The Andhra Agric. J 59(1): 83-86, 2012

Effect of Inoculation Methods, Inoculum Concentration of *Colletotrichum capsici* and Fruit Ripening Stage on Chilli Fruit rot.

G Darvin, K V M Krishna Murthy, P Anil Kumar and K L Narasimha Rao
Department of Plant Pathology, Agricultural College, Bapatla, Andhra Pradesh 522 101

ABSTRACT

Hypodermic injection method of inoculation of *Colletotrichum capsici* was found to be the best in producing the large lesions and per cent disease index. Fully ripened red stage chilli fruits were the most susceptible producing large lesions and per cent disease index. Inoculum concentration of 10^6 conidia/ml of *C. capsici* was the most effective concentration, producing maximum lesion size and per cent disease index.

Key words :*Colletotrichum capsici*, Chilli, Inoculation method , Inoculum concentration.

Chilli (*Capsicum annum* L.), one of the spice crops belonging to the family, Solanaceae, is a well-known commercial crop used both as condiment or culinary supplement and vegetable. Chillies are excellent source of Vitamin, A, B, C and E with minerals like molybdenum, manganese, folate, potassium, thiamin, and copper. Chillies produce alkaloids, capsaicinoids and carotenoids which make chilli hot and pungent.

Many of the diseases have been reported to affect chilli crop. Among those diseases, dieback and fruit rot caused by *Colletotrichum capsici* (Syd) Butler and Bisby is prevalent in all chilli growing states of India and causes losses ranging from 10 to 60 %. Although only ripe fruits turning red are most frequently affected. In Assam, 12-32% fruits are found to be affected by this disease (Chowdhury, 1957).

MATERIAL AND METHODS

Effect of inoculation methods on chilli fruit rot infection:

In order to evaluate the efficacy of different inoculation methods of *C. capsici*, chilli fruits were inoculated with spore suspension of *C. capsici* by different inoculation methods *viz.,* spore spray method, hypodermic injection method and cork borer method.

Spore spray method:

Colletotrichum capsici isolated from infected chilli fruits was maintained on potato dextrose agar at 25^0C for 10 days. The surface of fungal culture was scraped from the plate with addition of 2 to 5 ml of sterilized distilled water using a sterile scalpel. Mycelial mat and conidia were separated using double layered cheese cloth. The concentration of the conidial suspension was adjusted to 10^6 conidia/ml with sterilized distilled water using a haemocytometer. The conidial suspension was sprayed on surface sterilized chilli fruits by using hand atomizer.

Hypodermic injection method:

The conidial suspension of *C. capsici* prepared as explained above was injected into surface sterilized healthy chilli fruits vsing cork born using a sterilized hypodermic syringe at two equidistant points on the fruits.

Cork borer method:

Three mm sized discs cut from the periphery of colony of *C. capsici* by were inserted in the centre of the wound made by cutting 3 mm size disc on the rind of surface sterilized fruits and covered by placing small wad of sterilized and moistened cotton on surface of the wounded tissue.

In all the inoculation methods, the artificially inoculated fruits were incubated in moist chambers at a temperature of 25 ± 1^0C for five days. Each inoculation method was replicated five times and for each replication ten uniformly sized chilli fruits were used. At the end of incubation, the size of lesion on inoculated fruits was measured and severity ratings were assigned. Per cent disease severity was calculated and expressed as per cent disease index (PDI). The per cent disease index (PDI) was

Table 1. Chilli fruit rot lesion size and PDI in different *C. capsici* inoculation methods on chilli fruits.

Inoculation method	Lesion Size (mm)	PDI
Cork borer method	10.31	35.3 (36.4)
Hypodermic injection method	11.38	36.3 (37.1)
Spore spray method	0.00	0.0 (2.5)
CD at 5% level	0.67	1.02

Table 2. Chilli fruit rot lesion size and PDI at different *C. capsici* inoculum densities

Inoculum concentration (No. of spores per ml)	Lesion Size (mm)	PDI
10^2	0.00	0.0 (2.5)
10^3	9.45	11.9 (20.2)
10^4	13.95	19.3 (26.1)
10^5	17.79	30.4 (33.5)
10^6	21.49	34.1 (35.7)
Check (Sterile distilled water)	0.00	0.0 (2.5)

Table 3. Chilli fruit rot lesion size and PDI on inoculated chilli fruits of varying maturity stages.

Stage of the chilli fruit	Lesion Size (mm)	PDI
Dark green stage	12.84	34.8 (36.2)
Transition stage (from green to red)	17.29	38.5 (38.4)
Fully ripened red stage	22.22	46.7 (43.1)
CD at 5% level	0.72	0.97

computed using the formula of

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings X Maximum disease grade}} X\ 100$$

Relationship between inoculum concentration of *C. capsici* and chilli fruit rot infection:

Chilli fruits at transition (from green to red) stage were surface sterilized with 1.0% sodium hypochlorite and inoculated by hypodermic injection method with spore suspension of the *C. capsici* at different concentrations *viz.*, 10^2, 10^3, 10^4, 10^5 and 10^6 spores/ ml of water. At the end of incubation lesion size on inoculated fruits was measured, rating assigned and per cent disease severity was calculated and expressed as per cent disease index (PDI).

Relationship between ripening stage of chilli fruit and fruit rot infection:

Surface sterilized chilli fruits at dark green, transition (from green to red) and ripened red stages of ripening were artificially inoculated with spore suspension (10^6) of *C. capsici* by hypodermic injection method and incubated in moist chambers for five days at 25±1^{0}C temperature. Each growth stage was replicated five times and in each replication ten uniformly sized chilli fruits were used. At the end of incubation, lesion size on inoculated fruits was measured, rating assigned and per cent disease severity was calculated and expressed as per cent disease index.

RESULTS AND DISCUSSION

Effect of different inoculation methods of *C. capsici* on chilli fruits:

The hypodermic injection method of inoculation was found to be the best in producing the largest sized lesions (11.38 mm) compared to other methods. However in terms of per cent disease index (PDI), hypodermic injection method (36.30) and cork borer method (35.30) did not differ significantly (Table 1). However, cork borer method caused mechanical injury on the fruits to a greater extent than the hypodermic injection method, leading to the rotting of fruit which could not be attributed to the effect of pathogen alone. Since spore spray a method did not cause any lesion, it can be presumed that a slight injury is needed for the in fection to occur. Natural wounds that occur on the fruits infield may serve as portals of entry for the pathogen. Essentiality of slight injury for chilli fruit rot infection was also demonstrated by Verma (1973), Singh *et al.* (1993) and Bhale *et al.* (1999) who used pin-prick method. Kenchaiah (1975) reported that direct inoculation i.e of fruits to be a more appropriate method than indirect inoculation i.e. of seed and standing crop.

Effect of inoculum concentration of *C. capsici* on chilli fruit rot infection

Conidial concentration of 10^6/ml caused significantly highest PDI (34.07) and the largest mean lesion size (21.49 mm). Decrease in the inoculum concentration tapered off the lesion size as well as PDI. Reduction in the inoculum concentration also delayed appearance of the symptoms. Chilli fruits inoculated with 10^6 conidia/ ml developed the symptoms very rapidly. Inoculum concentration of 10^2 conidia per ml was not sufficient to produce the infection (Table 2).

The findings of the present study are in conformity with the observations of Hong and Hwang (1998) who reported an increase in severity of anthracnose of hot pepper with increase in inoculum density from 10^3 to 10^6 conidia/ ml. Dillard (1989) reported that disease severity of anthracnose of tomato was increased with increasing inoculum density from 10^1 to 10^6 conidia/ml/fruit, reaching the greatest at 10^6 conidia/ml/ fruit.

Effect of ripening stage of chilli fruit on fruit rot infection:

The results of the present study (Table 3) revealed that, the highest PDI (46.70) and mean lesion size (22.22 mm) were recorded on fruits inoculated at fully ripened red stage. The fruits at transition (from green to red) stage showed 38.5 PDI with mean lesion size of 17.29 mm. Lowest PDI (34.80) and mean lesion size (12.84 mm) were observed in the fruits at dark green stage. The susceptibility of ripened red fruits was reported to be due to the production of endo-PGTE (Polygalacturonate *trans*-eliminase) enzyme which would increase the permeability of chilli fruits leading to leakage of electrolytes in chilli fruits (Thirupathaiah and Subramanian, 1979). This effect is greater in red ripened fruits which are susceptible to rotting than in the green chilli fruits. High content of phenols and waxes in the green chillies may impart resistance to the fruits at green stage.

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(Received on 04.06.2011 and revised on 29.08.2011)