North Eastern part of Nigeria and lies between latitude 7° and 11° N of the equator and between longitude 11° and 14° E of the Greenwich meridian in the Northern Guinea Savanna (Adebayo, 1999

Source of Sample and Isolation of the Pathogen

The pathogen (*Colletotrichum capsici*) was isolated from infected pods (showing typical brown blotch lesions) obtained from Bole (III) of Yola South L.G.A. in the month of December 2012. Under aseptic conditions, diseased portions of the pod were sectioned into 5mm square piece with a heat sterilized scalpel. Pieces were picked with flamed, then cooled pair of forceps. These portions were transferred into 0.1% mercuric chloride solution contained in a sterile 9cm Petri-dish for surface sterilization for 30 seconds. The sterilized portions were then washed in three changes of sterile distilled water and dried between sterile filter papers. With a flamed and cooled forceps a sterile piece of pod was then plated out on sterile solidified potato dextrose agar (PDA) and incubated at temperature of 28 ± 2°C in the month of August 2013 for seven days. Pure isolate of *Colletotrichum capsici* was obtained from hyphal tip of growing colonies by using a sterile needle and repeated sub-culturing on sterile potato dextrose agar until pure cultures were obtained. Pure culture was stored on agar slants in Mc Cartney bottle for future use. Potato Dextrose Agar (PDA) was prepared using the method of Smith and Onion (1983).

Sterilization

The Petri dishes used were sterilized in an oven at 160°C for 6 hours in a sterilizing can. The inoculating needles and the cork borer where sterilized by flaming over a Bunsen burner and cooled by dipping them into ethanol. The prepared media was autoclaved in an autoclave for 15 minutes at 101 Lbs pressure at 121°C and allowed to cool. The inoculation of the organisms was done in a sterile environment in the inoculation chamber. The table in the inoculation chamber was wiped with 95% ethanol and then ultra violet (UV) light was switched on for 30 minutes for sterilization before carrying out all inoculations.

Pouring

Media was poured in an inoculation chamber. Twenty mills of the media were poured into Petri dishes, adopting the method of Smith and Onion (1983).

Source of Garlic Bulb and Preparation of Garlic and Oil

White garlic was purchased from the garlic seller at Jimeta Main Market, and the dry outer peel was removed. Essential oil was extracted through steam distillation using stove-still apparatus. A 500g portion of the garlic bulb was placed in a plant chamber and pressurized by steam for three hours. The heat of the steam forced the tiny intercellular pockets that hold the essential oil to evaporate and together with the steam molecules travel through a tube into the still-condensation chamber. As steam cooled it formed a film on the surface of the water which was then dried over anhydrous sodium sulphate.

In-vitro Control Experiment with Garlic

Petri dishes containing PDA were incubated with garlic oil at three concentration levels of 1.0ml, 0.5ml, and 0.2ml per 20ml of PDA. Approximately 0.1ml from *Colletotrichum capsici* spore suspensions (conc.1 × 106 spores/ml) were dispensed at the center of the amended PDA media. The inoculated plates were then sealed with a masking tape and then incubated at 28±2°C for 72 hours in the Month of August. The Petri-dishes without the garlic oil as control. The experiment was performed under aseptic conditions in a completely randomized design and replicated four times.

Colony diameter of the radial growth was measured from 72 hours (3 days) to 6 days. The inhibition zone (P), was measured using the formula of Francisco (2010):

P = C - T/C ×100. Where C is the colony cm^2 of the control and T is the treatment

In-Vivo Control Screen House Experiment

Sandy-loam soil was sterilized in an oven at 160°C for one hour in aluminum foil and placed in one liter plastic buckets. The weight of the soil in the plastic was measured on a beam balance, which gave exactly one kilogram and were labeled; GOT and ICC (Garlic oil Treatment and Inoculated Control respectively). Soil in the plastics was inoculated 10ml/kg of suspension of the pathogen in each plastic rubber a day soil is filled up in the plastics using the method of Rumusi (2006).

Two seeds of cowpea were planted 3cm deep in each plastics containing the sterilized soil a day after inoculating the soil with the pathogen. Garlic oil was added after two days at varied levels of 2.0, 5.0 and 10ml. Twenty mills of water were added to the soil after every three days as adopted by Rumusi (2006). Set up was a completely randomized design in four replicates. This experiment was carried out, at the Screen house constructed in the Biological garden of Modibbo Adama University of Technology, Yola.

Assessments of growth characters were done after four and seventh day, growth characters included;

(i) Count of seed emergence and percentage emergence was obtained using the formula of Rumusi (2006), E= No of emerged seedlings ×100/ total no of seed planted.

(ii) Ruler measurement of seedlings height (mm)

(iii) Count of leaf number

(IV) Measurement of stems girth (mm)

Disease characters were assessed and included:

(i) Disease incidence through count of brown blotch lesions on seedling leaves

(ii) Disease severity through ruler measurement of brown blotch lesion size (mm)

This experiment was conducted for ten days and was repeated three times to obtain

The best result.

Experimental Design and Data Analysis

Statistical tool for Applied Sciences (SAS) was used for this analysis and the experimental design used was Completely Randomized Design (CRD), each treatment was replicated four times. Data collected was analyzed using ANOVA according to Gomez and Gomez, (1984) and means that were significant were separated using the Least Significant Difference (LSD) according to Scheffer (1953).

RESULTS AND DISCUSSION

In vitro test

Effect of garlic oil on the colony growth and percentage inhibition of *Colletotrichum capcisi*

Analysis of variance for effect of garlic oil on the colony growth and inhibition of *Colletotrichum capsici* was significantly different from that of control at $P<0.01$ as shown In Table1. Garlic oil treated culture had the colony diameter of 12.76mm and inhibition zone of 65.66% while that of control was 32mm with zero inhibition zone (Table 1). The result from the present study has shown that garlic oil is capable of controlling the colony growth of *Colletotrichum capsici in-vitro and* thus an addition to the list of plant extracts with efficacy on this pathogen. Shukla and Tripathi (1987)

observed that the oil of anise (*Pimpenella anisum*) at 100mg/l exhibited total lethality on *Colletotrichum capsici.*

Table1.*Colony Diameter and Percentage Inhibition of Colletotrichum capsici Treated with Garlic oil*

Treatment	Colony Diameter (mm)	Inhibition (%)
Garlic	12.76	65.66
Control	32.00	0.00
LSD (0.01)	0.52	6.80

Analysis of variance for the effect of the different concentration levels of garlic oil on colony radial expansion of *Colletotrichum capsici* showed a significant difference among all the concentrations levels (P<0.01). Increase in concentration of garlic oil resulted in decrease in colony diameter of the pathogen. Concentration of 0.2ml gave the least control with the colony growth of 20.00mm, and 0.5ml concentration level gave 13.75mm colony growth and also treatment with 1.0ml produced the highest control of the pathogen with colony growth of 8.13mm. While the analysis of variance for percentage inhibition showed no significant difference between concentration level of 0.5ml and 0.2ml, but differed significantly with 1.0ml. The concentration of 1.0ml produced the highest percentage inhibition zone of 65.89%, while 0.5ml and 0.2ml had percentage inhibition zone of 56.17% and 54.79% respectively (Table 2).

Table2. *Colony Diameter and Percentage Inhibition In vitro of Colletotrichum capsici at various ncentrations of Garlic oil*

Concentration (ml)	Colony diameter (mm)	Inhibition (%)
1.0	8.13	65.89
0.5	13.75	56.17
0.2	20.00	54.79
LSD (0.01)	0.45	5.89

Effect of Garlic Oil on *Colletotrichum capsici*, In-vivo Growth characters

The growth characters of cowpea seedlings analyzed were: cowpea seedling emergence (percentage emergence), stem height of cowpea seedlings (millimeters), stem girth of cowpea seedlings (millimeters), cowpea seedling leaf number and cowpea seedling leaf size (millimeters).

Analysis of variance (P<0.01) for percentage emergence of cowpea infected seeds treated with garlic oil showed a significant difference between garlic and control. Treatment with garlic yielded the percentage emergence of 59.83%. Control showed a lower emergence of 55.50% (Table 3). Alice and Rao (1987) observed that *A. sativum* extracts significantly reduced seed infection by *Drechslera* on rice and treated seeds had significantly higher viability. Garlic extract treatment of wheat seeds was reported to have also significantly reduced the incidence of seed-borne fungi, increased seed germination, the number of healthy seedlings and vigour index (Grozav and Foarce, 2005; Khalaf *et al*., 2011).

Garlic oil was reported by Milner (2001) to have antimicrobial substance such as volatile oil; these volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic. Garlic contains at least 33 sulfur compounds like aliin, alicin, ajone, allypropl, dially, sulfide, sallysteine, vinylthiines, sallymercaptocytein, and others. Allicin is reported to be the most important and the most active substance and it is found in the fresh extract of *Allium* (Vasile *et al*., 2012).

Table3. *Growth Characters of Infected Cowpea Seedlings Treated with Garlic Oil*

Treatment	Emergence	Height (mm)	Stem girth (mm)	Leaf No.	Leaf size (mm)
Garlic oil	59.83	57.50	6.70	45.00	34.50
Control	55.50	30.00	3.20	22.50	17.50
LSD (0.01)	3.09	30.00	0.18	9.50	16.40

Analysis of variance for seedling height growth at P<0.01 showed significant difference between garlic oil and control with seedling height of 57.50mm and 30.00mm respectively.

For stem girth, the analysis of variance at P<0.01 also showed significant difference between garlic oil with control. Control trial result showed the stem girth of untreated seedlings (control) was lower (3.20mm) than for garlic oil (6.70mm). Analysis of variance (P<0.01) for seedling leaf number showed a significant difference between garlic oil (produced a higher leaf number of 45.00) and control with lower leaf number of 22.50.

For seedling leaf size, analysis of variance revealed a highly significant difference between garlic oil and control (34.50mm and 17.50mm respectively) at P<0.01.

As shown in Table 4, the analysis of variance (P<0.01) for concentration level of garlic oil showed no significant difference between concentration levels of 5ml/kg and 2ml/kg. But 10ml/kg has the lower emergence of 55.50%.

Based on the concentration of the treatments, 10.0ml/kg of treatment produced the highest seedling height of cowpea followed by 5.0ml/kg and the least is observed in 2.0ml/kg control treatments. It is observed that seedlings treated with 10.0ml/kg high of the garlic oil have more height compared to lower concentration. This is to show that increase inconcentration increases the height of cowpea seedlings. From this study, cowpea seedlings treated with lower concentration of garlic oil had higher emergence of seedling. As the concentrations increases the emergence of cowpea decreases. Different concentration levels at P>0.01 on seedling height, showed no significant difference between 5ml/kg and 2ml/kg, but differs significantly with 10ml/kg having higher height of 60.00mm. Test showed that (P<0.01) there is no significant difference among different concentration levels on seedlings stem girth. Test also showed for leaf number, there was no significant difference between the different concentration levels of treatments at P<0.01. Significant difference was observed between the concentration levels of 5ml/kg and 2ml/kg on leaf size, while there was no significant difference between 10ml/kg and 5ml/kg (Table 4).

Applications of treatments such as plants oil which solidifies at lower temperature turn to bind the soil particles together. This may affect the permeability of water and oxygen which are factors responsible for seed germination, but after emergence the seedlings with higher concentrations were noted to have more vigor (Table 4) compared to low level concentration of the treatments. This may be ascribed the superior inhibitory effect of the increased concentration. The concentration of 10ml of garlic/1kg of soil produced lower vigor generally than that of 5ml/1kg. This suggests that higher doses may not be tolerated by cowpea seedlings.

Screen house findings also revealed similar results with those of *in vitro* tests. Both growth and disease characters indicated that garlic oil has a high propensity for control of brown blotch disease of cowpea. The anti-fungal properties of garlic oil have earlier been reported (Atia, 2011 reported that date palm fruit treated with garlic oil reduced the rate of pathogens). Its efficacy for control *has* been adduced to chemical substances that are present in garlic oil which have antimicrobial activities. Hiland and Sfeir (1994) found that geranoid and eugenol were effective in suppressing most fungal strains growth.

Table4. *Growth Character of Infected Cowpea Seedlings Treated with Garlic oil at Different Concentration in ml/kg*

Concentration (ml)	Emergence	Height (mm)	Stem girth(mm)	Leaves no.	Leaves size (mm)
10	55.50	60.00	5.80	33.60	33.10
5.0	60.3	41.30	6.20	40.60	33.80
2.0	61.38	41.30	5.30	33.10	25.90
LSD (0.01)	2.68	15.20	1.60	8.20	7.40

Disease Character

Disease characters in this study included; disease incidence and disease severity, analysis of variance on disease incidence (P<0.01) of cowpea seedlings showed a significant difference between garlic oil and control. The control showed higher disease incidence of 10.80 while that of garlic was 2.50% (Table 5) Analysis of variance at P<0.01for disease severity also showed a significant difference between garlic oil and control. The control treatment differed from the treated significantly by showing higher disease severity of 17.50mm compared to 4.50 of garlic oil (Table 5).

Table5. *Effect of Garlic Oil on Disease Incidence and Severity of Cowpea Seedlings*

Treatment	Disease incidence	Disease severity
Garlic	2.50	4.20
Control	10.80	17.50
LSD (0.01)	4.00	9.80

Obagwu (2003) had reported a successful control of this pathogen with the aqueous extract of garlic. Similarly, in field trials he obtained a better with the aqueous garlic extract used in combination with benomyl. On barley in green house and field experiments, allicin used as elicitor was as effective as fungicide against the leaf spot severity caused by *Bipolaris sorokiniana* (Silva *et al*., 2001; Rodrigues *et al*., 2002; Rodrigues and Bach, 2003; Antoniazzi *et al*., 2008).

Significant difference is observed between the concentrations level of 10ml/kg and 2ml/kg of garlic oil on disease incidence at P<0.01. Concentration of 10ml/kg has better control on *Colletotrichum capsici* to the rest of the concentrations. Analysis of variance on disease severity revealed that there was no significant difference among all the concentration levels of garlic oil (Table 6).

Tabl6. *Effect of Concentration levels of Garlic Oil on Disease Incidence and Severity of Cowpea Seedlings*

Concentration (ml/kg)	Diseaseincidence	Diseases severity
10	2.50	6.90
5.0	3.80	5.00
2.0	6.30	8.80
LSD (0.01)	3.70	8.50

CONCLUSION

Garlic oil has been found effective against *Colletotrichum capsici*, referred to as an unspecified pathogen of many plants as well as been the pathogen of cowpea brown blotch in the savanna zone. Both *in vitro* and *in vivo* control trials present a strong promise of being a remedy for the infection this notorious pathogen incites.

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