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INHIBITORY ACTIVITY OF PLANT EXTRACTS ON THE EARLY BLIGHT PATHOGEN *Alternaria Solani* GROWTH

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ABSTRACT

This study evaluated the effect of two plant extracts, *Ricinus communis* and *Chromolaena odorata* on the control of the early blight pathogen, *Alternaria solani* (Ell. and Mart.). The study was conducted in the Laboratory of the Crop Production and Horticulture Department, Federal University of Technology, Yola, Adamawa State, Nigeria. A Completely Randomized Design (CRD) replicated three times was used. Three concentrations (25%, 50%, and 100%) of each plant extract were determined for inhibitory activity of *A. solani* growth. From the radial growth results, it revealed that *Ricinus communis* at 100 % concentration was recorded for the lowest radial growth rates of 1.43 cm, 2.00 cm and 2.72 cm at 24, 48 and 72 hours were recorded, respectively. It was concluded that the plant extracts used at different concentrations showed promising prospects for control *Alternaria solani* growth *in vitro*. However, it was recommended that there is a need to evaluate the inhibitory function of the plant extracts in the field to ascertain their effectiveness.

KEY WORDS: Plant Extracts, Radial Growth, *Ricinus communis, Chromolaena Odorata,* Inhibition.

INTRODUCTION

The attempt to improve crop yield in order to produce enough food for consumption in the face of increasing population is a decision in the right direction although it is being hampered by many constraints (Amusa *et al.,* 1994).

One of the most important and interesting problem encountered by scientists is how to drastically reduce or wholly prevent plant diseases. Even though, there is increasing control methods such as resistant varieties and chemicals to control the diseases, the control has become a continual battle because the pathogens normally attack the crops suddenly (Akinbode and Ikotun, 2008).

Efforts have been made by researchers to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants and one of such resources is folk medicines (Okereke and Wokocha, 2007). Using systematic screening, novel effective compounds have been discovered (Tomoko *et al.,* 2002). The increasing prevalence of resistant new strains of bacteria, fungi and the recent appearance of strains has reduced susceptibility to chemical control and raises the specter of bacterial and fungal infections which consequently result in search for new disease-fighting strategies (Sieradski *et al..* 1999). Furthermore, it has been reported that more than one hundred species of plant pathogens have become resistant to fungicides while some resistant varieties have become susceptible (Zitter *et al.,* 2005). The effect of these pathogens with resistant traits now has negative consequences on the crop producer. *Alternaria solani* has in no small measure contributed to the reduction in the yield of tomato both in the field and after harvest (Damicone *et al*., 1986).

Several attempts have been made to control early blight of tomato through cultural, physical, chemical, and biological methods (Adekunle *et al.,* 2001). However, because the early blight fungi of tomato have very large host range, cultural control methods have been advocated and are the most widely acceptable means of controlling the disease as one species has many hosts (Amusa *et al.,* 1994). More so, absence of disease resistance tomato cultivars in the field has been a major problem, and has been attributed to the high levels of virulence in pathogen populations (Ghaffer, 1988). A second limitation stems from the fungicides themselves when they are used intensively, they place enormous selection pressure on the fungi, and the pathogens rapidly develop resistance (Alabi *et al.,* 2005). In the recent past control of plant-parasites, essentially, involves the use of synthetic pesticides. However, apart from its very high cost, indiscriminate and unsafe use, increased concern for the environment and the inherent danger pose to man and his livestock calls for caution in its utilization (Adegbite and Adesiyan, 2005).

In view of the foregoing, it becomes necessary to use plant extracts to ascertain their effectiveness in the control of early blight disease in tomato. The study therefore was aimed at identifying the plant extract that would control early blight of tomato in Yola, Adamawa State. And this was intended to be achieved through the following objectives: To determine the effectiveness of some plant extracts in the control of the early blight of tomato induced by *Alternaria solani in vitro* and, to determine the most effective concentration of the plant extracts in controlling the organism.

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Data Analysis

The data collected were subjected to analysis of variance (ANOVA) for a completely randomized design using SAS (1999) statistical package. The treatment means that were significantly different were compared using the Duncan's Multiple Range Test (DMRT) at P=0.05.

MATERIALS AND METHOD

Experimental Sites and Experimental Design and Layout

This experiment was carried out in the Laboratory of the Crop Production and Horticulture Department, Federal University of Technology Yola, Adamawa state. The experiment was laid in a Completely Randomized Design (CRD) using Castorbean plant *(Ricinus communis)* and Siam weed *(Chromolaena odorata)* at concentrations (25%, 50%, and 100%) of each extract and a control replicated three times, bringing the total number of treatments to (7) seven which were assigned completely at random to each replicate.

Collection of Plant Materials and Preparation of extracts

The plant materials, *Ricinus communis* and *Chromolaena odorata* were collected within and around the Federal University of Technology, Yola. The castor bean plant and siam weed were rinsed and washed with 10 % sodium hypochlorite (NaOCl) which was prepared by adding 1 part bleach to 9 part water, air dried and later packed in brown envelopes before oven drying at 70 ^{o}C for 20 minutes, according to Akinbode and Ikotun (2008). Thereafter they were ground using pestle and mortar, sieved through a 40 mm mesh and 200 g of the respective plant powder (castor bean plant and siam weed) was added to 500 ml of distilled water in a 1000 ml flat bottom flask. The suspension was then allowed to stand for 24 hours and the content was filtered using a muslin cloth and kept in glass bottles until needed. The extracts at different concentrations were prepared by mixing 25 ml, 50 ml and 100 ml of the stock with (100 ml of distilled water) to give final concentrations of extract at 25 %, 50 % and 100 % respectively.

Isolation of *Alternaria solani*

Diseased leaves of tomato with the symptoms of *Alternaria solani* infection were collected from farms within the university. A small piece of an advancing margin of a lesion was cut with a sterile pair of scissors after sterilizing with 10 % sodium hypochlorite according to Larone, (1995). The tissues were then washed thoroughly several times with sterile distilled water and placed aseptically into 9 cm diameter Petri dishes containing 15 ml of molten Potato Dextrose Agar (PDA). The medium was impregnated with streptomycin to prevent bacterial contamination, and cultured for 7 days at room temperature (28-30 ^{o}C) in a sterile fume cupboard. Distinct colonies present on the plates were selected, purified by repeated culturing and maintained on PDA slants. The identification of the fungal isolate was carried out using the macroscopic and microscopic identification guide according to Larone (1995). The isolate (*A. solani*) was stored in an agar slant for other experiments.

Identification of *Alternaria solani*

The *Alternaria* early blight was identified through microscopic and macroscopic features. *Alternaria solani* can grow rapidly on PDA and the colony size reaches a diameter of 3 to 9 cm after incubation at 25°C for 7 days. The colony is flat, downy to woolly and covered by grayish, short, aerial hyphae in time. The colony surface is grayish white at the beginning which later turns dark and finally greenish black or olive brown with a light border. The reverse side is typically brown to black due to pigment production (Larone, 1995). *Alternaria* spp. has septate, brown hyphae. Conidiophores are also septate and brown in color, occasionally producing a zigzag appearance (Larone, 1995). They have simple or branched large conidia (7-10 x 23-34 µm) which have both transverse and longitudinal septations. They are ovoid to obclavate, darkly pigmented, smooth or roughened. The end of the conidium nearest the conidiophore is round while it tapers towards the apex. This gives the typical beak or club-like appearance of the conidia as described by Zitter *et al.* (2005).

Preparation of Growth Medium and Inoculation

About 39 g of PDA powder (Sigma GMBH) was dissolved in 1000 ml of distilled water and the content was stirred and autoclaved for 25 minutes at 115 ^{o}C, using the method described by Awale, (2001). The medium was allowed to cool down and was then aseptically poured into 25 ml flavour bottles. Thereafter, 5 ml of the plant extract prepared was poured into the Petri dishes. About 15 ml of molten PDA at 45-50 ^{o}C was poured aseptically onto the plant extract in the Petri dish and swirled round five times for even dispersion of the extract into the agar and allowed to solidify, before the pathogen was inoculated (introduced) into the middle of the 'poisoned agar'. A mycelial plug of 5 mm diameter from a 3-days-old fungus was cut using a 5 mm sterile cork borer and transferred to the PDA plate in the centre of the Petri dish, and then kept in a sterilized fume cupboard kept at room temperature of 28 - 30 ^{o}C.

Data collected

Measurement of the radial growth in centimeters (cm) was done using a ruler and the radial growth rate was determined by using the formula K_r (Reeslev and Kjoller, 1995).

$$\text{Radial growth rate } (K_r) = \frac{(R_1 - R_0)}{(t_1 - t_0)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where R_0 and R_1 are the colony radii at time t_0 and t_1 respectively, determined at 24, 48 and 72 hours interval.

RESULTS

Radial Growth of *Alternaria solani* at 24, 46 and 72 hours

The result of the mean values of plant extracts on radial growth during the first and second laboratory experiments was highly significant (P<0.5) at 24, 48 and 72 hours after inoculation (Table 1). The highest radial growth values of 3.53 cm, 4.09 cm and 4.59 cm were recorded in the control while the lowest values, 1.57 cm, 2.16 cm and 2.88 cm for 24, 48 and 72 hours respectively were recorded for *Ricinus communis* with 100 % concentration (Table 1).

In the second experiment the treatment means also showed that *Ricinus communis* 100 % concentration recorded the lowest radial growth with 1.30 cm, 1.83 cm and 2.55 cm at 24, 48 and 72 hrs respectively. In addition the control recorded the highest radial growth with mean of 3.40 cm, 3.98 cm and 4.43 cm at 24, 48 and 72 hrs respectively (Table 1).

In addition, the combined results in Table 2 revealed that at 24, 48 and 72 hours *Ricinus communis* 100 % concentration had the lowest means of 1.43 cm (Fig 1), 2.00 cm and 2.72 cm respectively, followed by *Ricinus communis* 50 % concentration with 1.83 cm, 2.32 cm and 2.93 cm respectively. While the control which recorded the highest radial growth with 3.47 cm, 4.04 cm and 4.51 cm (Fig 2) followed at 24, 48 and 72 hours respectively.

Table 1: Effects of plant extracts on radial growth (cm) of *Alternaria solani* mean of first and second laboratory experiments

Treatment	24 h 1st experiment	24 h 2nd experiment	48 h 1st experiment	48 h 2nd experiment	72 h 1st experiment	72 h 2nd experiment
†Chord25	2.38cdef	2.58d	3.10bcd	2.70ef	3.75bcde	3.67bc
Ricom25	2.27efg	1.85f	2.66def	2.76ef	3.35efg	3.33d
Chord50	2.20fg	2.30e	2.95cde	2.65f	3.72cde	2.90e
Ricom50	1.75gh	1.91f	2.43ef	2.20g	2.95fg	2.90e
Chord100	2.28defg	2.30e	2.90cde	2.33g	3.57def	3.63c
Ricom100	1.57h	1.30g	2.16f	1.83h	2.88g	2.55f
Control	3.53a	3.40a	4.09a	3.98a	4.59a	4.43
Mean	2.28	2.23	2.90	2.64	3.54	3.34
Prob. F	**	**	**	**	**	**

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test ** = highly significant (P=0.01); †Chord = *Chromoleana odorata*; Ricom = *Ricinus communis*

Table 2: Combined means of the effect of plant extracts on radial growth (cm) of *Alternaria solani* in the laboratory

Treatment	24 hrs	48 hrs	72 hrs
Chord25	2.48de	2.90def	3.71def
Ricom25	2.06fg	2.71ef	3.34g
Chord50	2.25ef	2.80def	3.31g
Ricom50	1.83g	2.32g	2.93h
Chord100	2.29ef	2.62fg	3.60efg
Ricom100	1.43h	2.00h	2.72h
Control	3.47a	4.04a	4.51a
Mean	**2.26**	**2.77**	**3.45**
Prob. F	**	**	**

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test, ** = Highly significant (P=0.01)

Chord = *Chromoleana odorata*; Ricom = *Ricinus communis*;

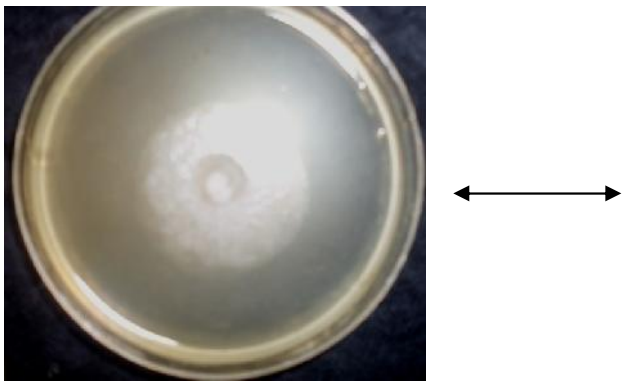

Fig 1: The lowest radial growth in *Ricinus communis* 100 %

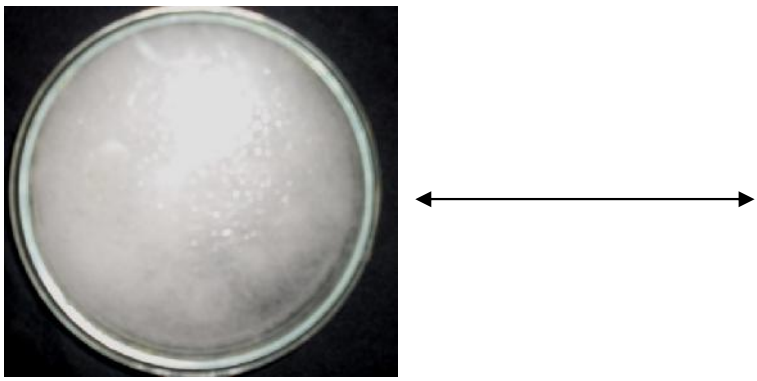

Fig 2: The highest radial growth in control

DISCUSSION

Different effect of plant extracts on *A. solani* radial growth rate has been observed among different plant extracts and concentrations. This investigation revealed that the lowest mean radial growth rate was obtained from *Ricinus communis* extracts at 100% concentration and could be attributed to the presence of ricin and ricinine in the extracts (Ukpabe, 2002). The author found that leaf extracts of *R. communis* can inhibit the growth of *Fusarium oxysporum*. *Chromolaena odorata* has the inhibitory activity on *A. solani* radial

growth after *R. communis* as was earlier reported by Ngane *et al.,* (2006) who found that aqueous ethanol extract of *C. odorata* leaves and some of its fractions examined has antifungal properties by dilution methods on solid and liquid media, using yeasts and filamentous fungi. They authors reported that both extract and fractions can inhibit *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum*. Chemical analysis of the extract and fractions showed the presence of biologically active constituents such as coumarins, flavonoids, phenols, tannins and sterols which they are attributed to the fungicidal activity (Ngane *et al.,* 2006). Asthana *et al*. (1986) found that the leaf extract of *Ocimum adscendens* has fungitoxic effect against *Aspergillus flavus.* This result is similar to the results of this study which showed that extracts of *R. communi,* and *C. odorata* inhibited the radial growth of *A. solani in vitro.*

Comparing the rate of radial growth in *R. communis* with that of the control; it could be deduced that the pathogen grew freely and penetrated the medium, establishing itself and using up the food as compared with the "poisoned food" in the PDA containing the plants extracts. The inhibitory effects of the extracts might be due to the ricinoleic acid and β-sitosterol present in the plant, and ricin a protein toxin (Lowery *et al.,* 2007). Similarly *Ricinus communis* had been reported to have inhibited number of eggs and percentage hatch in 100 % concentration of root extracts in *Meloidogyne incognita*. Equally, siam weed and neem gave maximum inhibition of egg hatching (100 %) and larval mortality followed by lemon grass and castor bean with 95 and 93 % inhibition of egg hatch respectively (Adegbite and Adesiyan, 2005). This therefore suggests that the plant extracts (*Ricinus communis* and *Chromolaena odorata*) showed inhibitory effects on the growth of the fungus.

Results on inhibition percentage of the plant extracts as observed in this study showed that *Ricinus communis* at higher concentration inhibited radial growth by as much as 41 % to 59 % in the combined results. This finding is in agreement with that of Baldrian and Gabriel (2002) who reported that *Piptoporus betulinus* growth was found to be concentration dependent as fungal growth was inhibited at higher concentration. This could be inferred that *Ricinus communis* could inhibit the growth of *Alternaria solani* under laboratory condition. In addition, the fungus is much more sensitive to *Ricinus communis* in affecting radial growth than to the other plant extract, due to the ricin, ricinoleic acid and β-sitosterol present in the plant.

CONCLUSION

In conclusion, the evaluation of the various plant extracts at different concentrations tested on *Alternaria solani* showed promising prospects for the utilization of natural plant extracts in plant disease control. *In vitro* experiments showed that *Ricinus communis* 100 % could reduce radial growth in *Alternaria solani.* Therefore, plant extracts can be used as a potential source of sustainable ecofriendly botanical fungicides, after successful completion of wide range trials.

RECOMMENDATIONS

Based on the findings of this study it was found that *Ricinus communis* 100 % inhibited the radial growth of *Alternaria solani* under laboratory condition and is probably fungitoxic, and so could be field tested.

REFERENCES

Adegbite, A. A. and S.O. Adesiyan., 2005. Root Extracts of Plants to Control Root-Knot Nematode on Edible Soybean. *World Journal of Agricultural Sciences* 1(1): 18-21.

Adekunle A. T., Cardwell, K. F., Florini, D. A., and T. Ikotun., 2001. Seed treatment with *Trichoderma* species for control of Damping - off of Cowpea caused by *Macrophomina phaseolina*. *Biocontr.* Sci. Technol. 11: 449-457.

Akinbode, O. A. and T. Ikotun., 2008. Evaluation of some bioagents and botanicals in *in vitro* control of *Colletotrichum destructivum. African Journal of Biotechnology* 7(7): 868-872.

Alabi, D. O., Onibudo, M. Z., and N. A. Amusa., 2005. Chemical and nutritional composition of four botanicals with fungitoxic properties. *World Journal of Agricultural Sciences* 1(1): 84-88. Retrieved from http://www.idosi.org/wjas/wjas 1(1)118.pdf.

Amusa, N. A, Ikotun, T, and Y. O. K. Osikanlu., 1994. Screening Cowpea and Soybean Cultivars for resistance to anthracnose and Brown blotch diseases using Phytotoxic metabolites. African Crop Science Journal 2 (2): 221- 224.

Asthana, A.; Tripathi, N. N. and S. N. Dixit., 1986. Fungitoxic and phytotoxic studies with essential oil of *Ocimum odscendens*. Journal of Plant Pathology, 117:152-159

Awale, H. E., 2001. Inoculum inoculation and media preparation of anthracnose, caused by *Colletotrichum lindemuthianum*. Michigan State University, EL, MI 48824. Accessed from www.msu.edu.

Baldrian, P. and J. Gabriel., 2002. Intraspecific variability in growth response to cadmium of the wood-rotting fungus *Piptoporus betulinus. Mycologia*, 94(3): 428-436.www.mycologia.org/misc/terms.

Damicone, J. P., Conway, K. E. and L. Brandenberger., 1986. Common Diseases of Tomatoes Part I, Diseases caused by Fungi. Oklahoma Cooperative Extension Service-EPP 7625.Retreived from http://.osufacts.okstate.edu/PDF.

Ghaffer, A., 1988. Biological control of sclerotial diseases. in: *Biocontrol of Plant Diseases*. Vol. I. K. G. Mukerji and K. L. Garg, (eds.) pp 153-175. CRC Press: Boca Raton, FL, U.S.A.

Larone, D. H., 1995. Medically Important Fungi - A Guide to Identification, 3rd ed. ASM Press, Washington, D.C.

Lowery, C., Auld, D., Rolfe, R., McKeon, T., and J. Goodrum., 2007. Barriers to commercialization of a Castor Cultivar with Reduced Concentration of Ricin. Reprinted from: Issues in new crops and new uses. 2007. J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA.

Ngane, A. N., Etame, R. E., Ndifor, F., Biyiti, L., Amvam Zollo, P. H. and P. Bouchet., 2006. Antifungal Activity of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) of Cameroon. *Chemotherapy* 52:103-106.

Okereke, V. C and R. C. Wokocha., 2007. *In-vitro* growth of four isolates of *Sclerotium rolfsii* Sacc. In the humid tropics. African Journal of Biotechnology **6** (16): 1879-1881.

Reeslev, M. and A. Kjøller., 1995. Comparison of Biomass Dry Weights and Radial Growth Rates of Fungal Colonies on Media Solidified with Different Gelling Compounds. Applied and Environmental Microbiology 61 (12): 4236–4239

SAS., 1999. Statistical Analysis Software Package Version 8. SAS Institute Inc., Cary, NC, USA.

Sieradski, K., Roberts, R. B., Haber, S. W. and A. Tomasz., 1999. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. New England Journal of Medicine 340: 517–523.

Tariq, V., 2009. Fungi online: Growth kinetics. British Mycological Society. www.britmycolsoc.org.uk.

Tomoko, N., Takashi, A., Hiromu,T., Yuka, I., Hiroko, M., Munekazu, I., and W. Kazuhito., 2002. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. Journal of Health Science 48: 273–276.

Tongle, H., Wang, S, Cao, K., and H. R. Forrer., 2002. Inhibitory effects of several Chinese medical herbs against *Phytophthora infestans. ISHS. Acta Horticulturae 834*. 3rd International Late Blight Conference Proceedings. http://www.actahort.org/.

Ukpabe, R., 2002. Effect of four plant crude extracts on the growth of *Fusarium oxysporum f.sp lycopersici*. Student Project Report, University of Ibadan, Ibadan, Nigeria (unpublished).

Zitter, T. A., Drennan, J. L., Mutschler, M. A., and M. J. Kim., 2005. Control of early blight of tomato with genetic resistance and conventional and biological sprays. *ISHS Acta Horticulturae* 695: I International Symposium on Tomato Diseases. http://www.actahort.org/.