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Biological control of Tomato rotting molds by Healthy Tomato Microbial flora

Shrimali Ragini[1*], Masih Usha[2], Iqbal Sanjeeda[3] and S.M.A. Naqvi[4]
[1]Dept. of Microbiology Islamia Karimia College Indore, MP, India
[2]M.J.B. Govt. P.G. Girls College, Indore, MP, India
[3]Govt. Holkar science college, Indore, MP, India
[4]Islamia Karimia College, Indore, MP, India
ragini.dashore@gmail.com

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Abstract

Post harvest decay in harvested tomatoes is worldwide major battle. Post harvest decay recognized persistent problem. Many fungicides are available to control rotting but they are injurious to human health. Biological controllers are eco friendly alternative remedies instead of fungicides. Antagonism process of biological control stops sever loses of post harvest tomatoes. For the antagonism effect of isolated healthy tomatoes flora organisms were applied against rotten mould flora isolated from the rotten tomato. In the antagonism study analyzed Lactobacillus sp. against Fusarium sp. (Inhibition zone 18 mm.), Alternaria sp. (Inhibition zone 15 mm.), Rhizopus sp. (Inhibition zone 20 mm), and Penicillium sp. (Inhibition zone 30 mm.). Pseudomonas sp. antagonism against Fusarium sp., Alternaria sp., Rhizopus sp., and Staphylococcus sp. against three moulds were Aspergillus sp., Alternaria sp., and Penicillium sp. Other isolated bacteria shown antagonism against two rotting moulds. Healthy tomato micro flora Rhizopus sp. against four rotting moulds Aspergillus sp., Fusarium sp., Alternaria sp. and Penicillium sp. tested but Fusarium sp., Penicillium sp. against only two and one rotting moulds.

Keywords: Biological control, Fungicides, Antagonism. Inhibition zone (I.Z.), Microbial flora.

Introduction

Tomato *Solanum lycopersicum* is worldwide edible fruit/berry. Nutritious tomato contains carbohydrates, sugar, dietary fibers, fat, proteins, vitamin C and other vitamins in high amount and has approximate 17% trace elements like Magnesium (Mg), Manganese (Mn), Phosphorous (P), Potassium (K). Natural antioxidants Carotene and Lycopene also present in tomato. It helps to prevent prostate cancer. Tomatoes also improve the skin's ability to protect against harmful ultra violet rays. The Lycopene in tomatoes might help in managing human neurodegenerative disease[1]. Tomatoes has no effect on the risk of developing diabetes but may help relief the oxidative stress of people who already have diabetes[2]. Our important diet part of tomato is very perishable. Post harvest loss of tomatoes is increasing day by day, main cause of post harvest loses is microbial spoilage as rots. Two third of the spoilage of tomatoes is caused by moulds. In vegetables and fruits rots most

Decomposers are opportunistic pathogens. They are already presence in fresh vegetables and fruits flora. Microorganisms are part of the saprophytic surface micro-flora of postharvest fruits and vegetables and many will be present at the time of consumption. The majority of bacteria found on the surface of Fresh fruits and vegetables have differed Gram-negative and Gram positive bacteria. It is depended on soil water and other postharvest techniques but some opportunistic bacteria. Many of these organisms are normally non-pathogenic for human**,** non pathogenic microbes interact with other non pathogenic microbes that produce associated microbial communities which inhibits pathogens found on fruits and vegetables surface[3,4]. Composition of microbial communities varies from variety of reasons in this reasons one is environmental reason including pH and moisture. The treated harvested product transport or storage for a long time is maintained after treatment[5]. But fresh fruits and vegetables microbial flora mostly have Enterobacteriaceae, Oxalobacteraceae, and Bacillaceae bacterial families. Tomatoes are the most important vegetables with great market demand. Tomato (*Solanum lycopersicum*) also known as popular as a Tamator and it is a widely cultivated crop in India[6]. Every tomato grower, dealer, whole seller and retailer are faced with the great lose by the fungal and bacterial decomposition, which are most common and causes big damage to the tomato fruit[7]. Synthetic fungicides primarily used to for control of post harvest lose but in recent trend this control shifting on more eco-friendly alternative method is BCPD (Biological control post harvest decay). Microbial antagonism is most effective in controlling postharvest decay on fruits and vegetables. Our research express that in further research, the potential of the above micro organisms better the mode of action of rotting micro organisms, antagonist and host interactions, to increase the potential of bio-control helping to become a real alternative of synthetic postharvest fungicides[8].

Materials and Methods

Collection of samples: After collecting lots of healthy tomatoes from the different local markets and Sabji Mandies in Indore (M.P.). Samples were washed by collected drinking water and dried by sterilized napkins.

Isolation and Pure culture preparation of healthy fruits and vegetables microbial flora organisms: Some moulds and bacteria which were isolated from healthy tomato and were cultivated on PDA (Potato Dextrose Agar Medium) and NAM (Nutrient Agar Medium). Isolated moulds were identified by microscopic examination with cotton blue stain. Moulds conformation done by used moulds differential media is Martine rose bengal agar media (MRBA). After bacteria isolation gram staining was performed and identified by biochemical test series. For bacterial conformation used selective media culture technique.

Isolation and Pure culture preparation of rotting organisms: All the methods of isolation, purification, identification and biochemical tests were performed as above.

Antagonistic effects on spoiling organisms: Well diffusion method: Fresh Nutrient agar medium plates and Potato dextrose agar plates were prepared for bacteria and fungi cultures respectively. 1 ml. pure culture of rotten organism was spread on plates. Sterilized rubber borer was used to form a well per plate. One ml. pure culture which was isolated by healthy fruits and vegetables had been poured in the wells and treated plates were incubated at favorable conditions (30 to 35^{O}C). Then inhibition zone diameter was measured in mm. scale after 48 hours.

Results and Discussion

The antagonism study of *Lactobacillus sp*. showed high antagonistic effect against the isolated micro flora of rotten samples in respect to *Aspergillus sp*. Results showed that and the antagonism effect is higher in case of *Pseudomonas sp*. against rotting fungi as compare to *Staphylococcus sp., E-coli sp., and Salmonella sp.*

Comparative result shows that *Fusarium sp, Penicillium sp*. and *Rhizopus sp*. has shown better results but *Lactobacillus sp*. is best biological controller.

Conclusion

Comparative result concluded that *Pseudomonas sp., Staphylococcus sp., Rhizopus sp., Penicillium sp*., and *Fusarium sp*. has shown better results. But *Lactobacillus sp*. is best biological controller to tomato rotting.

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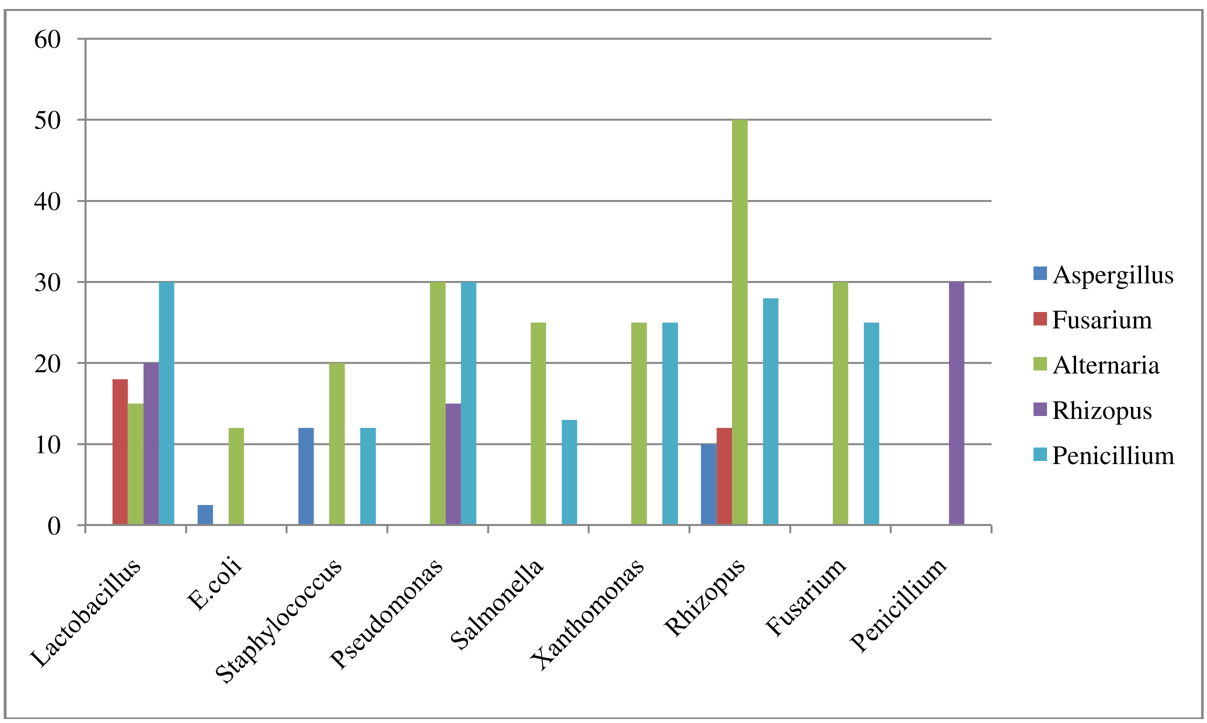

Figure-1
Comparative study of antagonism

Table-1
Identification table of isolated fresh tomato's bacteria and mould cells

Bacterial cell identification (Isolated from Fresh Tomatoes)			Fungal cell identification (Isolated from Fresh Tomatoes)			Fungal cell identification (Isolated from Spoiled Tomatoes)		
Positive biochemical Tests	Special Media for isolated organism	Result	Cultural characters	Microscope observation of Fungus	Result	Fungal cells Isolation	Microscope observation of Fungus	Result
Catalase Negative	On deMan Rogasa Sharpe's Agar Medium (MRS Hi Medium M641), small Milky White colonies are observed.	*Lactobacillus*	On Martin Rose Bengal Agar Medium (MRBA) White Cottony fluffy growth which turns dirty white on ageing.	Long slender mycelia with rhizoid node	*Rhizopus*	On Martin Rose Bengal Agar Medium dry powdery growth in black shade	Conidiophore arise from foot cell are long un branched and ending in vesical on which sterigmata are radially in chain.	*Aspergillus*
Indole and Methyl Red positive	On Eosin Methylene blue agar medium (EMB HiMedium M317), Green metallic shiny colonies are appeared.	*E.coli*	On Martin Rose Bengal Agar Medium (MRBA) Pink fluffy growth observed.	Mycelium septate,conidiop-hore simple, observed spherical short micro conidia	*Fusarium*	Isolation on Martin Rose Bengal Agar Medium (MRBA),Pink fluffy growth observed	Mycelium septate, conid-iophore simple, observed spherical short micro conidia	*Fusarium*
Catalase positive	Baired Parker agar medium (HiMedium M043)Gray black shiny colonies	*Staphylococcus*	On Martin Rose Bengal Agar Medium Green velvety smooth in growth folds	Septate mycelia, conidiophores bears sterigmata which possess conidia in chain giving palm like appearance.	*Penicillium*	On Martin Rose Bengal Agar Medium (MRBA), White Cottony fluffy growth which turns dirty white on ageing	Long slender mycelia with rhizoid node	*Rhizopus*
Citrate utilization positive	Cetrimide Agar Medium (HiMediaMM024) Pigment less, mucoidand florescence at 250nm. Ultra Violet rays	*Pseudomonas*				On Martin Rose Bengal Agar Medium Green velvety smooth in growth folds	Septate mycelia, conidiophores bears sterigmata which possess conidia in chain giving palm like appearance.	*Penicillium*
Red slant and yellow butt on T.S.I. slant with H_2S production	Deoxycholate citrate Agar (Hi Media MMo65). colour less with black canters	*Salmonella*				On Martin Rose Bengal Agar Medium Woody brown growth	Septate mycelium, Branched conidia in chain of three conidia, conidia multi cellular with tapering ends	*`Alternaria*

Table-2
Study of Antagonism average effect between isolated fresh microbial flora and isolated rotted moulds

Bacterial cells which isolated from Fresh Tomato	Fungi which isolated from rotted tomato	Inhibition zone in mm.	Bacterial cells which isolated from Fresh Tomato	Fungi which isolated from rotted tomato	Inhibition zone in mm.
Lactobacillus	*Aspergillus*	*NIZ.	*Salmonella*	*Aspergillus*	NIZ.
Lactobacillus	*Fusarium*	18	*Salmonella*	*Fusarium*	NIZ.
Lactobacillus	*Alternaria*	15	*Salmonella*	*Alternaria*	25
Lactobacillus	*Rhizopus*	20	*Salmonella*	*Rhizopus*	15
Lactobacillus	*Penicillium*	30	*Salmonella*	*Penicillium*	13
E.coli	*Aspergillus*	2.5	*Xanthomonas*	*Aspergillus*	NIZ.
E.coli	*Fusarium*	NIZ.	*Xanthomonas*	*Fusarium*	NIZ.
E.coli	*Alternaria*	12	*Xanthomonas*	*Alternaria*	25
E.coli	*Rhizopus*	NIZ.	*Xanthomonas*	*Rhizopus*	NIZ.
E.coli	*Penicillium*	NIZ.	*Xanthomonas*	*Penicillium*	25
Staphylococcus	*Aspergillus*	12	*Rhizopus*	*Aspergillus*	10
Staphylococcus	*Fusarium*	NIZ.	*Rhizopus*	*Fusarium*	12
Staphylococcus	*Alternaria*	20	*Rhizopus*	*Alternaria*	50
Staphylococcus	*Rhizopus*	NIZ.	*Rhizopus*	*Penicillium*	28
Staphylococcus	*Penicillium*	12	*Fusarium*	*Aspergillus*	NIZ.
Pseudomonas	*Aspergillus*	NIZ.	*Fusarium*	*Alternaria*	30
Pseudomonas	*Fusarium*	NIZ.	*Fusarium*	*Rhizopus*	NIZ.
Pseudomonas	*Alternaria*	30	*Fusarium*	*Penicillium*	25
Pseudomonas	*Rhizopus*	15	*Penicillium*	*Aspergillus*	NIZ.
Pseudomonas	*Penicillium*	30	*Penicillium*	*Fusarium*	NIZ.
			Penicillium	*Alternaria*	NIZ.
			Penicillium	*Rhizopus*	30

*NIZ.=No Inhibition zone

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