BIOINFOLET 18 (3) : 386 - 389, 2021

EFFECT OF AMINO ACIDS AND VITAMINS ON ANTAGONISTIC ACTIVITY OF TRICHODERMA VIRIDE

Pratima. P. Kamble and Shivaji. S. Kamble

Mycology and Plant Pathology Research Laboratory, Department of Botany, Shivaji University, Kolhapur - 416 004 (M. S.)

ABSTRACT

The antagonistic activity of *Trichoderma viride* was evaluated against *Pythium aphanidermatum (*Edson) Fitzp. causing turmeric rhizome rot by using Dual culture technique. To enhance antagonistic potential of this bio-agent physical and chemical mutagenesis of *T. viride* was carried out by using UV light and Sodium azide (SA) respectively. From these, 2 mutants namely T_{VUV-48} and T_{VSA-56} were selected for present study. In the present investigation, effect of physiological factors such as amino acids and vitamins on antagonistic activity of three isolates of *Trichoderma viride* ($T_{VW,}$ T_{VUV-48} and T_{VSA-56}) were determined. Highest percent inhibition was recorded when Isoleucine (70.0%) was used in case of $T_{VW,}$ and Proline (72.2%) and Histidine (66.3%) for T_{VUV-48} and T_{VSA-56}respectively. Remarkable results for vitamins (Nicotinic acid, Riboflavin and Ascorbic acid) were recorded in case of $T_{VUV-48,}$ among which Nicotinic acid was most effective for all *Trichoderma viride* isolates.

Key Words: Vitamins, Amino acids, *Trichoderma viride*, Antagonistic activity

Introduction:

Rhizome rot of turmeric (*Curcuma longa* L.) caused by the fungal pathogen, *Pythium aphanidermatum* (Rao et al.1993) affects commercially important rhizomesof the plant(Muthulakshmi and Saveetha, 2009). It causes up to 50-60% yield loss both in field as well as under storage conditions (Nirmal et al. 1992; Rajlakshmi et al. 2016).

Use of pesticides is traditional and most extensively used method of disease management. However, their excessive and continuous use by farmers resulted in the development of resistance of pathogens (Ford, et al., 1987; Fletcher and Locke, 1993). In view of this, development of eco-friendly disease management strategies are being developed. For this purpose, use of biological control agents is one of the most promising and extensively used technology. Among the bio-agents *Trichoderma* species is the most commonly used fungal antagonist against wide range of fungal plant pathogens including *Phytophthora, Pythium, Rhizoctonia, Aspergillus, Sclerotinia* and *Fusarium* sp. (Singh et al., 2018).

To maximize antagonistic potential of *Trichoderma* sp. physical and chemical mutation experiments were performed by using UV light and Sodium azide (SA) mutagens respectively (Patil and Kamble, 2011: Singh, 2016) As, various factors affect growth, sporulation and antagonistic activity of *Trichoderma* sp. its physiological studies were undertaken. During present investigation, effect of amino acids and vitamins on antagonistic potential of *Trichoderma viride* (wild and mutants) was studied.

Material and Methods:

Infected rhizomes of Turmeric exhibiting rhizome rot symptoms were collected from turmeric fields from different districts of Maharashtra. Those were surface

sterilized, cut down into 4-6 mm pieces and inoculated on petri-plates containing Czapek Dox agar medium (Santa Maria, 2017). The test pathogen (*Pythium aphanidermatum*) was identified following *Pythium* monograph (Plaats-Niterink, 1981) and purification of culture was carried out by Hyphal tip technique (Tuite, 1996).

Isolation of *Trichoderma viride* from soil samples, of turmeric rhizospheres was carried out by using soil plate method (Warcup, 1947), followed by serial dilution technique (Aneja, 2005). Identification of *Trichoderma* sp. was done by referring key given by Bissette (1991). Physical and Chemical Mutation of *T. viride* was performed by using UV light (Kumar, 2015) and Sodium azide mutagen respectively (Pellett, 1964) and the mutants obtainedwere tested for their antagonistic activity against *P. aphanidermatum.* Mutants with highest antagonistic activity i. e T_{VUV-48} & T_{VSA-56} were selected for further experimental work (Kamble and Kamble, 2020).

Different amino acids viz., Alanine, Arginine, Cysteine, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Methionine, Aspartic Acid, Proline, Serine, Threonine, Tryptophan Tyrosine and Valine were incorporated in CDA medium at 0.02% concentration, while vitamins such as Nicotinic acid, Riboflavin and Ascorbic acid were amended into CDA medium at the concentration of 0.1%. Petri-plate without either amino acid and or vitamin was regarded as control. On the petri-plates containing above mentioned medium, the pathogen and *Trichoderma viride* were inoculated at polar ends and incubated for 4 days at 28±2^{0}c in the BOD Incubator. Average linear growth of both fungi were measured after completion of incubation period and inhibition percentage was calculated by using theformula given by Vincet (1947).: Inhibition Percentage (%) I =[(C - T) × (100)] / [C], Where, I = Percent Growth Inhibition (%).C = Colony Diameter in Control plate and T = Colony Diameter in treatment plate.

Results and Discussion:

Physiological factors such as incorporation of different amino acids and vitamins into culture medium influenced growth and antagonistic activity of *Trichoderma viride* wild (T_{VW}) as well as its mutants (T_{VUV-48} and T_{VSA-56}). Highest inhibition percentage against *Pythium aphanidermatum* was recorded with the supplementation of Isoleucine (70%), Proline (72%) and Histidine (66.3%). Reduced antagonistic activity was observed in Lysine (54.4%) and (56.6%) for T_{VW} and T_{VUV-48} respectively. In case of T_{VSA-56} Arginine (55.2%) recorded lowest antagonistic potential (Table).

In case of vitamins, all *Trichoderma viride* strains (i. e. $T_{VW,}$ T_{VUV-48} and T_{VSA-56}) showed promising growth and antagonistic activity against the rhizome rot pathogen *Pythium aphanidermatum*. Among all vitamins, Nicotinic acid was found to be most effective in enhancing antagonistic potential of *T. viride* with inhibition percentage of 67.7%, 68.2% and 62.2 % respectively. Treated petriplates showed more favourable results as compared to control (i. e. 61.1%, 65.5%, 57.7%) in case of three mutants $T_{VW,}$ T_{VUV-48} and T_{VSA-56}respectively(Table 1).

Jayswal et al. (2003) reported that *Trichoderma* species can utilize diverse nutritional sources. Cochrane (1958) also stated that, in presence of amino acids and vitamins, growth of *Trichoderma* species increases. According to Chattopadhyay and Nandi (1981) amino acids are good nutrientsources for growth of fungi. Aspartic acid and Asparagine were the best amino acids for growth and spore formation in *Trichoderma viride.*(Jayswal, et al. 2003).

Table 1 : Effect of different Amino acids and vitamins on the antagonistic activity of *Trichoderma viride* (wild and mutants):

Sr. No.	Amino acid (0.02%)	(Tv_w)		(Tv_{UV-40})		(Tv_{SA-56})	
		Length (mm)	Inhibition percent (%)	Length (mm)	Inhibition percent (%)	Length (mm)	Inhibition percent (%)
1.	Alanine	34.5	60.5	37	58.8	34.6	61.5
2.	Arginine	32.0	64.4	35	60.5	30.3	55.2
3.	Cysteine	38.0	57.7	36	60.0	36.0	60.0
4.	Glutamic acid	35.5	60.5	35	61.1	313	65.2
5.	Glycine	35.0	61.1	34	62.2	34.3	61.8
6.	Histidine	30.0	66.6	30	66.6	30.3	66.3
7.	Isoleucine	27.0	70.0	31	65.5	34.6	61.5
8.	Leucine	37.5	58.3	32	64.4	39.3	56.3
9.	Lysine	41.0	54.4	39	56.6	38.0	57.7
10.	Methionine	39.0	56.6	35	60.5	38.0	57.7
11.	Aspartic acid	29.5	67.2	36	60.0	32.0	64.4
12.	Proline	30.5	66.1	25	72.2	36.3	59.6
13.	Serine	34.5	61.6	37	58.8	30.3	66.3
14.	Threonine	35.0	61.1	35	61.1	31.6	64.8
15.	Tryptophan	30.5	61.1	30	66.6	31.6	64.8
16.	Tyrosine	38.5	57.2	33	63.3	31.6	64.8
17.	Valine	40.0	55.5	38	57.7	33.6	62.6
18.	Control	41.0	54.4	36	60.0	38.0	57.7
Sr. No.	**Vitamins, (0.01%)**	**Length (mm)**	**Inhibition percent (%)**	**Length (mm)**	**Inhibition percent (%)**	**Length (mm)**	**Inhibition percent (%)**
1.	Nicotinic acid	29.0	67.7	28.6	68.2	34.0	62.2
2.	Riboflavin	30.6	66.0	30.0	66.6	37.3	60.5
3.	Ascorbic acid	32.6	63.6	30.0	66.6	36.6	59.3
4.	Control	35.0	61.1	31.0	65.5	38.0	57.7

Acknowledgements:

The authors are grateful to the Head of the Department of Botany, Shivaji University, Kolhapur for providing necessary laboratory and library facilities.

References:

Aneja., K. R. (2005) " *Experiments in Microbiology, Plant Pathology and Biotechnology "*. New Age Publishers, New Delhi, pp:69.

Bissett, J. (1991). *Canadian Jounal of Botany.***69:**2357.

Chattopadhyay, N. C. and Nandi, B. (1981).*Mycologia***73**:401.

Cochrane, V. W. (1958). "*Physiology of fungi*". John Wiley, England.

Fletcher, J. and Locke, T. (1993).*British Society for Plant Pathology Newsletter*, **22**:25.

Ford, M.G., Hollomon, D.W., Khambay, B.P.S. and Sawicki, R. M. (1987).“*Ellis Horwood series in biomedicine.”*Eliis Horwood, Chichester.

Jayswal, R. K., Singh, R. and Lec. S. Y. (2003). *Mycobiology* **31(1):**36.

Kamble, P. P. and Kamble, S. S. (2020). *Bioinfolet*.**17(A):** 591.

Kumar, A. K. (2015). *Bioresources and Bioprocessing*.**2:** 22.

Muthulakshmi, P. and Saveetha, K. (2009).*Journal of Biological Control.***23(2):** 181. Nirmal, B. K. Samsuddin, K. and Ratnambal, M. J. (1992).*Plant, cell tissues and organ culture,* **29:** 71.

Patil, V. B. and Kamble, S. S. (2011). *International Journal of Academic Research*.**3(1):**702.

Pellette, H. M. (1964).*“Digital Depository capstones”,* Iowa State University. Thesis and Dissertations :26.

Plaats-Niterrink A. J. Van der. (1981). *Studies in mycology***21:** 242.

Rajalakshmi, J., Durgadevi, D., Harish, S. and Raguchander, T. (2016).*International Journal*

of Environment, Ecology, Family and Urban Studies, **6(4):***1.*

Rao, T. G. N., Reddy, B.N. and Saikumr, B. (1993).*Spice India*. **6:**8.

Santa Maria. (2017). '*CZAPEK-DOXAGAR'*.CA. Hardy Diagnostics.

Singh, J., Kumar, V. Srivastava, S., Kumar, A., and Singh, V. P. (2018).*Plant Pathology Journal*.**17:** 59.

Singh, R., Maurya, S. and Upadhyay, R. S. (2016). *Brazilian Journal of Microbiology*.**47:**10.

Tuite, J. (1996).”*Plant Pathological methods Fungi and Bacteria”* Burges.Pub. Co., Minneapolis Min. USA, pp: 293.

Vincet, J. M. (1947). *J. Soc. Chem. Ind.,* **66**: 149.

Warcup, J. H., (1950). *Nature,***166**: 117.