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In Vitro Evaluation of Bioagents against Post Harvest Disease Causing Fungi of Mandarin

Pallavi. J. Mahajan[1], K. S. Raghuwanshi[2] and R. A. Raut[3]

Abstract: *Isolation carried out from infected mandarin fruits and recorded association of three fungi viz; Aspergillus niger, Penicillium digitatum and Rhizopus stolonifer and pathogenecity of all three isolates were proved. The pathogen Aspergillus niger produced brown to black lesions on fruits, Penicillium digitatum produced greenish spots on fruits while Rhizopus stolonifer produced blackening thereafter inducing severe rotting. In the study on effects of bioagents, bioagent Trichoderma harzianum significantly suppressed the growth of Penicillium, Aspergillus while others were not effective.*

Key words: *Mandrin orange, bioagents, bio-efficacy, post harvest.*

INTRODUCTION

Orange (*Citrus reticulata* Blanco) is most common among citrus fruits in India and occupies nearly forty percent of the total area under citrus cultivation. India is the third largest producer of orange in the world. Although, India is second in area and third in production of orange in the world, the productivity/hectare is very low as compared to the US, Indonesia, Turkey and other countries where the crop is grown commercially. The area under orange cultivation in India is 31.12 mha and the production is 290.63 mt during 2013. In terms of productivity, India ranks 64th in the world with only 9.23 t/ha. Maharashtra is the leading orange (Mandarin) producing State with 37.0 mt production in 2013 accounting for 40% of total production with productivity of 2.8 mt/ha. The area under cultivation of mandarin orange in Maharashtra is 13.3 mha (Anonymous, 2013). The seasonal disease profile of mandarin orange fruit clearly marked two distinct peak periods of fungal rot spoilage. In the first peak, the major monthly fruit rot loss was inflicted by green and blue mold rots caused by *Penicillium digitatum* (1.78-2.44%) and *P. italicum* (0.73-1.30%). The second peak period of fruit spoilage inflicted by *Aspergillus niger* was observed during the summer months of May (5.27%) and June (6.05%). The cumulative rottage losses during the entire marketing season were 34.30% (Verma, 2007). Several species and varieties of citrus are subjected to various diseases caused by fungi, bacteria, viruses, phytoplasma and other pathogenic entities. In fungal post harvest diseases of mandarin (*Citrus reticulata* Blanco), fruit spoilage or decay after harvest is caused by many fungal pathogens such as *Penicillium italicum* and *Penicillium digitatum* causing green mold and blue mold, black core rot by *Aspergillus spp*, *Rhizopus* rot by *Rhizopus spp*, *Colletotrichum gloeosporioides* causing anthracnose and fruit rot in storage. Most of these fungal pathogens are carried out from the orchard soils, contaminated handling practices and storage

[1] Ph.D Scholar, Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri, Maharashtra, India- 413 722;

[2] Associate Professor, Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri, Maharashtra, India- 413 722

[3] Jr. Plant Pathologist, Regional Fruit Research Station, Vengurle, *E-mail: pallavi.mahajan90@gmail.com*

conditions after harvest. Hence, it was felt necessary to study the *in vitro* evaluation of bio agents against post harvest disease causing fungi of mandarin.

MATERIAL AND METHODS

Isolation of pathogens associated with infected fruits of mandarin orange

The micro-organisms responsible for spoilage of mandarin fruits were isolated on potato dextrose agar medium by employing tissue isolation method. Fungi isolated from diseased samples were identified on the basis of morphological characters observed under microscope and identified as *Aspergillus, Penicillium* and *Rhizopus*.

In vitro evaluation of bioagents

Bio efficacy of antagonist against test pathogens

The antagonistic potential of two species of Trichoderma *viz.*, *Trichoderma viride* and *Trichoderma harzianum* was assessed against the test pathogen viz., *Aspergillus, Penicillium* and *Rhizopus* by the dual culture method. The mycelial disc of 5mm diameter were cut from the margin of 7 days old culture of both these pathogens and antagonistic biocontrol agents and placed opposite to each other on PDA in petri plates. The inoculated discs were placed 30 mm away from each other. The petri plates inoculated with disc of test fungus alone served as control. The inoculated plates incubated at 27±2 °C in BOD incubator for 7 days. The radial growth of test fungi was measured to assess the antagonistic potential of *Trichoderma spp*. against the test pathogen. The percent growth inhibition of intersecting colonies was calculated as per formula given below

$$Growth\ inhibition\ \% = \frac{Colony\ growth\ in\ control\ plate - colony\ growth\ in\ intersecting\ plate}{Colony\ growth\ in\ control\ plate}$$

Or by using following formula (Padule and Shinde, 1986)

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

Where,

I = Per cent inhibition of fungal growth.

C = Growth of the test fungus in control.

T = Growth of test fungus in treatment i.e. bio-agents.

Bio efficacy of Yeast *(Saccharomyces* spp.) and *Pseudomonas* (P. *fluorescens)*

Antagonastic potential of bioagent i.e. Yeast (*Saccharomyces* spp.), Pseudomonas (*Pseudomonas fluorescens*) and some strains of Yeast were assessed against the test pathogen *viz*., *Aspergillus*, *Penicillium* and *Rhizopus* by dual culture metohod. Mycelial discs of 5 mm diameter were cut from the margin of seven days old culture of test pathogen and placed at the centre on PDA in petri plates. Similarly two days old culture of bioagents viz., Yeast (*Saccharomyces* spp. strain-1) and *Pseudomonas fluorescens* were stick around the pathogen and incubated in BOD incubator at 27+1°C temperature. Radial growth of test pathogens were measured and compared with the growth of test pathogen obtained in control plate.

RESULTS AND DISCUSSION

In vitro evaluation of bio agents against post harvest disease causing pathogens

The efficacy of five bioagents *viz*., *Trichoderma viride, Trichoderma harzianum,* yeast *Saccharomyces cerevisiae* strain 1, some new unknown strains of yeast and *Pseudomonas fluorescens* were evaluated *in vitro* for the control of post harvest pathogens of mandarin orange and the results are presented in Tables 1, 2 and 3.

1. Aspergillus niger

The results of *in vitro* evaluation of bioagents against *Aspergillus niger* presented in Table 1. Significant differences amongst different bioagents evaluated. It was observed that among 8 bioagents only *Trichoderma harzianum* showed cent percent inhibition of fungus. While Yeast *Saccharomyces cerevisiae* strain-1 showed significantly low colony diameter (41.67mm), more than 50 % inhibition over control.

The bioagents Yeast 1, 2 and 3, *Peudomonas fluorescens* and *Trichoderma viride* were either not effective or less effective against *Aspergillus niger*. Growth and sporulation of pathogen were not observed in *Trichoderma harzianum* treatment while growth and sporulation of pathogen were medium to good in remaining bioagents treatments.

Thus, results indicated that growth of pathogen was suppressed by *Trichoderma harzianum* only through competition mechanism.

Table 1
Effect of bioagent over growth and sporulation of pathogen, *Aspergillus niger.*

Sr. No.	*Treatment*	*Mean colony diameter(mm)*	*Per cent inhibition over control*	*Growth*	*Sporulation*
1	Yeast strain-1	41.67	53.7	+++	+++
2	Yeast – 14	90.00	00.00	++++	++++
3	Yeast – 18	90.00	00.00	++++	++++
4	Yeast – 21	88.33	1.85	+++	+++
5	Yeast – 22	88.33	1.85	+++	+++
6	*P.fluorescens*	90.00	00.00	++++	++++
7	*T.harzianum*	0.00	100.00	-	-
8	*T.viride*	88.33	1.85	+++	+++
9	Control	90.00	00.00	++++	++++
	SEm (±)	**1.32**			
	CD @1%	5.32			

- = No growth, ++ = Poor, +++ = Medium, ++++ = Good

2. Penicillum digitatum

In vitro evaluation of bioagents (Table 2) against *Penicillium digitatum* revealed that among 8 bioagents only *Trichoderma harzianum* showed cent per cent inhibition of fungus followed by *Trichoderma viride* (46.33mm).

The bioagent Yeast *Saccharomyces cerevisiae* strain-1, Yeast-1, 2, 3 and 4, *Pseudomonas fluorescens* were not effective against *Penicillium digitatum* (Table 2). Growth and sporulation of pathogen were not observed in *Trichoderma harzianum* treatment. While growth and sporulation of pathogen were medium to good in *Trichoderma viride*. Thus, the results indicated that the growth of pathogen was only suppressed by *Trichoderma harzianum*.

Table 2
Effect of bioagents over growth and sporulation of pathogen, ***Penicillium digitatum***

Sr. No.	*Treatment*	*Mean colony diameter (mm)*	*Per cent inhibition over control*	*Growth*	*Sporulation*
1	Yeast strain-1	90.00	00.00	++++	++++
2	Yeast – 14	90.00	00.00	++++	++++
3	Yeast – 18	90.00	00.00	++++	++++
4	Yeast – 21	90.00	00.00	++++	++++
5	Yeast – 22	90.00	00.00	++++	++++
6	*P.fluorescens*	90.00	00.00	++++	++++
7	*T.harzianum*	0.00	100.00	-	-
8	*T.viride*	48.33	46.3	++	++
9	Control	90.00	00.00	++++	++++
	SEm (±)	**0.50**			
	CD @1%	2.00			

- = No growth, ++ = Poor +++ = Medium, ++++ = Good

3. Rhizopus stolonifer

In vitro evaluation of bioagents against *Rhizopus stolonifer* (Table 3) indicated that among 8 bioagents, only *Trichoderma harzianum* showed significantly low colony diameter (68.33) over control and percent inhibition over control was 24.07% only. The bioagents Yeast *Saccharomyces cerevisiae* strain-1, Yeast 1, 2, 3 and 4, *Peudomonas fluorescens* and *Trichoderma viride* were not at all effective against *Rhizopus stolonifer*. Thus, the results indicated that growth of pathogen was only suppressed by *Trichoderma harzianum*.

Table 3
Effect of bioagents over growth and sporulation of pathogen, *Rhizopus stolonifer.*

Sr. No.	*Treatment*	*Mean colony diameter(mm)*	*Per cent inhibition over control*	*Growth*	*Sporulation*
1	Yeast strain-1	90.00	00.00	++++	++++
2	Yeast – 14	90.00	00.00	++++	++++
3	Yeast – 18	90.00	00.00	++++	++++
4	Yeast – 21	90.00	00.00	++++	++++
5	Yeast – 22	90.00	00.00	++++	++++
6	*P.fluorescens*	90.00	00.00	++++	++++
7	*T.harzianum*	68.33	24.07	+++	+++
8	*T.viride*	90.00	00.00	++++	++++
9	Control	90.00	00.00	++.++	++++
	SEm (±)	**0.50**			
	CD @1%	2.00			

- = No growth, ++ = Poor +++ = Medium, ++++ = Good

The results are in confirmation with the findings of Diaz-Borras and Vila-Aguilar (1990) who studied the antagonistic nature of *T. viride* and reported that *T.viride* was found to be the most effective in controlling *Penicillium digitatum*, the causal organism of post harvest rot of citrus fruits. Sivakumar *et al.* (2000) also showed that both, antibiosis and direct parasitism, have also been suggested to be the mode of action of *T. harzianum* in reducing the incidence of post harvest stem end rot (*Botrydiplodia theobromae*), anthracnose (*Colletotrichum gloeosporoides*) and brown spot (*Gliocephalotrichum microclahmydosporum* on rambutan fruits. Adekunle *et al.* (2001) reported that *Trichoderma harzianum* was effective to control of several pathogens.

Maharshi *et al.* (2008) reported eco-friendly management of blue mould rot (*Penicillium italicum*) of kinnow (*citrus deliciosa*) fruits using biocontrol agents including an unrecorded yeast (*Sporidiobolus pararoseus*) from Rajasthan, India. Management of blue mould rot (*Penicillium italicum*) of Kinnow fruits explored using biocontrol agents viz. *Trichoderma, Gliocladium, Bacillus, Pseudomonas, Debaryomyces*, and *Sporidiobolus* spp. revealed variable effects *in vitro*. Palazon *et al.* (1988) reported that *Trichoderma viride* was strongly antagonistic to *Alternaria alternata*, *Botrytis cinera* and especially to *Rhizopus nigricans.* On inoculating apples with *Trichoderma viride*, rots due to *Alternaria alternata, Botrytis cinerea* and *Rhizopus stolonifer* were reduced by 20-40 %.

CONCLUSION

In the *in vitro* evaluation of bioagents, it was observed that *Trichoderma harzianum* showed maximum inhibition of the growth of *Aspergillus niger* , *Penicillium digitatum* and *Rhizopus stolonifer* than *Trichoderma viridae*. In case of Yeast *Saccharomyces cerevisiae*- strain 1 was able to restrict the growth of *Aspergillus niger In vitro.* While, other four strains of yeast and *P. fluorescence* were failed to inhibit the growth of all three post harvest pathogens i.e. *Aspergillus niger, Penicillium digitatum* and *Rhizopus stolonifer.*

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