

EFFICACY OF DIFFERENT FUNGICIDES, BIOCIDES AND BOTANICAL EXTRACT SEED TREATMENT FOR CONTROLLING SEED-BORNE COLLETOTRICHUM SP. IN CHILLI (*CAPSICUM ANNUUM* L.)

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KEYWORDS

Anthraxnose
Colletotrichum capsici
Seed treatment
Seedling vigour index.

Received on :

21.10.2012

Accepted on :

19.02.2013

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ABSTRACT

Chilli anthracnose caused by *Colletotrichum capsici* (Sydow) Butler and Bisby is one of the major constrain in the production of chilli in tropical and sub-tropical regions. Present research was aimed at studying the efficacy of various chemical and biocidal agents on the germination and seedling vigour of *C. capsici* infected chilli seeds. Among different seed treatments, *Safeda* leaves extract, Neem seed extract, *tulsi* leaves extract, Thiram, Captan and Bavistin were found superior and significantly higher seed germination as compared to other treatments and control. Among different bioagents used for the experiment, maximum per cent seed germination (82.35%) was recorded in seed treated with *T. viride*. Pre and post emergence mortality was minimum in case of *T. polysporum* (2.65% and 6.10%) followed by *T. viride* (6.00% and 6.80%). Bavistin was most effective with 93.00% (68.00% in control) seed germination with no pre or post emergence mortality and increased seedling vigour index of 506.85 which was 306.00 in control. Maximum per cent seed germination and seedling vigour index was observed in seed treated with leaf extract of *E. tereticornis* with germination percent of 94.00 and seedling vigour index of 540.05. Among all treatments maximum root/shoot length and vigour index was found by *T. viride*, *Safeda*, Bavistin and Thiram treated seeds and found superior to others.

INTRODUCTION

Chilli (*Capsicum annum* L.) has its unique place in the world diet in its ripe dried form (as a spice) as well as green fruit (as vegetable). It has several medicinal properties and also used as counter irritants in lumbago, neuralgia, rheumatic disorders and also useful in atomic dyspepsia (Pruthi, 1993). Chilli production suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also by abiotic stresses. Chilli suffers from several seed borne fungal fruit rot disease viz., *Alternaria* fruit rot, *Aspergillus* rot, Black rot, Black fruit rot, Die-back, Ripe fruit rot, *Cladosporium* fruit rot, Charcoal fruit rot, Grey mold rot, *Phytophthora* and *Rhizopus* rot caused by *Alternaria alternata* (Fr.) Keissler, *Alternaria solani* (Ellis and Martin) Jones and Grout, *A. tenuis* Auct., *Aureobasidium pullulans* (De Bary), *Aspergillus niger* (van Tieghem), *Botrytis cinerea* (De Bary), *Brachysporium senegalensis* (Speg.), *Cladosporium herbarum* (Pers.) Link ex Gray, *Colletotrichum coccodes* (Wallr), *C. gloeosporioides* (Penz.) Penz. and Sacc., *C. Dematium* (Fr.) Grove, *C. Piperatum* (E. and E.) E. and H., *C. multisetorum*, *C. lindemuthidnum* (Sacc. et Magnus) Briosi et Cav, *C. acutatum* Simmonds, *C. lini* (Manns) Bolley, *Drechslera qustralinesis* M. B. Ellis, *D. halodes* (Drechsler) Subram. and B.L. Jain, *D. rostrata* (Drechsler) M. J. Richardson and E. M. Fraser, *Fusarium moniliforme* J. Sheld., *F. solani* W. C. Snyder, *Macrophomina phaseolina* (Tassi) Goid., *Periconia byssoides* Pers., *Phomopsis capsici* (Magnaghi) Sacc., *Phytophthora capsici* Leonian and *P. parasitica* Dastur (Mirdha and Siddiqui,

1989; Basak *et al.*, 1994; Khodke and Gahukar, 1995; Prabhavathy and Reddy, 1995; Kulshrestha *et al.*, 1976 and Suryawanshi and Deokar, 2000). *Colletotrichum capsici*, which causes varied disease symptoms viz., damping off, anthracnose or fruit rot, die back in chilli, is one of the major production constraints in tropical and subtropical areas. This fungus causes severe damage on chilli fruits in both pre and post harvest stages and these infections together account for more than 50% of the crop losses (Pakdeevaporn *et al.*, 2005). The fungus is both internally and externally seed-borne (Ramachandran *et al.*, 2007). The black wound found on infected fruits expands very quickly under high moisture condition, especially in tropical conditions. Fruiting bodies and spores of *Colletotrichum* will be abundantly produced on those black lesions. Sowing such contaminated seeds results in pre emergent and post emergent damping-off of seedlings in nursery and in transplanted field.

Vegetable growers generally apply fungicides as preventive measure to control this devastating disease on chilli in the region. One of the option overcome this problems is to replace the unilateral chemical approach with the search for alternative control methods. The control of chilli anthracnose fruits rot has, for many years, relied on chemicals and resulted in many undesirable problems. There is a need to incorporate alternative control components which will be effective at farm level. There was no systematic study has been conducted on this seed borne pathogen to control at farm level using

fungicides as well as local available plant extracts. So, the present study was therefore conducted to examine the efficacy of various fungicides, plant products and bioagents on the germination of chilli seeds contaminated with *C. capsici*.

MATERIALS AND METHODS

Isolation and Pathogenicity test of the pathogen

C. capsici, a causal agent of anthracnose was isolated from two hundred anthracnose infected chilli seeds obtained; from Department of Horticulture, Agricultural Research Station (ARS), Durgapura (Jaipur), Rajasthan. Anthracnose fungus was isolated by Blotter Method and Agar Plate Method described by ISTA (1976). After the pathogenicity test, isolates of *C. capsici* were cultured on PDA for 3 days. Then a most pathogenic isolate was used for further study.

Preparation of plant extracts

Leaf extraction of *Eucalyptus tereticornis* Sm. (Safeda), *Azadirachta indica* A. (Neem), *Ocimum sanctum* Linn. (Tulsi), *Lawsonia inermis* L. (Mahendi) and *Datura stramonium* L. (Datura) was done following the method described Jacob and Sivaprakasam (1994) with slight modifications. All the plant leaf was collected from Research farm of SKN college of Agriculture, Jobner (Jaipur) Rajasthan. Collected leaves were washed using distilled water, chopped into fine pieces and then overnight soaked in cold distilled water to soften the tissues. After that overnight soaked leaves were grinded using a pestle and mortar with water at the ratio of 1:2 (1 part of leaf: 2 part of water). Semi liquid extract was used as seed dresser.

Sources of bioagents and fungicides

Three *Trichoderma* strains namely; *T. viride*, *T. harzianum* and *T. polysporum* (10^8 cfu/mL) and one stain of *Pseudomonas fluorescens* (48h old culture) were used in this study which was collected from Department of plant pathology, Agriculture Research Station, Durgapura (Jaipur) Rajasthan. Four fungicides namely; Thiram, Captan Indofil M-45 and Bavistin were used in study was collected from Department of Plant Pathology, SKN college of Agriculture, Jobner (Jaipur)

Rajasthan.

Methods of seed treatment and observations

For studied the efficacy of plant products, bioagents and fungicides as seed protectants against *C. capsici*, apparently healthy sterilized seeds of chilli were artificially contaminated with isolated pathogenic species of *C. capsici*. Hundred such contaminated seeds were then treated (dressing/soaking) with plant products and bioagents at the rate of 5mL/10g seeds and at the rate of 2.5g/kg seeds with fungicides for 30 minutes in above solutions. The treated seeds were fine shade dried before sowing. The treated and untreated inoculated seeds were then sown in earthen pots for further observations. Observations were recorded after 10 days on seed germination, pre-and post emergence mortality and root/shoot length.

Statistical analysis

Seedling vigour was also calculated by formula suggested by Abdul Bakshi and Anderson (1973).

Vigour index = Germination (%) × (Root length + shoot length)
All other data were analyze using the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

During the present investigation it was evident that all the seed treatments (fungicidal, biocidal and leaf extract) have resulted in higher seed germination and also enhanced seedling vigour compared to control (Table 1). Among seed treated with different treatments, Safeda leaves extract, Neem seed extract, tulsi leaves extract, Thiram, Captan and Bavistin were found superior and significantly higher seed germination as compare to other treatments and control. Seed treatment with *P. fluorescens*, *T. polysporum* and *Datura* had no significant enhancement on seed germination infected with *C. capsici*. Alam *et al.* (2002) tested the effect of ten plant extracts on conidial germination of *C. gloeosporioides* and recorded that *Tagetes erecta* (leaf) and *Azadirachta indica* extracts were most effective in inhibition of conidial germination at 5:1.5 (w/v) concentration. Extract of different parts of plants also reported

Table 1: Effect of different seed treatment against *C. capsici* on seed germination, pre- and post emergence mortality and vigour index

Treatments	Per cent germination Pre-emergence	Per cent mortality Post-emergence	Root length(cm) *	Shootlength(cm)*	Vigour index	
Safeda (<i>E. tereticornis</i>)	94.00 ^a (75.82)	0.00 ^x (0.00)	0.00 ^x (0.00)	3.00 [@] (9.98)	2.75 [§] (9.46)	540.05 ^{LM}
Neem (<i>A. indica</i>)	92.00 ^a (73.57)	8.50 ^y (16.95)	9.50 ^y (17.95)	3.10 [@] (10.14)	2.70 [§] (9.46)	538.20 ^{LM}
Tulsi (<i>O. sanctum</i>)	90.50 ^a (72.05)	4.50 ^{yz} (12.25)	9.36 ^y (17.76)	2.80 [§] (9.63)	2.20 [#] (8.53)	452.50 ^{LM}
Mahendi(<i>L. inermis</i>)	85.50 ^b (67.62)	0.00 ^x (0.00)	0.00 ^x (0.00)	2.65 [§] (9.28)	2.10 [#] (8.33)	406.12 ^{MN}
Datura (<i>Datura stramonium</i>)	77.75 ^c (61.82)	15.50 ^z (23.19)	14.50 ^z (22.38)	2.25 [#] (8.53)	1.70 [#] (7.49)	307.11 ^N
Thiram	90.50 ^a (72.05)	8.75 ^y (17.16)	4.45 ^{yz} (12.11)	3.00 [@] (9.98)	2.50 [#] (9.10)	497.75 ^{LM}
Captan	91.00 ^a (72.54)	3.80 ^{yz} (11.24)	9.26 ^y (17.76)	2.75 [§] (9.46)	2.50 [#] (9.10)	477.75 ^{MN}
Indofil M-45	77.75 ^c (61.68)	15.90 ^z (23.50)	14.05 ^z (21.97)	2.50 [#] (9.10)	2.00 [#] (8.13)	349.87 ^{MN}
Bavistin	93.00 ^a (74.66)	0.00 ^x (0.00)	0.00 ^x (0.00)	2.95 [#] (9.81)	2.50 [#] (9.10)	506.85 ^{LM}
Vitavax	84.75 ^{ab} (66.97)	0.00 ^x (0.00)	0.00 ^x (0.00)	2.70 [§] (9.46)	2.10 [#] (8.33)	406.80 ^{MN}
<i>Trichoderma viride</i>	82.35 ^{bc} (65.12)	6.00 ^y (14.18)	6.80 ^y (15.12)	3.30 [@] (10.47)	3.65 [@] (10.94)	572.33 ^L
<i>T. harzianum</i>	81.50 ^{bc} (64.52)	8.75 ^y (17.16)	9.75 ^y (18.15)	3.15 [@] (10.31)	2.70 [§] (9.46)	476.77 ^{LM}
<i>T. polysporum</i>	77.50 ^c (61.68)	2.65 ^{yz} (9.28)	6.10 ^y (14.30)	2.45 [#] (8.91)	2.15 [#] (8.53)	356.50 ^{MN}
<i>Pseudomonas fluorescens</i>	71.56 ^c (57.73)	10.50 ^y (18.91)	10.50 ^y (18.91)	2.50 [#] (9.10)	2.25 [#] (8.53)	339.91 ^{MN}
Control	70.00 ^c (56.79)	20.00 ^z (26.56)	15.00 ^z (22.79)	2.50 [#] (9.10)	2.30 [#] (8.72)	325.00 ^N

*Mean of 10 seedlings; Figures given in parentheses are angular transformed values; Means in a column followed by the same letter(s) are not significantly different according to CRD (P=0.05).

to be effective in inhibiting the radial growth of *C. capsici* (Bagri *et al.*, 2004 and Shivpuri *et al.*, 1997). After the germination there was no pre and post emergence mortality in case of Bavistin, Vitavax, Safeda and Mahendi treated seeds. Among different bioagents used for the experiment, maximum per cent seed germination (82.35%) was recorded in seed treated with *T. viride*. Pre and post emergence mortality was minimum in case of *T. polysporum* (2.65% and 6.10%) followed by *T. viride* (6.00% and 6.80%) was most effective with 93.00% (68.00% in control) seed germination with no pre or post emergence mortality and increased seedling vigour index of 506.85 which was 306.00 in control (Table 1). *Trichoderma* species have been applied to control *Colletotrichum* species in chilli (Boonratkwang *et al.*, 2007) and also found effective in *in-vitro* control *C. truncatum* causing anthracnose of soybean (Jagtap *et al.*, 2012). Maximum per cent seed germination and seedling vigour index was observed in seed treated with leaf extract of *E. tereticornis* with germination percent of 94.00 and seedling vigour index of 540.05. Leaf extract of *E. tereticornis* (Safeda) also reported to effective in reducing the incidence of damaging off of brinjal with improving seedling emergence (Jacob and Sivaprakasam, 1994). Seed treatment with fungicides is well known for the control of seed-borne fungi (Mills and Wallace, 1970 and Abou-Heilan, 1984). Bavistin followed by Vitavax and Thiram have been also been reported to be best seed dresser against seed-borne *C. capsici* and other fungi (Mridha and Siddique, 1989; Mirdha and Choudhary, 1990; Mishra, 1988). Seed treatment with different biocides (bioagents and plant leaf extracts) have been reported to increase seed germination and vigour index by reducing the pre- and post emergence mortality in several crops including chilli (Prasad and Simlot, 1982; Farzana and Ghaffar, 1991; Haque and Ghaffar, 1992; Rahman *et al.*, 2011). Among all treatments maximum root/shoot length and vigour index was found by *T. viride*, Safeda, Bavistin and Thiram treated seeds and found superior to others. Biological control is an effective, ecofriendly and alternative approach for any disease management practice. The results of seed treatment of artificially contaminated chilli seeds with isolated pathogenic species of *C. capsici* by various bioagents and leaf extracts revealed that all the plant extracts and antagonists had high significance on germination percentage, pre and post emergence mortality and also on seedling vigour. Alternative use of fungicides like Bavistin for seed treatment may also be followed for controlling seed borne chilli anthracnose.

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