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Antifungal activity by resistance inducing salicylic acid and plant extracts against postharvest rots of tomato

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Abstract

The utilization of resistance inducing salicylic acid (SA) and botanicals could be valuable alternatives for the management of postharvest diseases of tomato. The aim of this study was to assess the antifungal activity of salicylic acid and plant extracts against sour rot, pink mold rot and *Rhizopus* soft rot of tomato. Antifungal activity of all botanicals was tested by media amendments and detached fruit assays. The harvested tomato fruits were sprayed with 2, 4, 6, 8 and 10mm salicylic acid and dipped in 25%, 50% and 75% (w/v) extracts of the three plant species. The tomato fruits were inoculated with 10^5/ml conidia of each tested fungi. The results revealed that SA concentration of 6mM completely retarded the radial growth of all plant pathogens and extract of kinnow fruit peel showed significantly ($P<0.05$) high antifungal activity and also completely inhibited mycelium growth of tested pathogens. Garlic extract was effective in reducing mycelium growth of *G. candidum* and *T. roseum*, while mentha leaves extract proved effective against the mycelial growth of *R. oryzae*. Postharvest application of SA significantly reduced mycelia growth and lesion diameter on artificially inoculated fruits. Incidence of pathogenic fungi was significantly reduced at 50% concentration when harvested tomatoes were dipped in kinnow peel extract. In conclusion, resistance inducing SA and natural botanicals have potential to manage of postharvest fungal diseases of tomato and can be the alternatives of health hazardous chemical pesticides.

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Introduction

Sour rot, pink mold rot and *Rhizopus* soft rot in tomato are caused by *Geotrichum candidum*, *Trichothecium roseum* and *Rhizopus oryzae*, respectively (Fajola, 2006; Bartz *et al*., 2010; Hamid *et al*., 2014). These fungal pathogens cause severe economic losses in field and after harvest during transportation and storage. Severity of fruit losses due to these pathogens greatly varied and depends upon environmental conditions, production area and postharvest handling practices. These pathogens are opportunistic pathogens and enter into tomato fruits through wounds during preharvest and postharvest stage. As a result of *G. candidum* infection, the tomato fruits turn soft with light to yellow-brown coloration as the decay enlarges it moves downward may indulge whole fruit and results in vinegar like sour smelling (Thornton *et al*., 2010; Yaghmour *et al*., 2012). *T. roseum* results in rotting of fruits with circular and water soaked lesion. The fungus produced mycelium and spores on the surface of host and result in severe rotting within tomato fruits (Hamid *et al*., 2014). Whereas, fruits infected with *R. oryzae* showed water soaked lesion increasing with time in diameter and fruit become fully rotted after 36-48 hour of infection. Grey hyphae in the form of web originate from water soaked lesion comprised of sporangia, sporangiophores, stolons and rhizoids (Kwon *et al*., 2011).

Postharvest pathogens are main menace to tomato fruits; especially *Rhizopus* spp. caused significant damage after harvest in stored conditions. Currently, postharvest diseases are controlled with the use of synthetic fungicides, for instance, imazalil, sodium ortho-phenylphenate and thiabendazole (Ismail and Zhang, 2004; Kinay *et al*., 2007). These synthetic chemicals are efficient for management of latent pathogens, or prevent from infection although development of resistance by pathogen has been reported in packing houses (Eckert, 1990). In addition, environmental pollution, health hazards, pathogens resistance and high cost, diverted researches to find potential alternatives of these synthetic chemical fungicides (Li and Xiao, 2007; Mari *et al*., 2007). Therefore, it is necessary to develop such management strategies which are safe for consumer health and environment friendly.

Among an alternative that is more eco-friendly is the appliance of such chemicals that provoke resistance in host against the plant pathogens. Basically, induce resistance produce in plants is the result of harmless compounds that trigger plant defense systems (Olivieri *et al*., 2009). Salicylic acid is a plant resistance hormone present in plant cells (Hayat and Ahmad, 2007). It is primarily involved in defense mechanism of plant and induces resistance in fruits against post-harvest plant pathogens (Hussain *et al*., 2014). Moreover, SA has also been reported to have antifungal action against important pathogens (Zainuri *et al*., 2001; Cao *et al*., 2006). SA has also effectively prevented the rotting of fruits, for instance, SA considerably reduced diameter of lesion on muskmelon inoculated by *T. roseum* at room temperature (Cun-fei *et al*., 2012). In another study, SA completely inhibited *R. stolonifer* mycelium growth at 5mM concentration and also significantly reduced rotting lesion diameter on peach fruits (Panahirad *et al*., 2012).

Plant extracts have been gained scientific interest as a substitute of synthetic fungicides to manage postharvest plant diseases due to their antimicrobial action (Verástegui *et al*., 2008; Santas *et al*., 2010). Plants synthesize secondary metabolites such as phenol, quinines, flavonoids, tanninsand these compound produced by plants with phenolic structures like eugenol, carvacrol and thymol have the ability to inhibit pathogens (Cowan, 1999; Arif *et al*., 2009). These groups of compounds showed antifungal activity and involved in defense mechanisms of plants (Das *et al*., 2010). Garlic (*A. sativum*) extract have shown significant inhibitory effect against *Penicillium digitatum* (Obagwu *et al*., 1997). The antifungal property of garlic is the presence of compound allicin, which is the main ingredient in *A. sativum*. Mentha (*M. spicata*) is also reported to have antifungal action in respect to citrus postharvest diseases (du Plooy *et al*., 2009).

Inhibitory property has been accredited to constitutive components of citrus peel. Thus, we carried out research on the development of plant extracts from garlic, mentha and kinnow peel as a potential alternative of synthetic fungicides to manage postharvest diseases of tomato.

The key objectives of this study plan were to investigate the alternatives ways of synthetic chemical fungicides to manage postharvest fungal rots for tomato fruits. Resistance inducing salicylic acid and some botanicals were evaluated to inhibit the common postharvest rots by using different concentrations which proved to be an alternative strategy by lowering the human health risks and environmental friendly.

Materials and methods

Fungal cultures

The isolates of *G. candidum*, *T. roseum* and *R. oryzae* were obtained from infected fruits of tomato and used in present study. Each fungus was cultured on potato dextrose agar (PDA) (LAB M Limited, United Kingdom) medium at 24±1^{o}C. Fungal pure colonies were maintained on Potato dextrose agar medium and stored at 4^{o}C for further study.

Preparation of spore suspension

The suspension of spore of pathogens prepared by addition of 10ml distilled water into pure colony of 10 days old culture and glass rod was used to remove conidia from mycelium. The spore concentration was adjusted to 1×10^{5} spores/ml through hemocytometer and Tween 20 (0.05% (v/v)) was used to prepare spore suspension.

In-vitro assessment of SA on the growth of pathogens

The efficacy of SA on the mycelial growth of *G. candidum, T. roseum and R. oryzae* were assessed according to methodology adopted (Yao and Tian, 2005). SA was primarily dissolved in 1% ethanol and then subsequent required concentrations were prepared in distilled water. Five concentrations of SA (0 to 10mm) were prepared and amended into PDA medium. When PDA medium solidified, 5mm discs of*G. candidum, T. roseum* and *R. oryzae* inoculated into the middle of each Petri dish and after that plates incubated at 24±1^{o}C. The data of mycelium growth was recorded at 48, 72 and 96, 120h for *R. oryzae*, 48, 96, 144 and 192h for *G. candidum* and additionally 240h for *T. roseum*. The treatment without amendment was allocated as control. The present trial was conducted with three replications and each replication was consisting of three Petri dishes.

The mycelium growth of pathogens was calculated by using formula: {(colony width of treatment – colony width of control) ÷ (colony width of control)} × 100 (Juliano *et al*., 2000). Fresh and healthy tomato fruits of same size and maturity without any fungicide treatment for present study were collected from greenhouses. All the fruits were of same size and maturity. Selected fruits were sanitized with sodium hypochlorite (1%) solution for 5 mins subsequently three to four washing with distilled water and subject to natural air drying at room temperature for further studies. Surface sterilized fruits were dipped into SA solution (2, 4, 6, 8 and 10mm) for 30 minutes and control fruits were in sterilized water.

The inoculation was done by inserting 30µl spore suspension of each pathogen into injured fruits (0.5mm diameter, 5mm depth). The data was recorded at 24, 48 and 72h. The experimental composed of three replications and each replicate consist of three fruit.

Plant material and preparation of extract

Garlic, mentha and kinnow were harvested from plants within the territory of Sargodha region and dipped in sodium hypochlorite (1%), followed by washing with the help of distilled water and subjected to natural drying. Leaves of mentha were homogenized in distilled water and crude plant extract (100%) obtained through a muslin cloth. Similarly, Garlic bulb and kinnow peel was crushed in blender with water 1:1 (w/v) and then squeezed through muslin cloth. All plant extracts were heated at 40^{o}C for 15 minutes to keep away from contamination and further diluted to requisite concentrations.

In vitro evaluation of plant extracts against pathogens

The efficacy of plant extracts against *G. candidum*, *T. roseum* and *R. oryzae* were evaluated using food poison technique. Plant extracts of all concentrations (25%, 50%, and 75%) were amended into molten potato dextrose agar media at the rate of 1:4 (v/v) and poured into Petri plates. An agar disc (5mm) from pure cultures of *G. candidum*, *T. roseum* and *R. oryzae* was placed in the centre of each Petri plate containing PDA media and incubated at 24**°**C. PDA plates without plant extract served like control.

The experimental plan was included three replications with three Petri plates of PDA medium in each replication. The tomato fruits were disinfected with 70% ethanol for 2 minutes and followed by drying under sterile condition at room temperature.

After that fruits were dipped in different concentration (25%, 50%, and 75%) of plant extracts for 30 minutes and allowed to dry all dipped fruits at room temperature. The twenty five microliters of 10^5 conidia/mL of each pathogen were inoculated into injured fruits then placed in plastic bags and stored at 24**°**C for 3 days. Each treatment having three fruits and replicated for three times.

Statistical Analysis

Statistical analysis performed using R.3.0.3-Statistical program. Two factor factorial analysis was used for the assessment of results. Duncan's Multiple Range (DMR) test was used to compared treatment means (P = 0.05). Each fungus was evaluated individually.

Results

Effect of SA on mycelium growth of pathogens

The mycelium growth of *G. candidum*, *T. roseum* and *R. oryzae* was entirely retarded by ≥6mm of SA. The concentration of 2 and 4mm SA considerably reduced mycelial growth, when compared with control of all pathogens. The fungal mycelium growth was initiated on second day and clear difference in growth diameter of all pathogens was easily observable on the third day. The delay in growth perhaps due to the injury of hyphae while transferring to the newly prepared PDA media. The 2 and 4mm concentration of SA proved to be significantly effective and provided 51.5% and 75% growth inhibition of *G. candidum*, respectively after 8 days per inoculation. The growth of *T. roseum* was inhibited more than 64% as compared to control at 2mm concentration of SA after 10 days per inoculation followed by 74.3% at 4mm concentration. The level of *R. oryzae* growth inhibition after 5 days per inoculation at 2 and 4mm concentration was 26.8% and 53.1%, respectively (Table 1, 2, 3). The result obtained from 2 and 4mm conc. showed significant difference ($P \leq 0.05$) in mycelium inhibition of pathogen, while SA concentrations (6, 8 and 10mm) were significantly same in inhibiting mycelium growth.

The high inhibition concentration ≥6mm of SA was equally effective *in vitro* as the fungicides to inhibit *G. candidum*, *T. roseum* and *R. oryzae*. In the control treatments, *R. oryzae* showed faster mycelium growth and covered whole Petri plates after 120h while *G. candidum* and *T. roseum* reached to the same level after 192 and 240h, respectively

Table 1. Mycelium growth and percentage inhibition rates of *T. roseum* under different concentrations of salicylic acid in plate assay.

Treatments	48h^{A}			96h^{B}			144h^{C}			192h^{D}		
	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)
2Mm^{B}	5.66±0.29^{b}	1.24±0.3^{a}	54.4	10.8±0.32^{b}	27.7±0.3^{a}	61	15.1±0.26^{b}	42.4±0.4^{a}	64	22.0±0.37^{b}	68.1±0.3^{a}	67.7
4Mm^{C}	2.88±0.26^{c}		76.8	5.11±0.20^{c}		81.6	8.55±0.17^{c}		79.8	14.4±0.29^{c}		78.9
6Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100
8Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100
10Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100

The treatments mean were compared using Duncan multiple range test (p≤0.05), TG= Total Growth; CG= Control Growth; IP= Inhibition Percentage.

Table 2. Mycelial growth rate and percentage inhibition of *G. candidum* at different concentrations of salicylic acid in plate assay

Treatments	48h^{A}			96h^{B}			144h^{C}			192h^{D}		
	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)
2Mm^{B}	6.00±0.37^{b}		59.5	15.1±0.26^{b}		52.8	26.0±0.37^{b}		50.9	40.8±0.46^{b}		51.5
4Mm^{C}	2.88±0.26^{c}		80.5	6.77±0.54^{c}		78.8	13.4±0.38^{c}		74.7	21.0±0.66^{c}		75.0
6Mm^{D}	0.00±0.00^{d}	14.8±0.2^{a}	100	0.00±0.00^{d}	32.0±0.2^{a}	100	0.00±0.00^{d}	52.9±0.5^{a}	100	0.00±0.00^{d}	84.2±0.6^{a}	100
8Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100
10Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100

The treatments mean were compared using Duncan multiple range test (p≤0.05),TG= Total Growth; CG= Control Growth; IP= Inhibition Percentage.

Table 3. Mycelial growth rate and percentage inhibition of *R. oryzae* at different concentrations of salicylic acid in plate assay

Treatments	48h^{A}			72h^{B}			96h^{C}			120h^{D}		
	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)
2Mm^{B}	10.6±0.50^{b}		60.9	24.3±0.52^{b}		44.0	40.8±0.66^{b}		37.2	65.3±1.00^{b}		26.8
4Mm^{C}	4.00±0.29^{c}		85.2	11.8±0.36^{c}		72.8	24.0±0.55^{c}		63.1	41.8±0.64^{c}		53.1
6Mm^{D}	0.00±0.00^{d}	27.1±1.3^{a}	100	0.00±0.00^{d}	43.4±0.7^{a}	100	0.00±0.00^{d}	65.0±0.8^{a}	100	0.00±0.00^{d}	89.2±0.4^{a}	100
8Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100
10Mm^{D}	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100	0.00±0.00^{d}		100

The treatments mean were compared using Duncan multiple range test (p≤0.05), TG= Total Growth; CG= Control Growth; IP= Inhibition Percentage.

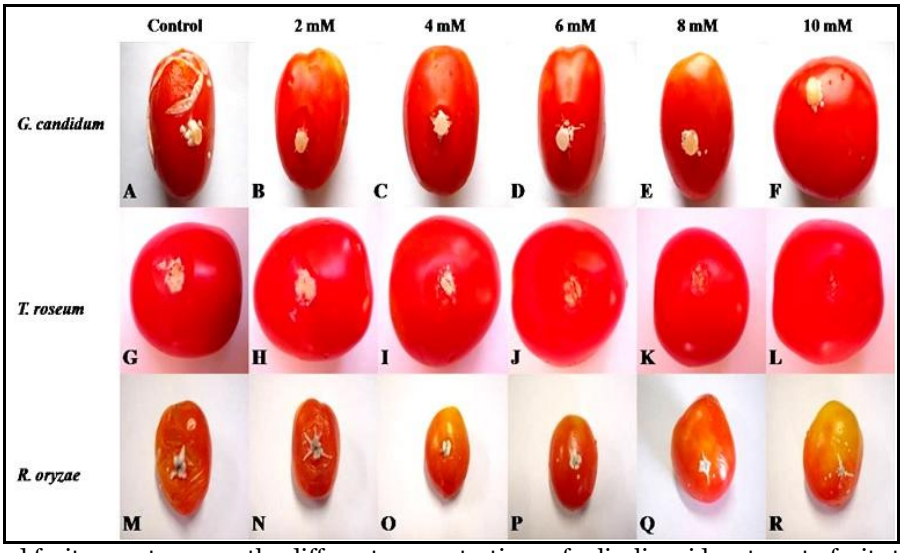

Fig. 1. Detached fruit assay to access the different concentrations of salicylic acid on tomato fruits to suppress the postharvest pathogens.

Effect of postharvest application of SA

All the concentration of SA on harvested tomato by dipping method reduced lesion diameter considerably (P< 0.05) as compared to control. Application of SA on harvested fruits at 8mm concentration provided effective control of sour rot, pink mold rot and *Rhizopus* soft rot up to 48h after inoculation at ambient temperature. SA at 10mm concentration showed least lesion diameter of *Rhizopus* rot and pink mold rot on tomato fruits while completely control sour rot on day 3. The mean lesion diameter inhibition of *G. candidum* after 72h of inoculation was 70.9, 94.5 and 100% on the tomato fruits treated with 6, 8 and 10mm, respectively and was significantly greater than the control. For *T. roseum* the mean lesion diameter inhibition percentage was 44.1, 65.1 and 81.2% at 6, 8 and 10mm concentration, while at the same SA concentrations the inhibition rate of *R. oryzae* was 69.2, 86 and 95% after 72h of inoculation. Experiment showed that SA significantly.

Reduces the lesion diameter on fruits but its efficacy greatly varies for each pathogen and it decreases with passage of time.

Evaluation of plant extract on mycelium growth of postharvest pathogens

The extracts of garlic bulb, mentha leaves and kinnow peel were effective in reducing mycelium development of *G. candidum*, *T. roseum* and *R. oryzae* at all tested concentrations. Results showed that, extracts of *A. sativum*, *C. reticulata* and *M. spicata* completely inhibited pathogens growth at 100% concentration except in case of *T. roseum* by *M. spicata*. The percentage growth inhibition of *G. candidum* by *A. sativum, C. reticulata* and *M. spicata*at concentration of 50% was 78.9, 94.72 and 68.08%, whereas for *T. roseum* the inhibition percentage was 70.73, 86.68 and 59.03% while in case of *R. oryzae* it was 69.61, 88.17 and 82.69%. *A. sativum* and *C. reticulata* were effective to reduce *G. candidum*, *T. roseum* and *R. oryzae* hyphal growth by 90% at 75% concentration, more than the extract of *M. spicata* leaves at the same concentration (Fig 2). In general all three extract were established as the most effective plant extracts to control *G. candidum*, *T. roseum* and *R. oryzae in vitro*.

Table 4. Lesion diameter and inhibition percentage of *T. roseum* after different time interval on tomato fruit.

Treatments	24h[A]			48h[B]			72h[C]		
	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)	TG(mm)	CG(mm)	IP(%)
2Mm[B]	4.88±0.22[b]	6.88±0.4[a]	29.1	11.7±0.29[b]	15.44±0.3[a]	24.2	16.9±0.35[b]	15.44±0.3	24.2
4Mm[C]	3.33±0.17[c]		51.6	8.22±0.22[c]		46.8	15.2±0.32[c]		46.8
6Mm[D]	1.44±0.18[d]		79.1	5.88±0.31[d]		61.9	11.9±0.31[d]		61.9
8Mm[E]	0.00±0.00[e]		100	3.33±0.24[e]		78.4	7.44±0.38[e]		78.4
10Mm[F]	0.00±0.00[e]		100	0.00±0.00[f]		99.3	4.00±0.40[f]		99.3

The treatments mean were compared using Duncan multiple range test (p≤0.05), TG= Total Growth; CG= Control Growth; IP= Inhibition Percentage.

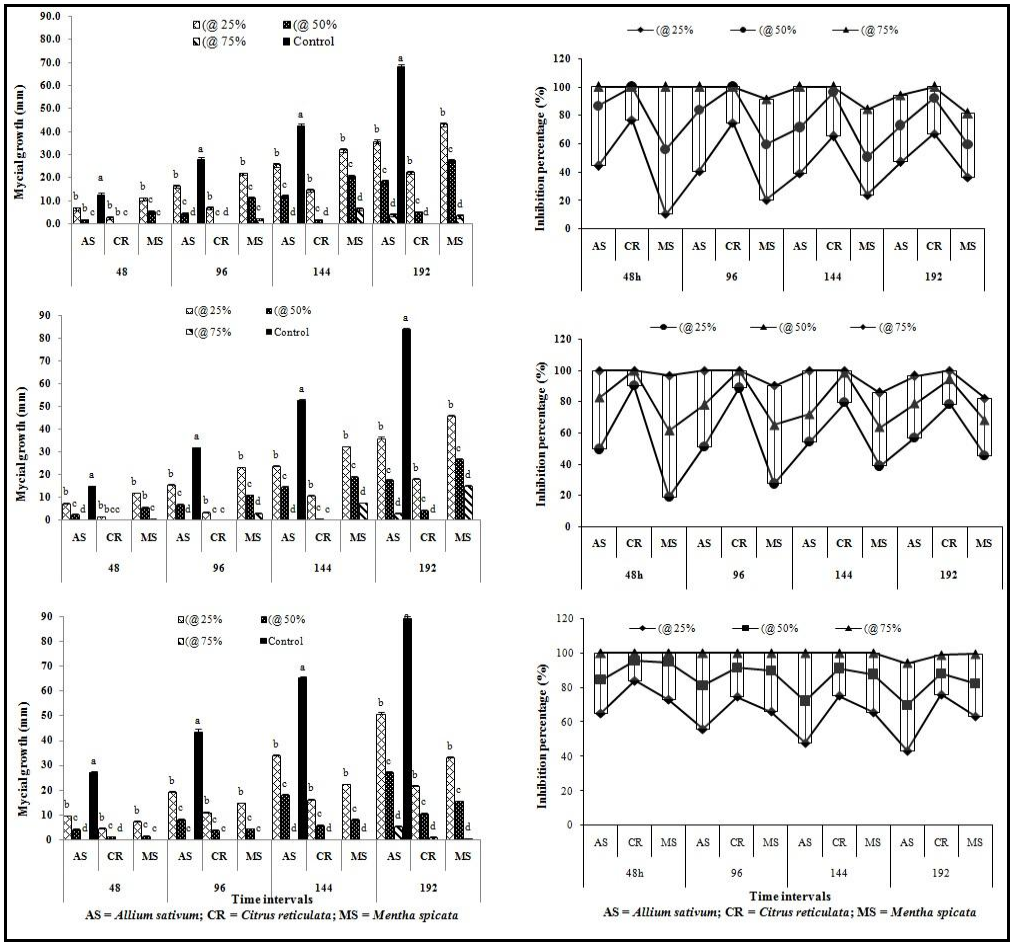

Fig. 2. Effect of different concentrations of plant extracts on the mycelial growth of postharvest pathogens of tomato on potato dextrose agar at 24±1ºC. Significant differences (P< 0.05) between means were indicated by different letters above histogram bars.

Effect of plant extracts on artificially inoculated fruits

Plant extracts proved significant in reducing growth of disease on artificially inoculated tomato fruits. Sour rot caused by *G. candidum* was completely inhibited by *C. reticulata* at 75 and 100% concentration after 3 days at 24±1°C. Moreover, plant extracts of *A. sativum* and *M. spicata* in tomato fruits inhibited *G. candidum* lesions diameter up to or more than 90% at 75 and 100% concentration. Extract of *C. reticulata* completely inhibited *T. roseum* at 75 and 100% concentration while percentage inhibition of *T. roseum* by *A. sativum* was 78.15 and 97.93%, respectively. The efficacy of *M. spicata* against *T. roseum* was moderate with percentage inhibition of 64.08 and 87.52% at 75 and 100% concentration, respectively (Table 5). For the control of *R. oryzae*, extracts of *M. spicata* and *C. reticulata* showed very promising result at 100% concentration and completely inhibit lesion diameter on inoculated tomato fruits. All extracts showed more than 50 percent inhibition of lesion diameter at 50% concentration. In general peel extract of *C. reticulata* proved to be more valuable in controlling lesion diameter of *G. candidum*, *T. roseum* and *R. oryzae*, followed by extract of *A. sativum* and *M. spicata*.

Table 5. Effects of different concentrations of plant extracts on lesion diameter of tomato fruit caused by *T. roseum*, *G. candidum* and *R. oryzae* applied as dip application method and incubated at 24±1°C.

Treatments	Doses	*Allium sativum*			*Citrus reticulata*			*Menthaspicata*		
		Time intervals								
		24	48	72	24	48	72	24	48	72
T. roseum	25%	5.11±0.20^{b} (25.5)	11.3±0.29^{b} (26.6)	16.6±0.33^{b} (22.1)	1.11±0.31^{b} (83.9)	4.78±0.22^{b} (68.9)	9.67±0.47^{b} (54.6)	5.78±0.15^{b} (16.1)	14.3±0.24^{b} (7.14)	18.8±0.46^{b} (11.6)
	50%	1.33±0.17^{c} (50.7)	5.67±0.17^{c} (63.2)	11.4±0.24^{c} (46.5)	0.00±0.00^{c} (100)	1.22±0.43^{c} (92.1)	3.56±0.47^{c} (83.3)	3.22±0.15^{c} (53.3)	7.78±0.15^{c} (49.5)	12.7±0.17^{c} (40.4)
	75%	0.00±0.00^{d} (100)	1.67±0.24^{d} (89.2)	4.87±0.28^{d} (78.1)	0.00±0.00^{c} (100)	0.00±0.00^{d} (100)	0.00±0.00^{d} (100)	0.00±0.00^{d} (100)	3.56±0.10^{d} (100)	7.67±0.17^{d} (64.0)
	Control	6.89±0.33^{a}	15.4±0.29^{a}	21.3±0.53^{a}	6.89±0.33^{a}	15.4±0.29^{a}	21.3±0.53^{a}	6.89±0.33^{a}	15.4±0.29^{a}	21.3±0.53^{a}
G. candidum	25%	2.00±0.24^{b} (61.7)	5.22±0.46^{b} (77.1)	21.8±0.49^{b} (51.3)	0.00±0.00^{b} (100)	2.22±0.22^{b} (90.3)	8.11±0.31^{b} (81.9)	4.56±0.18^{b} (12.6)	11.9±0.26^{b} (47.8)	25.4±0.41^{b} (43.3)
	50%	0.44±0.18^{c} (91.6)	2.67±0.20^{c} (88.3)	10.1±0.48^{c} (77.5)	0.00±0.00^{b} (100)	0.00±0.00^{c} (100)	1.56±0.18^{c} (96.5)	2.56±0.18^{c} (50.9)	5.56±0.18^{c} (75.6)	14.1±0.20^{c} (68.5)
	75%	0.00±0.00^{c} (100)	0.44±0.18^{d} (98.1)	3.33±0.29^{d} (78.1)	0.00±0.00^{b} (100)	0.00±0.00^{c} (100)	0.00±0.00^{d} (100)	0.33±0.17^{d} (93.7)	1.67±0.17^{d} (92.7)	4.67±0.17d (89.6)
	Control	5.22±0.22^{a}	22.5±0.46^{a}	44.8±0.62^{a}	5.22±0.22^{a}	22.5±0.46^{a}	44.8±0.62^{a}	5.22±0.22^{a}	22.5±0.46^{a}	44.8±0.62^{a}
R. oryzae	25%	11.7±0.24^{b} (24.0)	22.3±0.47^{b} (22.0)	34.0±0.67^{b} (30.8)	7.56±0.18^{b} (50.9)	13.3±0.33^{b} (53.5)	17.6±0.24^{b} (64.2)	7.56±0.18^{b} (50.9)	14.8±0.22^{b} (48.3)	23.4±0.18^{b} (52.3)
	50%	5.22±0.15^{c} (66.1)	11.7±0.41^{c} (59.1)	17.6±0.24^{c} (64.2)	3.56±0.24^{c} (76.9)	6.67±0.24^{c} (76.6)	11.4±0.24^{c} (76.9)	3.22±0.32^{c} (79.1)	7.11±0.35^{c} (75.1)	13.8±0.28^{c} (71.9)
	75%	0.78±0.28^{d} (94.9)	2.56±0.34^{d} (91.0)	7.44±0.29^{d} (78.1)	1.33±0.17^{d} (91.4)	2.22±0.15^{d} (92.2)	4.11±0.39^{d} (91.6)	1.22±0.15^{d} (92.1)	3.67±0.17^{d} (87.2)	6.44±0.18^{d} (86.9)
	Control	15.4±0.33^{a}	28.6±0.44^{a}	49.1±0.68^{a}	15.4±0.33^{a}	28.6±0.44^{a}	49.1±0.68^{a}	15.4±0.33^{a}	28.6±0.44^{a}	49.1±0.68^{a}

Values followed by the same letter are not significantly different at the 5% level by DMRT (Values in the parenthesis are inhibition percentage (%).

Discussion

The results showed that SA was very effective in reducing mycelium development of *G. candidum*, *T. roseum* and *R. oryzae* significantly. The treatment ≥6mm of SA completely inhibited the mycelium growth of tested pathogens. The decrease in mycelial growth may be due to antifungal activity of SA. In recent studies, antifungal effect of SA has been published against different postharvest pathogens like *A. alternata*, *M. fructicola*, *P. digitatum*, *P. expansum*, and *P. italicum* in citrus (Qin *et al.*, 2003; Yao and Tian, 2005; Wang and Li, 2008; Iqbal *et al.*, 2012). It was studied that SA between 2 to 5mm concentrations inhibited 50% of *in vitro* fungal mycelium growth of pathogens while concentration (0.5mm) had no effect on the development of pathogens (Strobel and Porter, 2005). The overall findings of the present study also demonstrated that SA at 2mm, 4mm concentration caused more than 50% inhibition of *G. candidum* and *T. roseum*, while in case of *R. oryzae* 2mm concentration was least effective but 4mm inhibited 50% of mycelium growth. It is apparent from present study that SA has potential to control *G. candidum*, *T. roseum* and *R. oryzae* by reducing mycelium growth and conidial germination thus inhibiting ability of pathogens to cause primary infection.The trials on harvested fruits against *G. candidum*, *T. roseum* and *R. oryzae*are according to hypothesis that exogenous appliance of SA significantly reduces disease. In general

application of SA after harvesting of tomato fruits at 8 and 10mm concentration inhibited the growth of pathogens on wounded sites. It was published that SA treatment at 2 to 10mm was moderately effective against pathogens and also recommended the inclusion of synergistic antifungal agents (Strobel and Porter, 2005). It was discussed before, SA directly control postharvest pathogens due to its fungitoxic activity. Therefore inoculation of pathogen in tomato fruits after treatment with SA induce resistance in fruits (Verberne *et al.*, 2000) but in addition the contact between pathogens and SA solution results in direct fungicidal influence and induce inhibitory effect (Panahirad *et al.*, 2012).

In conclusion, the dipping of fruits in SA solution instantly after harvest provided effective control of sour rot, pink mold rot and *Rhizopus* soft rot of tomato. This study provides an effectiveness of SA for the control of tomato postharvest diseases. Exercise of natural compounds that enhance resistance in harvested fruits against fungal infection has become more widely accepted and an efficient control strategy for disease management (Caccioni *et al.*, 1998; Cerioni *et al.*, 2009). The efficacy of SA in reducing or minimizing the incidence of *G. candidum*, *T. roseum* and *R. oryzae* under different physiological conditions needs research prior to their exploitation on a large and commercial scale.

The result of the botanical extracts of *A. sativum, C. reticulata* and *M. spicata* significantly inhibited mycelium development of *G. candidum*, *T. roseum* and *R. oryzae in-vitro* at all concentration as compared to control. The mycelium inhibition percentage was increased with the increase in level of plant extract concentration. Timothy *et al.*, (2012) also reported a dose dependent antimicrobial action of *Cassia alata* against some pathogenic fungi. Plant extract of garlic has been reported to entirely inhibited *Botrytis cineria* spore germination (Wilson *et al.*, 1997). The active compound in garlic extract that is involved in the antimicrobial activity is allicin (Curtis *et al.*, 2004). The fungitoxic effect of *M. spicata* has been already studied against *Fusarium oxysporum* f. sp. *radicis-cucumerinum* in cucumber (Nosrati *et al.*, 2011). In this study,kinnow peel significantly inhibited mycelium growth of tomato postharvest pathogens. Rodov *et al.*, (1995) reported that lemon peel contain an active compound citral that play significant role in growth retardation of fungal postharvest pathogens.

In present study, we observed that pathogens growth at 75% concentration were not initiated in early days of incubation but as the time progressed mycelium growth was also observed on same concentration. The response of extract to pathogen growth is directly related to its potential use to reduce pathogen inoculums. Hence, it was recommended that every pathosystem should search out separately to evaluate the viability of utilizing plant extracts to manage plant diseases (Curtis *et al.*, 2004).

We also observed that plant extracts of *A. sativum*, *C. reticulata* and *M. spicata* significantly reduced lesion diameter of *G. candidum*, *T. roseum* and *R. oryzae* on tomato. As mentioned above, allicin is the active compound of garlic and this compound approximately constituent 70 to 90% of the overall sample (Combrink *et al.*, 2011). Mechanism of allyl chain is still under debate but it is possible that the antifungal action of garlic extract may be due to allyl chain. In our study, leaves extract of mentha was limited to completely inhibit the development of *G. candidum* and *T. roseum* on tomato fruits, beside to perform efficiently *in vitro*. Carvalho *et al.*, (2011) reported that plant extracts except *Anadenantheracolubrina* proved less effective against *Alternaria alternata* during *in vivo* trial on fruits of Murcotttangor, although all plant extracts performed very effective *in vitro*. Plaza *et al.*, (2004) reported that essential oils were unable to control *Penicillium* spp. when oil directly applied to artificially inoculated fruits. Results of our study clearly demonstrated the fungitoxic effect of *Allium sativum* (garlic), *Citrus reticulata* (Kinnow) and *Menthaspicata* (mentha) against sour rot, pink mold rot and *Rhizopus* rot of tomato respectively. In future, our main focus will be on the extraction of the main active compounds from these extracts with high antifungal potential.

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