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Tamin

Department of Plant Pathology,
IGKV, Raipur, Chhattisgarh,
India

AS Kotasthane

Department of Plant Pathology,
IGKV, Raipur, Chhattisgarh,
India

RK Dantre

Department of Plant Pathology,
IGKV, Raipur, Chhattisgarh,
India

Vivekanand Uraiha

Department of Plant Pathology,
IGKV, Raipur, Chhattisgarh,
India

Effect of fungicides on mycelial growth and morphogenetic pathway of *Colletotrichum truncatum*

Tamin, AS Kotasthane, RK Dantre and Vivekanand Uraiha

Abstract

Genus *Colletotrichum* attacking over 3,200 species of monocot and dicot plants. During the field survey in kharif 2017-18, *Colletotrichum* spp. will be isolated from naturally infected soybean pods collected from different locations of Raipur (C.G.). Most characteristic symptom is the development of black fruiting structures (pycnidia) of the fungus arranged in linear rows on the stems, petioles, and pods. *In vitro* evaluation of hexaconazole, NF, thifluzamide and azoxystrobin were highest percent inhibition of 60% at 50 ppm of NF and lowest percent inhibition of 19.5% at 500 ppm of azoxystrobin. In case of interference of fungicides in infective morphogenetic pathway azoxystrobin, thifluzamide and hexaconazole completely inhibited spore germination at all the concentrations except 50 ppm of NF and thifluzamide.

Keywords: Soybean, *Colletotrichum*, Morphogenetic pathway, Chemical control

Introduction

Soybean (*Glycine max* L. Merrill) is a species of legume native to East Asia. The plant is classed as an oilseed rather than a pulse. Soybean is one of the world's most important sources of oil and protein. Besides its economic value, it is highly nutritious food to human being as its 40 per cent protein content, 20 per cent carbohydrates and 23 per cent oil, holds a great promise in meeting most of the need in human diet (Chandel, 2002) [2]. Soybean contains valuable amino acids. In addition, it contains a good amount of minerals (Ca, Mg, P and Fe), salts and vitamins (Thiamine and Riboflavin and D).

In Chhattisgarh, soybean is grown over an area of 1.47 lakh ha with a production of 1.346 lakh MT and productivity of about 816 kg/ha (SOPA, 2014). The state productivity (816 kg/ha) and national productivity (959 kg/ha) are low in comparison with world average (2206 kg/ha). Diseases play a major role in yield reduction. More than 100 pathogens are known to affect soybean of which 66 fungi, 6 bacteria, 8 viruses and 7 nematodes are involved. The world loss of more than 7 million tons of soybean is due to diseases alone. Soybean diseases cause reductions in yield to the tons of 10 to 30% in most of the areas. The most important disease reported to cause economic losses to the soybean crop is anthracnose incited by *Colletotrichum truncatum* (Schw) Andrus and Moore causing yield losses of 16-100 per cent. Chemicals are necessary at present, but are not a long term method to crop health. Several chemicals have been used for achieving disease control of various plant species.

Methods and Materials***In vitro* evaluation of fungicides against *C. truncatum* by Poison food technique**

Four fungicides (Azoxystrobin 23%SC, NF 180% SC, Thifluzamide 24% SC, Hexaconazole 5% EC) were tested against *C. truncatum* on the potato dextrose agar medium using poison food technique (Joshi and Waingikar, 1978; Ekbote *et al.*, 1996; Hingole and Kurundkar, 2004) [10, 3] under *in vitro* condition. The fungicides were evaluated @ 50, 150, 300, 400 and 500 ppm. The list of fungicides used along with their chemical and trade names are given below. The fungus was grown on PDA medium for 8 days prior to setting up the experiment. The PDA medium was prepared and melted. The required quantity of fungicide was added to the melted medium to obtain the required concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates and suitable check was maintained without addition of fungicides. To avoid bacterial contamination, a pinch of streptomycin sulphate was added to the medium at the time of pouring. A 5 mm mycelial disc was taken from the periphery of 8 days old colony of *C. truncatum* and placed in the centre of petriplate. The inoculated plates were incubated at 27 ± 1 °C and 3 replications were maintained for each treatment. Diameter of

Corresponding Author:**Tamin**

Department of Plant Pathology,
IGKV, Raipur, Chhattisgarh,
India

the colony was measured when maximum growth of the *C. truncatum* was reached in any of the treatments and the

observations were recorded and per cent inhibition was calculated by using the formula of Vincent (1947).

Table 1: Common name, Chemical name and trade name of fungicides

S. No	Common name	Chemical name	Trade name
1	Hexaconazole	RS-2-(2, 4-D)-1-(1H-1, 2, 4 Trizole-1-yl) hezan 2-ol	Trigger 5% EC
2	Azoxystrobin	(2E)-2-(2-[[6-(2-Cyanophenoxy)-4-pyrimidinyl]oxy]phényl)-3-methoxyacrylate de methyl	Amister 23% SC
3	Thifluzamide	N-(2,6-Dibromo-4-(trifluoromethoxy)phenyl)-2methyl-4-(trifluoromethyl)thiazole-5-carboxamide	Pulsor 24% SC
4	NF	New formulation	NF 180% SC

$$I = \frac{(C - T)}{C} \times 100$$

Where,

I: Per cent inhibition
C: Mycelial growth in control
T: Mycelial growth in treatment

Fungicidal interference in infective morphogenetic pathway of *C. truncatum*

The conidia of *C. truncatum* were taken from 10 days old culture and conidial suspension was made with 1% of glucose solution separately for each concentrations (50, 150, 300, and 500 ppm) of different fungicides *i.e.*, Azoxystrobin 23% SC, NF 180% SC, Thifluzamide 24% SC, Hexaconazole 5% EC. These suspensions (10 ml) were taken in sterilized watch glass. 3µl of fungicide suspension and 3 µl of the conidial suspension were mixed and the mixtures were added to the surface of dried depression slides and kept in moisture chamber at 25 °C for 24 hours of incubation. Then a remove mixer drop by blotter paper and add oil drop on the slides. The slides were examined under the microscope (Nikon Eclipse 50 i attached with Nikon Digital sight screen to capture the image) of high power (100 X) for recording the inhibition of conidial germination of *C. truncatum*.

Result and discussion

***In vitro* evaluation of fungicides against *C. truncatum* by Poison food technique**

It is evident from the data that *C. truncatum* varied considerably in their sensitivity to different concentrations of

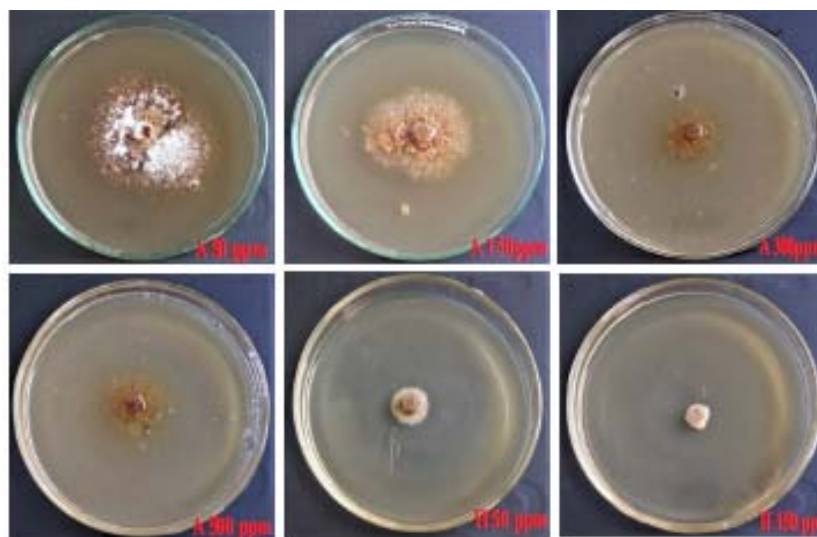
the fungicide. Growth inhibition of *C. truncatum* increased with corresponding increase in concentration of the fungicides.

All the four fungicides used in the study were effective in arresting the growth of *C. truncatum* at all the concentrations. These fungicides were tested at all concentrations to know the sensitivity of specific fungicide at specific concentrations and also to ascertain the variation in specificity and the results are presented in table 4.6. All the fungicides arrested the growth of the pathogen at all concentrations significantly over uninoculated control. The results in the data revealed that, there was a significant difference between the fungicides and concentrations.

At 50 ppm, significantly less mycelia growth of *C. truncatum* was observed in hexaconazole (41.5 mm), thifluzamide (54.5 mm), azoxystrobin (55.5 mm) and NF (60 mm). At 150 ppm, mycelia growth of *C. truncatum* was observed in hexaconazole (32.5 mm), NF (40.5 mm), azoxystrobin (45.5 mm), thifluzamide (46 mm).

Similarly at 300 ppm azoxystrobin (21.5 mm), thifluzamide (40.5 mm), hexaconazole and NF show zero mm growth. The percent inhibition at 500 ppm observed in azoxystrobin (19.5 mm) hexaconazole, thifluzamide, and NF showed ingeminate the mycelia disc. The highest percent inhibition of 60% at 50 ppm of NF and lowest percent inhibition of 19.5% at 500 ppm of azoxystrobin.

Propiconazole, hexaconazole and chlorothalonil have shown affectivity against *C. capsici* (Elliott and Patterson, 2000; Jagtap *et al.*, 2013) [4, 9]. Ingle *et al.* (2014) [8] have proved the sensitivity of tebuconazole, hexaconazole and propiconazole against pod blight of soybean caused by *C. truncatum*.



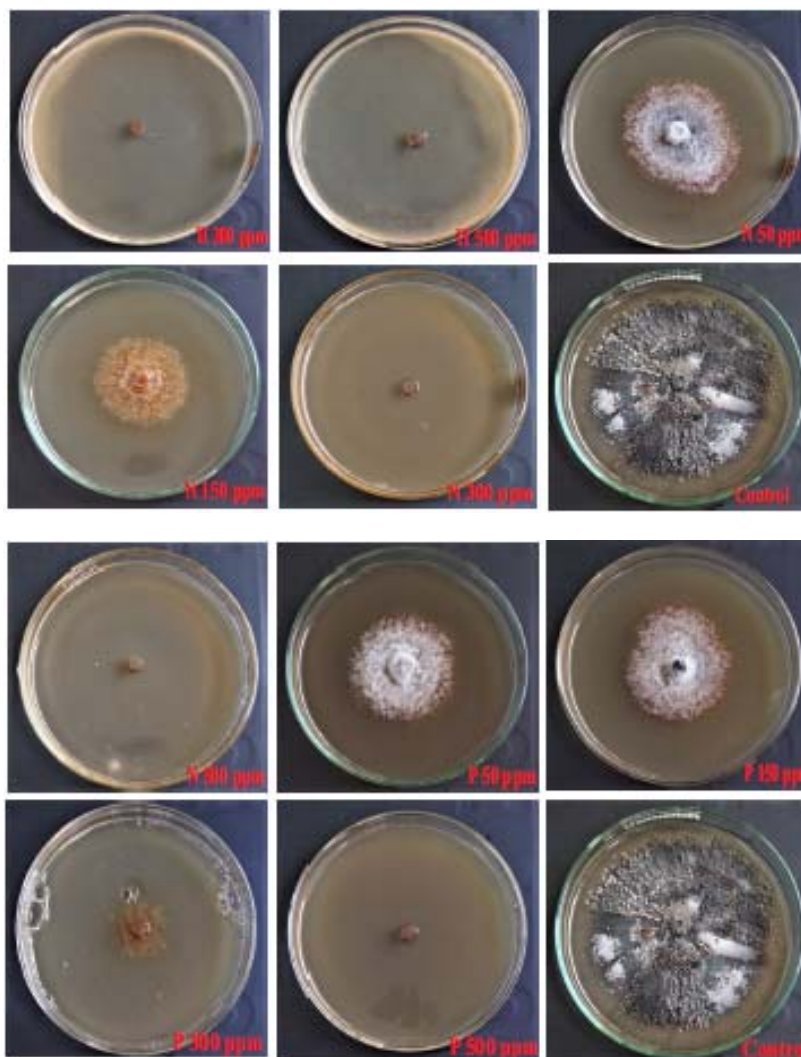


Fig 1: *In vitro* evaluation of fungicides at different concentration against *Colletotrichum truncatum*
Where N- NF (New formulation), A- Amister (Azoxystrobin)
H- Hexaconazole (Trigger), P- pulsor (Thiifluzamide)

Table 2: Effect of fungicides on radial growth of *Colletotrichum truncatum*

S. No	Fungicide	Radial growth (mm) 6DAI				Inhibition%				Average Inhibition%
		Concentration (ppm)				Concentration (ppm)				
		50	150	300	500	50	150	300	500	
1	Thiifluzamide					39.44	48.89	47.4	100	58.9325
		54.5	46	40.5	0	(38.91)	(44.36)	(43.75)	(90)	(53.06)
2	Hexaconazole					53.89	63.89	100	100	79.445
		41.5	32.5	0	0	(47.23)	(53.06)	(90)	(90)	(67.6825)
3	Azoxystrobin					38.33	49.44	76.11	78.33	60.5525
		55.5	45.5	21.5	19.5	(38.25)	(44.68)	(60.74)	(62.26)	(51.4825)
4	NF					33.33	47.4	100	100	70.1825
		60	40.5	0	0	(35.26)	(43.75)	(90)	(90)	(62.3625)
5	Control	70								
	CD	2.245								
	SE(m)	0.738								

Effect of fungicides on infective morphogenetic pathway of *C. truncatum* conidia

Appressoria single or in loose groups, medium to dark brown, smooth-walled, clavate, ovate, pear shaped or irregular outline, the edge entire or lobate, sometimes undulate. Conidiophores hyaline to pale brown, smooth-walled, septate, branched. Conidiogenous cells hyaline to pale brown, smooth-walled, cylindrical to ampulliform, sometimes intercalary (necks not separated from hyphae by septum).

It is evident from results that azoxystrobin, thiifluzamide and hexaconazole were effective at all the concentrations in suppression of germination and interfering in the morphogenetic pathway of *C. truncatum* at variable concentrations. Among the different concentrations of fungicide, at 50 and 150 ppm spore germination, germ tube elongation and appressoria formation was observed, at 400 and 500 ppm germination and germ tube elongation was

observed but formation and melanization of appressoria was inhibited.

Hexaconazole, a triazole fungicide inhibits one specific enzyme, C14- demethylase, which plays a role in sterol production. Sterols, such as ergosterol, are needed for membrane structure and function, making them essential for the development of functional cell walls. Therefore, these fungicides result in abnormal fungal growth and eventually death. Each triazole compound may act in a slightly different part of the biochemical sterol-producing pathway. While the results are similar in various fungi--abnormal fungal growth and death--there are great differences in the activity spectra of these fungicides. Triazoles have no effect against spore germination because spores contain enough sterol for the formation of germ tubes. Some spores even have enough sterol to produce infection structures so, in some cases, triazoles may not be effective against infection of the host tissue. At 50, 150, 300 and 500 ppm conidia show abnormal shape and granulation.

Some triazole fungicides have anti-sporulant properties, which mean they inhibit spore production and therefore help to slow disease development. According to Gopinath *et al.* (2006) [6] propiconazole, a triazole fungicide as superior to inhibit mycelia growth, biomass production, sporulation and spore germination of *C. capsici* at 0.1 μ /ml.

Azoxystrobin is to prevent the respiration of fungi due to the disruption of electron transport chain, preventing ATP synthesis. This occurs as the azoxystrobin binds to the Qo site of complex III within the Mitochondrion. Therefore, these

fungicides result in abnormal shape of conidia, granule formation within spore, abnormal fungal growth and unmalanized appressoria. At 300 and 500 ppm best for check the melanization of appressoria.

Thiifluzamide fungicide was effective in inhibiting the spore germination at all the concentrations except in 50 ppm. It belongs to carboxamide systemic fungicide (Carboxanilide group). It inhibits the succinate dehydrogenase in the tricarboxylic acid cycle (TCA cycle). Therefore, these fungicides result check the development of germ tubes, formation of appressoria, growth of mycelia and browning the conidia except 50 ppm.

NF was effective for produce swell conidia and small granulation. It's all concentrations not more effective as compare other fungicide.

All the fungicides inhibited spore germination. The present investigations are in accordance with the findings of Hassan and Khan (1979) who reported that captan, mancozeb and COC as best to inhibit spore germination of *C. truncatum*. Gawade *et al.* (2009b) [5] showed the effectivity of bavistin at 1000 and 1500 ppm in inhibiting the spore germination of *C. capsici*. Imtiaz *et al.* (2005) [7] found mancozeb and ridomil at 500 and 1000 ppm as most effective to inhibit the conidial germination of *C. gloeosporioides*. Gopinath *et al.* (2006) [6] also reported propiconazole (0.1 μ /ml), a triazole fungicide superior to inhibit growth an sporulation. Bavastin at 150 ppm was found to be most effective in inhibiting the spore germination of *C. gloeosporioides* as reported by Azad *et al.* (2014) [1].



Fig 2: Sequence of events in infection structure formation by *Colletotrichum truncatum*

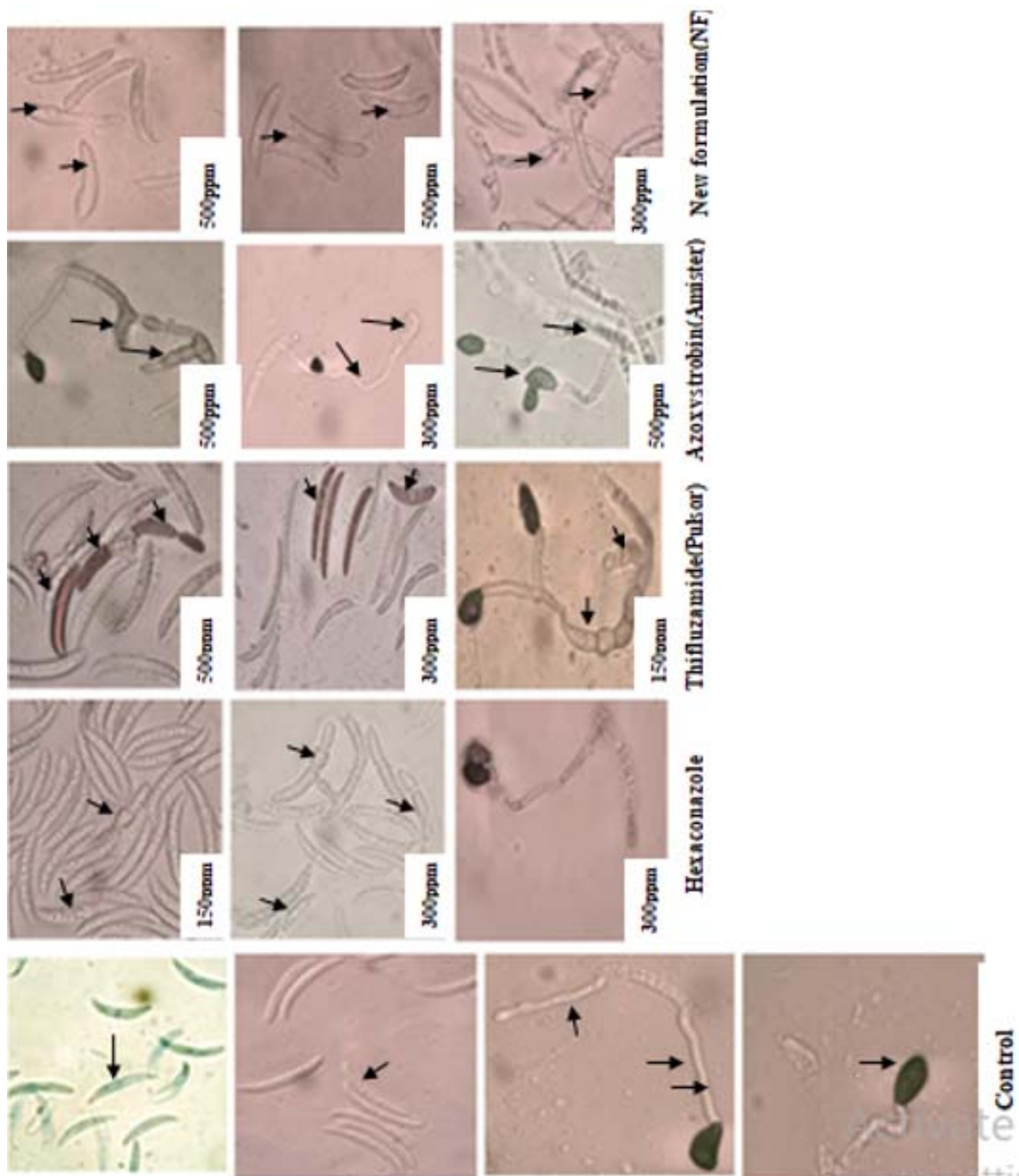


Fig 3

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