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Effect of propiconazole and difenoconazole on the control of anthracnose of chilli fruits caused by *Colletotrichum capsici*

K. Gopinath[a], N.V. Radhakrishnan[a,b], J. Jayaraj[a,c,*]

[a]*Department Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar 608 401, India*
[b]*Department of Plant Pathology, Coconut Research Station, Kerala Agricultural University, Balaramapuram, Thiruvananthapuram - 695 509, Kerala, India*
[c]*Department of Biological Sciences, 8888 University Drive, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*

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Abstract

Anthracnose of chillies caused by *Colletotrichum capsici* is a serious disease affecting the yield and quality of fruits. In vitro, greenhouse and field experiments were conducted to evaluate the efficacy of propiconazole, difenoconazole and carbendazim at different concentrations to control the pathogen and disease incidence. Among the fungicides, propiconazole exhibited the highest level of inhibition of in vitro mycelial growth, biomass production, sporulation and spore germination at concentrations as low as 0.1 $\mu g\,ml^{-1}$. Enzyme (PG, PGTE, PTE and cellulases) production by *C. capsici* was significantly reduced by incorporation of fungicides into the growth medium. The highest degree of enzyme production inhibition was observed with propiconazole, followed by difenoconazole and carbendazim. Greenhouse and field experiments were conducted to study disease control by spraying propiconazole (0.1%, 0.05%, 0.025% a.i.), difenoconazole (0.05%, 0.025% a.i.) and carbendazim (0.1% a.i.). Application of propiconazole at 0.1% caused a dramatic reduction of disease incidence by 70% when compared to difenoconazole at 0.05% (58%) and carbendazim at 0.1% (44%). Additionally the fruit yield increased in the range of 86%, 63% and 60% for propiconazole, difenoconazole and carbendazim, respectively, when compared to unsprayed controls.

Keywords: Chilli-fruits; Anthracnose; *Colletotrichum capsici*; Fungicides; Efficacy; Disease control

1. Introduction

Chilli (*Capsicum annum* L.) is an important cash crop grown under both tropical and subtropical conditions. India is the largest grower, consumer and exporter of chilli, currently exporting dry chilli and chilli products to over 90 countries around the world (Singal, 1999). Anthracnose disease of chilli caused by *Colletotrichum capsici* Butl. and Bis. has been a serious problem for chilli cultivation in India. The fungus is distributed throughout the tropics and very commonly occurs in chilli growing areas of India resulting in disease incidence levels ranging between 66% and 84%, and incurring yield loss up to 12–50% (Thind and Jhooty, 1985; Bagri et al., 2004; Sharma et al., 2005). The disease causes extensive damage in field, storage and transit when conditions are favourable (Thind and Jhooty, 1985; Jayalakshmi et al., 1999). Widespread occurrence of this disease was reported in India as early as 1957 (Chowdhury, 1957). Disease symptoms are observed in three forms (i) seedling blight or damping-off, that is prevalent in the nursery, (ii) leaf spotting and die back, which is initiated at different stages of growth; die back infection starts from the growing point of secondary branches, gradually advancing downwards and inwards infecting the entire branch, and (iii) fruit spotting and rotting where mostly ripe fruits are infected (Siddiqui et al., 1977). Two types of fruit rot symptoms; dry rot and soft rot are observed (Datar, 1995).

In the absence of resistant cultivars, chemical control offers the only viable solution for disease management.

*Corresponding author. SFU-Biology, 8888 University Drive, Burnaby, BC, Canada V5A 1S6. Tel.: +1 604 291 3090; fax: +1 604 291 3496.
E-mail address: jaya@sfu.ca (J. Jayaraj).

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Under field conditions, only organo-sulphur and copper oxychloride compounds are extensively used. Practical limitations of these fungicides are: (i) they are preventive and not curative, and (ii) require several applications up to fruit ripening stage (Thind and Jhooty, 1987). Although the unit cost of these fungicides is relatively low, multiple applications cause an increase in overall cultivation cost, and above all leads to accumulation of toxic residues in the crop and ecosystem (Bagri et al., 2004). Although the use of systemic fungicides simplifies the management strategy, not many systemic fungicides are practically in use on chilli. There is a strong need to find alternative systemic fungicides to the existing chemical carbendazim, which is the only systemic fungicide currently used in chilli fields. Carbendazim application at 0.1% concentration controlled the incidence of anthracnose and ripe fruit rot of chilli (Biswas, 1992; Ebenezer and Alice, 1996). However, repeated application of carbendazim in turmeric and grapevine has resulted in the development of resistant strains of *C. capsici* (Hemantha Kumar et al., 2005) and *Gloeosporium ampelophagum* (Thind et al., 2000) respectively. The target site of carbendazim was identified as β-tubulin synthesis in fungal cells and resistance is ascribed to amino acid substitutions in β-tubulin (Davidse, 1986). Azole fungicides show a broad antifungal activity and are used either to prevent or cure fungal infections (Zarn et al., 2003). They are primarily sterol demethylation inhibitors (DMIs), which represent the most important group of systemic fungicides, and are applied commonly for the control of rusts, powdery mildews and scabs (Buchenauer, 1995). The triazole fungicides, propiconazole and difenoconazole have both protective and curative activity and are extensively used for control of diseases of cereals, grapevines, banana and peanut (Munkvold et al., 2001), and also for the control of anthracnose of strawberry (Smith and Black, 1991). However they have not been tested in chilli crops against anthracnose. In the present study we evaluated the efficacy of these two triazole fungicides for chilli anthracnose management through extensive greenhouse and field trials.

2. Materials and methods

2.1. Fungal culture and plant material

Colletotrichum capsici-infected chilli fruits were collected from chilli fields in and around Annamalainagar, India. The pathogen was isolated and maintained on potato dextrose agar (PDA) medium (Himedia, India). The virulence and pathogenicity of the isolates was verified by inoculating chilli fruits with a spore suspension of the pathogen (Bansal and Grover, 1969). Seeds of the chilli cultivar Guntur-4 were obtained from Tamilnadu Agricultural University, Coimbatore, India. Seeds were disinfected in 20% sodium hypochlorite for 15 min and washed in sterile distilled water before planting.

2.2. In vitro evaluation of fungicides

2.2.1. Effect of fungicides on the biomass, growth and sporulation of C. capsici

The fungicides used for in vitro evaluation were carbendazim (BASF, Mumbai, India), difenoconazole and propiconazole (Novartis India, Mumbai). The test fungicides were suspended in sterile distilled water and added to Czapek's broth (50 ml per 250 ml Erlenmeyer flask) to a final concentration of 0, 0.1, 0.5, 1, 5, 10, 25, 50 μg active ingredient (a.i. ml^{-1}). Fungal mycelial disks (5 mm diameter), prepared from the periphery of 7-day-old cultures of *C. capsici* were transferred to four replicate flasks and dry mass of mycelial mats were determined after 20 days of incubation under 16 h light photoperiod at 28 °C.

This same experiment was also conducted in agar medium on petri plates. Twenty-five millilitres of Czapek's Agar medium was amended with the fungicides at the same concentrations and poured into petri plates. Four replicate plates per treatment were inoculated at the centre with fungal discs. Plates were incubated at 28 °C under 16 h light photoperiod for 20 days and colony diameters were periodically measured.

Czapek's broth (25 ml) was dispensed in 100 ml Erlenmeyer flasks and autoclaved. The medium was amended with individual fungicides at the same concentrations as above. The flasks were inoculated with 9 mm mycelial discs of *C. capsici* and incubated at 28 °C under 16 h light photoperiod for 20 days. At the end of incubation, 100 μl of Tween 20 was added to the flask and the contents of the flask were vigorously shaken in a vortex shaker for 5 min to harvest spores. The fluid phase was decanted and filtered through a nylon mesh (60 μm) and centrifuged at 3000 *g* for 5 min. The pellet containing spores was suspended sterile distilled water and spore concentration was evaluated by using a Neubauer's chamber haemocytometer (Neubauer-ruled Bright Line counting chambers; Hauser Scientific, Horsham, PA), according to the classical procedure. Four replicates were performed and the experiment was repeated once. Fungicide unamended medium served as control (Jayaraj and Radhakrishnan, 2003).

2.2.2. Effect of fungicides on the in vitro spore germination of C. capsici

A spore suspension of the pathogen was prepared in sterile distilled water from a 20-day-old culture of *C. capsici*. The concentration of spores was adjusted to $1 \times 10^3\ ml^{-1}$ by diluting in sterile distilled water. Petri plates filled with fungicide-amended medium amended at the same concentrations were inoculated with 100 μl of spore suspension spread evenly with a sterile glass rod. Plates containing no fungicide served as controls.

Inoculated plates were incubated under darkness for 4 days at 28 °C. The average number of *C. capsici* colonies in 10 microscopic fields was recorded for each plate at 48 and 96 h after incubation (Lange et al., 1998). There were four