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CHEMICAL CONTROL OF FUNGAL DISEASES OF STORED *SOLANUM LYCOPERSICUM* FRUIT BY POTASSIUM BICARBONATE AND CALCIUM CHLORIDE

^aGulshan Irshad*, ^aZeeshan Haider, ^bZahid Ikram, ^aAsfand Iqbal, ^aSajjad Hyder, ^aMuhammad Inam-ul-Haq

^a Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.

^b Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University Rawalpindi, Pakistan.

ABSTRACT

The present study was carried out to see the effect of potassium bicarbonate and calcium chloride to control post-harvest diseases caused by fungi in stored tomato (*Solanum lycopersicum* L.). Samples of tomato fruit were collected from different places of Rawalpindi district. Eight fungal species namely *Alternaria alternata*, *Aspergillus niger*, *Cladosporium fulvum*, *Fusarium oxysporum*, *Penicillium oxalium*, *Rhizopus stolonifer*, *Mucor racemosus* and *Botrytis cinerea* were found in the samples. It was found that among the eight fungal species, *M. racemosus* and *B. cinerea* were responsible for significant spoilage and weight loss of stored tomato. Potassium bicarbonate and calcium chloride were applied to these samples at different concentrations viz. 1.5, 2.5 and 3%. It was found that potassium bicarbonate at 2.5% concentration resulted in 6.66% spoilage and 11% weight loss. While it was found that when concentrations of calcium chloride (CaCl₂) was increased from 1.5-3.5%, the degree of spoilage and weight loss decreased significantly as well. The results revealed that gradual increase in concentration of applied chemical decreased the degree of spoilage and weight loss in stored tomato.

Keywords: Calcium chloride, potassium bicarbonate, spoilage, tomato, weight loss.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a summer vegetable crop belongs to family Solanaceae (Saleem *et al.*, 2013a). It constitutes chiefly vitamin A, C, E and minerals i.e. Ca, P and Fe (Dhaliwal *et al.*, 2003). It is rich in antioxidants such as carotenoids (lycopene and β -carotene) (Berry, 2007) and phenolics (Rai *et al.*, 2012). It is used as an essential ingredient in preparation of several dishes and sauces (Saleem *et al.*, 2013b). In 2011 it was grown on an area of 52.3 thousand hectare in Pakistan. The 20% of the total area under vegetable cultivation constitutes *S. lycopersicum*. During last decade, the average productivity of tomato crop was 9.5-10.5 ton/ha in Pakistan (Anonymous, 2011). In modern agricultural areas the average productivity of tomato crop 33.6 ton/ha (Anonymous, 2013). There are many yield limiting constraints in *S. lycopersicum* crop production. In Pakistan, major factors for yield losses are lack of quality seed, biotic stresses (early blight, late blight, cucumber

mosaic virus, aphid, fruit borer etc.), abiotic stresses (frost, heat, drought etc.) (Akhtar *et al.*, 2012). Disease and insect pest attack owing to imported tomato seed. So there is a need to grow and develop the pest resistant varieties (Saleem *et al.*, 2013b).

The fungal diseases during postharvest stage can cause 50% yield loss of entire product (Ippolito *et al.*, 2005). The fungal species which are responsible for diseases in stored tomato fruit are *Alternaria alternata* (Feng and Zheng, 2007), *Botrytis cinerea* (Lee *et al.*, 2006) and *Rhizopus stolonifer* (Schena *et al.*, 1999) *Monilinia* spp., *Cladosporium* spp., *Penicillium expansum* and *Mucor* spp. (Ogawa *et al.*, 1995). *R. stolonifer*, *A. alternata* and *B. cinerea* are most common fungal species (Wang *et al.*, 2008). *A. alternata* causes black rot at high frequency in tomato (Akhtar *et al.*, 1994). *B. cinerea* is commonly known as grey mould which is a serious fungal disease in tomato worldwide (Elad *et al.*, 1995). *B. cinerea* results destructive leaf, infections in vegetative parts, which eventually results yield losses (Menziés and Jarvis, 1994). It was reported that *R. stolonifer* spreads rapidly from the infected to adjacent fruits when the temperature is higher

* Corresponding Author:

Email: gulshanirshadpp@gmail.com

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than 5°C (Ogawa *et al.*, 1995). Chemical control of *B. cinerea* involves the use of dicarboximides and benzimidazoles, but their intensive use is sometimes difficult and incomplete. The reason is the development of resistance of *B. cinerea* towards these chemicals (Elad *et al.*, 1992; Holmes and Eckert, 1999).

Sometimes use of synthetic fungicides i.e. 1- MCP (Guillen *et al.*, 2007), chitosan (Liu *et al.*, 2007), essential oils (Tzortzakis, 2007) and microbial antagonist (Xi and Tian, 2005) are used to control postharvest decay of tomato fruit. It was reported that use of fungicides is successful for control the postharvest vegetable loss but its use for a long period of time results in development of resistant strains (Rosslenbroich and Stuebler, 2000; Wang *et al.*, 2008). To meet the public demand of tomato we must decrease the intensive pesticide use and search for alternative control strategies to control fungal diseases (Mónaco *et al.*, 2013). There are many biocontrol agents which are effective against *B. cinerea* on tomato and other vegetable crops (Nicot *et al.*, 2000). These include *Trichoderma* spp. and *Gliocladium* spp., *Fusarium* sp., *Ulocladium atrum*, *Cladosporium* spp. and *Penicillium* spp. (Nicot *et al.*, 2000). Sometimes natural substances (Ippolito and Nigro, 2003), physical treatments (Juneja and Thayer, 2001) and organic or inorganic salts (Palou *et al.*, 2002) are also used.

Graham (1983) found that nutrients like calcium (Ca), boron (B) and phosphorus (P) are very important components to protect tomato plants against bacterial and fungal pathogens. It is necessary to use integrated control strategy against fungal species rather than single approach (Conway *et al.*, 2005). Bushra *et al.* (2013) reported that postharvest losses due to pest attack can be managed when good storage practices are adapted. Therefore, the present study was carried out to see the effect of potassium bicarbonate and calcium chloride to control postharvest diseases caused by microflora in stored tomato.

MATERIALS AND METHODS

Sample collection: Healthy and fully ripened tomato fruits were collected from market area of Pirwadae, Bakramandi and Saawan. From each market area 25 tomato samples were collected by using cluster random sampling technique.

Isolation by humid chamber technique: All the experiments were carried out in Department of Plant Pathology, Pir Mer Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan. Five sterilized humid chambers for

each location were used for fungi isolation. Hundred milliliter autoclaved distilled water was added in sterilized humid chambers to make humid environment in it. Five tomatoes were placed in each sterilized humid chamber without any washing. These chambers were placed at 25°C temperature for 5day interval. After 5 days, fungal growth was observed on fruit samples.

Fungi isolation was carried out by blotter method described by ISTA (ISTA, 1985). In this method sterile laminar flow was used. The infected tissues from periphery of each rotten tomato were cut in to 4 pieces (3-5 cm) by fine sterilized scalpel. These pieces were cultured out on potato dextrose medium (PDA) and Malt extract medium plates. The inoculated plates were incubated at 25 °C for 24 hr. Fungi produced was isolated and cultured to media plates to obtain pure culture and incubated for 3 days at 25 °C.

Detection of fungal flora: The incubated plates containing isolated fungal flora were examined under microscope at 10, 20, 40x for specie identification. The identification key derived by Samson *et al.* (2002) was followed.

Pathogenicity test: Pathogenicity test was performed by the method described by Kutama *et al.* (2007). Pathogenicity test was carried out for identification of pathogenic and nonpathogenic mycoflora. Healthy and fully ripened tomato fruits were used for incisions by using sterile 4mm cork borer. For inoculation of fungal mycelia in healthy tomatoes, Akin sterile cork borer was used to cut pallets of potato dextrose agar medium containing identified fungal cultures. Hole present on one side of each replicate was filled with identified fungal isolates in a laminar flour chamber. The inoculated gash was sealed with petroleum jelly to avoid being contaminated by opportunistic microorganisms. The incisions of water on healthy tomato fruits were inoculated as control. The inoculated material was placed in a clean sterilized polythene bags (three fruits/bag). Water soaked absorbent cotton was used to craft a humid environment and incubated for 5 days at 25°C. The inoculated fruits were observed after 5 days. The causal agents were re isolated from infected fruits and their morphological characteristics were compared with original isolates.

Chemical treatment: The efficacy of Potassium bicarbonate (KHCO₃) and calcium chloride (CaCl₂) formulations was tested by method described by (Bombelli and Wright, 2006). These chemical

formulations were applied on fungal isolates (*Mucor racemosus* and *Botrytis cineria*) on tomato fruit samples. The solutions were allowed to air dry for 3 minutes. Then, inoculation was applied with a suspension of *M. racemosus* and *B. cineria* conidia which was prepared in autoclaved distilled water by using 10 day old PDA cultures and inoculums. The PDA cultures and inoculum load was adjusted to 6.5×10^5 conidia ml/L. Each sample was inoculated by puncturing the pericarp on two opposite sides of fruit equatorial region. For inoculation, sterilized tooth picks were allowed to submerge in the conidial suspension and introduced for incisions at a distance around 3mm spaced out (Yabuki *et al.*, 1997; Sato *et al.*, 1997). Weight of all treated fruits was measured and placed in sterilized humid chambers contained 100 ml autoclaved distilled water. The treated material was incubated at 25 °C, and one daily observation was taken till 14th day. The efficacy of potassium bicarbonate and calcium chloride was measured on the basis of these observations.

Amount of spoiled fruits: The fruits which have abnormalities on their surface more than 10% due to *M. racemosus* and *B. cineria* attack were considered and counted as spoiled fruits (Aharoni *et al.*, 1997).

Loss in fruit weight: The fruit samples were weighted prior and after the chemical treatment.

Statistical Analysis

Data regarding to chemical treatment, fruit weight and spoilage was subjected to statistical analysis using ($p \leq 0.05$) level of probability. DMR test was applied and

means are separated using software “Statistical analysis system” (Freund *et al.*, 1986).

RESULTS AND DISCUSSIONS

Fungal prevalence at different locations: During isolation and identification, eight fungal species were identified on 25 samples in three replications from each site (Table 1). These fungal species were *A. alternata*, *A. niger*, *Cladosporium fulvum*, *F. oxysporum*, *P. oxalium*, *Rhizopus stolonifer*, *Mucor racemosus* and *Botrytis cinerea*. Baiyewu *et al.* (2007) reported that when *Aspergillus spp.* and *Rhizopus spp.* were isolated from tomato fruit, these species can cause soft rot in tomato. Efiuvwerwere (2000) found and identified the *Cladosporium* and *Alternaria* fungal species on tomato fruit. Onyia *et al.* (2005) isolated *F. oxysporum*, *A. niger* and *Rhizopus stolonifer* from rotten tomato. Chuku *et al.* (2008) reported the incidence of three fungal species i.e. *Fusarium*, *Penicillium* and *Mucor* in postharvest tomato fruits.

Postharvest loss assessment: About 50% weight loss was found due to attack of different fungal genera in Peerwadhai. But in Bakramandi and Sawan, the percent weight loss was up to 38% and 17% respectively.

Fungal species identified from isolates: Out of 109 isolates, 8 fungal species were characterized, 6 isolates were resembled to *A. alternata*, 5 isolates were related to *A. niger*, 6 isolates were associated to *Cladosporium fulvum*, 9 isolates were the member of *F. oxysporum*, 11 isolates were found as the member of *Penicillium oxalium*, 8 isolates shown the characteristics of *Rhizopus stolonifer*, 29 isolates have the identification characters of *Mucor racemosus*, 35 isolates were associated with *B. cinerea* (Table 2).

Table 1. Prevalence of Fungal Genra at different locations.

S/No.	Pathogens	Peerwadhai (%)	Bakramandi (%)	Saawan (%)	Total Mean (%)
1	<i>Alternaria alternata</i>	16	8	0	8
2	<i>Aspergillus niger</i>	20	0	0	20
3	<i>Cladosporium fulvum</i>	24	0	0	24
4	<i>Fusarium oxysporum</i>	28	0	8	12
5	<i>Penicillium oxalium</i>	20	4	20	14.6
6	<i>Rhizopus stolonifer</i>	24	0	8	10.6
7	<i>Mucor racemosus</i>	60	28	28	38.6
8	<i>Botrytis cinerea</i>	80	36	24	46.6
	Total Mean (%)	34	12	11	

Site Comparison: The data regarding to occurrence of fungal mycoflora in three sites was subjected to Analysis of variance in a complete randomized block design using ($p \leq 0.05$) and their means were compared. It was found that (Site1) was statistically significantly different from (Site 2) and (Site 3) with mean value 9.12 (Table 3). The

(Site 1) indicated the higher degree of prevalence of fungal mycoflora as compared to (Site 2) and (Site 3). It was found that prevalence of *M. racemosus* and *B. cinerea* were statistically at par to each other with mean value 3.11 and 3.33 respectively (Table 3). While other fungal species including *A. alternata*, *A. niger*, *C. fulvum*,

F. oxysporum, *P. oxalium*, *R. stolonifer* were statistically low as compared to *M. racemosus* and *B. cinerea*. The results revealed that *M. racemosus* and *B. cinerea* fungal species were dominant as compared to other six isolated fungi. The mycoflora which was identified from test

sample is normally present in vegetable garbage and spoiled fruits. It was found that a lot of damp fruits were present in the (Site 1). Hameed *et al.* (2008) found that when unhygienic or messy water is used for irrigation of vegetables, the fungal mycoflora can be established.

Table 2. Fungal species identified from isolates of different locations.

S/No.	Species identified	Isolates
1	<i>Alternaria alternata</i>	6 isolates (AsPrw1, AsPrw2, AsPrw3, AsPrw4, Aabk1, Aabk2)
2	<i>Aspergillus niger</i>	5 isolates (AsPrw1, AsPrw2, AsPrw3, AsPrw4, AsPrw5)
3	<i>Cladosporium fulvum</i>	6 isolates (Clprw1, Clprw2, Clprw3, Clprw4, Clprw5, Clprw6)
4	<i>Fusarium oxysporum</i>	9 isolates (Fuprw1, Fuprw2, Fuprw3, Fuprw4, Fuprw5, Fuprw6, Fuprw7, FuSu1, FuSu2)
5	<i>Penicillium oxalium</i>	11 isolates (Pnprw1, Pnprw2, Pnprw3, Pnprw4, Pnprw5, PnBk1, PnSw1, PnSw2, PnSw3, PnSw4, PnSw5)
6	<i>Rhizopus stolonifer</i>	8 isolates (Rhprw1, Rhprw2, Rhprw3, Rhprw4, Rhprw5, Rhprw6, RhSw1, RhSw2)
7	<i>Mucor racemosus</i>	29 isolates (Muprw1, Muprw2, Muprw3, Muprw4, Muprw5, Muprw6, Muprw8, Muprw9, Muprw10, Muprw11, Muprw12, Muprw13, Muprw14, Muprw15, MuBk1, MuBk2, MuBk3, MuBk4, MuBk5, MuBk6, MuBk7, MuSw1, MuSw2, MuSw3, MuSw4, MuSw5, MuSw6, MuSw7)
8	<i>Botrytis cinerea</i>	35 isolates (BoPrw1, BoPrw2, BoPrw3, BoPrw4, BoPrw5, BoPrw6, BoPrw7, BoPrw8, BoPrw9, BoPrw10, BoPrw11, BoPrw12, BoPrw13, BoPrw14, BoPrw15, BoPrw16, BoPrw17, BoPrw18, BoPrw19, BoPrw20, BoBk1, BoBk2, BoBk3, BoBk4, BoBk6, BoBk7, BoBk8, BoBk9, BoSw1, BoSw2, BoSw3, BoSw4, BoSw5, BoSw6)

Pathogenicity of the isolates: The pathogenicity was calculated by the method described by Kutama *et al.* (2007). It was found that eight fungal species isolated namely *A. alternata*, *A. niger*, *C. fulvum*, *F. oxysporum*, *P. oxalium*, *R. stolonifer*, *M. racemosus* and *B. cinerea* tend to show different level of pathogenicity. The fungal isolates namely *M. racemosus* and *B. cinerea* were pathogenic among eight fungal species. The fungal species *M. racemosus* and *B. cinerea* were able to produce same spoilage signs when re inoculated. While *A. alternata*, *A. niger*, *C. fulvum*, *F. oxysporum*, *P. oxalium*, *R. stolonifer* were found to be non-pathogenic.

Efficacy of applied Chemicals: Potassium bicarbonate (KHCO₃) and calcium chloride (CaCl₂) were applied at

different concentrations in test samples. About 100% spoilage (soft rotting) and weight loss was calculated in untreated tomato samples within 14 days. Purwadaria and Sinaga (2007) tested the efficacy of potassium bicarbonate at 2.5% concentration. They found significant results with 6.66% spoilage and 11% weight loss. Bombelli and Wright (2006) reported that 3.5% concentration of potassium bicarbonate applied can give the more significant results as compared to 1.5% concentration. But concentration must be increased up to a certain level. Table 4 shows that when the 3.5% of chemical concentration was applied, the deformity in fruit morphology and a significant weight loss was resulted.

Table 3. Comparison of three different locations in collection of mycoflora.

Isolate	Site 1	Site 2	Site 3	Grand Mean Isolates
1	5.33	2	0.33	2.556a
2	5.67	0.33	0.33	2.111a
3	22	8.67	7.67	12.778b
4	5.67	0	0.33	2a
5	7.33	0.67	1	3a
6	15.33	7.33	7.67	10.111b
7	6	1	2.33	3.111a
8	5.67	1.67	2.67	3.333a
Grand Mean Sites	9.125b	2.708a	2.792a	

Site LSD =0.735; Fungi LSD =3.556

Table 4. Effect of calcium chloride treatment on Spoilage and weight loss.

CaCl ₂ (%)	<i>Botrytis cineria</i>		<i>Mucor racemosus</i>	
	Sp (%)*	Wg (%)**	Sp (%)*	Wg (%)**
0	100	100	100	100
1.5	86.66	90	93	99
2.5	46.66	51	60	68
3.5	33.33	39	46.66	52

Sp**= Mean % of tomato fruit; Wg**= Mean % weight loss of tomato fruit

It was found that when concentration of calcium chloride (CaCl₂) is increased from 1.5-3.5%, the degree of spoilage and weight loss decreases significantly (Table 4). Mahmud *et al.* (2008) reported that calcium chloride (CaCl₂) applied at different concentrations gave the significant results to control fungal species in stored fruits.

It was found that the potassium bicarbonate (KHCO₃) formulations applied at 2.5% concentration gave the significant results to control *M. racemosus* and *B. cinerea* for 14 days. While calcium chloride (CaCl₂) formulations applied at 3.5% gave the significant results to control *M. racemosus* and *B. cinerea* for 14 days (Table 4). These concentrations can retain the quality of tomato fruit commercially. When potassium bicarbonate (KHCO₃) was applied at 2.5% concentration, similar significant results were found in pepper (Fallik *et al.*, 1997) and melon fruit (Aharoni *et al.*, 1997).

CONCLUSION

Spoilage and rotting are important factors responsible for postharvest losses in stored tomato. Many disease controls have been devised yet but not in reach of common tomato producer and seller. Though, the only reliable and safe method to control postharvest diseases caused by fungal microflora is use of potassium bicarbonate (KHCO₃) and calcium chloride (CaCl₂). These chemicals are also safer to human health as well as increase the shelf life of stored produce.

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