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Research Article

In vitro screening of *Trichoderma viride* against *Albugo candida* - white rust pathogen of rapeseed-mustard

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Abstract: Field experiments on white rust of rapeseed-mustard were conducted at Kakching located 45 km away from Imphal, Manipur during two consecutive *rabi* seasons (2014-15 & 2015-16) under organic farming. Using dual culture technique of *Trichoderma viride* and *Albugo candida* resulted in significant effect in all the varieties except in between M-27 (V_3) & Ragini (V_4). The percent inhibition of mycelial growth over control was found to be highest in V_4 (58.78%) and lowest in local cultivar, Lamtachabi (V_2) i.e. 33.17%.

Keywords: Antagonist, Dual Culture, Rhizosphere, White Rust.

1. Introduction

Rapeseed-mustard is an oil yielding crop cultivated across the state. The crop is raised under organic condition using farmyard manure (FYM). This crop plays an important role to the marginal farmers for their domestic consumption. Rapeseed-mustard is incited by various fungal diseases, among them white rust was found to be most prominent. White rust caused yield losses of 23 - 54.5% [1]. As white rust, which is caused by *Albugo candida* is a common and prominent disease of the crop, it is necessary to manage the crop to the threshold level. Rhizosphere is an abode of many microorganisms, including foes and friends to the plants. It triggered the present study under the organic farming system in Manipur to investigate the effect of rhizosphere microflora (*Trichoderma viride*) against *Albugo candida*, the white rust pathogen of rapeseed-mustard under *in vitro* condition.

2. Material and Methods

2.1 Field trials

Field experiments were conducted at Kakching, an agricultural hub of diverse crops, located about 45 km away from Imphal, Manipur during *rabi* seasons (2014-15 & 2015-16). The experimental varieties used were two cultivars of mustard namely *Brassica juncea* (L.) Czern. & Coss. cv. Local Yella (V_1) and *B. juncea* Czern. & Coss. cv. Lamtachabi (V_2) and two varieties of rapeseed namely *B. rapa* (L.) var. M-27 (V_3) and *B. rapa* (L.) var. ragini (V_4). The crop was sown in last week of October in plots (2.2 x 1.3m^2) with three replications and laid out in a randomized block design (RBD). A spacing of 30 x 10cm^2 with row to row and plant to plant distances were maintained. Normal agricultural practices such as irrigation, thinning and weeding were also done from time to time to raise the crop.

2.2 Isolation of soil microorganisms

Fortnightly monitoring was conducted and at 45 DAS (days after sowing) after the appearance of disease, sample collection was done. Soil samples were collected from white rust infected plants which were selected randomly from the experimental plots. 5 plants were uprooted for each variety and brought to the lab using sterile poly bags. Isolation of soil fungi was done using the method as suggested by [2]. 10gm of soil was taken in a 250ml conical flask containing 100ml sterile distilled water. It was shaken for 10 – 15 minutes to make the solution homogenized. The dilution in the flask was treated as 1: 100. Subsequently, dilution was made up to 1: 10000 (10^{-4}) for isolation of soil microorganisms. Then 1ml of prepared solution was inoculated in the respective Petri plates using PDA [3]. Three replications were maintained. The plates were incubated for 5 days at 25 ± 1°C inside incubator.

2.3 Isolation of the pathogen and identification

The fungal pathogens were isolated by following the method [4] in the laboratory. The infected plant

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tissues along with unaffected tissue were cut into pieces (size: 2-5mm^2). With the help of sterilized forceps, they were transferred to sterile Petri plates containing 0.1% mercuric chloride solution and later they were surface sterilized for 30 seconds. The sterilized pieces @ 3-5 per plates were inoculated in BNPRA [5] medium. The inoculated Petri plates were incubated at 25-27 ± 1°C and observed after 5 days.

After the incubation period, the growths of fungi were studied under microscope and data were recorded. Identification of the isolated rhizosphere microflora and pathogen were done using standard literature [6,7 & 8].

2.4 *In vitro* interaction and data analysis

Among the rhizosphere microflora, *T. viride* was selected for antagonistic effect against *A. candida*. The antagonistic effect of *T. viride* against *A. candida* was tested using dual culture technique [9]. Wells (5mm diameter) were made using borer on PDA 3cm away from rim of the Petri plates (9cm) on opposite sites. The actively growing mycelia discs were taken out using borer from the respective Petri plates. Then they were inoculated using the pathogen and antagonist on opposite sides in the wells of the plates. The Petri plates containing only pathogen and inoculated at the centre served as control. Three replications were maintained. The percent inhibition of the pathogen by *T. viride* over control was assessed by the formula [10] as follows:

$$I = \frac{C-T}{C} X\ 100 \qquad (1)$$

Where, I = Percent growth inhibition;
C = Radial growth of mycelium in control;
T = Radial growth of mycelium in treatment.

The data obtained from radial growth of *Albugo candida* in dual culture for different varieties of rapeseed-mustard were subjected to one – way ANOVA (analysis of variance) to test any significant variance among the varieties.

3. Results and Discussion

The *in vitro* screening of *Trichoderma viride* against *Albugo candida* showed (Table 1) significant effects among the experimental varieties tested except in between M-27 (V_3) and ragini (V_4). It may be due to varietal differences of the crop. The highest inhibition over control was found in V_4 (58.78%) and lowest in V_2 (33.17%). These results revealed variability in inhibition of the pathogen using *T. viride* by virtue of degree of pathogenicity towards the varieties used. The antifungal activity of *T. viride* in the present study corroborated with the findings of [11] who reported out of the 110 *Trichoderma* isolates tested, 32% of them showed antifungal antagonistic activity under *in vitro* conditions against *Pythium aphanidermatum* the causal agent of damping – off of beans. Thus the bioagent-*Trichoderma viride* can be used in field trials to manage white rust of rapeseed-mustard organically.

Fig. 1a. Growth of the pathogen in control.

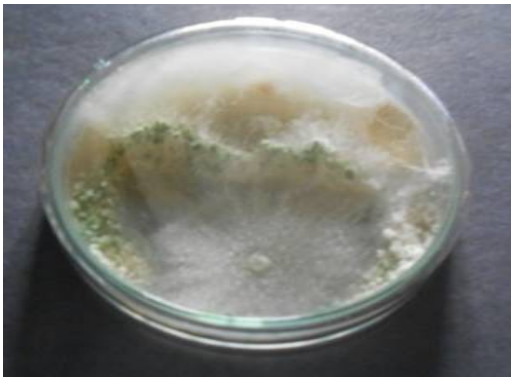

Fig. 1b. Growth of the pathogen with *T. viride* in dual culture.

Table 1. Effect of *Trichoderma viride* against *Albugo candida* over control.

Varieties	*Radial growth of *A. candida* in dual culture (cm)	*Radial growth of *A. candida* in control (cm)	Percent inhibition over control
V_1	2.44	4.10	40.48
V_2	2.74		33.17
V_3	1.81		55.85
V_4	1.69		58.78
C.D. at 5% level	0.32		

*data are mean of three replications

4. Conclusion

In vitro interaction of *T. viride* and *A. candida* using dual culture technique resulted in significant effect in all the varieties except in between M-27 (V_3) and ragini (V_4) varieties. The highest percent inhibition over control is 58.78% (V_4). It can thus be highlighted that *T. viride* can be used as a biocontrol agent for managing white rust of rapeseed-mustard under organic systems.

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References

[1]. Saharan, G.S., Kaushik, C.D., Gupta, P.P. & Tripathi, N.N. (1984). Assessment of losses and control of white rust of mustard. *Indian Phytopathol*., 37: 397 (Abstr).

[2]. Timonin, M.I. (1940). The Interaction of Higher Plants and Soil Micro-Organisms: I. Microbial Population of Rhizosphere of Seedlings of Certain Cultivated Plants. *Canadian Journal of Research*, 18c(7): 307-317. https://doi.org/10.1139/cjr40c-031.

[3]. Riker, A.J. & Riker, R.S. (1936). Introduction to Research on Plant Diseases. John Swift and Co., St. Louis, MO.

[4]. Narayanasamy, P. (2010). Microbial Plant Pathogens-Detection and Disease Diagnosis: Fungal Pathogens. Vol. 1, Springer Science & Business Media, p. 9.

[5]. Masago, H., Yoshikawa, M., Fukada, M. & Nakanishi, N. (1977). Selective Inhibition of *Pythium* spp. on a Medium for Direct Isolation of *Phytophthora* spp. from Soils and Plants. *Phytopathology,* 67: 425-428. DOI: 10.1094/Phyto-67-425.

[6]. Barnett, H.L. & Hunter, B.B. (1998). *Illustrated genera of imperfect fungi.* 4th Edition. APS Press, St. Paul (Minn.), pp. 218.

[7]. Joseph C. Gilman (1957). *Manual of Soil Fungi*. 2nd Edition (Rev.). Oxford & IBH Publishing Co., Calcutta. pp. 450.

[8]. Singh, R.S. (2009). *Plant Diseases*. 9th Edition. Oxford & IBH Publishing Company Pvt. Ltd. India, pp. 173-393.

[9]. Coşkuntuna, A. & Özer, N. (2008). Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. *Crop Protection,* 27: 330 – 336.

[10]. Vincent, J.M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature,* 159: 850. https://doi.org/10.1038/159850b0.

[11]. Kamala, T. & Indira, S. (2011). Evaluation of indigenous *Trichoderma* isolates from Manipur as biocontrol agent against *Pythium aphanidermatum* on common beans. *3 Biotech,* 1(4): 217–225. doi: 10.1007/s13205-011-0027-3.