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Morphological and Physiological Analysis of Bio-Control Agent (*Trichoderma viride*)

R.P. Mishra[1*], Manjul Pandey[2] and Mukesh Srivastava[1]

[1]Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur-208002, India
[2]Department of Plant Pathology, Krishi Vigyan Kendra, Banda, India

**Corresponding author*

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A B S T R A C T

Trichoderma are free-living fungi and found commonly in soil - root ecosystem. They are highly interactive in root, soil and foliar environments. They produce or release a variety of compound that induces localized or systemic resistance responses in plant. In this investigation ten different synthetic media (TSM, PDA, Asthana & Hawkers, Richard's agar, Sabouraud's dextrose, Rose Bengal agar, Czapex Dox, Beef extract agar, Cooke rose Bengal and Pikovaskys agar) used to study the morphological and cultural characters of the bio-control agent. Potato dextrose agar (PDA) medium supported the best growth of the bio-agent followed by *Trichoderma* specific medium (TSM), Pikovakyas agar and Rose Bengal medium. Beef extract agar medium supported the lowest growth of this fungus. Excellent sporulation obtained on Potato Dextrose Agar and Trichoderma Specific Medium. It was good on Pikovakyas Agar, Rose Bengal Agar and Sabouraud Agar, fair on Asthana & Hawker, Cooke Rose Bengal Agar, Czapak-dox Agar and Richard Agar, while poor on Beef Extract Agar medium. The maximum colony growth of the mycelium was observed at 25^0C. The statistical analysis showed that isolate T1 (8 CP) exhibited the highest growth which was significantly superior to other isolates. Rests of the isolates were at par with each other. The maximum dry weight recorded as 208.81mg, 205.80mg, 209.17mg, 207.11mg, 206.00mg, and 208.14mg at pH 7.0. The minimum dry weight recorded at pH 4.0. Thus, excellent sporulation was recorded at pH 6.5 and 7.0. It has observed that neither alkaline nor acidic conditions are congenial for the growth and sporulation of the bio-agent.

Introduction

Trichoderma strains have been recognized as biological agent for the control of plant diseases. It has ability to increase root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients are its important characteristics. *Trichoderma* sp. are free-living fungi that are common in soil and root ecosystems (Kubicek and Penttilame 1998). They are highly interactive in root, soil and foliar environments. They produce or release a variety of compound that induces localized or systemic resistance responses in plant (Harman *et al.*, 1996). *Trichoderma*

commonly available in soil and root ecosystem has gained immense importance since, last few decades due to its biological control ability against several plant pathogens Devi *et al.,* (2012) studied the morphological characters with antagonistic ability of *Trichoderma* species. Based on morphological and cultural characteristics, the *Trichoderma* isolates identified as *T. virens* (11 isolates), *T. asperellum* (15 isolates), *T. harzianum* (14 isolates) and *T. longibrachiatum* (32 isolates). Mustafa *et al.,* (2009) were examined on five different culture media including Potato Dextrose Agar, Waksman Agar, Agar-Agar, Czapeck's Dox Agar and Corn Meal Agar. The medium had a significant effect on growth rate and population of the three *Trichoderma* species. Potato Dextrose Agar was the best medium in terms of growth, spore production and biomass. Jayaswal *et al.,* (2003) recorded optimum growth and sporulation of *Trichoderma viride* between pH 4.5 to 5.5. The growth and sporulation decreased with either decreasing the pH below 4.5 or above 5.5 respectively, above pH 6.0 with increasing the pH, the growth and sporulation decreased proportionately. At pH 8.0 and 8.5 the growth and sporulation were very poor. At pH 9, no growth of *T. viride* was observed. *Trichoderma viride* showed a high range of temperature tolerance, it grew and sporulated well between temperatures 20 to 35^0C.

Materials and Methods

Effect of different media on growth of bio-control agent

Following ten different synthetic media (*Trichoderma* specific medium (Elad *et al.,* 1981), Potato Dextrose Agar (Lawrence C. Parks, 1997), Asthana & Hawkers (Dandge V. S, 2012), Richard's agar (Williams *et al.,* 2000), Sabouraud's Dextrose agar (Guinea *et al.,* 2005), Rose Bengal agar (Smith *et al.,*1944), Czapeks Dox (Thom and Church, 1926), Beef extract agar (Vanderzant *et al.,* 1992), Cooke rose Bengal agar (Cooke, 1954) and Pikovaskys agar medium (Sundara Rao . and Sinha, 1963) used to study the morphological and cultural characters of the bio-control agent. The studies carried out to determine the linear growth rate of the identified bio-agent *Trichoderma viride*. 20ml each sterilized media poured in to 90 mm size Petri dishes. The plates inoculated with 5mm disc of inoculums cut with the help of sterilized cork borer from seven-day-old culture in three replications. The plates incubated at $23+2^0$C for 7 days. After incubation of seven days linear growth of the bio-agent recorded in two directions regularly and then, average linear growth of three replicated Petri plates of each bio-agents were taken for statistical analysis.

Effect of temperature on growth of bio agent

To find out optimum and suitable temperatures for the growth of bio control agent (*Trichoderma* sp.), the micro-organism grown at five different temperatures on Potato dextrose agar medium. Inoculation of pure culture of *Trichoderma viride* with help sterile cook borer in Petri plate. The inoculated Petri plates kept at 15, 20, 25, 30 and 35^0C in BOD incubator and the daily observation recorded. For each temperature petri plates were inoculated with seven days old culture of bio-agent (*T. viride*) with help of sterile cook borer and kept at 15, 20, 25, 30 and 35^0C in BOD incubator and data recorded after daily observation.

Effect of pH on growth of the bio-agent

To find out optimum as well as suitable pH for the growth of *Trichoderma viride*. The set of different pH values (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) was adjusted by adding appropriate amount of citrate phosphate buffer

before sterilization. For each pH value three replications used. The spore count was recorded by using hemocytometer as 1 x 10^5 spore/ml. 1ml of this spore suspension was added in Potato Dextrose broth of different pH ranges and then kept in BOD incubator at 23 + 2^0C for 7 days. After 7 days, mycelium mat harvested by sterilized filter paper. The harvested mycelium was kept in hot air oven at 60^0C for 48h for dry weight and final dry weight of mycelium was measure in mg.

Results and Discussion

Morphological studies of bio control agent

Isolated fungus showed typical fast growth at 25-30^0C but will not grow at 35^0C. Colonies are transparent at first on Potato dextrose agar medium. A yellow pigment secreted in medium. Under the study of such microorganism showed highly branched conidiophores and main branches of the conidiophores produce lateral branches that paired or not, the longest branches distant from the tip.

The secondary branches often paired and longest secondary branches being closet to the main axis. All primary and secondary branches arise at or near 90^0 with respect to the main axis. Sheila and Odhiambo (2009) isolated green fungus from 120 soil samples.

Colony characteristics, growth rate in culture and morphological characters are used for identification. Nashwa *et al.,* (2008) collected soil samples for isolation of *Trichoderma* spp. from the rhizosphere of healthy been plants using dilution plate techniques and purified by the single spore method. They observed that conidia of *Trichoderma* are ellipsoidal and typically smooth. Synamorphos are formed and recognized by their solitary conidiophores that are vertically branched and bear conidia at the tip of each phialide. Chlamydospores produced by all species of *Trichoderma* but not all species produces Chlamydospores on PDA at 20^0C within 10 days. Chlamydospore were typically unicellular sub-globose and short hyphae; they formed with hyphal cells.

Effect of different media on growth of bio-agent

The bioagent (*Trichoderma viride*) grown on the solid states of ten media for 10 days at 28±1^0C temperature. The average diameter of the colonies, the nature and spoliation on different media recorded and the data presented in table 1 are summarized.

It is evident from the table that Potato dextrose agar medium supported the best growth of the bio-agent followed by *Trichoderma* specific medium, Pikovakyas agar and Rose Bengal medium. Beef extract agar medium supported the lowest growth of the fungus. It is also obvious from table that excellent spoliation obtained on Potato Dextrose Agar and Trichoderma Specific Medium.

It was good on Pikovakyas Agar, Rose Bengal Agar and Sabouraud's Dextrose Agar, fair on Asthana & Hawker, Cooke Rose Bengal Agar, Czapak-dox Agar and Richard Agar, while poor on Beef Extract Agar medium. Mustafa *et al.,* (2009) examined on five different culture media including Potato Dextrose Agar, Waksman agar, Agar-agar, Czepak's agar and Corn Meal agar.

The medium had a significant effect on growth rate on population of the three *Trichoderma* species. Potato Dextrose Agar was the best medium in terms of growth spore production and biomass yield. Farooq *et al.,* (2005) were studied *In vitro* effect of culture media on mycelial growth of *F. oxysporum* f. sp. *ciceri*. The fungus grew the best on Czapek dox agar and chickpea seed-meal agar media among eight culture media that were tried.

Table.1 Effect of different media on growth of bio-agent

S. No	Media	8 CP	11 CP	17 CP	33 CP	34 CP	35 CP	66 CP	89 CP	102 CP	119 CP	Sporulation
1	Potato dextrose agar	6.8	6.1	6.7	6.8	6.6	6.1	6.3	6.6	6.0	6.1	Excellent
2	Trichoderma selective media	6.1	5.9	6.5	6.6	6.1	6.0	6.5	6.2	6.1	5.9	Excellent
3	Rose Bengal agar	5.5	5.1	5.1	5.7	5.6	5.5	5.7	5.3	5.8	5.5	Good
4	Pikovakyas agar	5.8	5.3	5.6	6.0	5.0	4.8	5.8	5.6	5.5	5.8	Good
5	Sabouraud dextrose agar	5.0	5.1	4.6	4.9	4.0	4.2	5.0	5.2	5.5	5.2	Good
6	Asthana & Hawker	4.5	4.2	4.3	4.3	4.1	3.9	4.9	5.0	5.0	4.8	Fair
7	Cooke rose Bengal agar	4.0	4.1	4.1	4.8	4.7	4.2	4.0	4.2	4.1	4.4	Fair
8	Czapak dox agar	4.0	3.9	4.2	4.0	3.9	3.8	3.9	4.3	4.0	4.1	Fair
9	Beef extract agar	3.8	3.5	4.0	3.9	4.0	3.9	3.7	3.1	3.6	3.5	Poor
10	Richard agar	4.1	4.5	4.0	4.1	4.0	4.1	4.0	4.3	4.2	4.4	Fair

CD at 5% = 0.290

Table.2 Effect of different temperatures on growth of bio-agent

Temperature (^{0}C) *T. viride* isolates	Mycelial weight (gm)				
	15	20	25	30	35
08 CP	20.34	40.13	64.59	59.00	34.54
11 CP	19.45	38.23	58.64	60.15	33.05
17 CP	21.15	41.07	62.09	60.10	31.00
33 CP	20.89	39.22	61.20	59.00	30.50
34 CP	23.10	41.18	62.47	57.10	27.90
35 CP	22.12	40.33	60.00	56.74	28.20
66 CP	23.00	40.13	62.30	59.72	29.52
89 CP	22.41	41.00	62.52	59.31	30.14
102 CP	22.35	40.27	61.08	60.64	31.28
119 CP	23.18	40.57	62.73	61.20	31.77

CD at 5% = 1.048

Table.3 Effect of pH on growth of the bio-agent

pH	8 CP	11CP	17CP	33CP	34CP	35CP	66CP	89CP	102CP	119CP
4.0	114.73	116.66	112.50	111.57	117.90	114.75	113.97	111.29	112.08	112.37
4.5	128.42	135.64	126.87	125.40	125.07	124.68	125.38	125.67	124.87	126.01
5.0	159.54	161.30	154.73	151.29	149.37	145.27	158.23	159.12	160.43	149.00
5.5	178.67	185.00	176.14	175.67	169.20	161.46	168.03	169.33	169.82	170.20
6.0	185.31	189.25	182.29	188.63	181.19	179.00	172.91	171.38	172.11	173.61
6.5	192.46	191.70	191.52	190.32	190.66	186.10	189.00	190.03	190.68	191.11
7.0	208.81	205.80	209.17	207.11	206.00	208.14	207.91	208.89	209.13	207.14
7.5	197.27	198.32	197.23	196.20	196.09	195.19	192.33	193.45	194.30	194.56
8.0	123.44	121.41	129.00	127.30	128.00	123.00	122.62	121.50	123.51	124.90

CD at 5% =13.158

Physiological studies

Effect of different temperatures on growth of bio-agent

In order to find optimum as well as suitable temperature for the growth of bio-agent, they were grown at five different temperatures on Potato dextrose agar medium. After 10 days incubation the mycelia dry weight recorded and presented in table 2.

It is evident from the table that the growth of the microorganism increased up to 25^0Ctemperature, there after the growth starts decreasing. The maximum colony growth of the mycelium was observed at 25^0C. The growth of the bio-agent significantly affected with either increase or decrease in temperature. The statistical analysis shows that isolate T1 (8 CP) exhibited the highest growth which was significantly superior to other isolates. Rest of the isolates was at par with each other. Choi *et al.*, (2003) conducted study to investigate physiological characteristics of *Trichoderma* spp. isolated from *Pleurotus* sp. The optimal growth temperature of *Trichoderma* spp. was 27~30^0C. Although *T. longibrachiatum* was able to grow at 37^0C. *Trichoderma viride* showed a high range of temperature tolerance, it grew and sporulated well between temperatures 20 to 35^oC according to Jayaswal *et al.*, (2003)

Effect of pH on growth of the bio-agent

The observations standardized $1x10^5$ spores/ml presented in table 3.

It is evident from the table that the maximum dry weight recorded as 208.81mg, 205.80mg, 209.17mg, 207.11mg, 206.00mg, and 208.14mg at pH 7.0. The minimum dry weight recorded at pH 4.0. Thus, excellent sporulation was recorded at pH 6.5 and 7.0. It has observed that neither alkaline nor acidic conditions are congenial for the growth and sporulation of the bio-agent. Jayaswal *et al.*, (2003) recorded optimum growth and sporulation of *Trichoderma viride* between pH 4.5 to 5.5. The growth and sporulation decreased with either decreasing the pH below 4.5 or above 5.5 respectively, above pH 6.0 with increasing the pH, the growth and sporulation decreased proportionately. At pH 8 and 8.5 the growth and sporulation were very poor. At pH 9, no growth of *T. viride* could be observed. Farooq *et al.*, (2005) were studied *In vitro* effect of pH levels on mycelial growth of *F. oxysporum* f. sp. *ciceri*. He found the most suitable pH level for growth of fungus was 7.0 and 6.0.

References

Choi, Young; Gi-Tae, J., Joung-Sik, C. and Yeong-Geun, C. (2003). Physiological characteristics of green mold

(*Trichoderma* sp.) isolated from oyster mushroom, *Mycobiology*, 31(3): 139-144.

Cooke (1954). Antibiot. Chemother. 4:657.

Dandge V. S. (2012). Effect of some fungicide on growth (in mm) of *Curvularia lunata*, *Fusarium equiseti*, *Phoma vulga*ris and *Botryodiplodia theobromae*. *Journal of Experimental Sciences* 3(3): 19-20.

Devi, Prameela; Prabhakaran, N., Kamil, Deeba; Pandey, Pankaj and Jyoti, L. B. (2012). Characterization of Indian native isolates of *Trichoderma* sp. and assessment of their bio-control efficiency against plant pathogens, *African Journal of Biotechnology*, 11(85): 15150–15160.

Elad, Y., Chet, I., and Henis, Y. (1981). A selective medium for improving qualitative isolation of *Trichoderma* sp. from soil, *Phytoparasitica*, 9: 59-67.

Farooq, Sajid; Muhammad Iqbal and Rauf, Abdul (2005). Physiological Studies of *Fusarium oxysporum* f. sp. *ciceri*, *International Journal of Agriculture & Biology*, 7(2): 275–277.

Guinea, J., Peláez, T., Alcalá, L. and Bouza, E. (2005). Evaluation of Czapeck agar and Sabouraud dextrose agar for the culture of airborne *Aspergillus* conidia. *Diagnostic Microbiology and Infectious Disease* 53 (4): 333–334.

Harman, G. E., Latorre, B., Agosin, S., San Martin, R., Riegel, D. G., Nielsen, P. A., Tronsmo, A. and Pearson, R. C. (1996). Biological and integrated control of Botrytis bunch rot of grape using *Trichoderma* sp. *Biological control*, 7: 259-266.

Jayaswal, R. K., Singh, R. and Yu Su Lee (2003). Influence of physiological and environmental factors on growth and sporulation of or antagonistic strains of *Trichoderma viride*RSR-7, *Microbiology*, 31(1): 36-41.

Kubicek, C. P. and Penttiläme (1998). Regulation of production of plant polysaccharide degrading enzymes by *Trichoderma*. *Trichoderma* and *Gliocladium*. *Enzymes, Biological Control and Commercial Applications*. 49-71.

Lawrence C. Parks (1997). *Handbook of Microbiological Media*, second edition, Atlas, R.M.

Mustafa, A., Khan, M. A., Inam-ul-Haq, M., Pervez, M. A. and Ummad-ud-Din, U. (2009). Usefulness of different culture media for *in vitro* evaluation of *Trichoderma*, *Phytopathol.*, 21(1): 83-88.

Nashwa M. A., Sallam, KAM abo-Elyouusr, M. A. and Hasan, E. (2008). Evaluation of *Trichoderma* sp. as bio-control agents for damping off and wilt dieses of *Phaseolus vulgaris* L. and efficacy of suggested formula. *Egypt. J. Phytopathol*., 36(1-2): 81-93.

Sheila A., Okoth and Odhiambo, J. (2009). Influence of soil chemical and physical properties on *Trichoderma* spp. Occurrence in Taita region, Tropical and Sub tropical Ecosystems, 11: 403-413.

Smith, N. R. and V. T. Dawson. (1944). The bacteriostatic action of Rose Bengal in media used for the plate counts of soil fungi. Soil Sci. 58:467-471.

Sundara Rao, W. V. B. and Sinha, M. K. (1963). *Ind. J., Agric. Sci*., 33:272.

Thom and Church (1926). The *Aspergilli*: 39.

Vanderzant, C. and Splittstoesse, D. F. (1992). Compendium of methods for the microbiological examination of food, 3rdEd. American Public Health Association, Washington, D.C.

Williams, Peter W. and Phillips, Glyn O. (2000). "Chapter 2: Agar". *Handbook of hydrocolloids*. Cambridge: Woodhead, 28pp.

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