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Full Length Research Paper

Control of Fungal Leaf Spot disease of mango (*Mangifera indica. l*) with extracts of *Azadirachta indica* (neem) and *Zingiber officinale* (Ginger)

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Fungal leaf disease of Mango (Mangifera indica) caused by fungal pathogens, has been reported to reduce the quality of mango fruit. Three pathogenic fungi, Penicillium sp., Mucor sp. and Aspergillus sp. which are well known saprophytes of dead plant materials were isolated from diseased leaves and were thereafter confirmed to be the causal agents by pathogenicity test after symptoms developed 3-5 weeks after inoculation of healthy leaves. Mixtures of two plant materials namely Zingiber officinale (Ginger) and Azadirachta indica (Neem) were set up to control fungal leaf spot which gave effective control at high concentration (20-100%). The findings from this study are significant as it contributes vital information on the control of fungal leaf spot disease of mango (Mangifera indica) using natural herbs.

Keywords: Mangifera indica,Pathogen,Pathogenicity, Zingiber officenale, and Azadirachta indica.

INTRODUCTION

The mango is an erect, branched, medium to long-sized tree alternately arranged evergreen or nearly evergreen leaves, with a wide crown and inflorescences having numerous flowers (Van , 1999; Morton, 1987; Samson, 1980).The species which belong to the family Anacardiaceae is native of Asia, particularly eastern India, Burma and Andaman islands from where it spread to other parts of the world. But while the Persians introduced it to East Africa in the 10th century, the Portuguese were responsible for its introduction to West Africa in the 16th century (Morton, 1987). Mango came to Nigeria in the 20th century through itinerants merchant missionaries and colonialist, where it has become an integral part of indigenous cropping system (Aiyelaagbe, 2002; Nyishir, 2004). The guinea and Sudan Savanna zones of Nigeria are credited with producing greater percentage of the fruit in Nigeria (Olaniyan, 2004); with Benue state topping the list (Avav and Uza, 2002). Unfortunately, the history of mango production in the state is not very clear. Reports however indicate the improved mango varieties were introduced to Yandev farm centre by early agricultural officers from Zaria and Ibadan in the 1950s (Nyishir, 2004). Mango is the most popular and commonly eaten fruit among millions of people in tropical areas and especially the developed countries (Diedhiou, 2007; Crane and Cambel (1994). Universally, Mangoes are considered as one of the most important fruit in the world (Diedhiou, *et al.*, 2007). The crop is grown in over 87 countries in the world (Sauco, 2002). Mango has become an essential fruit crop in Asia, Southern and Central America as well as many parts of Africa (Diedhiou, *et al*., 2007). For this reason, fruit quality in terms of colour, taste and physiological damage is a vital importance. Also, because of diverse production conditions and the vast area grown, mango suffers a number of disease, some of them taken a heavy toll on the crops and representing limiting factor (Diedhiou*et al*., 2007).

The mango tree and most especially the fruits and leaves are hosts to a large number of pathogens among which fungal could be a major agent of fruit rot and leaf spot disease. It has been reported that *Macrophoma Mangiferae* causes foliage blight of young seedlings

and young grafted plants, while *Botryodiploidia Theobromae* was the agent of die-back and bark canker, and the gray blight of leaves is caused by *Pestalotiopsis Mangifera* (Verma, *et al.,* 1991).The attention of mycologist is presently focused on control measures of mango disease (Verma, *et al.,* 1991; Okigbo, 2001). The use of chemicals to control plant pathogens, especially foliage pathogens has only limited success in the past in Africa due to lack of suitable methods of application, or lack of effective chemical and prohibitive cost. There is also added concern about chemical residue in the environment and the development of resistance by the pathogen (Spotts and Cervantes, 1986; Korsten, 1995; Osuinde, *et al.,* 2001).

Aim and objectives

To use the extracts of Neem (*Azadirachta indica*) and Ginger (*Zingiber officinale*) to control leaf spot disease of mango, to isolate and identify the fungi responsible for mango leaf spot disease and to confirm the fungal disease isolated using pathogenicity test.

Azadirachta Indica (NEEM)

Neem is a Hindu noun derived from Sanskrit Nimba (Henry and Burnell, 2013; Hobson-Jobson, 1999; Encarta world English dictionary, 1999). Neem is a key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. Neem seeds are grounded into powder that is soaked overnight in water and sprayed onto the crops. To be effective, it is necessary to applied repeatedly, at least every ten days. It acts as an antifeedant, repellent, and egg-laying deterrent protecting the crop from damage. Neem also suppresses the hatching of pest insects from their eggs. Neem cake is often sold as a fertiliser (Bhaskara, 2010).

Phytochemical compounds

Ayurveda was the first scientist to bring the anthelminthic, antifungal, antibacterial and antiviral constituents of the neem tree to the attention of natural product chemists (Koul, *et al*., 1990), (Chattarjee and Pakrashi, 1994). In 1942, he extracted three bitter compounds from neem oil, which he named as nimbin, nimbinin and nimbidin respectively. The process involved extracting the water insoluble components with ether, petrol ether, ethyl acetate and dilutes alcohol. The provisional naming was nimbin (sulphur free crystalline product with melting point at 205^0C, empirical composition $C_7H_{10}O_2$), nimbinin (with similar principle, melting at 192^0C) and nimbidin (cream-coloured containing amorphous sulphur, melting at $90\text{-}100^0$C). Siddiqui identified nimbidin as the main active antibacterial ingredient, and the highest yielding bitter component in the neem oil. These compounds are stable and found in substantial quantities in the neem. They also serve as natural insecticides (Bhaskara, *et al.*, 2010). Neem coated urea is been used as alternative to plain urea fertilizer in India. It reduces pollution, improves fertilizer's efficacy and soil health (Retrieved from https://timesofindia.indiatimes.com/).

Ginger (*Zingiber officinale*) is a flowering plant in the family *Zingiberaceae* whose rhizome, ginger root or simply ginger is used as a spice or a folk medicine. It is an herbaceous perennial which grows annual stems about a metre tall, bearing narrow green leaves and yellow flowers. Ginger is indigenous to south China, and was spread eventually to the spice island, other parts of Asia and subsequently to West Africa and the Caribbean (Retrieved from "[abc]" Spices: Exotic flavours and medicines: Ginger", 2014).Preliminary research indicates that nine compounds found in ginger may bind to human serotonin receptors which may influence gastrointestinal functions. Research conducted in vitro test show that ginger extracts might control the quantity of free radicals and the peroxidation of lipids. Preliminary studies involving the effect of ginger on nausea occurring with pregnancy suggest that intake of ginger may cause belching and ingestion (Ernst and Pittler, 2012). In a 2010 study, daily consumption of ginger was shown to help ease muscle pain associated with exercise by 25%. (Wood and Pittler, 2012).In limited studies, ginger was found to be more effective than placebo for treating nausea caused by sea sickness, morning sickness and chemotherapy although ginger was not found superior to placebo for pre-emptively treating post operative nausea. Other preliminary studies show that ginger may affect arthritis pain or have blood thinning and cholesterol lowering properties, but these effects remain unconfirmed (Chen *et al.*, 2007).Advance glycation end-product are possibly associated in the development of diabetic cataract for which ginger was effective in preliminary studies, apparently by acting through antiglycating mechanisms(Rhode *et al.*, 2007).

MATERIALS AND METHODS

Materials

Petri dishes, beakers, test tubes, measuring cylinder, filter paper, spatula, forceps, conical flask, wire loop, non-absorbent cotton wool, aluminium foil, sterile cellophane, sprayer, syringe and needle, mortar and pestle, microscopic slide and clip, nose mask and hand gloves, Sabouraud Dextrose Agar.

Equipment include: Autoclave, Beam balance, Incubator, Compound microscope, Centrifuge, Refrigerator, distilled water, lactophenol, ethyl alcohol and 70% ethanol. Diseased leaves were collected from different sites in Campus 1 of Delta State Polytechnic, Ozoro. *Azadirachta indica* (neem leaves) were collected from locations in Ozoro town in Isoko North Local Government Area of Delta state. Ginger roots were purchased from Ozoro main market.

Method

Sterilization of materials

Sterilization of Petri-dishes, beakers, test tubes, filter papers, spatula and forceps, conical flask, cork-borers and slides previously washed with soap and water in hot air oven at a temperature of 160^{o}C for 1 hour. The wire loops were also sterilized by heating over an alcohol lamp until red hot and allowed to cool. Conical flask containing culture media were tightly plugged with non-absorbent cotton wool covered with aluminium foil and autoclaved at 121^{o}C and 15ps for 15 minutes (Agrios, 2005). Disinfectants used were ethyl alcohol, 70% ethanol; to wipe the walls tops and window to prevent contamination.

Preparation of culture media

Sabouraud Dextrose Agar (SDA) was prepared using the standard method. Commercially prepared Sabouraud dextrose Agar was measured using a beam balance at 32.5g and 250ml of distilled water was added to the SDA in a conical flask; corked with non-absorbent cotton wool covered with aluminium foil and the preparation were then autoclaved following strictly instructions by the manufacturers.

Isolation of fungi from mango leaf

Applying the technique used by Okigbo (2001), 2cm of infected mango leaf tissue was excised with a sterilised cork-borer and surfaced sterilised by dipping in 70% ethanol solution for 1.5 to 2.0 minutes. The tissues were then rinsed in three changes of sterilised distilled water and comminute onto SDA (Sabouraud Dextrose Agar) in Petri dishes. These were incubated at room temperature (25±28^{o}C) under light for 5 days to enhance fungal growth and sporulation. Subcultures were made until pure cultures were obtained.

Identification of fungi

The wet mounts of isolates in lacto phenol in cotton blue were examined microscopically and identified based on their colonial morphology, mycelia structure, spores and associated structures according to Alexopoulos, *et al*., (2002).

Pathogenicity test

Pathogenicity test was carried out for all the isolates from the mango leaf tissues using techniques of (Okigbo, *et al*., 2001). Spore suspensions of the isolates were prepared by centrifuging and re-suspending the spores in three changes of sterile distilled water. Healthy leaves on a young mango tree were surface sterilized with ethyl alcohol and then rinsed with sterile distilled water. The lower epidermis of the leaves was sprayed separately with a spore suspension from each of the fungi, using a sprayer. A mixture of the spore suspension of all the fungi was also used as inoculum by mixing 50ml of each suspension. One healthy branch was sprayed with distilled water to serve as control. All branches were covered with sterile cellophane bags for 5 weeks until symptoms of the disease were apparent, at which the bags were removed to expose the leaves to natural conditions. The leaves were inspected daily to check for symptoms or any other effect of the pathogens on the leaves.

Preparation of leaf extracts

The processing of plant materials of Abalaka, (2012) was used. The neem leaves and ginger were air-dried in Delta State Polytechnic, Ozoro; Biology Laboratory for two weeks and then grinded into powder using a sterile mortar and pestle. The grinded *Azadirachta indica* and *Zingiber officinale* were extracted using the process adopted by Abalaka, (2012). The extracts ofthe leaves and root were soaked for 24 hours in 50ml of water and ethanol. The extracts were filtered using Whatman No1 filter paper and stored in a refrigerator at 4^{o}C for subsequent use. Then two different extracts were obtained in water and ethanol.

Preparation of extract concentrations

Applying the techniques of (Arikpo, *et al.,* 2013). The extracts in ethanol were reconstituted in sterile distilled water to obtain various concentrations of the extracts as follows: 5ml of the extract was reconstituted in 5ml of sterile distilled water to obtain 100 percent concentration of the extract. Also, 4ml of the 100 percent concentration was diluted with 1ml of sterile distilled water to obtain 80 percent concentration. The serial dilution procedure was continued to obtain lower concentrations of the extract as follows; 100, 80, 60, 40 and 20.

Table 1: Percentage occurrence of pathogens isolated from leaf spot infection of mango.

Slide	Fungal isolates	% of occurrence
A	*Aspergillus sp.*	60
B	*Penicillium sp.*	30
C	*Mucor sp.*	10

Table 2: Percentage area of mango leaf surface occupied by lesions of 3 weeks and 6 week after

Inoculum	Leaf area (%) covered with lesions after 3 weeks	Leaf area (%) covered with lesions after 6 weeks
Penicilliumsp.	17	25
Mucor sp.	19	23
Aspergillus sp.	39	60
Mixture of Pathogens	28	40
Control	0	0

Preparation of culture media using leaf extracts

Following the procedures of (Mukherjee, *et al.,* 2011) and (Arikpo, *et al.,* 2013); SDA culture media in conical flasks were sterilized in an autoclave at temperature of 121^{o}C for 15 minutes. After autoclaving, about 20ml of the medium was poured in each 9cm sterilized Petridishes. Five millilitres (5ml) from each of the concentration of the extract (0, 20, 40, 60, 80 and 100%) was dispensed into the Petri dish and agitated thoroughly with the melted SDA forming Sabouraud Dextrose Leaf Extract Agar (SDLA). Mycelial discs were prepared using a cork borer (5mm diameter) from the tip of 5 days old cultures of the isolates. One disc of the isolates was placed at the centre of a petridish after solidification of the SDLA. Each treatment was replicated for all concentrations of the extract. The medium without plant extract served as control. All the plates were incubated at 27^{o}C, after which the zones of inhibition on colony diameter was measured for five days. Percentage inhibition was determined according to Whipps (1987). Percentage Inhibition $=\frac{R1-R2}{R1} \times \frac{100}{1}$Where, R1 = Furthest radial distance of fungus in control plates (PDA only) R2 = Furthest radial distance of fungus in treatment plates. Results were expressed as mean ± S.D comparison of 3 determination, and results adjudged significant at P<0.05

RESULTS

The fungi isolated from leaf spot infection of mango leaves were: *Mucor sp., Penicillium sp.* and *Aspergillus sp.* The fungi and their prevalence of occurrence are shown in (Table 1). The results showed that *Aspergillus sp.* was most prevalent (60%) followed by *Penicillium sp.* (30%) while the least was *Mucor sp.* (10%).

The result of the pathogenicity tests showed that all the isolated fungi were pathogenic to the mango leaf. From visual observation, leaves of the mango sprayed with spores of the different fungal isolates started showing lesions after 3 weeks. The lesions were very conspicuous for the three fungi and the mixture of the spores (Table 2). Infections were established slowly, maybe as a result of the cold weather. Young leaves are generally exposed to infection on very dry seasons but may escape the disease during the wet season.

The antifungal activity of *Azadirachta indica* (neem) and *Zingiber officinale*(ginger) extracts on the test fungi is represented in (Table 3). The extracts inhibited the growth of all the fungi tested at various concentrations. Increased antifungal activity was observed with corresponding increase in the concentration of the two plant extracts studied. The highest percentage inhibition of mycelia growth of the fungi was recorded at 60, 80 and 100% extract concentration (94.47, 96.69 and 98.88% respectively) of the mixture of *A. indica* and *Z. Officinale* with the mycelia showing no sign of growth between 3-5 days. The antifungal activity of extract mixture recorded at 20% and 40% concentration was moderate after 3 days (88.75% and 91.11%) but decreased after 5 days (33.37% and 77.70% respectively). The control plate, without the incorporation of extract grew very well with no inhibition of the fungus.

DISCUSSION

The pathogens associated with Leaf spot disease of mango in this study were: *Mucor sp., Penicillium sp.*

Table 3: In-Vitro antifungal efficacy of extract mixture of *A. indica* and *Z. officinale* (incubated at 27oC for 3-5 days)

Extract conc.	% of inhibition of extract mixture after 3 days	% of inhibition of extract mixture after 5 days
Control (0) untreated	**0**	**0**
20	88.75	33.37
40	91.11	77.70
60	94.47	94.47
80	96.69	96.69
100	98.88	98.88

and *Aspergillus sp. Aspergillus sp.* and *Penicillium sp.* showed the highest occurrence while *Mucor sp.* was the least. The fungal organisms causing the leaf spot infection in the mango must have been present right from the seeds before manifesting in the field. This study has shown that *Mucor sp., Penicillium sp.* and *Aspergillus sp.* associated with the leaf spot disease of mango were inhibited by the ethanolic extracts of *Azadirachta indica* (neem) and *Zingiber officinale* (ginger) at high concentrations in vitro. These results also agreed with earlier works on the inhibitory action of plant products employed on the mycelia and spore germination of other pathogenic fungi (Ajayi and Olufolaji, 2008). This study corroborates the report of other workers (Owolade, *et al.,* 2000; Okigbo and Emoghene, 2003), that *Azadirachta indica* and *Zingiber officinale* are among important plants whose extracts are capable of checking the spread of many fungal diseases of food crops. The high fungitoxic effect of *Azadirachta indica* observed in this study could be due to the presence of the volatile oil of the seed which was reported to contain three bitter compounds; nimbin, nimbinin and nimbidin which serves as natural fungicides (Bhaskara, *et al.*, 2010). The extract mixture at 60, 80 and 100% concentration acted favourably in this study. The leaves and seeds of *A.indica* were also reported to contain antifungal and antibiotic activities. This gives credence to its traditional use for healthy hair, to improve liver function, detoxify the blood and balance blood sugar (http://www.tamilnadu.com). It is also use in the treatment of human skin disease like eczema, psoriasis etc. (Rahman, *et al.*, 2011) and (Horsbrugh, 2006).The fungicidal attribute evidenced in this study can further be developed pharmacologically for the control of leaf spot disease of mango caused by pathogenic fungi and other microbial agents of other crop diseases.

Conclusion

This work has implicated three fungi to be responsible for the leaf spot disease of mango. Plant materials previously reported as good agro chemicals have also been found capable of reducing the effects of fungal in agricultural produce. Ginger and neem used in this study are capable of inhibiting leaf spot disease of mango at high concentrations, since they are strongly effective for eliminating fungal pathogens causing this infection.

Recommendation

Therefore, it is recommended that fungicides containing high concentrations of the extracts of Neem leaves and Ginger root should be employed to inhibit the growth of fungi in mango especially at their early stages instead of the conventional ones, so as to reduce the prevalence of the infection and exposure of the plant to toxic chemicals. This will ensure good quality and increased production of mango in Africa.

REFERENCES

"abc" spices (2014). Exotic flavours and medicine; ginger.http://unitproj.library.uda.edu/biomed/spices/.

Abalaka ME (2012). The Antibacterial Activities of *Azadirachta indica* against some Bacterial Pathogens.

Agrios GN (2006). Plant Pathology, Academic Press, New York. 922pp.

Aiyelaagbe IOO (2002). Mango Production in Nigeria. HORT Magazine 1:9-10.

Ajayi,A.M., Olufolaji,D.B (2008): The Biofungical Attributes of Some Plant Extracts on *Colletohichumcapsicum*, The Fungal Pathogen of Brown Blotch Disease of Cowpea. Nigerian J. Mycol. 1 (1):59-65.

Alexopoulos CJ, Muns CW, Blackwell M (2002). Introductory Mycology (4th edition) John Wiley and Sons Inc. Singapore, pp.869.

Avav T, Uza DV (2002). Agriculture In: Pigeonniere, A.L (Ed) Africa Atlases; Nigeria, les Edition, J.A, Paris, Pp. 92-95.

Bhaskara MV, Pramoda SJ, Jeevikaa MU, Chandana PK, Shetteppa, G (2010): "Letters: MR Imaging findings of Neem oil poisoning". Am. J. Neuroradiol. (American Society of Neuroradiology) 31 (7):E60-E61. doi:10.3174/ajnr.A2146

Chatterjee, A, Pakrashi S(Eds). The Treatise on Indian Medicinal Plant. 3: 76.

Crane JH, Campbell CW(1994). The Mango Facts Sheet Hs-2, Horticultural Sciences, Department Florida Cooperative Extension Service, Institute of Food and Agricultural Science, University of Florida, Gainesville, Florida.

De Jussieu A (2009). Museum Hist. Nat. Paris. 19,220.
Department of Land Resource Management (2015): Neem Azadirachta indica (PDF): Retrieved, 2015.
Diedhiou PM, Mbaye NA, Drame A, Samb PI (2007): Alternation of Post Harvest Disease of mango (*Mangifera indica)* through Production Practices and Climatic Factors. Afr. Biotechnol. 6:1087-1094.
Encarta World Dictionary (1999): Neem, page 1210. St.Martin's Press, New York.
Ernst E, Pittler MH (2012). Efficacy of Ginger for Nausea and Vomiting; A Systematic Review of Randomized Clinical Trials, British J. Anesthesia, 84(3): 367-371.
Henry Yule, Burnell AC (2013). Hobson-Jobson (1999): Neem, page 622,
Morton JF (1978). Mango In: Fruits of the Warm Climates, Miami-Florida, Pp. 221-239
Mukherjee SK, Lit 2, Richard E (2011). Introduction to Botany and the Importance, in Lit3, Richard, E, The Mango Botany Production and Uses, walling ford, Oxon, Uki CAB International, Pp. 1-18.
Nyishir SA (2004). Mango Variety Production, Marketing and Consumption among Household in Benue State. PGD thesis, Department of Agricultural Extension, University of Nigeria, Nsukka.
Okigbo RN (2001). Occurrence, Pathogenicity and Survival of *Macrophoma mangiferae* in Leaves, Branches and Stem of Mango (Mangifera indica l): Plant Project. Sci 37: 127-142.
Okigbo RN, Emoghene O (2003).Fungi Associated with Peels of Post-harvestYam (*Dioscorea spp*) in Storage. Global J. Pure. Appl. Sci, 9:19-23.
Olaniyan AO (2004). General Information about Mango and Citrus Production in Nigeria. Paper Presented at the Mashav International Training on Mango and Citrus Orchard Growing and Management, Held in Maize Farms, Minna,Niger State; 22nd Nov-3rd Dec, 2004, Pp. 10
Osuinde RN, Ikediugwu FEO (2001). Biological Control of Tuber Surface Mycoflora of Yam (*Dioscorea spp*): Tropical Sci. 41:85-89.
Rahman MM Sarker D, Barman JC, Alan SN (2011): Bagging Practice to Control Mango Fruit Fly. Regional Hortic. Res. Station, BAR (nawabgon)J. pp.59-64.
Sauco V (2002). Mango Production and World Market: Current Situation and Future Prospects. Acta Horticulturae 645: 107-116.
Spotts RA, Cervantes IA (1986). Population, Pathogenicity and Benomyl Resistance of *Botrytis spp., Penicillium spp., Mucor pyriformis* in Packing Houses Plant Dis. 70:106-108.
Van Ee S (1999). Trees Growing in the Tropics, 2nd Ed. Agrodok-Series No.5 Agromisa Foundation, Pp.88.
Verma KS, Cheema SS, Kang MS, Sharma AK (1991). Hitherto Unrecorded Disease Problems of Mango from Punjab Plant Dis. Res., 6: 141-142.