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SHORT COMMUNICATION

EVALUATION OF *in vitro* AND *in vivo* MANAGEMENT STRATEGIES AGAINST STEM GALL OF CORIANDER CAUSED BY *Protomyces macrosporus* (Unger.)

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ABSTRACT

The present study was aimed to evaluate the effect of different biocontrol agents, botanicals and fungicides on crop yield and stem gall disease of coriander (*Coriandrum sativum* L.), caused by *Protomyces macrosporus* Unger. The effectiveness of plant leaf extracts, fungicides and bioagents were evaluated against the test fungus under *in vitro* conditions by using poisoned food technique and further evaluated under field conditions for disease management and crop yield improvement. Among the biocontrol agents and botanicals evaluated, *Pseudomonas fluorescens* and datura leaf extract were most effective in in inhibiting the pathogenic growth; whereas amongst fungicides, metalaxyl + mancozeb @ 0.2% and copper oxychloride @ 0.2% were significantly superior with 100% pathogenic growth inhibition under *in vitro* conditions. Under field conditions, seed treatment and foliar spray of metalaxyl + mancozeb and copper oxychloride @ 0.2% proved most effective in managing the disease as well as improved crop yield (1493.06 and 1284.72 kg ha^{-1}, respectively) in comparison to the control (729.17 kg ha^{-1}).

Keywords: Bioagents, botanicals, coriander, fungicide, stem gall

INTRODUCTION

Seed spices play an important role in Indian economy because of large domestic consumption and demand for export. In India, seed spices are mainly cultivated in Gujarat and Rajasthan with sizeable area in Haryana, Madhya Pradesh, Punjab, Uttar Pradesh, Andhra Pradesh and Karnataka. Amongst the 15 widely grown seed spices in India, coriander is the 2nd highest export earner next to cumin (Malhotra 2016). In India coriander production during 2017-18 was 8.61 lakh MT from an area of 6.64 lakh ha with 1.3 MT ha^{-1} productivity (*https://www.indiastat.com*). All parts of this plant are used as flavouring agent or as traditional remedies for the treatment of different disorders (Sahib *et al.*, 2012). Coriander is rich in vitamin A, niacin, vitamin K, vitamin C and minerals such as iron, calcium. Coriander also contains fats, carbohydrates, proteins, fibers and provides energy. The fruits of coriander contain volatile oils as active ingredient and the most important constituents of its seeds are fatty oil and essential oils (Coskuner and Karababa, 2007).

Coriander suffers from various fungal diseases like powdery mildew, wilt and stem gall and among these, the stem gall disease caused by *Protomyces macrosporus* is the most destructive (Bilgrami *et al.*, 1991). The disease manifests in the form of tumor like swellings on all the above ground parts of plant including seeds. It is both seed as well as soil borne disease and the pathogen survives in the form of chlamydospores. The first symptoms appear in the form of galls on lower part of stem which gradually extend upwards and finally the whole plant becomes infected. The infected seeds become hypertrophied depending upon the stage of infection ultimately lowering the crop yield and quality. This disease may lead damage up to 15-27% in seed yield (Khan and Parvin, 2016). In spite of development of several technologies, the quality yield production in India is still less in comparison to other countries due to the lack of stem gall resistant varieties and climate change which enhance biotic and abiotic stress. This requires high prioritized research on proper management aspects *viz*., use of botanicals/biocontrol agents with reduced use of pesticides. The stem gall of coriander appears continuously every year when grown in the field (Verma *et al.*, 2017). However, research on the management of stem gall of coriander using integrated approach is meagre. The aim of this study was to evaluate management strategies against stem gall of coriander and assess their effect on crop yield.

MATERIALS AND METHODS

In vitro evaluation of bioagents, botanicals and fungicides

The effectiveness of plant leaf extracts namely neem (*Azadirachta indica*), aloe vera (*Aloe barbadensis*), parthenium (*Parthenium hysterophorus*), eucalyptus (*Eucalyptus* sp.) and datura (*Datura stramonium*) at 10% concentration and five different fungicides namely copper oxychloride (Blitox), hexaconazole (Contaf), metalaxyl + mancozeb (Ridomil MZ) , carbendazim (Bavistin) each @ 0.2% concentration and propiconazole (Tilt) @ 0.1% were evaluated against test fungus (*Protomyces macrosporus*) under *in vitro* conditions using poisoned food technique (Vincent, 1947).

The efficacy of plant leaves extract was evaluated by preparing the aqueous solution of plant leaf extract in sterile distilled water. The extracts were prepared by crushing 100 g leaves of each plant species in 100 mL sterile distilled water using mixer. The supernatant was filtered by using muslin cloth followed by Whatman filter paper No. 1, centrifuged at 5000 rpm for 25 min and added aseptically to double strength PDA medium to attain a final concentration of 10% before pouring the medium into Petri-plates (100 mm × 15 mm**)**. The double strength potato dextrose agar medium (PDA) was prepared by doubling the amount of ingredients, except water, and sterilizing it for 20 min in an autoclave. The test fungicides were dissolved in sterile distilled water in different sets of flasks and added in double strength medium to attain final concentration

The medium was sterilized for 20 min at in an autoclave in 150 mL Erlenmeyer flask containing 50 mL double strength medium. An equal amount of water was also sterilized separately in 150 mL flasks containing 50 mL distilled water. Three replications were maintained for each treatment in a completely randomized design. The petri-plates were inoculated with small bits of 5 mm of 7 days old culture of test fungus *P. macrosporus* under aseptic conditions and incubated at 21±1˚C. The split growth of test pathogen was observed when the control petri-plates were completely full with test pathogen. The percent growth inhibition was measured by using the formula of Vincent (1947).

In vitro evaluation of bioagents

The effect of three available isolates of bioagents *viz., Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* [procured from the Department of Plant Pathology, CCSHAU, Hisar (India)] on the growth of test fungal pathogen [isolated from infected stem of coriander and identified on the basis of morphological characteristics] was evaluated *in vitro* by dual culture technique (Lahlali *et al.,* 2007). The test pathogen and bioagents were inoculated opposite to each other on PDA medium in petri-plates. The plates were incubated at 21±1°C and split growth of pathogen observed when the control plates were completely full with test pathogen. Growth inhibition was calculated as per Vincent (1947).

***In vivo* evaluation of fungicides, botanicals and bioagents**

The efficacy of two botanicals (neem leaves extract and datura leaves extract @ 10%), bioagents formulations of *T. viride* and *P. fluorescens* @ 0.4%, fungicides (copper oxychloride and metalaxyl + mancozeb @ 0.2%), adjudged best under *in vitro* studies was evaluated under field conditions at the Experimental Area of the Department of Plant Pathology, CCSHAU, Hisar (India) against stem gall of coriander. The seeds of coriander cv. 'DH-36' were initially made sticky with jaggery solution, then treated with selected fungicides/plant leaf extracts/formulation of bioagents as per treatment and sown in the month of November 2018 at a plant spacing of 30 cm × 20 cm (plot size 9.6 m^2) in a randomized block design with each treatment replicated three times. All the routine intercultural operations were followed to raise a healthy crop, except crop protection measures. The fungicides/ plant leaf extracts/bioagents were sprayed 50 and 70 days after seed sowing. Disease incidence (%) was calculated at maturity as per the following formula

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants examined}} \times 100$$

The seed yield was recorded at crop maturity. The statistical analysis of data was carried out by using opstat software [available on *http://hau.ac.in*].

RESULTS AND DISCUSSION

To evolve an effective management strategy for stem gall of coriander caused by *P. macrosporus* various fungicides, botanicals and bioagents were evaluated both under *in vitro* and *in vivo* conditions.

***In vitro* evaluation of botanicals and fungicides**

The *in vitro* studies revealed that all the evaluated plant leaf extracts were significantly superior over control in inhibiting the growth of gall pathogen (*P. macrosporus*) [Table 1]. The highest growth inhibition was observed in datura leaf extract (89.33%), followed by neem leaf extract (74.22%) while lowest growth inhibition was observed in parthenium leaf extract (46.26%). Mima *et al*. (2009) also observed the inhibitory effect of neem on the mycelial growth of *Taphrina maculans* causing leaf blotch of turmeric. Similarly, the extracts from neem leaves have been used to manage several other fungal plant pathogens (Wang *et al*., 2010; Meena, 2012).

Among the various fungicides metalaxyl + mancozeb proved most effective in inhibiting the mycelial growth of *P. macrosporus* (100%), followed by copper oxychloride (98%), propiconazole 25 EC (85.93%), hexaconazole 5 SC (68.84%) and carbendazim 50 WP (41.06%). Metalaxyl + mancozeb and copper oxychloride were at par but superior over botanicals. These results are supported by Piperkova (2014) who found mancozeb and copper oxychloride highly effective in inhibiting 79.2 and 50.2% growth.

Table 1: Efficacy of different fungicides and botanicals in growth inhibition of *P. macrosporus* under *in vitro* conditions

Treatments	Pathogenic growth inhibition (%)
Hexaconazole 5 SC @ 0.2%	68.84 (56.58)
Copper oxychloride 50 WP @ 0.2%	98.00 (85.26)
Propiconazole 25 EC @ 0.1%	85.93 (68.00)
Metalaxyl + mancozeb 68 EC @ 0.2%	100.00 (90.00)
Carbendazim 50 WP @ 0.2%	41.06 (39.70)
Neem leaf extract @ 10%	74.22 (59.97)
Aloe-vera leaf extract @ 10%	68.68 (55.97)
Eucalyptus leaf extract @ 10%	64.45 (53.39)
Parthenium leaf extract @ 10%	46.26 (42.83)
Datura leaf extract @ 10%	89.33 (70.96)
Control	0.00 (0.00)
$CD_{0.05}$	(8.89)

*The figures in parentheses are arc-sine transformed values

Table 2: Efficacy of bioagents in the growth inhibition of *P. macrosporus* under *in vitro* conditions

Treatments (@ 0.4%)	Growth inhibition (%)
Pseduomonas fluorescens	41.50 (40.76)
Trichoderma viride	38.86 (38.53)
Trichoderma harzianum	35.09 (36.30)
Control	0.00 (00.00)
$CD_{0.05}$	(2.36)

*****Figures in parentheses are arc-sine transformed values

Amongst the three bioagents evaluated under *in vitro* condition against *P. macrosporus*, the *P. fluorescens* was effective inhibitor of mycelial growth (41.5%), followed by *T. viride* (38.86%) and *T. harzianum* (35.09) [Table 2]. Khan and Parveen (2018) found *Trichoderma viride* superior in enhancing plant growth and yield as well as minimizing the stem gall intensity (44.37%) of coriander.

Evaluation of bioagents, fungicides and botanicals under field conditions

The efficacy of two best bioagents, fungicides and leaf extracts were assessed for the management of stem gall of coriander under field condition. Among the treatments, metalaxyl + mancozeb proved most effective with disease incidence of 6.91%, followed by copper oxychloride (disease incidence 9.67%) and both were at par (Table 3). The maximum seed yield was found in of metalaxyl + mancozeb treatment (1493.06 kg ha^{-1}), followed by copper oxychloride (1284.72 kg ha^{-1}). Singh (2010) have reported the foliar sprays of carbendazim (Bavistin @ 0.1%) at 60 DAS to be effective in reducing the disease intensity of coriander stem gall and improving the yield. Kumar *et al*. (2014) found hexaconazole as seed treatment (0.2%) and foliar spray after 40, 60 and 75 DAS (0.2%) more effective in managing coriander stem gall disease than seed treatment of bioagents *viz., T. viride* (0.4%) and foliar spray of *Trichoderma* species (0.4%) after 40, 60 and 75 DAS. In corroboration to our observations, Khan and Parveen (2018) found that the application of biocontrol agents/botanicals alone or in combination significantly reduced stem gall intensity (20.33-84.82%) as compared to control; and *T. viride* was superior in reducing stem gall intensity (44.37%) and in enhancing the growth and yield of coriander.

Table 3: Efficacy of bioagents, fungicides and botanicals in managing the stem gall and improving the yield of coriander under *in vivo* conditions

Treatments	Disease incidence (%)	Yield (kg ha^{-1})
Pseduomonas fluorescens @ 0.4%	10.05 (3.21)	1250.00
Trichoderma viridef @ 0.4%	15.61 (4.03)	1180.56
Metalaxyl + mancozeb 68 EC @ 0.2%	6.91 (2.77)	1493.06
Copper oxychloride 50 WP @ 0.2%	9.67 (3.26)	1284.72
Datura leaf extract @ 10%	11.63 (3.53)	1232.64
Neem leaf extract @ 10%	16.00 (4.12)	1076.39
Control	18.44 (4.39)	729.17
$CD_{0.05}$	(0.82)	168.16

*The figures in the parentheses are square root transformed values

Conclusion: The study revealed that among the various approaches evaluated to manage coriander stem gall under field conditions, seed treatment and protective sprays of metalaxyl + mancozeb and copper oxychloride (0.2%) were most effective. As futuristic approach, the integration of bioagents and botanicals with chemicals have scope for management of disease to lessen the residual toxicity and protect the environment.

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