The Efficacy of Neem Extracts on The Control of Brown Blotch of Cowpea in Mubi, Nigeria

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Abstract*: The effect of aqueous and ethanolic extracts of neem plant parts (leaves, stembark and roots) on Colletotrichum capsici the causal agent of brown blotch of cowpea was carried out in Mubi. The isolate C. capsici was identified and proven through a pathogenicity test to be pathogenic. Cowpea plant parts (leaves, and pods) with similar symptoms of brown blotch such as dark brown to black patches were collected from farmers' fields in four districts of Mubi North during November 2018. The locations included Mubi, Mayo Bani, Ba'a and Fali districts. A total of 240 samples of two plant parts (leaves and pods) were collected using a simple random sampling technique. The causal agent of brown blotch was isolated from an infected pod of cowpea collected from the periphery of Mubi North in November 2017. The in vitro control trial was carried out on the Potato Dextrose Agar (PDA) polluted with aqueous and ethanolic extracts of Neem (Leaves, stembark and roots) in a completely randomized design with duplicates of 3; a restrain mycelial growth was observed in all the treatments 0.60 cm for leaves and stembark, 1.22cm for roots extract as against 0% inhibition by control. There was a corresponding growth of the action of ethanolic extract as to aqueous in vitro in all the treatments (leaves, stembark and roots) 0.63cm, 0.57cm, 0.57cm as for the ethanolic extract and 0.63cm, 0.67cm and 1.88cm for aqueous treatments respectively. It may be concluded from this study that C. capsici is a common pathogenic fungus that causes brown blotch of cowpea in the Study Area; result from the pathogenicity test indicated that the isolated fungus is pathogenic and attributed to the cause of brown blotch of cowpea in Mubi North. The inhibitory effect of the extract from neem tree against fungal isolate could be due to the anti-fungal substances present in the extract. Higher inhibition of fungus growth was observed at a higher concentration of the ethanolic extract as recorded. The result also indicates that ethanolic extract has more inhibitory compounds than aqueous extracts. This shows a clear indication of the potentials of plant extracts in control of the fungal pathogens.

***Keywords*: Colletotrichum capsici, ethanolic extract, aqueous extract**

I. INTRODUCTION

Cowpea (*V. unguiculata* L. Walp*)* thrives in poor dry conditions growing well in soils up to 85% sand (Obatolu, 2013). The crop can be effectively intercropped with sorghum, millet, maize, cassava or cotton, (Ashfield-watt *et al.*, 2011). Cowpea is grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America (Madan and Thind, 2018). It was originated and domesticated in Southern Africa and was later moved to East and West Africa and Asia (Keagy *et al*., 2018). Cowpea is an important grain legume crop in Sub-Saharan Africa (Hopper and Lampi, 2013). The crop is very important for the livelihood of the poor in undeveloped countries and is accustomed for diverse purposes such as food crops, cash crops and animal feed (Atia, 2011). Emechebe (2016) reported that cowpea highly assigned as an important plant to mankind was tricked to a wide range of diseases.

A collaborative report (Adegbite & Amusa, 2008) assigned brown blotch disease of cowpea as one of the most damaging diseases of cowpea. In Burkina Faso and other African countries, cowpea production is subject to many biotic constraints such as soil-borne and seed-borne fungal pathogens including *Colletotrichum capsici*, the causal agent of brown blotch disease, which can result in yield losses of between 42 and 100% in tropical agriculture (Banerjee *et al.*, 2007; Torres-Calzada *et al.*, 2011). *C. capsici* is a major constraint to many crop productions causing severe losses both in pre-and postharvest decay (Chandra *et al.*, 2009; N'Guettia *et al.*, 2013). Infection by *C. capsici* in cowpea is particularly devastating due to its Hemi biotrophic nature (Hyde *et al.*, 2009). Additionally, the pathogen occurs in different races. For instance, Anderson and Bridges, (2018) identified eight races of *C. capsici* associated with brown blotch disease in Nigeria; four out of the eight races were specific to Guinea and Sudan Savanna while the remaining four were to the rainforest area. Francisco, (2015) reported 12 pathogenic groups of *Colletotrichum spp*. including *C. capsici* associated with brown blotch disease in Burkina Faso. Based on molecular characterization with a specific primer pair (Brown *et al*., 2019) identified four variants of *C. capsici* in Burkina Faso. In the Northern Guinea Savanna zone, the 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 (Grozav and Foarce, 2015) and most farmers obtain their seeds from markets other than unreliable sources and hardly treat them, it may be a reason for its widespread within the state. Channya (2011) also found that there are four variants in Adamawa State.

Considering the risk of using fungicide which might pollute the ambiance while accumulation of the chemical in the soil may support the development of chemical resistance

(Whalen *et al*., 2003), it has become imperative to search for residue free and environmentally friendly alternatives.

Azadirachta indica, also known as Neem, Nimtree and Indian Lilac is a tree in the mahogany family – *Meliaceae* (Hafiza, 2016). It is one of two species of the genus *Azadirachta*, and is native to India, Pakistan and Bangladesh, growing in tropical and semitropical regions. Neem is a very common tree in India (Sonia and Srinivasan, 2017). It is a large evergreen dense tree growing some 10 to 15-meter-tall with a girth of about 2- 3 meters. The leaves of this are divided into numerous leaflets, each resembling a full-grown leaf (Saradhajyothi and Subbarao, 2011). The Neem tree has played an important role in Ayurvedic medicines and agriculture since time immemorial. The earliest documentation of Neem mentioned the fruit, seeds, oil, leaves, roots and bark for their medicinal properties (Srivastava and Kumar, 2017). In the ancient document Carak-Samhita and Susruta-Samhita, the books at the foundation of the Indian System of natural treatment Ayurveda, the various parts of this tree have many uses so it is named in Sanskrit- *Sarva roga nivarini*, meaning the curer of all ailments (Saseed and Junaid, 2018). Its twigs provide a chewing stick and are widely used in the Indian sub-continent (Bourdon *et al.*, 2016). Earlier studies on Neem have shown that it contains active substances with multiple medicinal properties (Mohashine *et al*., 2017). Neem leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in residential premises (Mahmood *et al.*, 2010).

Neem (*Azadirachta indica* A. Juss. Family Meliaceae) has been a beneficial tree to the inhabitant of the central-northern zone of Nigeria for several decades ago (Khalid and Shad, 2012). It has a wide range of uses in the control of crop and household pests, for medicinal purposes and as shade trees. It is also a raw material for soap and charcoal production (Anjorin *et al.*, 2014). Its use for the protection of crops and homes against pests and pathogens in the area reviewed is linked to its folklores and tradition. Anderson *et al.* (2019) submitted that botanical pesticides are simple to prepare, locally renewable, user friendly and environmentally safe. This study aims to determine the efficacy of aqueous & ethanolic extracts of neem (*A. indica*) (leaves, roots and stem bark) on the fungal pathogen of a brown blotch of cowpea in Mubi North.

II. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in the Botany Laboratory of Adamawa State University Mubi in Adamawa State between January-July, 2019. 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, 2014). Mubi North is a town in Adamawa North Senatorial District. The region experiences rainfall from April to October with its peak between August and September (Anon, 2019). The climate of the area is tropical with an average temperature of 32°C and relative humidity ranging from 15% to 68% (Anon, 2015). Mean annual rainfall is about 1056 mm (Adebayo, 2014).

2.2 Isolation and Identification of Colletotrichum capsici

Colletotrichum capsici was isolated from an infected pod (judging typical brown blotch lesions), acquired from a farmer's field in Mubi North in November 2018. Under aseptic conditions, the disease portion of the pod was sectioned into 5mm square pieces with a heat sterilized scalpel. Pieces were picked with flamed, then cooled pair of forceps. These portions were transferred into a 0.1% mercuric chloride solution contained in a 9cm sterile petri dish for surface sterilization for 30 seconds. The germless portions were then washed in five different sterile distilled water and dried between sterile filter papers. With flamed and cooled forceps, a sterile piece of the pod was then plated out on sterile solidified Potato Dextrose Agar (PDA) which was supplemented with chloramphenicol (0.3 µg/l of PDA) to prevent bacterial contamination. The petri dishes were incubated at a temperature of 26+_ 2°C in July 2019 for seven days. A clean isolate of Colletotrichum capsici was acquired from the hyphal tip of growing colonies by using a sterile needle and repeated Sub-culturing on sterile Potato Dextrose Agar until the clean culture was acquired. The clean culture was then stored on an agar slant in a McCartney bottle for future use. Potato Dextrose Agar PDA was assembled using the method of Smith and Onion (2013). The slides of the organism to be isolated were prepared and stained with lactophenol cotton blue and was observed under the microscope and subsequently identified (Alexopoulus and Mius, 2016).

2.3 Phytochemical constituents of the leaves, roots and stem bark of the neem tree.

Plant samples (leaves, stem bark and roots) were collected in a sterile polythene bag then washed with tap water and then with distilled water. The samples were shade-dried and pulverized using mortar and pestle, into fine powder form. Two hundred grams (200 g) of the powdered samples were placed in a beaker and then sterilized in an oven for three hours at 160°C. One hundred grams (100 g) portion of the powdered samples was mixed with 120mls of ethanol and then filtered (Aveling *et al.,* 2001). The extract then was poured into a sterile conical flask plugged with cotton wrapped in aluminum foil and heated between 30-60 °C for ten minutes to avoid contamination. These were allowed to cool, wrapped in aluminum foil and kept until when required. The same procedure was repeated for the aqueous extraction of the phytochemical constituents of the leaves, stembark and roots extracts of the neem tree.

2.4 In-vitro assessment of the aqueous and ethanolic leaves, stembark and roots extracts

Sterile razor blade was used to cut from 7 days old culture of fungi (approximately 0.1mm) which was incubated at room temperature of 26 °C, the pathogen was placed at the center of

the amended dextrose media. Petri dishes were sealed with a masking tape, incubated at 25 °C in July, and observed from 24 hours to 10 days for the inhibitory action of the extract. The experiment was executed under aseptic conditions in a completely randomized design and was replicated three times for each concentration level of the extract method (Sing, 2014). The colony diameter of the radial growth was measured from 72 hours (3days) to 10 days. The zone of inhibition (P) was measured using the formula of Francisco (2015).

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

2.5 Statistical Analysis

The statistical layout was a completely randomized design and Data generated from this study were subjected to two ways analysis of variance (ANOVA), and where there was a significant difference, means were divided using least significant differences (LSD). The statistical package used was Statistical Package for Scientist and Engineers SPSE (2012) STAT. 9.1

III. RESULTS AND DISCUSSION

The result showed that all extracts significantly reduced colony expansion of the pathogen compared to control at $p<0.05$. Leaf and stembark extracts were however reduced the colony growth than roots extract with the mean value of 0.60cm respectively (Table 1).

Table 1; Effect of Neem Extract on Radial Growth of *C. capsici* (cm) *in vitro*

Extract	Radial expansion of C. capsici (cm) in vitro
Leaf	0.60^{a}
Stem bark	0.60^{a}
Root	1.22^{b}
Control	2.38^{c}

a, b, c. Mean in the same column having a different superscript(s) are significantly ($P<0.05$) different according to Duncan Multiple Range Test.

Comparison of the Effect of Aqueous and Ethanolic Extract of Neem (Leaves, Stembark and Roots) on Radial Growth of C. Capsici.

Comparison of the effect of aqueous and ethanolic extract of neem (leaves, stembark and roots) on radial growth of *C. capsici in vitro* was shown in Table 2. The result indicates that they were significantly different ($p< 0.001$) in their ability to reduce colony growth of *C. capsici.* From the result in Table 2, treatment of cowpea with ethanolic stem bark and ethanolic roots extract reduced the growth of *C. capsici in vitro* compared to aqueous stem bark and aqueous roots extract. Analysis of variance for radial growth of *C. capsici* showed that there was no significant difference in the effect of aqueous and ethanolic leaves extracts on the radial growth of *C. capsici in vitro*. From Table 2, treating *C. capsici* pathogen of brown blotch in cowpea with ethanolic stembark extract or ethanolic roots extract brings about the highest control of the pathogen with colony growth of 0.57cm respectively. There was no significant difference in the effect of ethanolic stem bark and ethanolic roots on the radial growth of *C. capsici in vitro* (Table 2). There was a significant difference in the effect of aqueous and ethanolic extracts of different parts of neem (leaves, stembark and roots), the result in Table 2 revealed that there was no significant difference between ethanolic stem bark and roots extract. Analysis of variance shows that ethanolic extract reduced the colony growth of *pathogen in vitro* in stembark and roots extract compared to aqueous stem bark and roots extract. Aqueous and ethanolic leaf extracts were not significantly different.

Table 2. Comparison of the Effect of Aqueous and Ethanolic Extracts of Neem (Leaves, Stembark and Roots) on Radial Growth of *C. capsici in vitro*

Treatments solvents		Ethanolic	Control
	Aqueous		
Leaves	0.63^{b}	0.63^{b}	2.38^{e}
Stembark	0.67^{c}	0.57^{a}	2.38^{e}
Roots	1.88^{d}	0.57^{a}	2.38^{e}

a, b, c, d, e. Mean in the same row bearing different superscript(s) are significantly different ($P<0.05$) according to Duncan Multiple Range Test.

(0.63cm expansion) while solvents varied significantly for both stembark and roots extract with ethanolic extract being more effective than the aqueous (0.57cm, 0.57cm and 0.37cm, 0.37cm respectively). There was no significant difference in the effect of various concentration levels of aqueous and ethanolic extracts of neem (leaves, stembark and roots) on the radial growth of *C. capsici in vitro.* The findings from this study have indicated that neem extract is capable of controlling the colony growth of that pathogen *in vitro*. It is in line with the observation of Mark and Channya (2016) who demonstrated the control of fungal rot of plantain in the southwest of Nigeria, ash extract also reduces the colony growth of *C. capsici* by providing a reasonable inhibition. The record shows both aqueous and ethanolic extract of neem (leaves, stembark and roots) reduces the colony growth of *C. capsici* compared to control. The test shows that ethanolic extract provides reasonable inhibition of *C. capsici in vitro* compared to the aqueous extract of neem (leave, stembark and roots). This may be a result of differences in the polarity of the solvents. As reported by Bautista *et al*. (2013) some plants extract containing those metabolites has been extracted in water or other solvents, depending on its polarity and in powder form. An organic solvent such as methanol and ethanol have been found to extract the active ingredients better than water. Gahukaar (2006) reported that limonoids are generally being highly soluble in alcohol such as methanol or ethanol. The extracted, purified bioassay-guided limnoids could be added to inert compounds to produce a neem product with a known stable *azadirachtin* concentration (Jaryrum et al. 2011). It was also reported by Mondali *et al.* (2009) positive

effect of *A. indica* leaves extract on the growth of fungal species *in vitro* culture medium. Effective of neem as a control for fungi was attributed to the neem nature of having some active components such as flavonoids, terpenoids, steroids, phenols, reducing sugar. Franzener et al. (2003) reported that extracts from plants commonly found in tropical Africa have been tested for in vitro fungistatic effects.

IV. CONCLUSION

It has concluded that Neem plant has been found effective against *Colletotrichum capsici* which has been the organism capable of brown blotch of Cowpea as evidenced in the reduction of mycelial growth seen in treated over control.

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