

IN VITRO STUDY OF THE EFFICACY OF SIX FUNGICIDES AGAINST ASPERGILLUS NIGER USING POISONED FOOD TECHNIQUE AND PAPER TOWEL METHOD

Reddypalli Poorna Chandra Reddy , Dr. Jai Prakash Mishra,

Master of Science , Agriculture (Plant Pathology)

Department of Agriculture, Uttaranchal College of Agricultural Science,

Uttaranchal University , Dehradun , (Uttarakhand) India.

ABSTRACT

The present study was carried out to evaluate the efficacy of six fungicides against *Aspergillus niger* using two invitro methods namely poisoned food technique and paper towel method. Poisoned food technique was carried out by making potato dextrose agar medium poisoned with different concentrations of fungicides followed by inoculation with actively growing culture of *A. niger*. Among the fungicides tested by using above method, tebuconazole at concentrations of 25, 50, 100 and 200 ppm and azoxystrobin at concentrations of 100 and 200 ppm, completely (100%) inhibited the mycelial growth of *A. niger* followed by carbendazim 25% + mancozeb 50% (Sprint) @ 200 ppm (94.38%). In paper towel method, two varieties of groundnut namely SG-99 and M-522 were tested. In this method, seeds from previously collar rot infected plants were taken and treated with fungicides, then wrapped between two paper towels and these were kept in incubator. The minimum seed per cent mortality and seed rots due to *A. niger* was observed in seed treatment with tebuconazole followed by azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) in both of the tested varieties. In both methods, three fungicides namely tebuconazole, azoxystrobin and carbendazim 25% + mancozeb 50% (Sprint) found most effective against *A. niger*.

INTRODUCTION

The peanut or groundnut (*Arachis hypogaea* L.), a species in the legume or "bean" family (*Fabaceae*) was probably first cultivated in the valleys of Peru (World Geography of Peanut, 2004). Because peanut (*Arachis hypogaea* L.) grows underground, it is commonly known as groundnut. Today, it is grown in more than 90 countries of the world (Virmani and Singh, 1985), in areas, where average rainfall is 500 to 1200 mm and mean daily temperatures are higher than 20°C (Pattee and Young, 1982). Groundnut is known by many other local names such as earthnuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Groundnut the 'king' of oilseeds is popularly called as wonder nut and poor men's cashew nut. While being a valuable source of all nutrients, it is a low priced commodity and is one of the most important food and cash crops of our country. Currently, China is the leading groundnut producer with a share of about 37.5 % of overall world production, followed by India, the United States of America (USA), Argentina and Vietnam (Fabra *et al.*, 2010).

India has the largest groundnut cultivation area, but has a low average yield of approximately 1.0 t/ha whereas, USA have the highest average yield of 3.8 t/ha (FAO 2011). In India, groundnut occupies an important place in oil seed production and is being grown in area of 6 million hectares contributing 6.25 million metric tons of seeds to the country's oil seed production.

In India, the major groundnut growing states are Gujarat, Andhra Pradesh, Rajasthan, Tamil Nadu and Punjab (Kumari and Singh, 2016). Groundnut oil is composed of mixed glycerides and contains a high proportion of unsaturated fatty acids, in particular, oleic (50-65%) and linoleic acid (18-30%) (El Naim *et al.*, 2010). Thus, groundnut offers both health and ecological benefits to the areas of the world where it is grown.

Its roots contain numerous nodules which harbor of bacteria *Rhizobium* and fixes about 80-160kg N/ha per season (Alam *et al.*, 1988). The oil cake derived after extracting oil is used as cattle feed and is an important protein supplement in cattle and poultry rations due to its nutritive value and palatability.

Collar rot is devastating on stem tissues near ground surface causing rotting, wilting and plant death (Pande and Rao, 2000). Collar rot prevalent in almost all groundnut growing states of India viz., Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, Karnataka and Orissa is a very common and destructive disease in India and the losses in terms of mortality of plants due to collar rot range from 28 to 50 per cent (Ghewande *et al.*, 2002).

MATERIALS AND METHODS

Effect of fungicides on the mycelial growth of *A.niger* (Poisoned food technique)

The efficacy of Pyraclostrobin 20% WDG was assayed at the conc. of 0.05, 0.10 and 0.15 per cent along with other fungicides (Carbendazim 50% WP @ 0.1%, Carboxin+Thiram @ 0.2% and Mancozeb 0.2% conc.) for comparison. The culture filtrate of the antagonist's viz., *T. viride* and *P. fluorescens* were added to the medium at 25 per cent conc. and tested.

. Agar well method (Thongson *et al.*, 2004)

Various concentrations of Pyraclostrobin 20% WDG (0.05, 0.10 and 0.15 per cent), comparison chemicals viz., Carbendazim 50% WP @ 0.1%, Carboxin+Thiram @ 0.2% and Mancozeb @ 0.2% conc. and the antagonists *P. fluorescens* and *T.viride* (25%) were prepared.

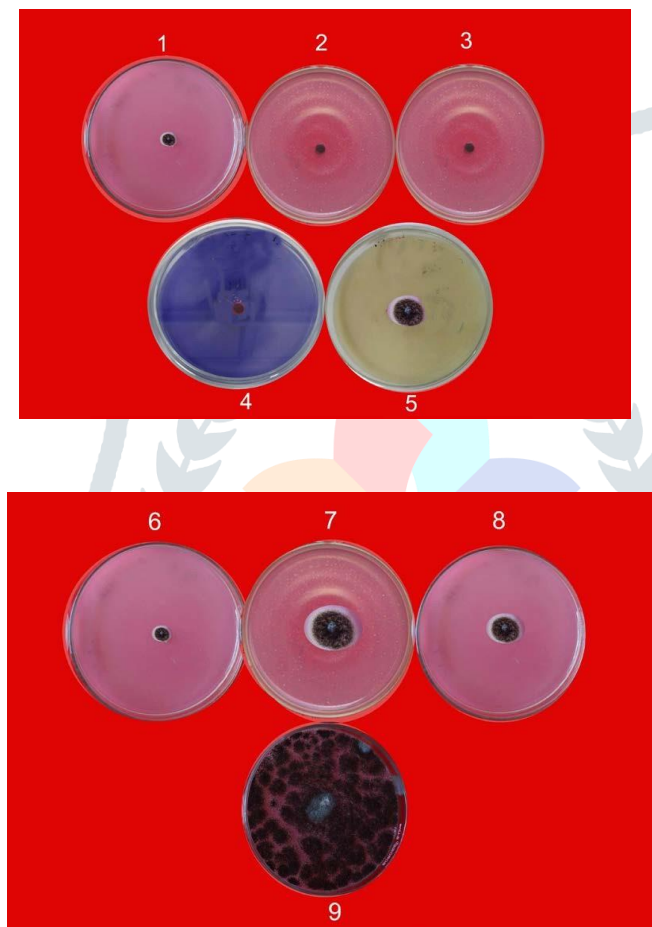
Plant growth-promotion-Roll Towel Method (ISTA, 1976)

The field efficacy of Pyraclostrobin 20% WDG along with comparison fungicides and bio agents were tested for their influence on the biometrics of groundnut and management of collar rot disease caused by *A.niger* under irrigated conditions (Rabi, 2012). The results showed that all the treatments significantly enhanced the biometrics of groundnut than the control. However, among the treatments, Seed treatment @ 1.5 g/kg of seed plus foliar spray of Pyraclostrobin 20% WDG @ 0.15 % (T3) recorded maximum biometrics with a root and shoot length of 11.80 & 25.06 cm, 11.62 g of biomass, 164.12 nodules per plant,

34.33 Pods per plant, 2320 kg/ha of Pod yield and maximum shelling per cent (74.52 %). The dosage level with Pyraclostrobin 20% WDG as seed treatment @ 1.0 g/kg of seed plus foliar spray at the conc. of 0.1 % recorded statistically at par results with T3 the best treatment in reducing the collar rot incidence to the minimum. These were followed by the treatments with (Carboxin 37.5% + Thiram 37.5% DS), carbendazim and Mancozeb in the decreasing order of merit days.

RESULT AND DISCUSSION

Efficacy of Pyraclostrobin 20% WDG against *A.niger* (Poisoned food technique)



- 1 - Pyraclostrobin 20% WDG@ 0.05% 2 - Pyraclostrobin 20% WDG @0.1%
 3 - Pyraclostrobin 20% WDG@ 0.15% 4 - Vitavax (Carboxin+Thiram 75%WS) @0.2%
 5 - Mancozeb (75% WP)@ 0.2% 6 - Carbendazim 50% WP @0.1%
 7 - Trichoderma viride@ 25% 8 - Pseudomonas fluorescens@25%
 9 - Untreated Control

Efficacy of Pyraclostrobin 20% WDG against *A.niger* (Paper Disc & Agar well assays)

A. niger with difference in the chemicals tested and their concentrations. Among the chemicals tested Pyraclostrobin 20% WDG at 0.15 % conc. produced the highest inhibition zone of 20.14 mm and 22.67 mm in paper disc assay and agar well method, respectively. It is followed by Pyraclostrobin 20% WDG at 0.1% conc with 19.76 and 21.24 mm in paper disc assay and agar well method, respectively (Table 9). The comparison chemicals at the irrespective concentrations and the culture filtrate of the biocontrol agents at 25% conc. also showed significant inhibition on the growth of *A.niger* when compared to control in both Paper disc and Agar well assays.

**Efficacy of Pyraclostrobin 20% WDG against *A.niger*
(Paper Disc assay) under *in vitro* condition**

Tr. No.	Treatments	Conc. of the chemical (%)	Paper disc assay	Agar well method
			Inhibition Zone (mm)	Inhibition Zone (mm)
1.	Pyraclostrobin 20% WDG	0.05	17.23	16.56
2.	Pyraclostrobin 20% WDG	0.10	19.76	21.24
3.	Pyraclostrobin 20% WDG	0.15	20.14	22.67
4.	(Carboxin 37.5% + Thiram 37.5% DS)	0.2	17.42	17.65
5.	Mancozeb 75% WP	0.2	13.20	16.54
6.	Carbendazim 50% WP	0.1	15.24	19.33
7.	<i>Trichoderma viride</i>	25.0	15.67	15.12
8.	<i>Pseudomonas fluorescens</i>	25.0	17.33	16.66
9.	Control		--	--
	S.Ed		0.12	0.11
	CD (P=0.05)		0.36	0.32

Effect of Pyraclostrobin 20% WDG on plant growth (Roll Towel Method)

Groundnut seeds were treated with different chemicals and bio control strains under in vitro condition and tested for their plant growth promoting ability using roll paper towel method. The results are summarized in Table 11. Among the various treatments, Pyraclostrobin 20% WDG at 1.5 g/kg seed was found to increase the seed germination (94.28%) and vigour index (1604.6) of groundnut seedlings to the maximum which was followed by Pyraclostrobin 20% WDG at 1g/kg seed. The least seed germination (72.30%) and vigour index of 942.79 was observed in untreated control. The comparison chemicals and biocontrol agent treatments also recorded significant increase in seed germination and vigour index when compared to control.

Effect of Pyraclostrobin 20% WDG on plant growth (Roll Towel Method)

Tr. No.	Treatments	Dosage g/Kg seed	Germination %	Shoot length(cm)	Root length(cm)	Vigour index
1.	Pyraclostrobin 20% WDG	0.5	90.78 (72.32)	6.66	08.88	1410.7
2.	Pyraclostrobin 20% WDG	1.0	92.77 (74.40)	7.12	09.27	1520.5
3.	Pyraclostrobin 20% WDG	1.5	94.28 (76.16)	7.33	09.69	1604.6
4.	(Carboxin 37.5% + Thiram 37.5% DS)	2	90.28 (71.84)	6.92	9.00	1437.2
5.	Mancozeb 3 g/kg (75% WP)	3	87.78 (69.54)	6.57	8.69	1339.5
6.	Carbendazim 50% WP	3	88.28 (69.98)	6.78	8.87	1381.5
7.	<i>Trichoderma viride</i>	4	87.33 (69.15)	6.98	9.27	1419.1
8.	<i>Pseudomonas fluorescens</i>	10	88.78 (69.54)	7.10	9.69	1490.6
9.	Untreated Control	--	72.30 (58.24)	4.82	5.66	942.79
	S.Ed		0.17	0.09	0.14	10.42
	CD (P=0.05)		0.51	0.27	0.41	31.25

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