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**Research Note**

# *In vitro* screening of the new strobilurin fungicide pyraclostrobin 20% WDG and biocontrol agents against *Aspergillus niger* (Van Tieghem) causing collar rot in groundnut

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**ABSTRACT:** The *in vitro* experiment revealed that the fungicide pyraclostrobin 20% WDG at 0.1%, 0.15% concentrations and carboxin + thiram 75% WS (Vitavax) at 0.2% conc. completely inhibited the mycelial growth of *Aspergillus niger* and accounted for 100% inhibition of the pathogen followed by carbendazim @ 0.1% ( 98.42% ), mancozeb @ 0.2% (94.51% ) and pyraclostrobin 20% WDG at 0.5% (98.46% ). *Pseudomonas fluorescens* recorded the higher inhibition of the pathogen as compared to *Trichoderma harzianum.* The present study clearly revealed that the fungicide pyraclostrobin 20% WDG at 0.1% and 0.15% completely inhibited the pathogen *A. niger.*

**KEY WORDS:** Pyraclostrobin, *Aspergillus niger,* groundnut, collar rot, screening

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In India, groundnut occupies an important place in oil seed production and is being grown in an area of 5.53 million hectares contributing 9.67 million metric tonnes of seeds to the country's oil seed production (Anonymous, 2014). Groundnut is affected by various diseases caused by fungi, bacteria and viruses. Of these pathogens, collar rot caused by *Aspergillus niger* is an important disease which is prevalent in almost all parts of the country, being more during kharif than rabi (Mayee, 1995). *Aspergillus niger* causes both seed and seedling rot and drastically reduces plant stand. The disease causes considerable loss in pod yield upto 50% (Pande and Rao, 2000; Kishore and Pande, 2005). Most of the groundnut cultivars are susceptible to this disease and complete management with biocontrol agents alone is not feasible. Management of *A. niger* using chemical fungicides has been the prevailing control method for over fifty years. Many seed dressing fungicides are reported to be effective against collar rot of groundnut (Gangopadhyay *et al.,* 1996; Karthikeyan, 1996). Considering these issues , it was thought to test the efficacy of new fungicide molecule 'Pyraclostrobin 20% WDG, a potent strobilurin compound having novel biochemical mode of action (Hewitt, 1998) along with potential biocontrol agents against *A.niger* under *in vitro* conditions.

The *A. niger* was isolated from infected groundnut plants using potato agar medium in the laboratory. Pure cultures of *A. niger* were maintained in slants of potato dextrose agar and the isolates were identified based on the colony and spore features (Klich, 2002).

The fungicide pyraclostrobin 20% WDG was tested at different concentrations *viz*., 500, 1000 and 1500 ppm. The efficacy of other chemicals *viz*., carboxin+thiram 75%WS (Vitavax), mancozeb (75% WP) @ 0.2 % conc. and Carbendazim 50% WP @ 0.1% conc. were also compared with pyraclostrobin 20% WDG. The commonly used biocontrol agents *viz*., *Trichoderma viride* and *Pseudomonas fluorescens* were collected from Tamil Nadu Agricultural University, Coimbatore and were also compared with all above mentioned chemicals. The efficacy of all those fungicides and biocontrol agents was tested by poisoned food technique using both solid and liquid media. One ml ($3\times 10^{10}$ cfu $ml^{-1}$) of log phase culture of *P. fluorescens* and *T. viride* was inoculated into 250 ml King's B broth and Potato Dextrose broth respectively and incubated at room temperature $28 \pm 2^{o}$ C. The contents were filtered through a bacteriological filter under vacuum after the incubation. The filtrate thus obtained was used for the efficacy studies.

Required quantity of fungicide was added separately into molten and cool potato dextrose agar so as to get the desired conc. of fungicide in the medium and 15 ml of the poisoned medium was poured into sterile Petri plates. The

biocontrol agents *viz*., the culture filtrates of *T. viride* and *P. fluorescens* were tested at 25 % conc. Each plate was inoculated at the centre with 9 mm culture disc (10 days old) of *A. niger* grown on PDA and incubated at room temperature for seven days. Each treatment was replicated thrice and a suitable control was maintained without adding any fungicide to the medium. The plates were incubated at room temperature and mycelial growth was measured at the end of the incubation period *viz*., seven days. The efficacy of the fungicide was expressed as per cent inhibition of mycelial growth over control (Vincent, 1929).

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

Where, I = percent inhibition, C = growth in control, T = growth in treatment

The efficacy of selected fungicides and bio agents were evaluated against *A. niger* and the inhibition of *A. niger* in solid medium (Table 1) and the results were presented. The experiment revealed the inhibition of the pathogen *A. niger* by the chemicals and as well as by the biocontrol agents. Poisoned food technique revealed that the pyraclostrobin 20% WDG at 0.1%, 0.15% and carboxin + thiram 75% WS (Vitavax) at 0.2% conc. completely inhibited the mycelial growth of *A. niger* and accounted for 100% inhibition of the pathogen followed by carbendazim @ 0.1% (98.42%), pyraclostrobin 20% WDG at 0.5% (98.46%) and mancozeb @ 0.2% (94.51%). Among the bioagents *P. fluorescens* recorded higher inhibition (94.61%) of the pathogen followed by *T. viride* (92.82%).

The results of the present experiment revealed that all the fungicides and bioagents reduced the mycelial growth of the pathogen significantly over control. However, the most effective fungicide in inhibiting the mycelial growth of *A. niger* was pyraclostrobin 20% WDG at 0.1% conc. The inhibition caused by the the fungicide carboxin + thiram 75% WS (Vitavax) @ 0.2% was at par with the pyraclostrobin 20% WDG at 0.1%. Similar findings on the *in vitro* efficacy of various fungicides against plant pathogens were reported by earlier workers (Imtiaj *et al*., 2005; Mondall *et al*., 2009; Taskeen-Un-Nisa *et al*., 2011)

Pyraclostrobin, a systemic broad-spectrum fungicide of the strobilurin group, inhibits spore germination and mycelial growth of wide range of plant pathogenic fungi. (Sendhil Vel 2003) studied the in vitro efficacy of azoxystrobin and pyraclostrobin against the spore germination of downy mildew of grapes and found that even at a concentration of 100 ppm, it was able to reduce the germination up to 90 per cent, with the increasing concentration of the chemical (250, 500, 750 and 1000 ppm) complete inhibition of germination. In the présent study also pyraclostrobin 20% WDG at 0.5% conc. showed 98.46% inhibition on the mycelial growth of *A. niger*. Karadimos *et.al.,* (2005) reported that pyraclostrobin at lower concentration was more effective than difenaconazole as pre inoculation and post inoculation against *Cercospora beticola* causing complete inhibition of spore germination and mycelial growth of the fungus. He also reported that the antisporulant activity of strobilurin fungicides, pyraclostrobin and trifloxystrobin was good.

**Table 1. *In vitro* screening of Pyraclostrobin 20% WDG and biocontrol agents against *Aspergillus niger***

| Tr.No | Treatments | Concentration of the chemical (%) | Mycelial growth of *A.niger (mm)** | % inhibition |
|---|---|---|---|---|
| 1 | Pyraclostrobin 20% WDG | 0.50 | 1.20 | 098.46 |
| 2 | Pyraclostrobin 20% WDG | 0.10 | 0.00 | 100.00 |
| 3 | Pyraclostrobin 20% WDG | 0.15 | 0.00 | 100.00 |
| 4 | Vitavax (Carboxin + Thiram 75%WS) | 0.20 | 0.00 | 100.00 |
| 5 | Mancozeb (75% WP) | 0.20 | 4.28 | 094.51 |
| 6 | Carbendazim 50% WP | 0.10 | 1.23 | 098.42 |
| 7 | *Trichoderma viride* | 25.0 | 5.60 | 092.82 |
| 8 | *Pseudomonas fluorescens* | 25.0 | 4.20 | 094.61 |
| 9 | Untreated Control | - | 78.0 | - |
| | | SE | 0.02 | - |
| | | CD (*P*=0.05) | 0.06 | - |

* Mean of three replication

The conidial germination, mycelial growth *in vitro* of *Alternaria alternata*, a predominant fungal pathogen responsible for mouldy –core in Red Delicious strains of apple were more sensitive to sygnum, a premix fungicide containing pyraclostrobin+nicobifen. Decay formation by *A. alternata* on mature detached fruits was also more affected by sygnum. (Reuveni, 2006).

*In vitro* screening of various fungicides against the mycelial growth of Eucalyptus leaf spot caused by *Quambalaria eucalypti,* showed high sensitivity to pyraclostrobin alone and pyraclostrobin with epoxiconazole. Also, the most effective inhibitors of conidial germination were pyraclostrobin, azoxystrobin and pyraclostrobin with epoxiconazole. (Ferraria *et al.,* 2008).

Amini and Sidovich, (2010) used six different fungicides, *viz*. benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin aganist phytopathogenic fungi *Fusarium oxysporum* f. sp. *lycopersici* and reported that all the fungicides were found effective. The fungicidal activity of pyraclostrobin results from the inhibition of mitochondrial respiration in fungi, which is achieved by the prevention of electron transfer between cytochrome b and cytochrome c (Becker *et al*., 1981). The bio agents also showed significant inhibition and it has already been proved that the various mechanisms including antibiosis, action of lytic enzymes etc., exerted by *T. viride* and *P. fluorescens* bring about the inhibition of mycelial growth of several fungi (Zaidi *et al*., 2004; Sreedevi *et al.,* 2011; Alice and Sundravadana, 2012). Thus, the results of the present study clearly revealed that the new chemical pyraclostrobin 20% WDG at 0.1% conc. inhibited maximum the *A. niger* and could be explored for the effective management of *A. niger* at field level.

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