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Effect of neem based plant products and plant extracts against anthracnose of chilli (*Capsicum annuum* L.)

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Abstract
The anthracnose of chilli is one of the most devastating disease. *C. capsici* was isolated from disease samples and the isolate of *C. capsici* was confirmed on the basis of disease symptoms, morphological and cultural characteristics. The various factors, viz., nutritional, physical and toxicological factors showed wide variation on growth and sporulation of *C.capsici.* Antagonistic ability of different isolates of *Trichoderma harzianum* was tested by dual culture technique. Among them *T. harzianum* isolate 5 was found most effective in inhibiting the growth of *C. capsici.* The toxicological factors like Neem Seed Kernel Extract (NSKE) was found most effective in inhibiting the growth of *C. capsici* compared with neem oil, combined application (neem, garlic, ginger, onion plant extracts) and garlic bulb extract.

Keywords: bio-agents, *Colletotrichum capsici*, fungicide, plant extracts/plant products

Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable as well as spice crop, cultivated worldwide. It is not only used in many cuisines but also found to have many medicinal properties. The genus *Capsicum* comprises about 20-25 species, out of which *C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens* are cultivated. *Capsicum annuum* is widely cultivated variety, second being *C. frutescens.* Commonly used term is Chilli, which refers to hot types of *Capsicum*. Though it was originated in the American tropics, it is widely propagated (Sahitya *et al.*, 2014) [12].

Chilli (*Capsicum annuum* L.) also called red pepper is an important cash crop in India. It is grown for its pungent fruits. Both green and ripe fruits are used to impart pungency to the food. As a condiment, it has become indispensable in every Indian house. It is also used medicinally, in chutneys and pickles. India has become world's largest producer and exporter of chilli by exporting to USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and and many other countries across the world. India contributes 25% of the world's total production of chilli. Some of the hottest chillies are grown in India. Indian chillies have been dominating international chilli market. In India, chilli is mainly grown in states of Andhra Pradesh, Maharashtra, Karnataka, Gujarat, Tamil Nadu and Orissa (Lydia and Zacharia, 2012) [13].

Chilli anthracnose was first reported in India on from the Coimbatore of Madras Presidency (Sydow, 1913) [17]. The disease has been identified in all the chilli producing regions of the world and has become a serious constraint to chilli production. Different species of *Colletotrichum*, namely *C. capsici*, *C. gloeosporioides*, *C. acutatum* are known to cause anthracnose in chilli in India. Anthracnose disease appears as small circular spots that coalesce to form large elliptical spots on fruits and leaves. Under severe conditions, defoliation of affected plants occurs. Kim *et al.* (2004) [10] reported that different species cause diseases of different parts of the chilli plant. The disease has been observed to occur in three phases; seedling blight or dumping off, leaf spot, die-back and anthracnose or fruit rot. Management of these diseases through agro chemicals alone is neither cost effective nor environmentally safe. Therefore, an integrated disease management (IDM) approaches, using chemical, cultural and eco-friendly bio-agents are needed for sustainable chilli production (Pandey and Satpathy, 2009; Lydia and Zacharia, 2012) [13, 14].

Among all the diseases, anthracnose disease is the major constraint to chilli production worldwide resulting in high yield losses. This fungal disease caused by *Colletotrichum* species drastically reduces the quality and yield of fruit resulting in low returns to farmers. 10-80% of marketable yield is reduced in Thailand, about 13% in Korea. This die back/ fruit rot/ anthracnose disease is seen on mature fruits resulting in both pre harvest and post-harvest fruit loss. In India, in severe cases, pre harvest and post-harvest losses comprise up more than 50%.

Significant yield losses were reported from Punjab and Haryana (20-60%) and Assam (12-30%) (Sahitya *et al.*, 2014) [12].

Among the fungal diseases, anthracnose caused by *Colletotrichum* spp. is considered to be the major constrain to increase chilli production. It occurs every year with varying intensities and inflicts considerable quantitative and qualitative losses of the crop in the fields as well as in the storage. Anthracnose is mainly a problem on mature leaves and fruits, causing severe losses due to both pre-harvest and post-harvest fruit decay {Hadden and Black (1989) [6], Bosland and Votava (2003)} [1]. Four species of *Colletotrichum*, *C. capsici*, *C. gloeosporioides*, *C. acutatum* and *C. coccodes* have been reported as causal agents of pepper anthracnose in many countries. The major species are *C. capsici and C. gloeosporioides* (Hadden and Black, 1987) [5].

Materials and Methods

The present investigation was carried out in the laboratory of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.) during *Rabi* season 2017. The experiment was conducted using Completely Randomized Design (CRD) in pots consisting of 7 treatments combination with 3 replicates. Treatments were randomly arranged in each replication divided into 21 petri plates. All glasswares were cleaned with 60.0 gm of potassium dichromate and 60.0 ml of concentrated sulphuric acid in one litre of water and gently washed with tap water. The glasswares were air dried and sterilized in hot air oven at 160-180˚C for two hours. The metallic instruments such as forceps, needle and other instruments were sterilized by dipping them in 90% alcohol and heating over the flame up to red hot before use. Sterilization of media was done by autoclaving at 15 lbs/inch^2 pressure for 20 minutes. Potato Dextrose Agar (PDA) medium is very commonly used for isolation of fungus as well as for its maintenance.

The anthracnose disease samples (just after initiation of the disease) were collected in polythene bags from various plants from research plot of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.).

Isolation and purification

The chilli leaves exhibiting disease symptoms were brought to the laboratory for isolation. The anthracnose infected parts were cut into small pieces by sterilized stainless steel blade and surface sterilized with 0.1% mercuric chloride for one minute followed by three washing with sterilized water. Anthracnose infected pieces were placed in petriplates containing 20 ml of solidified potato dextrose agar (PDA) medium mixed with streptomycin sulphate to avoid bacterial contamination. Plates were kept for incubation at 28±2˚C in an incubator. Fungal colonies appeared within 5-7 days, they were sub cultured in PDA slants and purified.

Morphological study

Infected leaves resembling the infection of *C. capsici* were brought to the laboratory. The microscopic slides were directly prepared from infected leaves and pure culture of *C. capsici* were mounted with cotton blue and observed in details for different morphological characters such as colony character, mycelium development, appearance of acervuli, setae, conidiophore and conidia formation. Observations were recorded under stereo binocular microscope.

Preparation of plant extracts

Different plant products named as Neem Seed Kernel Extract (NSKE) @ 4%/lit. of water and neem oil @ 0.05%/lit. of water were used. For preparation of plant extracts the plant materials (100 gm) were boiled with 100 ml water till they become soften and pulpy. This plant materials (100 gm) were taken in a clean blender and blended without water, the pulverized leaves tissue were filtered through three fold of muslin cloth and 100% pure filtrate was used as an extract.

The extracts of garlic bulb extract @ 3%/lit. of water and combine application of neem leaf extract, garlic bulb extract, ginger rhizome extract and onion bulb extract diluted with water @ 1:4 w/v dilution were used in the laboratory to test their efficacy against *C. capsici* and carbendazim @ 0.2 %/lit. was used for comparision.

Results and Discussion

The present study entitled, "Effect of neem based plant products and plant extract against Anthracnose of Chilli (*Capsicum annuum* L.)" was conducted in the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.) during 2016-2017. The effect of selected plant products and plant extracts was studied *in- vitro*.

The experimental results of the present investigation entitled "Efficacy of *Trichoderma harzianum* and Botanicals Against Anthracnose of Chilli (*Capsicum annuum* L.)" reveal that the two neem based plant products, viz., Neem Seed Kernel Extract (NSKE) and neem oil were inhibiting the radial growth and sporulation of *C. capsici* and the results are shown in Table 1 and figure 1. All the neem based plant products showed their influence on the mycelial growth and sporulation of the fungus. Neem Seed Kernel Extract (NSKE) inhibited the mycelial growth of the *C. capsici* the most as compared to neem oil and minimum as compared to control. Thus, neem oil was found highly effective in per cent inhibition of mycelial growth as well as sporulation and also all the neem based plant products adversely affect the growth and sporulation of *C. capsici*. The inhibitory effect of neem extract on mycelial growth and sporulation of *C. capsici* was also reported by Singh *et al*. (1997) [15], Singh and Korpraditskul (1999) [16], Hegde *et al*. (2001) [7] and Meera *et al*. (2004) [11], which supports the present findings.

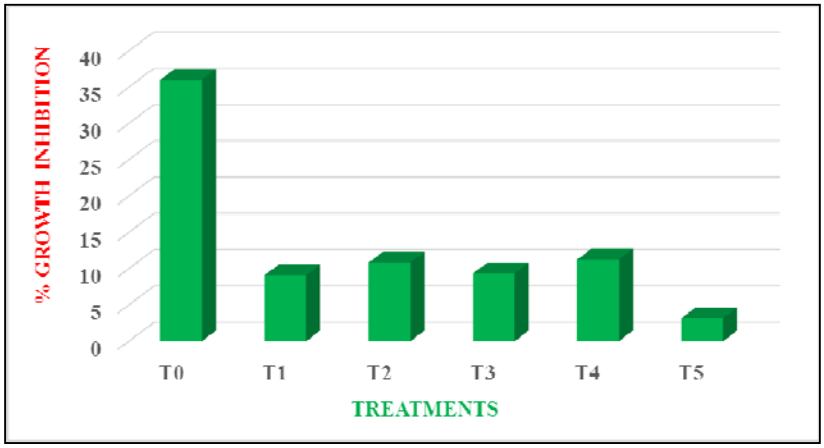
Different plant extracts were studied to evaluate their performance in inhibiting the radial growth and sporulation of *C. capsici* by poisoned food technique and the results are shown in Table 1 and figure 1. All the plant extract showed their inhibitory influence on the growth of *C. capsici*. The radial growth of *C. capsici* was minimum in combined application of neem, garlic, ginger, onion plant extract and garlic bulb extracts as compared to control. Thus all the extracts of plants of different species adversely affect the growth of *C. capsici*, among them combined application of neem, garlic, ginger, onion plant extract inhibited the mycelial growth as well as sporulation to the minimum of the fungus. Similar type of inhibition was also observed by Singh *et al*. (1997) [15], Singh and Korpraditskul (1999) [16], Hegde *et al*. (2001) [7] and Meera *et al*. (2004) [11].

Fungicide were assessed by poisoned food technique *in vitro* to find out the effective inhibitor for growth and sporulation of *C. capsici* and the results are presented in Table 1 and Figure 1. Minimum inhibition of mycelial growth of *C. capsici* was recorded with carbendazim (0.2 %), as compared

to all plant extracts and control. Minimum sporulation of *C. capsici* was observed under carbendazim. *In vitro* evaluation of fungicide showed minimum inhibition of *C. capsici* by carbendazim at their recommended doses. These observations are in confirmation with the reports of Joshi and Wangikar (1978) [9], Datar (1996) [2], Hegde (1998) [7] and Hegde *et al*. (2001) [7]. Carbendazim (0.2 %) was tested against anthracnose of chilli. The observations recorded in Table 1, clearly indicated that carbendazim (0.2 %) was effective in inhibiting the disease appearance and mycelial growth of *C. capsici*. The unsprayed check showed lesion length of anthracnose after inoculation. The results of following workers regarding efficacy of fungicides support in present observations {Thind and Jhooty (1987) [19], Datar (1996) [2], Ebenezar and Alice (1996) [4] and Deshmukh *et al*. (2004)} [3].

Table 1: Efficacy of neem based plant products, plant extracts and fungicide against *C. capsici* by poisoned food technique

Sr. No.	Treatments	Mycelial growth of the pathogen (mm)	Per cent growth inhibition
T_0	Control	36.00	-
T_1	Neem oil	9.08	74.77
T_2	Garlic bulb extract	10.83	69.91
T_3	Combine application of neem, garlic, ginger, onion plant extract.	9.33	74.08
T_4	Neem Seed Kernel extract (NSKE)	11.25	68.75
T_5	carbendazim (0.2%)	3.17	91.19
	S.Em± C.D. (at 5%)	0.902 2.779	



T_0- Control
T_1- Neem oil
T_2- Garlic bulb extract
T_3- Combine application of neem, ginger, garlic and onion plant extract
T^4- Neem Seed Kernel Extract (NSKE)
T^5- Carbendazim (0.2%)

Fig 1: Efficacy of neem based plant products, plant extracts and fungicide against *C. capsici* by poisoned food technique

Conclusions

In vitro evaluation of neem based plant products showed that Neem Seed Kernel Extract (NSKE) inhibited the mycelial growth of the *C. capsici* the most as compared to neem oil and minimum as compared to control. Neem oil minimum inhibited the growth and sporulation of *C. capsici* as compared to Neem Seed Kernel Extract (NSKE) and control. Combined application of neem, garlic, ginger, onion plant extract minimum inhibited the growth and sporulation of *C. capsici* as compared to garlic bulb extract. Garlic bulb extract inhibited the growth and sporulation to the maximum of *C. capsici* in comparison to control.

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