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Research Note

A rapid *in vivo* bioassay method for testing and selection of fungal antagonists of plant pathogens

R. K. SRIVASTAVA[1], R. D. PRASAD, R. RANGESHWARAN,
A. R. WASNIKAR[2], S. P. SINGH and N. S. RAO
Project Directorate of Biological Control (ICAR)
P. B. No. 2491, H. A. Farm Post, Hebbal
Bangalore 560 024, Karnataka, India
E-mail: ravulapalliprasad@yahoo.com

ABSTRACT: **Eight *Trichoderma* isolates were tested for their bioefficacy against seed, root and seedling rot incited by *Rhizoctonia solani* by adopting an *in vivo* test method (blotter test). Vigor index ranging from 169.5 to 2239.4 and disease incidence ranging between 10 to 86 percent were recorded in various treatments. All bioagents were graded based on disease grading key proposed for their rating. The clear differentiation of efficacy of various *Trichoderma* species against *R. solani* obtained with the *in vivo* bioefficacy test method adopted (blotter test) in the present study shows suitability of this method for routine screening of fungal biocontrol agents against seed and soil borne plant pathogens.**

KEY WORDS: Fungal antagonists, *in vivo* bioassay, plant pathogens

In recent years, the research on biological control has gained momentum for controlling serious soil borne plant pathogens like *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, *Pythium* and *Phytophthora* species employing *Trichoderma* and *Gliocladium* species and varied success has been achieved in controlling wilts, damping off, leaf and stem blight, crown and root rots in various crops (Dwivedi, 1984; Singh, 1991; Raghuchander *et al.*, 1993, Jeyarajan *et al.*, 1994). Before employing *Trichoderma* or any other antagonists as biological control agents, it is an essential pre-requisite to test their bioefficacy against the target pathogens for selection of potential isolates. Till now we are depending upon time consuming and laborious laboratory and field-testing methods for selection of potential bioagents. These types of lengthy selection procedures for bioagents have resulted in availability of very few bioagents for commercialization. Hence, there is a need to develop rapid bioassay methods for selecting highly antagonistic microorganisms against target plant pathogens. Keeping all these points in view, the roll paper towel method (ISTA, 1976) commonly used in seed testing with slight modification to screen bioagents taking chickpea-*Rhizoctonia*

[1] Crop Research Station, Narendra Deva University of Agriculture and Technology, Bahraich 271 801, U. P.
[2] Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur 482 004, M. P.

solani system as a model. The grading system for fungal bioagents was developed by using the proposed bioassay method.

Rhizosphere soil was collected from healthy plants of different crops, *viz.*, mustard, chickpea, capsicum and chilli grown around Bangalore. For isolation of *Trichoderma* one gram of rhizosphere soil along with root portions was plated on *Trichoderma* selective medium (Elad and Chet, 1983) after serial dilutions. Individual *Trichoderma* colonies were transferred to potato dextrose agar (PDA) slants. Five fungal bioagent so isolated were purified. Three isolates of *Trichoderma* namely *Trichoderma harzianum* (PDBCTH 10) and *T. viride* (PDBCTV 6 & 23) from fungal bioagent culture collection of Project Directorate of Biological Control (PDBC), ICAR, Bangalore were also used in the study. The test pathogen used was *R. solani* (not identified to anastomosis group), which was isolated previously from diseased chickpea (cv. Annigeri) and was multiplied on PDA plates.

Rhizoctonia mycelial suspension was prepared from PDA plates by scraping mycelium using a sterile scalpel while adding 50 ml of sterile water. The suspension so prepared contains around 1-2x 10^7 colony forming units (cfu) per ml. Seeds of chickpea (cv. Annigeri) were surface sterilized with sodium hypochlorite solution (2%) followed by serial washings with sterile water. Surface disinfected seeds were first inoculated with mycelial suspension of pathogen followed by various *Trichoderma* isolates separately. Bioagent inoculum was also prepared similarly as in case of pathogen and serial dilutions were carried out to obtain approximately $2x10^3$ cfu/seed. In one treatment fungicide (Captan at 2.5g/kg seed) was used to treat seed without bioagent. Seeds treated with pathogen mycelium alone served as check. For each treatment three replications were maintained.

A roll paper method (ISTA, 1976) regularly used for seedling vigor testing was used for testing bioefficacy of bioagents. Seeds treated as described earlier with each biaogent separately were placed on moist blotter sheets equi-spaced and covered with a moistened blotter and rolled. Twenty-five seeds were placed on each blotter. Three such rolls were kept on a butter paper sheet and rolled as a single bundle and incubated in growth chamber at 25°C and 80 percent relative humidity. Moisture in blotter sheets was maintained by applying sterile tap water whenever needed.

Disease grading key

Disease Incidence (%)	Description	Rating of bioefficacy of bioagents
0	Germination >90%, no seed rotting, seedlings healthy, root and shoot portions well developed	Highly Efficient (HE)
1-15	Germination 80-90%, infection on main as well as lateral roots, seedlings are well developed	Efficient (E)
16-30	Germination 70-80%, development of roots restricted and growth is less compared to Score 1. Infection occurred on roots. Shoot portions developed but growth retarded compared to Score 1	Moderately Efficient (ME)
31-45	Germination 60-70%, length of roots and shoots short compared to Score 1. Germination of seeds inhibited. 50% of root area infected. Shoot portions also showed infection.	Moderately Inefficient (MI)
46-60	Seed germination 50 to 60%. Development of roots and shoots greatly retarded. Shoot portions showed infection.	Inefficient (I)
above 60	Less than 50% germination and seed rotting	Highly Inefficient (HI)

Observations on seed germination and seedling growth (shoot and root length) were recorded after 10 days of incubation by following Abdul-Baki and Anderson (1973). The vigor index was calculated by multiplying the sum of root and shoot length with germination percent.

A disease grading key (mentioned below) was developed for rating efficacy of bioagents based on seed rotting, infection occurring on roots and shoots.

The results represented in Table 1 shows *in vivo* bioefficacy of bioagents screened using blotter tests. Germination of seeds in all treatments was significantly high compared to pathogen check. Germination ranging from 74 to 90.3 percent was obtained with bioagent seed treatments whereas in pathogen check it was 32.6 percent only. Maximum seed germination was obtained with *T. harzianum* (PDBCTH 10). There was a significant increase in shoot and root length in all bioagent treatments compared to pathogen check. *Trichoderma* isolate 2 and *T. harzianum* (PDBCTH 10) treatments recorded more shoot and root length compared to all other treatments. Manoranjitham *et al.* (1999) also observed increase in root, shoot length and dry matter production of chilli seedlings when talc based formulation of *T. viride* and *Pseudomonas fluorescens* were applied individually or in combination. Maximum vigour index (2239.4) was also recorded in *T. harzianum* (PDBCTH 10) treatment followed by *Trichoderma* isolate 2 (2226.3), which was isolated from capsicum rhizosphere. Vigour index ranging from 878.6 to 2239.4 was obtained with various bioagent treatments. Pathogen check recorded a vigor index of 169.4 only. All bioagent treatments and fungicide treatments have recorded significantly less disease incidence compared to pathogen check. The disease incidence was ranged between 10 to 28.6 per cent

Table 1. Effect of seed treatment with *Trichoderma* spp. on chickpea plant growth and disease incidence

Antagonist	Germination (%)*	Shoot length (cm)**	Root length (cm)**	Vigor index	Disease incidence (%) *
Trichoderma isolate 1	85.3 (67.50)	4.50	5.8	878.60	19.0 (27.90)
Trichoderma isolate 2	85.3 (67.50)	8.80	17.3	2226.30	10.0 (19.40)
Trichoderma isolate 3	82.0 (64.90)	6.50	7.0	1107.00	24.7 (34.40)
Trichoderma isolate 4	78.0 (62.00)	6.00	9.6	1216.80	28.6 (38.80)
Trichoderma isolate 5	74.0 (59.30)	6.40	9.6	1184.00	20.0 (28.40)
T. harzianum (PDBCTH 10)	90.3 (71.80)	8.50	16.3	2239.40	12.3 (20.20)
T. viride (PDBCTV 6)	86.6 (68.60)	4.90	7.8	1099.80	22.0 (15.70)
T. viride (PDBCTV 23)	87.2 (69.30)	6.30	12.3	1020.20	18.7 (13.60)
Fungicide (Captan at 2.5g/kg seed)	82.0 (64.90)	5.10	6.6	959.40	29.0 (16.1)
Pathogen check	32.6 (34.80)	2.70	2.5	169.50	86.0 (56.10)
C D (P=0.05)	(3.14)	0.65	0.95	80.42	(6.28)

* Figures in the parentheses are angular transformations.

** Average of 30 seedlings from each treatment

in bioagent treatments. Lowest disease incidence of 10 and 12.3 percent was recorded in *Trichoderma* isolate 2 and *T. harzianum* (PDBCTH 10) treatments respectively which were not significantly different. Fungicide treatment and pathogen check recorded a disease incidence 29 and 86 percent, respectively.

All bioagents were rated for their biocontrol efficacy based on disease grading index mentioned earlier. As per that grading *Trichoderma* isolate 2 and *T. harzianum* (PDBCTH 10) were rated as efficient and all remaining bioagents were rated as moderately efficient.

The clear differentiation in efficacy of various *Trichoderma* species against *R. solani* obtained with the bioefficacy test method adopted (blotter test) shows suitability of this faster *in vivo* bioassay method for routine screening of fungal biocontrol agents against seed and soil borne plant pathogens.

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