In vitro Efficacy of Fungicides and Bioagents Against Wilt of Pigeonpea Caused by Neocosmospora vasinfecta

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ABSTRACT

Background: Pigeonpea (Cajanus cajan) is one of the important leguminous crop of the tropics and subtropics and is infected by the wilt pathogen Neocosmospora vasinfecta in addition to Fusarium udum. Objective: Hence, the study was undertaken to see the in vitro effect of different fungicides (Thiram 75 WP, Carbendazim 50 WP, Chlorothalonil 75 WP, Metalaxyl MZ 72 WP, Thiram + Carbendazim (2:1), Carbendazim + Mancozeb 75 WP, Tricyclazole + Mancozeb 80 WP, Zineb + Hexaconazole 72 WP) and bioagents (Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis) against the pathogen. Methodology: The efficacy of fungicides was assayed by poisoned food technique and of bioagents was assayed by dual culture technique. Results: It was found that among eight fungicides tested carbendazim (0.1%), combination of carbendazim + mancozeb (0.2%) and thiram + carbendazim 2:1 (0.3%) exhibited 100% inhibition of N. vasinfecta, other fungicides were also significant over control. Whereas among bioagents tested, Trichoderma herzianum (50.30%) showed maximum per cent growth inhibition of the pathogen followed by Bacillus subtilis (41.47%). Conclusion: Thus it was proved that the fungicides viz. carbendazim, combinations of carbendazim + mancozeb and thiram + carbendazim as well as bioagent, T. herzianum were effective against Neocosmospora wilt of pigeonpea under in vitro condition.

Key words: Pigeon pea, wilt, fungicides, bioagents

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INTRODUCTION

Pigeonpea (Cajanus cajan L. Millsp) is widely adaptable to wide variety of climate and is one of the important legume crops of tropics and subtropics. It is an important source of protein in the cereal based vegetarian diet. It is largely consumed in the form of ‘Dal’. Pigeonpea plays an important role in farming system because it fixes the atmospheric nitrogen in the soil. But the crop is affected by more than hundred pathogens1. These includes fungi, bacteria, viruses, nematodes and mycoplasma like organisms. Major diseases of pigeonpea are, wilt (Fusarium udum Butler), sterility mosaic disease (virus), stem blight (Phytophthora drechsleri f.sp. cajan), stem canker (Phoma cajanii and Colletotrichum capsici), root rot (Macrophomina phaseolina), bacterial leaf spot and canker (Xanthomonas axonopodis pv. cajanii) and leaf spot (Cercospora indica). Among these diseases, wilt caused by Fusarium udum Butler, is the most destructive soil and seed born disease of pigeonpea in India leading to serious yield losses. In addition to the above, many workers have been reported that the wilt in pigeonpea was caused by Neocosmospora vasinfecta (Anamorph, Acremonium spp.). Sarojini2 reported the wilt of pigeonpea was caused by N. vasinfecta and observed the similar symptoms to those incited by F. udum and wilt incidence was observed in the range of 62-100%. Giri (Unpublished data) noted the association of N. vasinfecta, while studying the pathogenic variability among the isolates of F. udum from different locations of Vidarbha region. Recently, Vishwa et al.3 reported the wilt of pigeonpea caused by N. vasinfecta from experimental as well as farmers filed. Maximum 72.4% wilting was recorded at 60 days after sowing which indicates the strong virulence of pathogen.

Hence, the wilt caused by N. vasinfecta in pigeonpea is an emerging problem and further added to the existing problem of wilt caused by F. udum which may become wilt complex of both the fungi in future. Some fungicides are reported to have inhibition against the soil borne pathogens4,5,6,7 as well as bioagents8. So the study was undertaken to study the see the efficacy of fungicides/bioagents against the pathogen N. vasinfecta.
MATERIALS AND METHODS
The wilted pigeon pea samples were collected from wilt sick plot, Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and the isolation of the pathogen was made by tissue isolation method in Petri plates containing PDA medium and the culture was purified by following the hyphal tip method and maintained for experimentations in future. The in vitro efficacy of fungicides and bioagents was studied as follows.

Efficacy of fungicides against *N. vasinfecta* by poisoned food technique: Potato Dextrose Agar (PDA) medium was prepared, equally distributed measuring 100 in 250 mL conical flask and sterilized in autoclave. Requisite quantity of each of the fungicide was added in sterilized melted (45°C) PDA separately so as to obtained desired concentration. Flask containing poisoned medium were shaken well to have even and uniform distribution of fungicide. About 20 mL of melted poisoned PDA was poured in each sterilized petri plate and allowed to solidify. These petri plates were inoculated by test fungus separately. Six mm disc of one week old fungus lawn culture was cut with a sterilized cork borer, lifted and transferred aseptically in the center of a petri plate containing the medium poisoned with test fungicide. The control plates were kept where the culture disc were grown in same condition on PDA without fungicide. Treated plates were incubated at room temperature (27±2°C) for a period of seven days. Colony diameter was recorded in mm and per cent of mycelial inhibition was calculated as per formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusions were drawn by the methods given by Gomez and Gomez:

\[
PI = \frac{C - T}{C} \times 100
\]

Where:

- \( PI \) = Per cent inhibition
- \( C \) = Growth in control plate
- \( T \) = Growth in treatment plates

Efficacy of bioagents against *N. vasinfecta* by dual culture technique: Lawn culture of test fungi and the fungal bioagents i.e., *Trichoderma harzianum* were prepared in Petri plate. In sterilized Petri plate autoclaved melted Potato Dextrose Agar was poured and allowed to solidify for obtaining leveled surface. These plates were inoculated with the culture of test fungi and bioagents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bioagents, *Pseudomonas fluorescens* and *Bacillus subtilis* were prepared by inoculating a loopfull culture in sterilized conical flask containing 100 mL of nutrient broth. Broth culture was incubated at room temperature for three days. Autoclaved PDA was poured in each of the sterilized Petri plates and allowed to solidify. Four Petri plates of each bioagents were used for this study. Six millimeter disc of one week old test fungus and bioagent lawn culture was cut with the help of cork borer lifted and transferred in Petri plates.

In each Petri plate, four disc of bioagents were inoculated at four peripheral points of the plates and test fungi was placed in centre of Petri plates. In case of *P. fluorescens* and *B. subtilis*, a three days old culture was streaked around the test fungus at four sites. Control plates were kept where culture disc of test fungus were grown in same condition on potato dextrose agar without bioagents. All these plates were incubated at room temperature for seven days. After a expiry of incubation period, the mycelial inhibition was calculated as per formula mentioned in poisoned food technique.

Statistical analysis: Statistical analysis was carried as given by Panse and Sukhatme.

RESULTS AND DISCUSSION
The observations on colony diameter were recorded after seven days of incubation and presented in Table 1. Among the eight fungicides tested, carbendazim (0.1%), combination of carbendazim+mancozeb (0.2%) and thiram+carbendazim 2:1 (0.3%) exhibited cent per cent inhibition of *N. vasinfecta*. All the other fungicides were significantly superior over control in arresting the mycelium growth of fungus.

| Fungicides          | Conc. (%) | Mean of Mycelial growth (mm) | Mycelial inhibition (%) |
|---------------------|-----------|------------------------------|-------------------------|
| Thiram              | 0.3       | 11.54                        | 77.43                   |
| Carbendazim         | 0.1       | 0.00                         | 100.00                  |
| Chlorothalonil      | 0.25      | 14.01                        | 72.59                   |
| Metalaxyl-MZ        | 0.2       | 8.57                         | 83.23                   |
| Thiram+Carbendazim  | 0.3       | 0.00                         | 100.00                  |
| Carbendazim+Mancozeb (1:1) | 0.2 | 0.00 | 100.00 |
| Tricyclazole+Mancozeb (1:1) | 0.2 | 8.39 | 83.61 |
| Zineb+Hexaconazole (1:1) | 0.2 | 16.04 | 68.62 |
| Control              | -         | 51.11                        | -                       |
| F-test               | -         | Sig.                         | -                       |
| Mean±SE             | -         | 0.49                         | -                       |
| CD (p = 0.01)       | -         | 2.00                         | -                       |
Table 2: Efficacy of different bioagents against \textit{N. vasinfecta} by dual culture technique

| Bioagents                | Mean of mycelial growth (mm) | Mycelial inhibition (%) |
|--------------------------|-----------------------------|-------------------------|
| \textit{Trichoderma harzianum} | 19.87                       | 50.30                   |
| \textit{Pseudomonas fluorescens} | 33.76                       | 15.55                   |
| \textit{Bacillus subtilis}       | 23.48                       | 41.47                   |
| Control                  | 39.98                       | -                       |
| F-test                   | Sig.                        | -                       |
| Mean±SE                  | 0.46                        | -                       |
| CD (p = 0.01)            | 2.06                        | -                       |

Efficacy of carbendazim in present studies confirms the result of Raju et al.\textsuperscript{6} who have reported complete inhibition of mycelial growth of \textit{F. udum in vitro} at 100, 250 and 500 ppm. Efficacy of carbendazim, thiram and mancozeb against \textit{Fusarium} species (causing wilt diseases) for inhibiting mycelial growth in different crops were also reported\textsuperscript{4,5}.

Data presented in Table 2 indicated that maximum growth of inhibition of \textit{N. vasinfecta} recorded in \textit{Trichoderma harzianum} (50.30\%) followed by \textit{Bacillus subtilis} (41.47\%). \textit{Pseudomonas fluorescens} was found least effective against \textit{N. vasinfecta}.

The suppression of wilt pathogen of pigeonpea and chickpea with antagonist \textit{Bacillus subtilis} and \textit{Trichoderma harzianum} are reported\textsuperscript{8,11,12,13,14}. Findings of these workers thus support the present result in case of \textit{N. vasinfecta}.

Thus, it is concluded that among the eight fungicides tested by poisoned food technique, carbendazim (0.1\%), combination of carbendazim+mancozeb (0.2\%) and thiram+carbendazim 2:1 (0.3\%) have shown hundred per cent inhibition of mycelium of \textit{N. vasinfecta} whereas among the bioagents tested by dual culture technique, maximum growth of inhibition of \textit{N. vasinfecta} recorded in \textit{Trichoderma harzianum} (50.30\%) followed by \textit{Bacillus subtilis} (41.47\%).

REFERENCES

1. Nene, Y.L., V.K. Sheila and S.B. Sharma, 1989. A world list of chickpea (\textit{Cicer arietinum} L.) and pigeonpea (\textit{Cajanus cajan} L. Millsp) pathogen, legume pathology progress report. Patancheru India ICRISAT, 7: 1-23.
2. Sarojini, T.S., 1955. Soil conditions and root diseases IX \textit{Neocosmospora vasinfecta} Smith disease of \textit{Cajanus cajan}. Rev. Applied Mycol., 34: 727-727.
3. Vishwa, D., R.G. Chaudhary, S. Mishra and A.A. Khan, 2005. Occurrence of pigeon pea wilt caused by \textit{Neocosmospora vasinfecta}. Indian J. Pulses Res., 18: 254-255.
4. Chandel, S.S. and R. Katooch, 2001. Chemical control of \textit{Fusarium oxysporum} f. sp. \textit{dianthi}, an incitant of carnations wilt. Indian J. Microbiol., 41: 135-137.
5. Fravel, D.R., K.L. Deahl and J.R. Stommel, 2005. Compatibility of the biocontrol fungus \textit{Fusarium oxysporum} strain CS-20 with selected fungicides. Biol. Control, 34: 165-169.
6. Raju, G.P., S.V. Rao and K. Gopal, 2008. \textit{In vitro} evaluation of antagonists and fungicides against the red gram wilt pathogen \textit{Fusarium oxysporum} f. sp. \textit{Udam} (butler) Snyder and Hansen. Legume Res.-Int. J., 31: 133-135.
7. Upadhyay, R.S. and B. Rai, 1987. Studies on antagonism between \textit{Fusarium udum} Butler and root region microflora of pigeon-pea. Plant Soil, 101: 79-93.
8. Mandhare, V.K. and A.V. Suryawanshi, 2004. Application of \textit{Trichoderma} species against pigeonpea wilt. JNKVV Res. J., 38: 99-100.
9. Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons, New York, USA., ISBN-13: 9780471870920, Pages: 680.
10. Panse, V.G. and P.V. Sukhatme, 1957. Statistical Methods for Agricultural Workers. ICAR Publication, New Delhi, India.
11. Gholve, V.M. and B.P. Kurundkar, 2004. Efficacy of combined \textit{in vitro} inoculation of \textit{Pseudomonas fluorescens} and \textit{Trichoderma viride}. J. Mycol. Pathol., 34: 524-525.
12. Pandey, K.K. and J.P. Upadhyay, 2000. Microbial population from rhizosphere and non-rhizosphere soil of pigeonpea: Screening for resident antagonist and mode of mycoparasitism. J. Mycol. Plant Pathol., 30: 7-10.
13. Somaskhekhar, Y.M., A.L. Siddaramaiah and T.B. Anilkumar, 1998. Evaluation of \textit{Trichoderma} isolates and their antifungal extracts as potential biological control agents against pigeonpea wilt pathogen. Curr. Res., 27: 158-160.
14. Sumitha, R. and S.J. Gaikwad, 1997. Checking \textit{Fusarium} wilt of pigeonpea by biological means. Rev. Pl. Path., 76: 189-189.