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Crop Isolate Based Variations in Biological Attributes of *Verticillium lecanii* (Zimmermann) Viegas Native Strains

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ABSTRACT

Present study reveals that V.lecanii strains were isolated from different crop rhizospheres and were purified where the cultures obtained were designated by denoting crop and location from where they were collected. A total of 6 isolates viz., BCSH VSF CF (sunflower), BCSH VM CF (maize), BCSH VGN FF (ground nut), BCSH VRG SF (redgram), BCSH VJ AF (jowar)and BCSH VT AF (tomato) were evaluated for their biological attributes along with PDBC isolate as standard check and lab culture as local check for comparision. Among the test isolates, BCSH VT AF from tomato rhizosphere showed maximum radial growth (59.17 mm), highest conidial concentration (1.58 x10^{9}), highest percentage of conidial viability (94.24 per cent). Findings of the present investigation shared strain variation among the isolates collected from different crop rhizospheres.

Key words: *Isolates, Biological attributes, Verticillium lecanii.*

INTRODUCTION

Bio pesticides based on bacteria, viruses, entomopathogenic fungi and nematodes have considerable scope as plant protection agents against several[11]. Use of entomopathogenic fungi as biological control agents for insect species has increased the global attention during the last few decades. The myco insecticides based on *Beauveria bassiana* (Balsamo) Vuillemin[4,12], *Paecilomyces fumosoroseus* (Wize) Brown and Smith[1,3] and *Verticillium lecanii* (Zimm.) Viegas[5] have been used to control various insect pests in major crops.

Among the Entomopathogenic Fungi (EPF), *Verticillium lecanii* (Zimmermann) Viegas is one of the highly promising fungal bioagent causing infections mostly to soft bodied insects including whiteflies[7,8,9] coccids and aphid[6,10] considering the eco friendly benefits of biological control. *V. lecanii* is also known "White halo fungus" because of white mycelial growth on the body surface of infected host.

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Eventhough, *V. lecanii* has gained a good amount of commercial importance due to it's efficacy against sucking pests of important crops, there is still a need for generation of location based vital information pertaining to it's biological attributes, pathogenecity and virulency against the target insects. By keeping this, in the back ground, investigation pertaining to biological attributes *V. lecanii* isolates from different cropping systems.

METERIAL AND METHODS

The present investigations on "Crop based variations among different isolates for their biological attributes of *Verticillum lecanii* (Zimmermann) Viegas native strains" were carried out at AICRP on Biological Control, Agricultural Research Institute, Rajendranagar, Hyderabad during 2012-13. The materials used and the methods employed in this investigation are furnished here under.

Isolation, Identification and Maintenance of *V. lecanii* Strains

Collection of Cadavers and Soil sample

Insect cadavers infected by suspected fungal pathogens were collected from different crop ecosystems *viz.*, maize, sorghum, red gram, groundnut, sunflower and tomato. For collection of insect cadavers, plants were examined for insects infected by fungal pathogens in student farm, college farm, ARI farm and farmers' field to ensure the availability of sufficient number of fungal infected cadavers. Besides this, the soil samples from the rhizosphere of above mentioned crops were also collected. A total number of 25 samples were collected each for insect cadavers and rhizosphere soil samples.

Isolation and Purification of Entomopathogenic Fungi

The samples including different batches of insect cadavers were brought to laboratory and allowed for 24 hours for acclimatization and then transferred into brown paper packets and kept for 72 hours to allow absorption of the emanating moisture. The samples were separated and crushed in to fine powder and were plate cultured as per the standard micro-biological procedures. For isolation of fungi from soil samples, the samples were brought to lab and stored in brown paper packets for 72 hours; later, isolation and purification was done as per the microbiological protocols. After the growth of the colonies frequent sub culturing was done through Single Spore Isolation (SSI) method till pure culture was obtained.

Studies on the Biological Parameters of Isolated *V. lecanii*

After isolation and purification of test fungi, *V. lecanii* colonies of individual sample of entomopathogenic fungi were grown on the selective media. Approximately 7mm disc was cut from the individual culture plates of *V. lecanii* and then transferred to fresh media plates. The plates were incubated at 25 $\pm$ 1^0C and then were subjected for evaluation of growth parameters in comparison with already designated lab strains of the *V. lecanii.*

Preparation of Dilutions

Using aseptic techniques, one ml of the stock dilution was transferred to a 9 ml dilution blank. This was done for all the samples collected from different crops/crop rhizospheres. Solution was mixed well to obtain even distribution of organisms. With a sterile pipette, 0.1 ml of the dilution was mixed into a sterile media plated in petridish and with the same pipette, 1.0 ml was transferred to a 9.0 ml dilution blank. Approximately 15-20 ml of nutrient agar which has been steam-pressure sterilized (autoclaved), poured and cooled to 45^0C in a water bath was used. The plates were rotated to ensure mixing the inoculum and medium. When the agar was completely solidified, the plates were inverted and placed in an incubator for 2 days to dry off excess surface moisture at 25^0C or 28^0C for better incubation.

Pure culture

Pure cultures of different fungal species and subspecies were isolated from the highly complex mixed populations in soil sample by adopting standard purification procedures. Each discrete colony grown on a prepared dilution plate was assumed to be composed of cells, all of which are descendants of a single cell.

Studies on Biological properties of Different Isolates of *V. lecanii*

Preparation of Culture Media

The cultures were established on the grown media and the fungus was selectively grown on the SDAY media. The composition of SDAY media is given below.

Dextrose	-	40 g
Peptone	-	10 g
Agar agar	-	15 g
Yeast extract	-	5 g
Distilled water	-	1000 ml

The collected samples from crop rhizosphere were isolated, purified and the cultures thus obtained were designated by denoting crop and location from where they are collected. Among the collected samples the following 6 isolates were studied for the biological parameters by using Project Directorate on Biological Control (PDBC) isolate as standard check and lab culture as local check (sourced through PDBC) for the purpose of comparisions.

TREATMENTS

T1: BCSH VSF CF Isolate

T2: BCSH VM CF Isolate

T3: BCSH VGN FF Isolate

T4: BCSH VRG SF Isolate

T5: BCSH VJ AF Isolate

T6: BCSH VT AF Isolate

T7: PDBC Vl Isolate (Standard Check)

T8: Lab Culture (Local Check)

*BCSH= Bio Control Scheme Hyderbad

*VSF/VM/VGN/VRG/VJ/VT= *Verticillium lecanii* Sunflower /*V. lecanii* Maize / *V. lecanii* Groundnut / *V. lecanii* Redgram /*V. lecanii* Jowar / *V. lecanii* Tomato

*CF/SF/FF/AF = College Farm / Student Farm / Farmers Field / ARI Farm

To evaluate the variations in the isolates the fundamental biological parameters such as radial growth, conidial concentration and conidial viability were studied.

Radial Growth

SDAY medium as described earlier was prepared and sterilized in an autoclave. The medium was allowed to cool down after which it was poured in to sterile petriplates of 9.5 x 1.5 cm. About 100ml medium was poured evenly in five petriplates and allowed to solidify.

Circular discs of 10 mm diameter were cut from the vigorously grown cultures of different isolates using a sterile cork borer and were placed in the middle of each petriplate. All these steps were carried out under aseptic conditions inside an inoculation chamber sterilized with UV radiation. The petriplates were incubated at 25 $\pm 1^0$C. The radial growth of the fungus was measured using a measuring scale at 6 days after inoculation.

Conidia Per unit Area

Suspension of the fungal isolate was made and the conidial concentration of the samples collected from different crop rhizospheres was done by using Digital Colony Counter with standard scale for counting number of Colony Forming Units (CFUs).

The circular discs of 10 mm diameter were cut randomly from the two weeks old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml of distilled water. The spores present in the disc were allowed to disperse uniformly in water by rotating the test tube on a vertex for one minute. Proper care was taken to avoid spillage of the suspension while rotation. The suspension was serially diluted and the spores were counted with the help of an improved Neubaur Haemocytometer under a compound microscope at 40 X magnification and number of spores present per ml was calculated using the below mentioned formula[2].

No. of spores per ml = Total number of spores in 5 randomly selecte squares of Haemocytometer x 5 x 10^4

The readings thus obtained were computed to 10 ml to determine the number of conidia per unit area of 10 mm diameter disc.

Conidial Viability

The conidial viability was measured in terms of the viable conidia available in the sample suspensions collected from different crop rhizospheres.

The conidia were harvested from the uniformly grown culture plates with the help of a fine brush into sterile distilled water and filtered through double layered muslin cloth.

Approximately 500 µl of uniformly suspended spore solution was placed in the cavities of a cavity slide containing 100 ml of SDAY medium. The slides were placed in a petriplate containing a moistened filter paper at its bottom and were incubated at a temperature of 25 ± 1^0C and relative humidity of 95 per cent. The slides were observed after every hour under microscope till 50 per cent of the conidia visible in any of the focused region got germinated which was recorded as TG-50, the time taken for germination of 50 per cent of spores. The germination of conidia was recorded after 24 hours of incubation and the per cent spore germination was calculated using the formula.

$$G = \frac{N}{T} \times 100$$

Where G = Per cent spore germination.
N = Total number of spores germinated.
T = Total number of spores observed.

RESULTS AND DISCUSSION

The radial growth in different isolates (Table 1) has shown that there are clear cut variations among isolates collected from different crop rhizospheres. All the isolates from crop rhizospheres varied significantly in radial growth from lab culture (Local check) and also from PDBC isolate (Std. check). It is also seen that there are statistically significant variations amongst them highlighting the importance of influence of the crop from where the isolates were collected. Maximum radial growth of 59.17 mm was observed in BCSH VT AF isolate (tomato) followed by maize isolate (41.97 mm). BCSH VT AF differed significantly from all other isolates and also with standard check (40.23) as well as local check (39.50) there by highlighting its supremacy over the entire test isolates in terms of radial growth. Isolate from maize i,e., BCSH VM CF has recorded 41.97 mm radial growth which is significantly less than tomato isolate but found statistically on par with standard check and local checks. Isolate from red gram (BCSH VRG SF) recorded 33.53 mm radial growth which is significantly superior to all other isolates but was inferior to standard and local checks. BCSH VSF CF (sunflower) and BCSH VJ AF (jowar) isolates were found to be on par with each other with recorded radial growth of 28.70 and 24.97 mm, respectively. BCSH VGN FF (groundnut) was the isolate with 24.30 mm radial growth and was found with least radial growth recorded so far in different isolates.

The variations in conidial concentrations (Table 1) in different isolates of *V. lecanii* showed almost similar trends of results obtained as in case of radial growth. Tomato isolate (BCSH VT AF) maintained its supremacy by recording highest conidial concentration of 1.58×10^9 conidia per ml which is statistically different from other test isolates. Similarly, PDBC isolate (Std. check) and lab culture (local check) recorded conidial concentration of 1.45×10^9 and 1.46×10^9 conidia per ml, respectively. Despite being inferior to tomato isolates they were found to be superior to all other test isolates by recording higher conidial concentrations than all other test isolates. Maize isolate (BCSH VM CF) recorded the next highest conidial concentration (1.41×10^9 conidia/ ml) which is statistically superior to the remaining test isolates namely BCSH VRG SF (1.23×10^9 conidia/ml), BCSH VSF CF (1.22×10^9 conidia/ml) and BCSH VGN FF (1.18×10^9 conidia/ml).

Over all observations of the data obtained from different crop rhizosphere samples in terms of per cent conidial viability (Table 1) has reconfirmed the promising nature of *V. lecanii* isolate collected from tomato rhizosphere by registering highest percentage of conidial viability i.e., 96.48 per cent. The Tomato isolate is distinctly different from not only with other test isolates but also with standard and local checks. Among the standard and local checks unlike radial growth and conidial concentration there was significant difference between per cent conidial viability in standard check of PDBC (94.24 per cent) and lab culture (90.71 per cent).With regard to other isolates, maize isolate - BCSH VM CF recorded 90.58 per cent conidial viability which is significantly superior to the other isolates from red gram, jowar and groundnut. Among the dry land crops, red gram isolate (BCSH VRG SF) recorded 87.19 per cent conidial viability which is on par with sunflower isolate (BCSH VSF CF) with 85.13 per cent conidial viability. Sunflower isolate recorded 85.13 per cent conidial viability there by making itself on par with red gram isolate on one side and jowar isolate-BCSH VJ AF (93.09) on the other side. Jowar isolate has recorded least per cent of conidial viability which is only on par with sunflower but statistically inferior to all other test isolates.

Table 1: Isolate based variations in biological attributes of *V. lecanii* native strains

Treatment	Isolate	Radial Growth (mm)	Conidial concentration (x 10^9)	Conidial Viability (%)
T1	BCSH VSF CF	28.70^{d}	1.22^{e}	85.13^{de} (67.31)
T2	BCSH VM CF	41.97^{b}	1.41^{c}	90.58^{c} (72.11)
T3	BCSH VGN FF	24.30^{e}	1.18^{e}	81.02^{f} (64.16)
T4	BCSH VRG SF	33.53^{c}	1.23^{e}	87.19^{d} (69.01)
T5	BCSH VJ AF	24.97^{de}	1.33^{d}	83.09^{ef} (65.71)
T6	BCSH VT AF	59.17^{a}	1.58^{a}	96.48^{a} (79.27)
T7	PDBC Isolate (Std. Check)	40.23^{b}	1.45^{b}	94.24^{b} (76.12)
T8	Lab Culture (Local Check)	39.50^{b}	1.46^{b}	90.71^{c} (72.23)
SE(m)±		1.364	0.013	0.694
CD at 5%		4.125	0.040	2.099

Values are given in parentheses, which are angular transformed values
Figures indicated by same letter are not significantly different from one another as per DMRT

CONCLUSION

A total of 6 isolates from different crop rhizospheres *viz.,* BCSH VSF CF (sunflower), BCSH VM CF (maize), BCSH VGN FF (ground nut), BCSH VRG SF (redgram), BCSH VJ AF (jowar)and BCSH VT AF (tomato) were evaluated for their biological attributes.PDBC isolate was used standard check and lab culture was considered as local check for comparisions. Among the test isolates BCSH VT AF isolate from Tomato showed maximum radial growth (59.17 mm), highest conidial concentration (1.58 $x10^9$), highest percentage of conidial viability (94.24 per cent).The findings of the present investigation also gave an information pertaining to the strain variation among the isolates collected from different crop rhizospheres in terms of the above mentioned biological attributes. On the basis of the data obtained and also in the view of above inferences, BCSH VT AF isolate is found to be better and promising for further downstream processing and commercialization.

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