J. Mycopathol, Res, **50(2)** : 255-259 (2012)

In vitro evaluation of *Trichoderma* strains against *Macrophomina phaseolina* causing Fruit Rot of *Coccinia indica*

U. N. BHALE, V. S. SAWANT, V. S. CHATAGE AND J. N. RAJKONDA*

Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg, Tq. Tuljapur, Dist Osmanabad, 413602 (M.S.) India

**Department of Botany, Yeshwantrao Chavan College, Tuljapur, Dist. Osmanabad, 413601 (M.S.) India*

Received : 28.09.2011 Accepted : 20.04. 2012 Published : 29.10.2012

Fruit rot of *Coccinia indica* Wight and Arn is caused by *Macrophomina phaseolina* (Tassi) Goid. Integrated management practice based on biocontrol is considered to be safe and sustainable measure for management of plant diseases. This paper describe the efficacy of different strains of *Trichoderma* species against *M.phaseolina* by dual culture method under *in vitro* conditions. *Trichoderma viride* (12), *T. harzianum* (12), *T. virens* (10), T. *koningii* (07), and *T. pseudokoningii* (05) strains were isolated and used for antagonistic study. Among 12 isolates of *T. viride*, Tv_1, Tv_2 and Tv_9 isolates showed maximum whereas Tv_7 and Tv_{11} isolates showed minimum antagonism. In *T. harzianum* isolates, Th_1 and Th_5 showed indicative results as compared to others but only 50% inhibition was found in Th_2. In case of *T. virens*, significant inhibitions were found in Tvr_3, Tvr_4 and Tvr_8 isolates but Tvr_1 isolate was found less inhibitory. In *T. koningii*, only Tk_4 isolate was found eloquent to other strains. Out of five isolates of *T. pseudokoningii* Tp1 Tp_2 and Tp_3 were found maximal and Tp_5 minimal in inhibition.

Key words : *Macrophomina phaseolina*, strains of *Trichoderma* species, dual culture

INTRODUCTION

Plants have always been an exemplary source of drugs and many drugs currently available have been derived directly or indirectly from them. A vast majority of population particularly those living in villages depends largely on medicinal plants for treating and curing diseases. One such medicinal plant ivy gourd (*Coccina indica* Wight and Arn.) of the family *Cucurbitaceae* is most important vegetable and medicinal plant, distributed in Tropical Asia, Africa, Pakistan, India and Sri Lanka (Cooke, 1903; Sastri; 1950). It is a climber and trailer (Nasir and Ali, 1973). Different names of ivy gourd like the parwal, kundru, tondli are in market. It is native to Africa and has been growing in the Indomalayan region of Asia for many centuries (Singh, 1990). It has white flowers and small cucumber like fruits which turn bright scarlet red when ripened. Ivy gourd has vitamin A,β- carotene and is a good source of protein. The fruit of *Coccinia* is used as vegetable when green and eaten fresh when ripened into bright scarlet color. Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for skin diseases bronchial catarrh, bronchitis and unani systems of medicine (Behl *et al.*, 1993). It shows also hypoglycemic activities (Mukerjee *et al.*, 1972 and Nahar *et al.*, 1998). The juice of the roots and leaves are considered to be a useful in treatment of diabetes (Chopra *et al.*, 1925). A post and pre-harvest food loss constitutes a vast complex of physical and biological changes due to microorganisms like fungi and bacteria. Diseases are very important in reducing market quality of ivy gourd fruit and are primarily responsible for the post and pre harvest losses of 10-35%.

However, ivy gourd fruits during field and storage are attacked by *Macrophomina phaseolina* which is severe in Marathwada region of Maharashtra. Since, biocontrol agents for protection of seeds and control of seed borne diseases offer farmers an alternative source for chemical fungicides which is highly effective (Callan *et al.*, 1997). It is therefore necessary to develop alternative ways of control. One such alternative is biological control, in which

*Email : jnrajkonda@gmail.com

microorganisms are selected for their ability to antagonize pathogens. Various disease management methods have been implemented to combat and eradicate pathogenic fungi which include cultural, regulatory, physical, chemical and biological methods. All these methods are effective only when employed well in advance as precautionary measure (Kata , 2000). Therefore an investigation has been made to evaluate the different isolates of *Trichoderma* species against *M. phaseolina* inciting fruit rot of ivy gourd.

MATERIALS AND METHODS

Isolation and identification of test pathogen

Fruits showing symptoms of fungal infection were collected and symptomatology of the disease was studied under natural and laboratory conditions. Isolation of the pathogen was done from each distinct type of symptoms produced on fruits. Infected fruit parts were cut into small pieces by sterilized blade then surface sterilized with mercuric chloride (0.1%) for 1 min. These pieces were then washed thrice with sterilized distilled water and dried by sterilized blotting paper. These pieces were placed on potato dextrose agar (PDA) medium and incubated at 28 ±2°C. The fungus was isolated and identified as *M. phaseolina* (Ellis, 1971). The culture was deposited at Department of Botany, Arts, Science and Commerce College, Naldurg. The pathogenicity of test fungus was confirmed by inoculating ivy gourd fruits properly (Thompson, 1996).

Isolation of Trichoderma spp.

Rhizosphere soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra. From the rhizosphere soil samples, desired strain of *Trichoderma* species were isolated by using potato dextrose agar (PDA) and *Trichoderma* selective medium (TSM) by dilution plate technique. The isolated strains were identified by reculturing on another Petriplates containing sterilized TSM. The isolated strains were identified up to species level based on colony characters, growth of fungus and structure of mycelium, conidiophores and conidia (Kubicek and Harman, 2002). All *Trichoderma* spp. were purified by hyphal tip culture technique. The isolated strains of *Trichoderma* species were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

Dual culture experiment

Antagonistic efficacy of different isolates of *T. viride* (12), *T. harzianum* (12), *T. virens* (10), *T. koningii* (7), and *T. pseudokoningii* (5) were tested against the isolated pathogenic fungus by dual culture experiment (Morton and Stroube,1955). *Trichoderma* species and test fungus was inoculated at 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ±2°C for 9 days. Monoculture plates of both served as control. Nine days after incubation (DAI), radial growth of test fungus and *Trichoderma* isolates were measured. Colony diameter of test fungus in dual culture plate was observed and compared with control. The growth inhibition was calculated by using the formula: 100 X C - T / C, where C = growth in control and T = growth in treatment (Vincent, 1947).

Statistical analyses

Arcsine transformation of biological control (*Trichoderma* species) percentage was done by using following formula - $Y = \arcsin \sqrt{p} = \mathrm{Sin}^{-1}\sqrt{p}$ where, p is the percentage and Y is the result of transformation

Statistical analyses of the experiments were performed using the Handbook of Biological Statistics (McDonald, 2008) and Mungikar (1997).

RESULTS AND DISCUSSION

Isolation and identification of test pathogen

Fruits showing blackish, gray, black, grayish white, blackish gray containing symptoms were collected from different locations of Marathwada region of Maharashtra and ten isolates of *M. phaseolina* were isolated.

Isolation of Trichoderma spp.

Isolates of five species of *Trichoderma*, *T. viride* Pers. (12), *T. harzianum* Rifai, (12), *T. virens* J. Miller, Giddens and Foster (10), *T. koningii*

Table 1 : Influence of *Trichoderma viride* isolates on radial growth of *M. phaseolina.*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tv_1	Naldurg	12.00	86.60 (98.84)
Tv_2	Osmanabad	16.02	82.34 (95.80)
Tv_3	Latur	29.77	67.91 (80.03)
Tv_4	Nanded	31.12	65.55 (79.95)
Tv_5	Jalna	24.13	73.50 (87.67)
Tv_6	Aurangabad	34.00	60.17 (70.75)
Tv_7	Beed	43.00	52.25 (59.99)
Tv_8	Paranda	38.12	57.09 (70.31)
Tv_9	Ashti	15.19	83.70 (96.89)
Tv_{10}	Omerga	32.99	63.46 (74.58)
Tv_{11}	Ahmedpur	41.29	54.73 (63.85)
Tv_{12}	Hingoli	30.00	66.66 (79.44)
Control		89.46	--
SEm ±			7.71
CD (p=0.05).			10.34

Radial growth and per cent inhibition values are means of three replications Figures in parentheses are arcsine transformed values of % inhibition.

Table 2: Influence of *Trichoderma harzianum* isolates on radial growth of *M.phaseolina*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Th_1	Tuljapur	14.77	83.30(96.95)
Th_2	Kallam	45.11	50.01(55.68)
Th_3	Ausa	41.00	54.68(63.86)
Th_4	Nanded	22.00	75.81(90.21)
Th_5	Badnapur	13.11	85.68(98.32)
Th_6	Beed	38.17	57.96(67.72)
Th_7	Paithan	31.00	66.10(79.44)
Th_8	Paranda	34.77	62.41(72.90)
Th_9	Patoda	28.12	69.62(82.49)
Th_{10}	Nilanga	39.11	54.88(63.85)
Th_{11}	Udgir	33.12	63.45(74.59)
Th_{12}	Parbhani	19.00	79.13(94.24)
Control		90.00	--
SEm ±			3.43
CD (p=0.05).			7.54

Radial growth and per cent inhibition values are means of three replications.Figures in parentheses are arcsine transformed values of % inhibition.

Oud.(07) and *T.pseudokoningii* Rifai. (05) were isolated from irrigated and non-irrigated rhizosphere soil of Marathwada region of Maharashtra. Isolates are deposited at Department of Botany, Arts, Science and Commerce College, Naldurg.

Dual culture experiment

Among 12 isolates of *T. viride*, Tv_1 (78%), Tv_2 (74.11%) and Tv_9 (75.12%) showed maximum

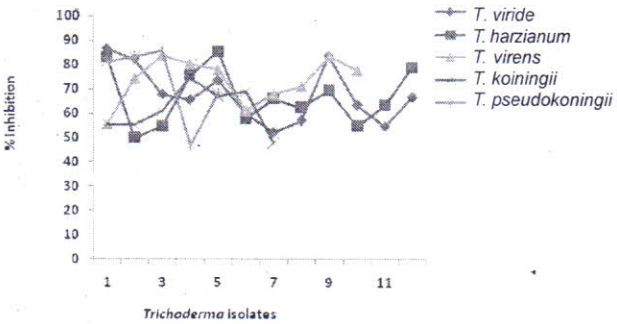

Fig.1: Influence of different isolates of *Trichoderma* species against *Macrophomina phaseolina.*

Table 3: Influence of *Trichoderma virens* isolates on radial growth of *M.phaseolina*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tvr_1	Naldurg	40.00	55.55 (65.15)
Tvr_2	Murum	23.00	74.44 (88.12)
Tvr_3	Beed	15.00	83.57 (97.12)
Tvr_4	Badnapur	18.00	80.24 (94.95)
Tvr_5	Kannad	20.11	77.90 (91.67)
Tvr_6	Parbhani	34.77	61.11 (72.06)
Tvr_7	Nilanga	30.99	67.91 (80.03)
Tvr_8	Udgir	36.02	71.11 (83.77)
Tvr_9	Nanded	15.11	83.46 (97.19)
Tvr_{10}	Hingoli	20.00	77.77 (92.23)
Control		89.00	--
SEm ±			2.98
CD(p = 0.05)			6.73

Radial growth and percent inhibition values are means of three replications.Figures in parentheses are arcsine transformed values of % inhibition.

antagonism as compared to others whereas, Tv_7 (47.03%) and Tv_{11} (49.26%) were reduced the antagonism (Table 1). *T. harzianum,* isolates Th_1 (83.3%) and Th_5 (85.68%) showed indicative results as compared to other isolates. But only 50% per cent inhibition was found in Th_2 (Table 2). Table 3 illustrated that, *T. virens* isolates i.e. Tvr_3 (83.57%), Tvr4 (80.24%) and Tvr_9 (83.46%) were found better antagonistic and followed by other isolates. In case of *T. koningii* isolates, only Tk_4 (74.56%) was found eloquent followed by Tk_6 (69.02%) and Tk_5 (66.97%) (Table 4). Out of five isolates of *T. pseudokoningii,* Tp_1 (81.01%), Tp_2 (83.34%) and Tp_3 (85.84%) showed maximal per cent inhibition (Table 5). Among all isolates of *Trichoderma* species, $Tv_{1,2 \text{ and } 9}$, $Th_{1,5 \text{ and } 12}$, $Tvr_{3,4 \text{ and } 9}$ and $Tp_{1,2 \text{ and } 3}$ isolates were found better but *T.koningii* isolates were failed to showed more antagonism (Fig.1).

The findings of workers have reported the use of

Table 4: Influence of *Trichoderma koningii* isolates on radial growth of *M. phaseolina*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tk_1	Naldurg	40.00	55.55(64.29)
Tk_2	Osmanabad	40.00	55.55(64.29)
Tk_3	Latur	45.00	61.11(71.16)
Tk_4	Aurangabad	23.11	74.56(88.05)
Tk_5	Jalna	30.00	66.97(79.05)
Tk_6	Parbhani	28.14	69.02(78.60)
Tk_7	Nanded	47.01	48.04(56.13)
Control		87.88	--
SEm ±			3.40
CD(p=0.05)			8.26

Radial growth and per cent inhibition values are means of three replications.Figures in parentheses are arcsine transformed values of % inhibition.

Table 5: Influence of *Trichoderma pseudokoningii* isolates on radial growth of *M. phaseolina*

Isolates	Locations	Radial growth of *M.phaseolina* (mm)	Inhibition %
Tp_1	Ausa	17.11	81.10 (95.42)
Tp_2	Tuljapur	15.00	83.34 (96.89)
Tp_3	Jalna	13.11	85.84 (98.32)
Tp_4	Sillod	47.66	46.81(53.14)
Tp_5	Ardhapur	29.12	67.91 (80.42)
Control		90.00	--
SEm ±			1.68
CD(p=0.05)			4.66

Radial growth and per cent inhibition values are means of three replications.Figures in parentheses are arcsine transformed values of % inhibition.

Trichoderma species as biological control against number of plant pathogenic fungi. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum* (Hesamedin Ramezani, 2008). Interaction implies that a single isolate of antagonist can be highly effective against an isolate of *M. phaseolina*, but may have only minimal effects on other isolates of *M. phaseolina* (Aly *et al.*, 2007). In *in vitro*, control of *R. solani, F. oxysporum* and *M. phaseolina* were achieved with *T. koningii, T. hamatum* and *T. harzianum* (Arora, 1990). Krishnaveni (1991) reported that seed treatment with *T.viride* was very effective in controlling charcoal rot of soybean and similarly, seed treatment with *T.viride* and *T.harzianum* also reported to be reducing incidence of charcoal rot of cowpea (Ushamalini *et al.*, 1997). The use of bioagents and oil cakes do not harm the environment but improve sustainably of field soils (Jaiman *et al.*, 2009). Deshmukh and Rout (1992) reported *T.harzianum* and *T.viride* were effective in inhibiting the mycelial growth of *M. phaseolina* and reducing the disease incidence in pot culture. Indra and Subbiah (2003) reported the least incidence of root rot in black gram (*M. phaseolina)* treated with *T.viride* and *Rhizobium*. In dual cultures, *T.viride, T.harzianum* and *Aspergillus vesicolor* were effective in inhibiting the growth of *M.phaseolina* (Choudhary *et al.*, 2010). Recently, Waghmare and Kurundkar (2011) reported efficacy of *Trichoderma* species against *Fusarium oxysporum* f. sp. *carthami* causing wilt of safflower. The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda *et al.*, 2011).

According to Papavizas and Lumsden (1980) the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably due to antibiosis, lysis, competition and mycoparasitism. Ayers and Adams (1981) indicated that interactions observed *in vitro* do not necessarily confirm their operation for decrease in pathogen populations and reduction in diseases observed in natural conditions.

The *in vitro* screening with our arbitrary system of bio-antagonists effective against soil borne pathogens is a simplistic approach to understand a small sector of biological system in disease control. Therefore, it may be more prudent to search for biological antagonists against specific pathogen and evaluate blends of antagonists for wider applications (Baker and Cook, 1974). Our results showed that although considerable success in biocontrol is achieved under laboratory conditions the outcome is also proportionate under field conditions. Hence, work is needed towards a better understanding and development of technologies that allow the biocontrol agent to spread and proliferate in soil. Papavizas (1985) suggested that the research should be directed towards the improvement of strains of biological agents that are more capable of becoming established and surviving under adverse field conditions. Thus, it is obvious that biological control offers durable, environmentally safe and cost effective alternative to chemicals for the efficient management of plant disease.

ACKNOWLEDGEMENT

Authors are thankful to the Director, Agharkar Research Institute (ARI) Pune for the identification

of *Trichoderma* species and also acknowledge to UGC, WRO Pune for the financial assistance.

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