



**ISOLATION, PURIFICATION AND CHARACTERIZATION OF BIO PESTICIDE
(*TRICHODERMA*) FROM BILASPUR DISTRICT CHHATTISGARH**

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ABSTRACT

Trichoderma spp. is one of the important biocontrol agents, as it grow well at all temperatures and pH, and also has higher antagonistic activity disease controlling potential caused by soil born pathogen. On the basis of temperatures (15^oC, 25^oC, 35^oC and 45^oC) on Mycelial growth of *Trichoderma* species was varied growth of all 28 isolates was faster at 25^oC whereas at low temperature 15^oC *Trichoderma* isolate no. T-14 grow at faster compared to other isolate. Isolate no. T-14, T-25, T-32 and T-37 are faster growth 25^oC, isolate no T-05, T-14, T-25 and T-47 faster growth at 35^oC and isolate no T-14, T-31, T-25 and T-43 faster growth at 45^oC can be successfully used bio-control conditions. different pH range influence of different pH (5.0, 6.0, 7.0 and 8.0) on Mycelial growth of *Trichoderma* species isolate no T-13, T-14 and T-41 was faster growth of pH-5.0 and isolate no T-14, T-32, T-39, T-42 and T-45 was fast growth of pH-6.0 and isolate no T-13 and T-41 are faster growth of pH-7.0 *Trichoderma* isolate no T-15 and T-31 is faster growth of pH-8.0 comparison than other isolates.

KEYWORDS: Bio Pesticide, *Trichoderma*, Antagonistic Activity.

INTRODUCTION

Trichoderma is a genus belonging to the filamentous Class Deuteromycetes. The members are generally found in all soils (Chet 1987; Samuels, 1996). The fungus is a valuable source for the commercial production of enzymes and helpful in recycling cellulosic waste materials while producing useful by products (Samuels, 1996). *Trichoderma* received the most attention as fungal antagonists not only of soil-borne pathogens but also of foliage pathogens such as *Botrytis cinerea* (Elad, 1994). *Trichoderma* can function at the same time both as microbial antagonists and plant symbionts (Lorito et al.2006).

The success of *Trichoderma* strains as BCAs is due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi, and efficiency in promoting plant growth and defense mechanisms.

MATERIALS AND METHODS

1. MATERIALS

1.1. Soil: Soil is the unique habitat which harbors a variety of micro flora and fauna and gives mechanical all types of microorganism are widely occurs in the soil so *Trichoderma spp.* widely distributed in the soil.

2. METHOD

2.1. soil sample collection

2.2. Characterization of soil sample

2.2.1. Soil pH

2.2.2. Soil EC (electrolytic conductivity)

2.2.3. Organic Matter of soil

2.2.4. Phosphorous (P) available in soil

2.2.5. Potassium (K) available in soil

2.3. Isolation of *Trichoderma* species

Trichoderma spp. after preparation of dilution series each soil sample strepatocycline (300ppm) and copper oxychloride (100ppm) added the in the petridishes having potato dextrose agar (PDA) medium and Petri dishes were incubated at 25^oC

2.4. Pure culture of *Trichoderma* species

After 15 days of incubated culture plates are cut with the help of borer single colony was transferred to another Petri dish in central area (7mm) and incubated at 25^oC for 7days.

2.5. Identification of *Trichoderma* species

Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species.

2.6. Effect of temperatures on the mycelium growth of *Trichoderma*: Temperature is the basis factor for growth & development of any micro-organism isolates pure cultured of *Trichoderma spp.* were used to study temperature.

2.7. Effect of different pH on the mycelium growth of *Trichoderma*: Effect of different pH rang (5.0, 6.0, 7.0, & 8.0) on the mycelia growth of *Trichoderma spp.* was studied 7 mm disc of *Trichoderma spp.* was inoculated on the medium (PDA) Petri dishes were incubated at 25°C using BOD incubator.

RESULTS

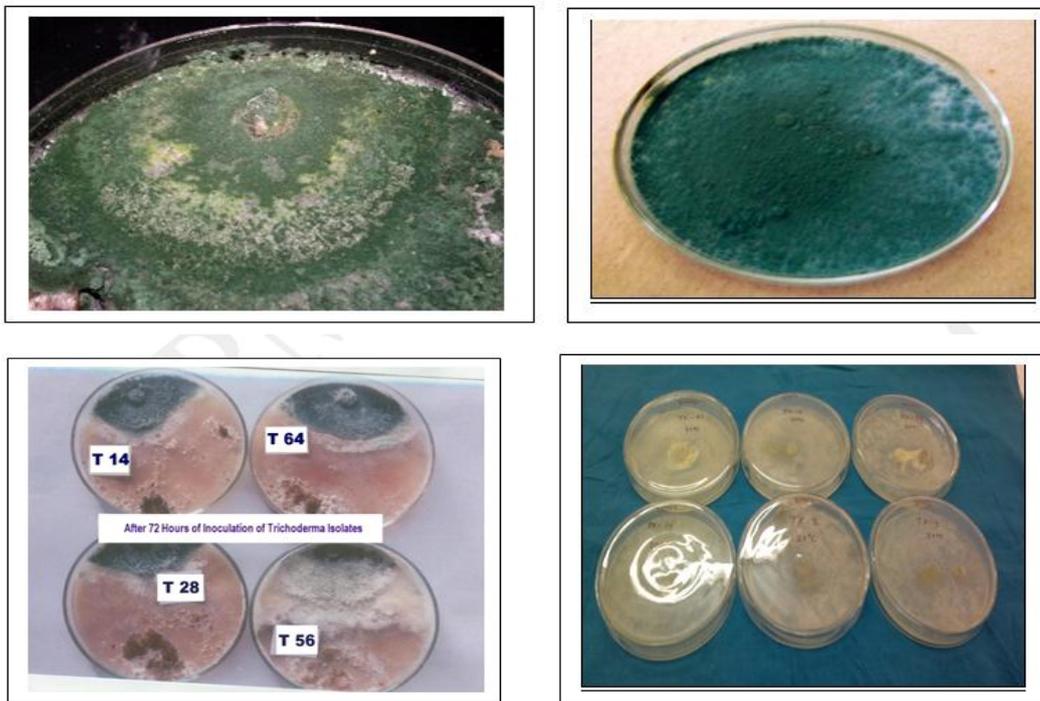


Fig. (a) and (b) Isolate *Trichoderma* Species. (c) Effect of Temperature, (d) Effect of pH.

Table. 1: Characterization of soil of pH, EC, OM, Phosphorous, Potassium Mg/Kg tests, Samples collected from different parts of Bilaspur district (Isolated *Trichoderma* sp.).

S.NO.	SAMPLE NO.	pH VALUE	EC VALUE	OM VALUE	P VALUE	K VALUE
1.	Sample T-02	5.13	0.03	0.64	0.35	1.10
2.	Sample T-04	4.13	0.05	0.65	0.26	1.12
3.	Sample T-05	5.26	0.04	0.73	0.26	34.12
4.	Sample T-10	5.18	0.03	0.59	0.62	3.36
5.	Sample T-11	5.01	0.03	0.59	0.67	1.12
6.	Sample T-13	5.41	0.03	0.63	0.62	1.12
7.	Sample T-14	3.09	0.06	0.67	0.54	1.12
8.	Sample T-15	4.06	0.03	0.61	0.44	1.12
9.	Sample T-17	5.04	0.03	0.43	0.04	29.12
10.	Sample T-24	5.01	0.04	0.64	0.17	25.76
11.	Sample T-25	4.09	0.03	0.71	0.22	23.52
12.	Sample T-29	4.59	0.04	0.23	0.04	31.98
13.	Sample T-31	5.15	0.03	0.45	0.17	30.00
14.	Sample T-32	5.11	0.05	0.09	0.35	30.24
15.	Sample T-35	4.10	0.05	0.58	0.71	2.24
16.	Sample T-36	4.02	0.04	0.35	0.62	1.12
17.	Sample T-37	5.03	0.03	0.62	0.76	1.12
18.	Sample T-39	5.09	0.04	0.56	0.85	2.24
19.	Sample T-40	4.12	0.05	0.59	0.80	1.12
20.	Sample T-41	4.20	0.04	0.57	0.98	2.24
21.	Sample T-42	4.25	0.05	0.61	0.89	3.36
22.	Sample T-43	4.17	0.05	0.41	0.67	1.12

23.	Sample T-44	4.09	0.04	0.33	0.71	1.12
24.	Sample T-45	4.49	0.05	0.31	0.67	1.00
25.	Sample T-46	5.21	0.05	0.49	0.80	2.24
26.	Sample T-47	4.00	0.05	0.50	0.80	2.24
27.	Sample T-48	5.19	0.02	0.54	0.85	3.36
28.	Sample T-50	5.79	0.02	0.48	0.62	26.88

Table. 2: Effect of Temperatures on the Mycelium Growth of *Trichoderma* at 15 °C, 25 °C, 35 °C, 45 °C.

S.NO.	Sample No.	15 °C (Maximum V Value)	25 °C (Maximum V Value)	35 °C (Maximum Value)	45 °C (Maximum Value)	Time Duration (HRS.)
1.	Sample T-24			17.00 mm		24 Hrs.
2.	Sample T-14			72.00mm		48 Hrs.
3.	Sample T-14, Sample T-15		90.00mm			72 Hrs.
4.	Sample T-14	90.00mm	90.00mm	90.00mm		96 Hrs.

Table. 3: Effect of pH on the Mycelium Growth of *Trichoderma* at pH – 5, 6, 7, 8.

S. NO.	Sample No.	pH – 5 (MAXIMUM V VALUE)	pH – 6 (MAXIMUM V VALUE)	pH –7 (MAXIMUM VALUE)	pH-8 (MAXIMUM VALUE)	TIME DURATION (HRS.)
1.	Sample T-31, Sample T-32			35.00mm		24 Hrs.
2.	Sample T-15, Sample T-41			90.00mm		48 Hrs.
3.	Sample T-13, Sample T-14 Sample T-42	90.00mm	90.00mm	90.00mm	90.00mm	72 Hrs.
4.	Sample T-14	90.00mm	90.00mm	90.00mm	90.00mm	96 Hrs.

DISSCUSSION

Samples Characterization of Soil

Availability of nutrient for plant depends on acidity and alkalinity of soil which is showed in pH scale. The fertility of soil can be assessed by soil pH. All 50 samples collected by different parts of Bilaspur in acidic in nature. Minimum pH recorded by soil sample no.14 (3.09), soil sample no 34 (3.70) and soil sample no. 19 (4.00). Maximum pH recorded by soil sample no. 50 (5.79), soil sample no. 28 (5.75) and soil sample no. 12 (5.72) comparatively.

Presence of higher salt quantity affects the plant growth specially plant termination. The water absorbing capacity of plant is also affected in soil electric conductivity. All 50 samples collected by different parts of Bilaspur in normal in nature. Minimum EC recorded by soil sample no. 12, 21 and 28 i.e. 0.01. Maximum EC recorded by soil sample no 41 (0.07) and soil sample no 14 (0.06) comparatively. All 50 samples collected by different parts of Bilaspur in high organic matter (OM) value. Maximum organic matter recorded by soil sample no. 5, 25, 6 and 20 i.e. 0.73, 0.71, 0.68 and 0.68. Minimum organic matter recorded by soil sample no 32 (0.09), soil sample no 29 (0.23) and soil sample no 27 (0.27) comparatively.

All 50 samples collected by different parts of Bilaspur in average phosphorus value. Maximum phosphorus

contains recorded by soil sample no. 5 (34.12), soil sample no 29 (31.98) and soil sample no. 32 i.e., 30.30 ppm/kg soil. Minimum phosphorus recorded by soil sample no 17, 18, 22 and 29 (0.04) comparatively. In case of potassium value maximum potassium recorded soil sample no. 5 (34.12ppm/kg), soil sample no. 29 (31.98ppm/kg) and sample no. 32 (30.30 ppm/kg) respectively. Minimum potassium value recorded by soil sample no. 19 (0.80), sample no. 45 (1.00) and soil sample no. 2 (1.00 ppm/kg soil).

Isolated *Trichoderma* Species

All soil samples were brought to the laboratory of end series method (Dhingra & Sinclar, 1992) one gram of soil sample was used for the isolation of isolates *Trichoderma spp.* Total 28 *Trichoderma* species isolated from 50 collected soil samples.

Effect of Temperatures on the Mycelium Growth of *Trichoderma* Species

Temperature is the basis factor for growth & development of any micro-organism isolates pure cultured of *Trichoderma spp.* were used to study temperature. Influence of different temperature effect (15°C, 25°C, 35°C, 45°C) on mycelia growth of *Trichoderma* species was studied. Seven mm disc cut with the help of borer at 10 days old culture of all 28 isolates of *Trichoderma* spp was incubated on the medium (PDA) Petri dishes were incubated of different

temp i.e., 15⁰c, 25⁰c, 35⁰c,45⁰c using BOD incubator. Mycelial growth (mm) was recorded after at an interval of 24 Hrs. the completion of growth (90mm).

Data presented in different table on the basis of temperatures (15⁰C, 25⁰C, 35⁰C and 45⁰C) on mycelia growth of *Trichoderma* species was varied growth of all 28 isolates was faster at 25⁰C whereas at low temperature 15⁰C *Trichoderma* isolate no. T-14 grow at faster compared to other isolate. Isolate no. T-14, T-25, T-32 and T-37 are faster growth 25⁰C, isolate no T-05, T-14, T-25 and T-47 faster growth at 35⁰C and isolate no T-14, T-31, T-25 and T-43 faster growth at 45⁰C can be successfully used bio-control conditions.

Effect of Different pH on The Mycelium Growth of *Trichoderma* Species: pH of soil also does part affect the growth of organisms. To know how pH regulates the growth of the organisms, this test of pH levels will be conducted. In this method Potato Dextrose Agar medium will be adjusted to pH 5.0, 6.0, 7.0 and 8.0 by buffer tablets and poured in Petri dishes. The plate's shell should be inoculated by 7 mm disc of 7-10 days old culture of *Trichoderma spp.* after inoculation, the plates shall be incubated in-side incubator at 25⁰C temperature. Mycelial growth will be recorded at an interval of 24 hrs. Data presented in the table of different pH range influence of different pH (5.0, 6.0, 7.0 and 8.0) on Mycelial growth of *Trichoderma* species isolate no T-13, T-14 and T-41 was faster growth of pH-5.0 and isolate no T-14, T-32, T-39, T-42 and T-45 was fast growth of pH-6.0 and isolate no T-13 and T-41 are faster growth of pH-7.0 *Trichoderma* isolate no T-15 and T-31 is faster growth of pH-8.0 comparison than other isolates. Some researcher reported that temperature and pH are very important for growth and development of the most.

CONCLUSION

It is concluded from the present study that *Trichoderma spp.* is one of the important biocontrol agents, as it grow well at all temperatures and pH, and also has higher antagonistic activity disease controlling potential caused by soil born pathogen. It can be used in commercial level for controlling soil born disease. It is better to use indigenous strain of *Trichoderma* rather than other region and characterized them so that the mass scale production of effective and efficient strain of *Trichoderma* can be taken up to distribute to the farmers of Bilaspur region.

Isolation of *Trichoderma* from the other parts of our state will lead to identification of more potential isolates and which can be useful in controlling the disease of different crops grown in Chhattisgarh.

Several species of the genus *Trichodesrma* received attention mainly due to their importance in biological control of soil borne plant pathogens. Antibiosis, mycoparasitism and competition for nutrients are the mechanisms involved in biological control. Recent

studies have shown that they are opportunistic, avirulent plant symbionts, as well as the parasites of other fungi. Many species of the genus *Trichoderma* have also been recognized for their plant growth promotion abilities.

In the present study, three species of *Trichoderma* isolate T-04, T-10 and T-50 from soil samples collected from Bilaspur i.e. Mopka, Bilha and Lakhram region have been investigated *in vitro* and *in vivo* for plant growth characters and bio-control properties.

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