TOXICITY OF COPPER (1) OXIDE METALAXYL FUNGICIDE AND SELECTED PLANT EXTRACTS TO COLLETOTRICHIUM LINDEMUTHIANUM (SENSU LATO) AND MANAGEMENT OF COWPEA ANTHRACNOSE DISEASE IN NIGERIA

M. J. Falade[1] and O.A. Borisade
[1, 2] Department of Crop Production, Horticulture and Landscape Design, Ekiti State University, Ado-Ekiti, Nigeria.
***Email**: tosoborisade@gmail.com*

Abstract
In vitro studies were conducted to evaluate the effect of three plant extracts, Datura stramonium, Ricinus communis and Jatropha gossypifolia at 30, 50 and 65% concentrations and Copper (1) oxide in metalaxyl based synthetic fungicide (Trade name: Tandem®) on Colletotrichum lindemuthianum growth characteristics. The plants extracts were applied in situ to assess their efficacy in the management of anthracnose disease on cowpea. The results showed statistically significant ($P<0.05$) variations in C. lindemuthianum growth depending on the extract and concentrations. D. stramonium at 65% concentration was the best in reducing the growth of the fungus and disease incidence in the field. The effect of 65% D. stramonium compared favourably with the standard application rate of the synthetic fungicide. The growth rate of C. limdemuthianum treated with 65% of D. stramonium extract and Tandem® were not statistically different, both were approximately 2.9 mm day-1. Similarly, disease incidence was reduced by 14% in the plots where either the extract or Tandem® was applied. The study demonstrated that the active compound in the extract of D. stramonium can potentially replace synthetic fungicides in the management of cowpea anthracnose disease.

Key words: anthracnose, Colletotrichum lindemuthianum, cowpea, mycelial growth plant extracts

1.0 Introduction

Cowpea, Vigna unguiculata (Fabaceae: Leguminosae), is widely cultivated in the tropical and subtropical regions. It is a major source of dietary protein for man and livestock and over six hundred people in Africa and Asia depend on it for food security and source of income (FAOSTAT, 2012). The world cowpea production in 2000 was 3.32 million metric tons (MT) and 75% of the total output was produced in Africa. (FAOSTAT, 2013). The major cowpea producing areas are in West Africa and the sub- humid to semi-arid zones, including Nigeria, Niger, Senegal, Ghana, Mali and Burkina-Faso are. (FAOSTAT 2000).

Cowpea is attacked by different fungal diseases which impair plant establishment, photosynthetic capabilities and plant vigour due to leaf blights, stem, root and flower rots, and abortion of flowers and fruit rots (Kareem and Taiwo, 2007). Cowpea is propagated by seed and since most of these pathogens are seed-borne, they are likely to multiply and spread from one production cycle to the other (Gideon and Anita, 2013).

Cowpea anthracnose is caused by the fungus, Colletotrichum lindemuthianum which is considered the most important fungal disease of field grown cowpea, capable of causing up to 75% yield reduction in the absence of effective management techniques (Enyiokwu et al., 2014). The disease affects all stages of the plant but more often the reproductive stages. Its symptoms include round brownish or purple specks which become darker and enlarge into lesions. Individual lesions are usually lenticular to circular, tan to brown coloration and the size and distribution depend on the degree of severity (Sharon and Douglas, 2011). The symptoms of anthracnose are visible on leaves and ripe fruits as water-soaked and sunken circular spots and appear as cankers on petioles and stems. (Davis, 2014).

Selective fungicide such as Benomyl (Methyl 1-butylcarbamoyl benzimidazol-2-ylcarbamate), as well as the contact/systemic type. Tandem® (65% Copper (1) Oxide in 12% Metalaxyl wettable powder, WP) are used for the management of fungal diseases of horticultural crops in Nigeria and they have been found effective. However, due to the increased awareness of side effects of these synthetic pesticides, particularly environmental concerns and the need to produce crops with acceptable residual pesticide levels, much attention is being focused on the alternative methods which includes the use of resistant varieties of cowpea where they exist, cultural removal of sources of pathogens and use of plant extracts.Leaf extracts of many plants have yielded promising outcomes when applied against fungal pathogens of horticultural crops. For example, the Purple princess (Cyathula prostrata), Black pepper (Piper nigrum), Clove basil (Ocimum gratissimium), Lemon (Citrus limon) Lemon grass (Cymbopogon citratus) have been tested against fungal infections in cowpea with varying degrees of success (Gideon

and Anita 2013; Amadioha and Obi, 1999; Amadioha, 2003). The potentials of botanicals for disease control have been recognized such that there is the need for evaluation of fungi-toxic properties of more tropical plants, such that their active ingredients can be developed into botanical fungicides in the future. .

Anti-fungal effects of the physic nut (Jatropha gossypifolia) (Igbinosa et al., 2009), castor oil (Ricinus communis) (Naz and Bano, 2012), jimson weed (Datura stramonium) (Usha et al., 2009) are well known but their toxicity to C. lindemuthianum and use for the management of cowpea anthracnose have not been studied. The objectives of this study were to compare the effects of extracts of these plants and the synthetic fungicide, Tandem® on mycelial growth and conidiation of a Nigerian isolate of C. lindemuthianum using invitro bio-toxicity trials

2.0 Materials and Methods

2.1 Preparation of Plant Leaves and Source of Fungicide

Leaves of D. stramonium, J. gossypifolia and R. communis (Figures 1a-c) were collected at Ekiti State University (Latitude 7 7212°N and longitude 5.2575°E) in the South western Nigeria during the month of April, 2013. The leaves were air-dried at ambient temperature (28±2°C) for 1-2 weeks. The dried leaves were powdered using a blender (Okapi®, Mixer-Grinder), packaged into sealable nylon and refrigerated at 4°C until they were required for bioassay. The synthetic fungicide Tandem,® containing 65% Copper (1) Oxide in 12% Metalaxyl as wettable powder (WP) was purchased from Agro-stores in Nigeria.

2.2 Preparation of Plant Extracts

Extracts were prepared by mixing equivalent grams of the prepared plant powder (65, 50 and 30) with 100 ml of distilled water in 500 ml flasks and kept in hot water bath-shaker at 70 °C for 2 hours. Thereafter, extract was separated from the shaft by vacuum filtration and stored at 4 °C in McCartney bottles. These were used as the stock solution from which 65%, 50% and 30% concentrations were prepared (Collin and Michael, 2000).

2.3 Preparation of Modified Media

Standard Potato Dextrose Agar (PDA,E. Merck, Darmstadt Germany) was modified either with different concentrations of the plant extracts or Tandem® at the recommended rate (0.1g/l) and autoclaved. Thereafter, the agar was allowed to cool to 50 °C and amended with 30 µg/L streptomycin Sulphate before it was poured into 9 cm sterile Petridishes (Sterilin® Product, UK) inside a laminar flow cabinet and left for 20 minutes to solidify.

(a) Jatropha gossypifolia

(b) Racinus communis

(c) Datura stramonium

2.4 Isolation and identification of C. lindemuthianum

Infected cowpea plants showing symptoms of anthracnose were collected from the cowpea fields at Ekiti State University Teaching and Research Farm, Ado-Ekiti. The leaves were cut into approximately 1cm2 sizes and surface sterilized by rinsing with sterile distilled water containing 0.2 % hypochlorite solution followed by two rinses in sterile distilled water in a laminar flow cabinet. Three leaf cuttings were placed on standard PDA media containing 30 µg/L streptomycin sulphate to suppress bacteria growth. 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 (MA) at x400 magnification of a compound microscope with reference to Zivkovic et al. (2010). Conidia suspension of C. lindemuthianum were sprayed on healthy cowpea plants and re-isolated to comply with Koch's postulate (Enikuomehin et al., 2010).

2.5 Evaluation of Growth

One centimeter agar disk of the pure culture was transferred unto the prepared plant extract- or Tandem® -modified PDA media. After 24 hours, measurement of colony diameter along pre-marked orthogonal axes at the bottom of the Petridishes was done and this continued until the surface of the plate was covered. The values of the colony diameter were averaged and the percentage inhibition of mycelia growth (PIMG) was calculated for each treatment relative to control (Equation 1)

<<equation 1>>

2.6 Effect of Plant Extracts and Tandem® on Disease Incidence

The field experiments were conducted at Ekiti State University Teaching and Research farm, Ado Ekiti (7.7129 °N, 5.2523 °E). The first trial was conducted in September, 2012 and repeated during the same period in 2013. The size of the plot was 2 m x 2 m and separated by boarder row of 1 m. The total area of the farm was 225 m2 and there were 540 stands of cowpea. Cowpea variety, Ife brown wassown at three seeds hole per hole and later thinned to two stand-1. The experiment was laid in a Randomised Complete Block Design (RCBD) with three replications. Three concentrations (30%, 50% and 65%) of extracts of D.

 ©Jomo Kenyatta University of Agriculture and Technology

stramonium, R. communis and J. gossypifolia at were sprayed on two weeks old plants in the first subplot. Tandem® was applied at the rate of 0.1 g liter-1 while the control plot was sprayed with distilled water. The cowpea plants were inoculated with C. lindemuthianum conidia suspension containing 1.0 x 106 conidia ml-1 after two weeks post-inoculation. Sampling was conducted on different plots to assess plants showing anthracnose symptoms and disease incidence was assessed by counting the numbers of plants infected (Equation 2).

The percentage disease incidence was calculated as:

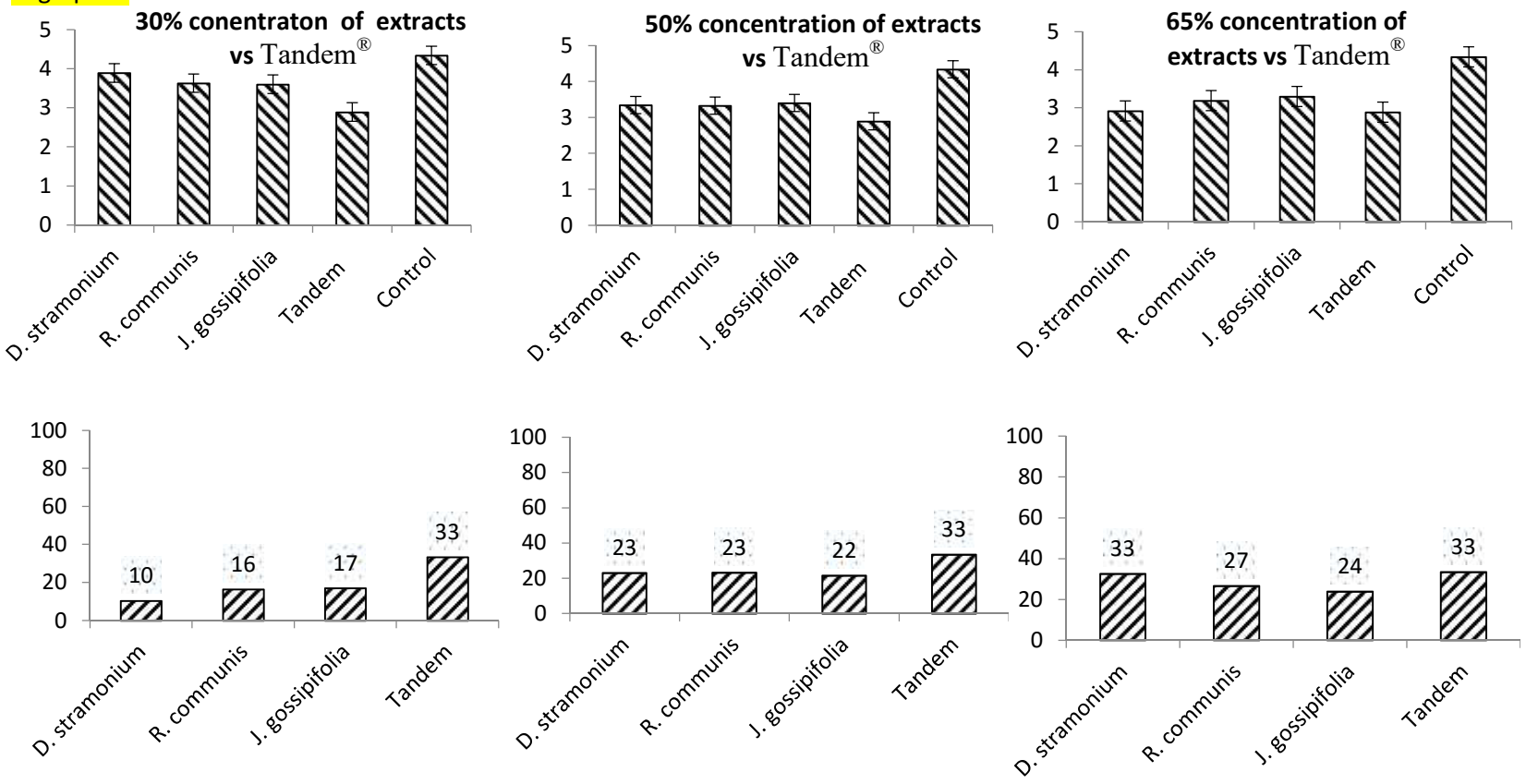
<<equation 2>>

3.0 Results

3.1 Inhibitory Effect of Plant Extracts and Tandem® on Growth

Figure 1 shows inhibitory effect of the plant extracts and Tandem® on C lindemuthianum growth. At 30% concentration, the growth rate of C. lindemuthianum on PDA containing extracts of D. Stramonium, R. communis and J. gossypifolia were 3.9, 3.6 and 3.6 mm day-1 respectively and these were not significantly different (P=0.05). At the highest concentration (65%) of D. stramonium, B. communis and J. gossypifolia extracts, growth rates were 2.9, 3.2 and 3.3 mm day-1 respectively. Rate of inhibition of growth increased as the concentration increased from 30-65%. The mean percentage growth inhibition at 65% concentration of D. stramonium was significantly (P<0.05) the highest being 33%, while R. communis and J. gossypifolia caused 27% and 24% inhibition. On the other hand, Tandem® reduced growth rate to 2.89 mm day-1 which corresponded with 33% inhibition

<<graph>>



©Jomo Kenyatta University of Agriculture and Technology

Effects of Plant Extracts on Disease Incidence

The effect of three plant extracts and Tandem® on anthracnose disease incidence in cowpea cultivar is shown in Figure 2. The percentage number of plants showing disease symptoms varied with the plant extracts and the applied concentrations. Tandem® treated plots and those sprayed with 65% concentration of *D. stramonium* had the lowest disease incidence among the treatments, which was approximately 14% in the respective plots. In the plots sprayed with *D. stramonium,* there was a significant change in disease incidence recorded as the concentration of the extract was increased from 30-65%, with the highest level control at 65%. In contrast, there was no significant difference in the disease incidence where *J. gossypifolia* was applied irrespective of the concentrations used. However, disease incidence in the control plots was significantly the highest.

Discussion

Cowpea production in the tropical agroecological regions is affected by anthracnose disease. In Nigeria, anthracnose disease is the major fungal disease influencing yield and productivity (Ogu and Owoeye, 2013). Management of anthracnose relies extensively on the use of synthetic fungicides which is often costly and beyond the reach of peasant farmers. Use of synthetic chemical fungicides may pose significant adverse effects on environment and consumers. Apart from this, development of resistant strains of pathogens is often associated with the use of chemical fungicides (Shilpa and Gokulapan, 2015). The environments in which crops will be grown in the next few decades is expected to change significantly with new pest and diseases becoming severe due to climate change (Borisade and Magan, 2015). In addition, disease problems have been predicted to become more severe in most agro-ecological regions of Sub-saharan Africa that have been identified as hot-spots for climate change (Apata et al., 2012). Problems of resistance of plant disease pathogens to conventional pesticides is expected to worsen due to climate change-associated temperature

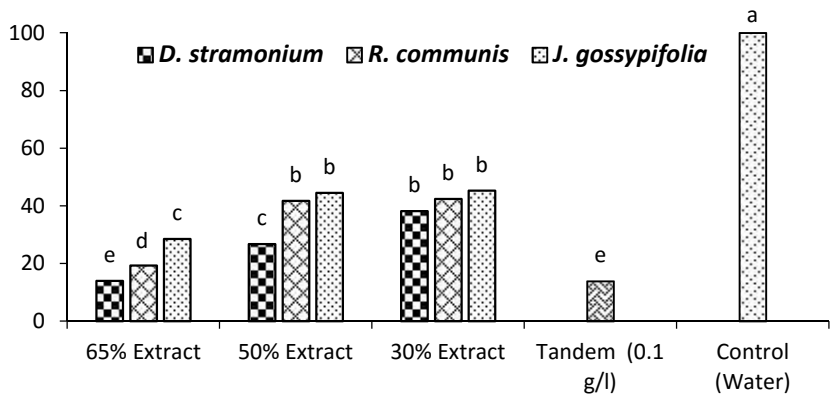


Figure 2. Effects of plant extracts and synthetic fungicide, Tandem® on incidence of cowpea anthracnose disease.

and humidity relations and their interactions with fungal growth characteristics and ecophysiology.

In this study, hot water extracts of D. stramonium, R. communis and J. gossypifolia and Tandem® were tested against C. lindemuthianum using invitro and field bio-efficacy trials.

All the plant extracts and the synthetic fungicide reduced mycelial growth of C. lindemuthianum. Higher inhibition of growth occurred at relatively higher concentrations of the plant extracts. Concentrations of active ingredients in plant extracts is known to influence their effectiveness. The higher inhibition rates observed may be due to increased availability of fungitoxic or fungistatic substances in the medium at higher concentrations. The mode of drying and extraction methods as well as the solvent influence the qualities of the extract. In this study, the leaves were air dried and powdered to increase the surface area between samples and extraction solvent. Water was used as the extractant at 70°C to ensure that the chemistry of the active substances were preserved. It has been reported that air dried plant materials are less fragile and do not tend to deteriorate-an advantage which it has over fresh leaf samples (Azwanida, 2015).

It appeared that D. stramonium contained contains higher fungitoxic constituents or compounds with greater antifungal activities. Significantly different inhibition of growth occurred at the three concentrations. At 65% concentrations, the antifungal activity of D. stramonium was comparable with Tandem-a systemic and fungal fungicide. Jatropha gossypifolia on the other hand showed no significant antifungal activity as the concentration was increased from 30%-65% and caused the least inhibition of growth among the three plant extracts. Although, J. gossypifolia have been reported as effective in the management of other phytopathogenic fungal species (Falade et al., 2006), its activity against C. lindemuthianum strain in this study was poor. There are possibilities that the strain of C. lindemuthianum being reported is resistant or tolerant to the bioactive substances in the extract. Probably higher concentrations could have shown a better performance. The results of this study are consistent with others where plant extracts were applied against many fungal pathogens of economic crops. Cao and Bruggen, (2001) reported reduction in mycelial growth of Phytophtora infestans at different concentrations of Allium sativum-modified PDA. Similarly, Enyiukwu and Awurum (2013) reported the control of Colletotrichum destructivum using extracts of Carica papaya, Piper guineenses and benomyl fungicide. The study showed thatP. guineense extract compared favourably with benomyl. Bautista et al., 2003 controlled the growth of C. gloeosporioides causing anthracnose of papaya fruit using plant extract and chitosan (a conventional fungicide) and the study showed that the extract compared favourably with the fungicide.

 ©Jomo Kenyatta University of Agriculture and Technology

Some of the extracts of the plants used on the field for the control of C. lindemuthianum were effective and the results were consistent with the outcome of the initial invitro evaluation. Pretorius et al. (2002) reported that extracts of some indigenous plants were effective invitro but failed to control the spread of pathogen on the field. Muthukuma et al. ((2010) reported that Pythium aphanidermatum, the causative organism of chilli disease of pepper, was controlled invitro and on the field with extracts of twenty-three medicinal plants. The result showed that A. sativum, A. cepa and T. procumbens extracts effectively controlled the disease. Similarly, Amadioha and Obi (1999), reported that alcohol and water extracts of Piper betle, Ocimum sanctum and Citrus limon significantly suppressed the mycelial growth of C. lindemuthianum invitro and reduced the spread of the disease in the field.

In the field, abiotic interactions such as temperature and solar radiation may interfere with the performance of active constituents in plant extracts. The stability of bioactive substances to temperature and ultraviolet radiation may vary with their chemical structure and this may be responsible for the failure under field conditions.

This study has demonstrated that cowpea anthracnose disease can be potentially controlled with the use of plant extracts. However, there is the need for further research on purification and identification of the main components which have antifungal properties, formulation to ensure stability of the constituents under field conditions where abiotic influences and the presence of other biotic factors may affect efficacy.

References

Amadioha, A. C. 2003. Evaluation of some plant leaf Extracts against Colletorichum lindemuthianum in Cowpea. Acta Phytopathologica. Hungaria: 38, 3-6.

Amadioha, A. C. and Obi, V. I. 1999. Control of Anthracnose disease of cowpea by Cymbopogon citratus and Ocimum gratissimum.Acta phytopathol. Entomol 34, 85-89.

Apata, T.A., Samuel, K.D. and Adeola, A.O. 2009. Analysis of Climate Change Perception and Adaptation among Arable Food Crop in South Western Nigeria. Contributed Paper Prepared for Presentation at the Association of Agricultural Economists 2009 Conference, Beijing, China, August 2009.

Azwanida N.N. 2015: A review of the extraction methods used in the medicinal plants, principles, strengths and limitation. Journal of Medicinal and Aromatic Plants. 4: 96-101

Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., & Wilson, C. L. 2003. Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit. Crop protection, 22(9), 1087-1092.

Borisade, O. A. and Magan, N. 2015. Resilience and relative virulence of strains of entomopathogenic fungi under interactions of abiotic stress. African Journal of Microbiology Research, 9(14), 988-1000.

Cao, K. Q. and Bruggen, V. 2001. Inhibitory efficacy of Several Plant extracts and Plant Products on Phytophthora Infestans. Journal of Agricultural University of Hebei. 2:90 – 96.

Davis, D. W., Oelke, E. S., Oplinger, J. D., Doll, C. V., Hanson, M. and Putman D. H. 1991. Alternative Field Crops Manual. University of Wisconsin. Wiley Publishers. http://www.hort.purdue.edu/newcrop/afcm/drypea.html

Enyiukwu, D. N., Awurum, A. N., Ononuju, C. C., and Nwaneri, J. A. 2014. Biology and management strategies of cowpea Anthracnose disease cause by Colletotichum species. Journal of Biochemistry and Biotechnology 101{2} 52 65

Enyiukwu, D.N. and Awurum, A. N. 2013a. Fungitoxic Principles and Antifungal Activities of Extracts from Carica papaya and Piper quineense on Covetotrichum desctructiuum O Gara. M.Sc Thesis, Department of Plant Health Management, Umudike, January 2011

Falade, M. J., Oso, A. A., & Borisade, A. O. 2006. Efficacy of Jatropha gossypifolia and Manihot esculenta seed extract for the Control of Sclerotium rolfsii and Fusarium oxysporum In South-Western Nigeria. Journal of Agriculture, Forestry and the Social Sciences, 4(2).

FAOSTAT. 2000. Food and Agriculture Organization of the United Nations.: http//www.fao.org/statistics

FAOSTAT. 2012. Food and Agriculture Organization of the United Nations. http//www.fao.org/statistics

FAOSTAT. 2013. Food and Agriculture Organization of the United Nations. http//www.fao.org/statistics

Gideon, K. O. and Anita 2013 In vitro control of Anthracnose disease of cowpea Vigna unguiculata L. Walp caused by C destructivum with Cyathula prostrata L and Diodia scadens S. W. Journal of Intercultural Ethnopharmacology 2 (1):29-36.

Igbinosa, O. O., E. O. Igbinosa, and O. A. Aiyegoro. 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn)." African Journal of Pharmacy and Pharmacology 3. 58-62.

Kareem, K. T. and Taiwo, M. A. C. 2007. Interaction of Viruses in cowpea: Effect on growth and yield parameters. Journal of Virology. 4: 15 – 23.

Muthukumar, A., Eswaran, A., Nakkeeran, S. and Sangeetha, G. 2010. Efficacy of plant extracts and biocontrol agents against Pythum aphanidermatum Inciting Chilli damping-off. Journal of Crop Protection 29: 1483 – 1488.

Naz, R. and Bano, A. 2012. Antimicrobial potential of Ricinus communis leaf extracts in different solvents against pathogenic bacterial and fungal strains. Asian Pacific journal of tropical biomedicine, 2(12): 944 – 947.

Ogu, G. E. and Owoeye A. E. 2013. Invitro control of anthracnose disease of cowpea (Vigna unguiculata L Walp) caused by Colletotrichum destructivum O Gara with Bioactive Compounds to be used as Nutraceuticals and Food Ingredients. 12.

Pretorius, J. C., Zietman, P. C., and Eksteen, D. 2002. Fungitoxic Properties of Selected South African Plant Species against Plant Pathogens of economic importance in Agriculture. Annual Applied Biology. 141: 117 – 124.

Sharon, T. and Douglas, M. 2011. Anthracnose Diseases of Trees. Agricultural Experiment Station. Department of Plant Pathology and Ecology.

http://www.ct.gov/caes/lib/caes/documents/publications/fact_sheets/plant_pathology_and_ecology/anthracnose_diseases_of_trees_06-03-11r.pdf

Shilpa, T. C. and Gokulapalan, C. 2015. In vitro study of fungicides and biocontrol agents against Colletotrichum capsici causing anthracnose of chilli (Capsicum annuum L.). International Journal of Applied Pure Science and Agriculture. 2394-5532

Usha, K. B., Singh, P., Praseetha, N., Deepa, D. K., Agarwal, R. and Nagaraja A, 2009. Antifungal activity of Datura stramonium, Calotropis gigantea and Azadirachta indica against Fusarium mangiferae and floral malformation in mango. European journal of plant pathology 124: 637-657.

Živković, S., Stojanović, S., Ivanović, Ž, Trkulja, N., Dolovac, N., Aleksić, G., & Balaž, J. 2010. Morphological and molecular identification of Colletotrichum trucutatum from tomato fruit. Pesticidi i fitomedicina. 25(3): 2 – 8.

Enikuomehin, O. A., & Oyedeji, E. O. 2010. Fungitoxic effect of some plant extracts against tomato fruit rot pathogens. Archives of Phytopathology and Plant Protection, 43(3), 233-240.