Control of tomato wilt disease caused by *Fusarium oxysporum f. sp. Iycopersici* using plant biological fungicides

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ABSTRACT
All plant extracts [i.e. extracts from *Portulaca oleracea* L., *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Zingiber zerumbet* (tuber) and *Codieum variegatum* (leaf)] used in this study significantly inhibited the spread and further development of the inoculum applied to leaves of the tomato plant. In this study, leaf extracts of *Codieum variegatum* significantly inhibited the proliferation of *Fusarium oxysporum* in both *in vitro* and the greenhouse. All biofungicides tested showed significant reduction in terms of radial diameter of *Fusarium oxysporum in vitro* after the treatment in comparison to the negative control (i.e. without any biofungicide), with the least inhibition of 64% by *Curcuma zedoaria* (tuber) extract and the highest inhibition of 89% exhibited by *Codieum variegatum* leaf extract. The other biofungucides *Portulaca oleracea* L., *Costus specious* (rhizome) and *Zingiber zerumbet* (tuber) also provided significant inhibition at 68%, 75%, and 81% respectively *in vitro*. Generally, the results of this study indicate a high inhibitory effect of all the biofungicides used on *Fusarium oxysporum* under *in vitro* and greenhouse conditions.

KEYWORDS: Biofungicide; Tomato wilt; Inoculum; Plant extract; Inhibition; etc.

1.0 INTRODUCTION

The efficacy of selected five (5) biofungicides in controlling the fungus disease, tomato wilt, caused by *Fusarium oxysporum f. sp. Iycopersici* was evaluated *in vitro* and under greenhouse conditions. Plant extracts from *Portulaca oleracea* L., *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Zingiber zerumbet* (tuber) and *Codieum variegatum* (leaf) were used as biofungicides. Biofungicides are naturally occurring substances, in most cases plant extracts, or microbial extracts that control plant diseases. Many of these biofungicides have been tested for efficacy while a good number is undergoing rigorous laboratory tests for suitability for crop and plant production. Extracts from higher plants (i.e. roots, stems, leaves, flowers and fruits) are known to contain antifungal substances in the form of alkaloids or prohibitins, which help in resisting the pathogens. Bandara and his colleagues (1988, 1989) at the University of Peradeniya in Sri Lanka evaluated three rhizomatous perennial herbs used in native medicine for their antifungal and antibacterial properties. The herbs tested were: *Acorns calamus* (*Araceae*), *Zingiber zerumbet* and *Curcuma longa (Zingiberaceae)*. Crude extracts of rhizomes at various dilutions were evaluated for their effect on growth and sporulation of *Cladosporium sp*., *Botryodiplodia theobromae, Fusarium solani, Phytophthora infestans, Pythium sp*. and *Pyricularia oryzae*. Their inhibitory action was compared to that of Benlate. Their findings revealed that extracts of *A. calamus and Z. zerumbet* had profound effect on growth of all fungi tested. Five antifungal substances that show activity against the Japanese pear pathotype of *Alternaria alternata in vitro* have been isolated from extract of *Portulaca oleracea* L. These antifungal substances are isobutyric acid, butyric acid, isovaleric acid, valeric acid and caproic acid (Park et al., 1986). The biological control is defined as the use of antagonistic organisms for the control of microorganisms, reducing the amount of inoculum that determines the extent of disease (Cook and Baker, 1983). Fungal diseases may be minimized by the reduction of the

inoculums, inhibition of its virulence mechanisms and promotion of genetic diversity in the crop (Strange and Scott, 2005).

2.0 MATERIALS AND METHOD

The efficacy of selected bio-fungicides in controlling the fungus disease was evaluated *in vitro* and under greenhouse conditions. Fungal pathogen was isolated by planting plant tissues (surface-disinfected with 1% sodium hypochlorite for 2 min) on PDA (Potato Dextrose Agar) and incubating them at 25°C for 5 days (Katan et al. 1991). Isolates were identified as *Fusarium. oxysporum* morphologically based on characteristics of the macroconidia, phialids, microconidia, chlamydospores, and colony growth traits (Messiaen and Cassini, 1968). The antifungal activities of the five (5) plant biofungicides on mycelial radial growth of the pathogen were determined by growing the fungus on a Potato Dextrose Agar containing the different biofungicides in petri plates. Plant extracts from *Portulaca oleracea L., Costus specious (*rhizome*), Curcuma zedoaria (*tuber*), Z. zerumbet (*tuber*) and Codieum variegatum* (leaf) were used as biofungicides both *in vitro* in the petri dishes and in the greenhouse experiments. Extracts were prepared from surfaced-sterilised plant material using an electric blender. Then, the aqueous extracts were filtrated and incorporated in PDA at 15%. Each bio-fungicide was added at recommended label rates to 100 ml sterilized Potato Dextrose Agar media at 60°C, which was then transferred into experimental petri plates. Each treatment was replicated 5 times. Control plates were made by replacing the quantity of biofungicides with the same quantity of sterile distilled water. A disc of mycelial culture of the fungus was aseptically transferred to the center of the solidified Potato Dextrose Agar media in plates. The plates were subsequently incubated for ten (10) days at 25°C (Hibar, 2002). For the petri dish experiments, after 2, 3, 4 and 5 days of incubation, the radial growth of pathogen isolates was measured and the average percentage of radial growth inhibition, with respect to fungal growth in controls, was calculated as follows: $L = [(C - T)/C] \times 100$, where L is inhibition of radial mycelial growth; C is radial growth of the pathogen in the control; T is radial growth of the pathogen, *Fusarium oxysporum* in the presence of each of the biofungicides from the plant extract. For the greenhouse experiments, the tomato seedlings were raised under same glasshouse condition by using a sterilized soil. Tomato, *Lycopersicon esculentum* L., seedlings were screened for the incidence of the tomato wilt disease caused by the fungus, *Fusarium oxysporum f. sp. Iycopersici*, and were found to be disease-free. The pathogen was isolated from tomato leaves collected from a different location. The pure culture of the isolate was developed via single spore subculture and was multiplied for pure culture. The pure culture of the isolate was then mixed with 500ml of distilled water and sprayed on the tomato seedlings using a hand water sprinkler. Tomato plants were then observed for the occurrence of the symptoms of the disease after 7 days from the date of the foliar spray. The biofungicides sprays were then applied to the affected plants and the biofungicides sprays were repeated twice at a 14days interval. Two weeks after the last biofungicides spray, the leaves of the inoculated plants were sampled and tested in order to confirm that the disease symptoms on the plants were caused by *Fusarium oxysporum*. The number of plants which showed the symptoms of the infection was recorded. The disease severity was recorded on 0 to 3 visual scale, in which 0 = no symptoms; 1 = light yellowing of leaves, light or moderate rot on taproot and secondary roots and crown rot; 2 = moderate or severe yellowing of leaves with or without wilting, stunting, severe rot on taproot and secondary

roots, crown rot with or without hypocotyls rot, and vascular discoloration in the stem; and 3 = dead seedlings (Vakalounakis and Fragkiadakis, 1999).

3.0 RESULTS AND DISCUSSION

All plant extracts [i.e. extracts from *Portulaca oleracea* L., *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Zingiber zerumbet* (tuber) and *Codieum variegatum* (leaf)] used in this study significantly inhibited the spread and further development of the inoculum applied to leaves of the tomato plant. In this study, leaf extracts of *Codieum variegatum* significantly inhibited the proliferation of *Fusarium oxysporum* in both *in vitro* and the greenhouse. All biofungicides tested showed significant reduction in terms of radial diameter of *Fusarium oxysporum in vitro* after the treatment in comparison to the negative control (i.e. without any biofungicide), with the least inhibition of 64% by *Curcuma zedoaria* (tuber) extract and the highest inhibition of 89% exhibited by *Codieum variegatum* leaf extract. The other biofungucides *Portulaca oleracea* L., *Costus specious* (rhizome) and *Zingiber zerumbet* (tuber) also provided significant inhibition at 68%, 75%, and 81% respectively *in vitro*. Generally, the results of this study indicate a high inhibitory effect of all the biofungicides used on *Fusarium oxysporum* under *in vitro* and greenhouse conditions. The results of this study is in consonance with the one obtained by (Naidu, 1988) as the author reported that *Alternaria alternata* and *Fusarium oxysporum* were inhibited by extracts of young and mature leaves of *Codieum variegatum* with the extracts of young leaves being more active against *A. alternata* and the old leaves against *F. oxysporum*. Extracts of *Portulaca oleracea* L. have been found to possess protective and therapeutic activities against leaf spot disease in maize caused by *Helminthosporium maydis* Nisik (Noriel and Robles, 1990). Aqueous extracts of the leaves of *Ocimum gratissimum* at 10, 25, 40 and 50% (w/v) concentrations induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls. The aqueous extracts also induced systemic resistance in cucumber against *Colletotrichum lagenarium*, reflected by reduction in disease incidence and an increase in chitinase production. Thirty-six medically used plant species have been screened for their activity against *Cladosporium cladosporioides*. Plant species whose extracts displayed significant activity were, *Butea monosperma* (stem, bark), *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Eupatorium riparium* (whole plant, root), *Pleisospermium alatum* (stem, bark, root, bark) and *Z. zerumbet* (tuber). The active constituent of *C. specious* has been isolated and identified as methyl 3-(4-hydroxyphenyl)-2(E)-propenoate. The compound's activity against *Penicillium sp. Aspergillus niger, C. cladosporioides* and *Curvularia sp*. has been evaluated and its activity against the two latter fungi has been comparable to that of the standard fungicide Benlate (Bandara et al. (1989). Many other authors have reported the tremendous success achieved in controlling different plant diseases using plant extracts from many plant species. For instance, Mishra et al. (1989), have isolated essential oils from leaves of *Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. grattissium*. These oils have demonstrated fungitoxicity against *Aspergillus flavus* at 200, 300, 400 and 500 ppm and most of them have shown to be more effective than synthetic fungicides viz; Agrosan G.N., Copperoxychloride, Ceresan, Thiovit *and* Dithane M45. Yegen et al. (1992) have studied the fungitoxic effect of extracts of six selected plants from Turkey. Results indicate that aqueous and essential oils of *Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia futicasa* and *Inula viscosa* are fungitoxic to *Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum and Phytophthora capsici*.

The use of microbial biofungicides has been reported by many authors. In this regard, biological disease control is a promising strategy for managing soil-borne or foliar diseases in a wide range of crops. Several commercial formulations of the biological control agents *Trichoderma harzianum, Bacillus subtilis, Streptomyces griseoviridis*, and *Gliocladium virens* have been reported previously for application against plant fungal pathogens in the field and greenhouse (Cook, 1993; Lo et al., 1996; Elad and Kapat 1999). With relation to the crude extract of lippia, the fungi *S. rolfsii, A. alternata* and *R. solani* presented inhibition to mycelial growth in concentration of 50%, what did not occur with *C. graminicola*, for which the best results were in concentrations above of 10%, and of *Phytophthora sp*., where the inhibition occurred in concentrations of 15%. When in the presence of rosemary and lippia extracts mixture, the results obtained showed that occurred inhibition for the mycelial growth of *C. graminicola, A. alternata* and *S. rolfsii* in concentration of 50%, while that for *R. solani* and *Phytophthora sp*. the inhibition was bigger in concentrations of 25 and 15%, respectively (Gasparin, et al., 2000). In naturally infested soils, wheat-bran preparation of *T. harzianum* inoculums significantly decreased diseases caused by *S. rolfsii* or *R. solani* in three field experiments with bean, cotton, or tomato, and these increased significantly the yield of beans (Elad et al., 1980).

4.0 CONCLUSION

Some substances naturally forming part of the plant cell composition have antifungal properties. These substances naturally occurring in plants which have antimicrobial properties inhibiting the growth and development of bacteria and fungi are referred to as phtytonzide. Plant extracts obtained from roots, barks, seeds, buds, leaves and fruits are important sources of antifungal compounds with low toxicity to mammals and safe to the environment which may serve as substitutes for synthetically produced fungicides.

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