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IN VITRO MANAGEMENT OF CURVULARIA LEAF SPOT OF MAIZE USING BOTANICALS, ESSENTIAL OILS AND BIO-CONTROL AGENTS

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ABSTRACT
Different plant extracts, essential oils and different strains of *Trichoderma harzianum* were evaluated *in vitro* against *Curvularia lunata*. Amongst the plant extracts, Lantana was highly effective @ 15 per cent (86.76 inhibition %) and 20 per cent (89.49 inhibition %) followed by Morphankhi @ 5 per cent (83.53 %) and 10 per cent (85.88 %) respectively. Among the essential oils, complete inhibition was recorded in Citronella oil at all 3 concentrations (2μL, 4μL and 8) and Peppermint oil at 4μL and 8 μL concentrations and least inhibition was observed in Palmaroza (65 %) at 2μL. Whereas, among different strains of *Trichoderma harzianum*, Th-13 shown maximum mycelial growth inhibition (83.82 %) followed by Th-9 (80.29 %) and Th-3(79.12 %).

INTRODUCTION

Maize (*Zea mays* L.) is one of the important cereal crops in the world agricultural economy as food, feed and energy source grown in more than 160 countries in tropical, sub-tropical and temperate regions. In India, maize is the third most important cereal crop after rice and wheat that serves as a source of raw material for developing hundreds of industrial products (Anon, 2007). But, maize is being plagued by an array of diseases which include the leaf spot of maize caused by *C. lunata* (Singh *et al.,* 2002) exhibiting symptoms as small chlorotic spots which gradually expand into round or oval-shaped lesion surrounded by a wide translucent straw yellow halo. A number of lesions can be connected leading to the formation of leaf necrosis. This cause significant damage to maize up to 60% due to great loss of photosynthetic area of the crop (Dia Hong-hai *et al.,*1995; Huang *et al.,* 2004; Li-FuHua *et al.,* 2006). This disease is an important seed and soil borne disease prevalent mostly in subtropical and tropical regions. Despite extensive damage caused by the pathogen, scanty literature is available. Currently studies pertaining to the use of botanicals in management of pathogens and related diseases are highly focused (Koche, 2013; Toppo, 2013; Mathad, 2013; Mathad, 2013; Mahapatra, 2013; Bisht, 2013). Keeping in view the destructive nature of the disease, the present investigation was under taken to evaluate the efficacy of plant extracts, essential oils and bio-control agents against *C. lunata* under *in vitro* conditions.

MATERIALS AND METHODS

Plant material and Isolation of fungus

The leaves of maize (cv.Gourav) showing symptoms of typical leaf spots were collected from Norman E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. The Fungus was isolated from the infected leaves on 2 per cent Potato Dextrose Agar (PDA) and incubated at 25 $\pm$ 1ºC.

Evaluation of plant extracts against test pathogen

Preparation of botanicals

The leaf and bulb extracts of Neem, Garlic, Eucalyptus, Onion, Castor, Thuja, Marigold, Ocimum, Bael and Lantana were prepared by cold water extraction method described by Shekhawat and Prasad (1971). The samples were washed separately in tap water and finally three changes in distilled water. They were crushed in motar and pestle by adding distilled water @ 2 mL/g fresh weight. The extracts were clarified by passing through two layers of cheese cloth and finally through Whatmann No. 1 filter paper. The filter sterilized extracts were quoted in the study as 100 % extract.

Bioassay procedure

The appropriate amount of plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. For bioassay, double strength concentrations of botanicals were prepared by dissolving 10, 20, 30 and 40mL of plant extract in 90, 80, 70 and 60mL of sterilized distilled water, respectively to get the final concentrations of 5, 10, 15 and 20 %. Later, poisoned food technique (plant extract amended PDA medium) was employed to screen different plant extracts *in vitro* and per cent inhibition in growth was determined with the help of

mean colony diameter and calculated by using the following formula (McKinney, 1923).

Per cent inhibition = X-Y/X ×100
(Where, X= colony diameter in check; Y = colony diameter on amended medium)

Evaluation of bio control agent against test pathogen

Seven strains of *Trichoderma harzianum viz.,* Th-1, Th-2, Th-3, Th-9, Th-13, Th-31 and Th-37 were obtained from Bio-control Laboratory, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar.

In vitro characterization of Trichoderma strains for antagonism against the test pathogen was carried out using dual culture method. The per cent inhibition in growth was calculated by the formula described by McKinney (1923).

Evaluation of essential oils against test pathogen

*In vitro*five essential oils *i.e.,* Peppermint oil, Geranium oil, Mentha oil, Palmroza oil and Citronella oil were used. Three concentrations *i.e.,* 2μL, 4μL and 8μL were prepared. First the discs were autoclaved and then placed on to sterilized petriplates and with the help of micropipette three different concentrations (2μL, 4μL and 8μL) of oil were put on the disc and fugal disc of 5mm were placed in petriplate having PDA media 4cm apart from each other. Then the petriplates were incubated at 25 $\pm$ 1ºC for 7 days. Each treatment was replicated thrice. After 7th day of incubation the growth of pathogen was measured.

RESULTS AND DISCUSSION

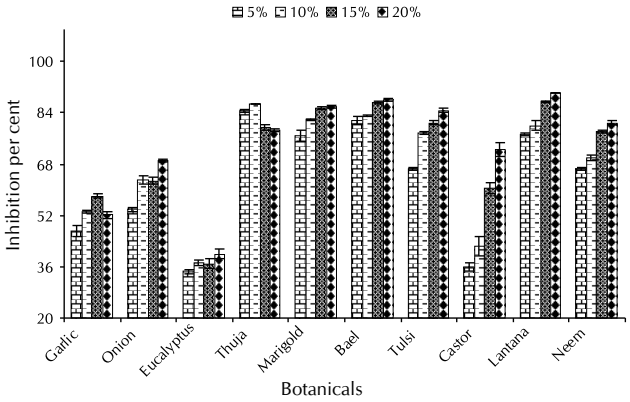
Effect of plant extracts on growth of the test fungus

Inhibition of mycelial growth varied significantly with different botanicals at different concentrations *viz.*, 5.0 , 10.0 , 15.0 and 20.0 per cent (Graph 1). At 5 per cent concentration, maximum inhibition in mycelial growth (83.53%) was recorded in Thuja followed by Bael (80.29%), Lantana (78.88%) and minimum inhibition in mycelial growth (4.12%) was recorded in Garlic. At 10 per cent concentration, maximum inhibition in mycelial growth (85.88%) was recorded in Thuja followed by Bael (82.06 %), Marigold (80.88%) and Lantana (78.82%) while minimum inhibition in mycelial growth (33.53 %) was recorded in Eucalyptus. At 15 per cent concentration, maximum inhibition in mycelial growth (86.76 %) was recorded in Lantana followed by Bael (86.47 %), Marigold (84.71%), Ocimum (79.71%) while least inhibition in mycelial growth (33.24 %) was recorded in Eucalyptus. At 20 per cent concentration, maximum inhibition in mycelial growth (89.49 %) was recorded in Lantana followed by Bael (87.35 %), Marigold (85.00%), Ocimum (83.82%) while minimum inhibition in mycelial growth (36.47 %) was recorded in Eucalyptus. Over all Lantana plant extract at 15 per cent and 20 per cent concentration was highly effective in inhibiting the mycelial growth of the test fungus. Though it was also effective at 5 per cent and 10 per cent concentrations but less effective than Thuja and Eucalyptus was found least effective at all the concentrations.

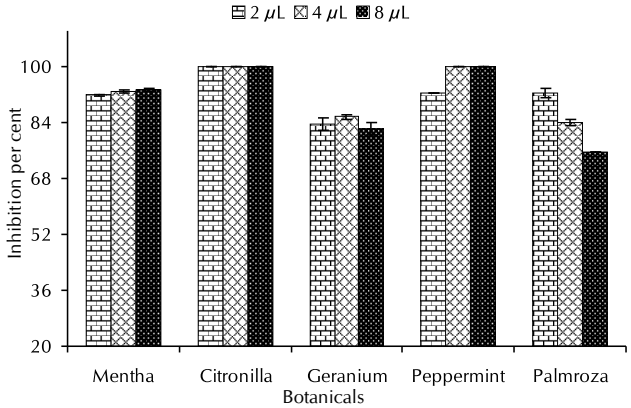
The plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics and many more. Plant extracts have played significant role in the inhibition of seed borne pathogen, *F. oxysporum* and in improvement of seed quality and emergence of plant seeds (Nwachukwu and Umechuruba, 2001). Kishore *et al.* (2001) reported that ethanol leaf extract of *A. indica* was highly inhibitory to *Phaeoisariopsis personate*, the causal organism of late spot of ground nut. Akinbode (2010) reported that extracts of the four plants *viz., Gliricidiasepium, Tithoniadiversifolia, Phyllanthusamarus*and *Morindalucida*e suppressed the growth of *C. lunata in vitro*.

Effect of essential oils on growth of the test fungus

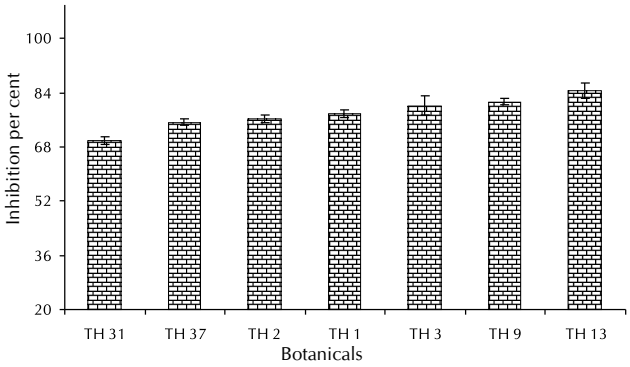
Inhibition of mycelial growth varied significantly with different essential oil at different concentrations *viz.,* 2μL, 4μL and 8μL (Graph 2). At 2μL concentration, complete inhibition of mycelial growth was recorded in Citronella oil followed by Peppermint oil (91.76%), Palmaroza oil (91.76%), Mentha oil (91.18%) while minimum inhibition in mycelial growth was recorded in Geranium (82.35%). At 4μL concentration, complete inhibition in mycelial growth was recorded in Citronella oil and Peppermint oil followed Mentha oil (92.35%) and Geranium oil (84.71%) while minimum inhibition in mycelial growth was recorded in Palmaroza oil (82.94%). At 8μL concentration, complete inhibition in mycelial growth was recorded by Citronella oil and Peppermint oil followed Mentha oil (92.65%) and Geranium oil (80.88%) while minimum inhibition in mycelia growth was recorded in Palmaroza oil



Graph 1: Effect of different botanicals on percent inhibition of radial growth of *C. lunata*at 25 $\pm$ 1ºC



Graph 2: Effect of different essential oils on percent inhibition of radial growth of *C. lunata*at 25 $\pm$ 1ºC



Graph 3: Effect of *T.harzianum*s trains on percent inhibition of radial growth of *C.lunata* at 25 ± 1ºC

(73.82%). From data it can be summarised that Citronella oil was highly effective at all the concentrations. The Higher concentration of some essential oils inhibits the mycelial growth of various fungi reported by Kzl *et al*. (2005) supports the present investigation on essential oils.

Effect of bio-agent on growth of the test fungus

Use of bio agents for controlling plant diseases is an old practice in India. In the last two decades great emphasis has been given to antagonistic organism and to assess their potential for control of plant diseases, particularly as one of the component of an integrated protection programme (Cook., 1982; Singh *et al.,* 2001; Chaube *et al.,* 2002) In the present investigation, different strains of *Trichoderma harzianum viz.,* Th-1, Th-2, Th-3, Th-9, Th-13, Th-31 and Th-37 were used to check the efficacy against the test fungus by using dual culture method. All the strains reduced the mycelia growth of the test fungus (Graph 3). Maximum per cent inhibition in mycelial growth was recorded in Th-13 strain (83.82%) followed by Th- 9 strain (80.29 %), Th-3 strain (79.12 %), Th-1 strain (76.47 %),Th-2 strain (75.00%), Th-37 strain (73.82%). While minimum per cent inhibition (68.24%) was recorded in Th-31 strain. The difference in per cent inhibition in mycelial growth indicates the difference in their efficacy against the pathogen. In the results, a clear cut zone of inhibition was observed with all the strains tested against the test fungus. This may be due to the mechanism of antibiosis of pathogens which has been reported by several workers (Dubey, 2001; Hyakumachiet *al.,* 2004; Chen *et al.,* 2005)

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