Research Article

Management of Citrus Green Mould through the Use of Allelochemicals and Salicylic Acid

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Abstract | The study was conducted to check the effect of different plant extracts /allelochemicals and salicylic acid on the growth of *Penicillium digitatum* causes citrus green mould by using 7 plant extracts alone and in combination with wax and 3 concentrations of salicylic acid alone and in combination with wax. The study revealed that in case of plant extracts maximum growth inhibition (35.90 %) was shown by Anar followed by Chabir (33.60%) and Garlic (29.16%), while minimum growth inhibition was recorded from Parthanium (17.43%) and Ginger (19.94 %). In case of plant extracts + wax maximum growth inhibition (44.36%) was shown by Anar+Wax followed by Chabir+Wax (42.78%) and Fungicide + Wax (37.45%), while minimum growth inhibition % was recorded from Fungicide (15.49 %) and Kortuma + Wax (17.48%). In case of salicylic acid concentrations maximum growth inhibition (34.73 %) was shown by Salicylic acid concentration of 6 mm followed by Salicylic acid (33.40 %) at 3 mm concentration and Salicylic acid (28.51%) at 9 mm concentration, while minimum growth inhibition % was recorded from SA + Wax (-13.37%). So it was concluded that allelochemicals + wax showed maximum growth inhibition of green mould.

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1. Introduction

Postharvest diseases cause substantial losses to harvested fruits during transportation and storage (Sharma *et al.*, 2009). Green mould caused by *Penicillium digitatum* (Pers. Fr.) Saac is the major postharvest disease of citrus which is accountable for causing severe losses up to 80% during favorable conditions which result in production of green spores and fruit shrink with the passage of time (Palou, 2009; Ballester *et al.*, 2010; Iqbal *et al.*, 2012).

Control of postharvest citrus diseases mostly depends upon the use of synthetic fungicides through different protocols used like dipping of citrus fruits in soak tanks, by spraying aqueous solution and as well as in the form of fumigants (Janisiewicz, 2004). Health and environmental hazards of these chemicals shifted the plant pathologists towards finding valuable alternatives of these chemicals (Leroux, 2003; Calvo *et al.*, 2007; Cunningham and Taverner, 2007). The other drawback of continuous and indiscriminate use of chemicals resulted development of resistant strains of the pathogens (Leroux, 2003).

Therefore, the most promising alternative to chemicals is the use of organic elicitors to induce resistance in plants and the use of allelochemical. Mostly, induce resistance is the application of chemical compounds that result in triggers of plant defense systems

(Olivieri *et al.*, 2009; Iqbal *et al.*, 2012). Allelochemical extracts isolated from the plants have been evaluated against various plant pathogens (Stephan *et al.*, 2005). Natural plant products are the most efficient and cost-effective alternatives of plant pathogens (Lira *et al.*, 2003; Jasso de Rodríguez *et al.*, 2007; Castillo *et al.*, 2010; Osorio *et al.*, 2010).

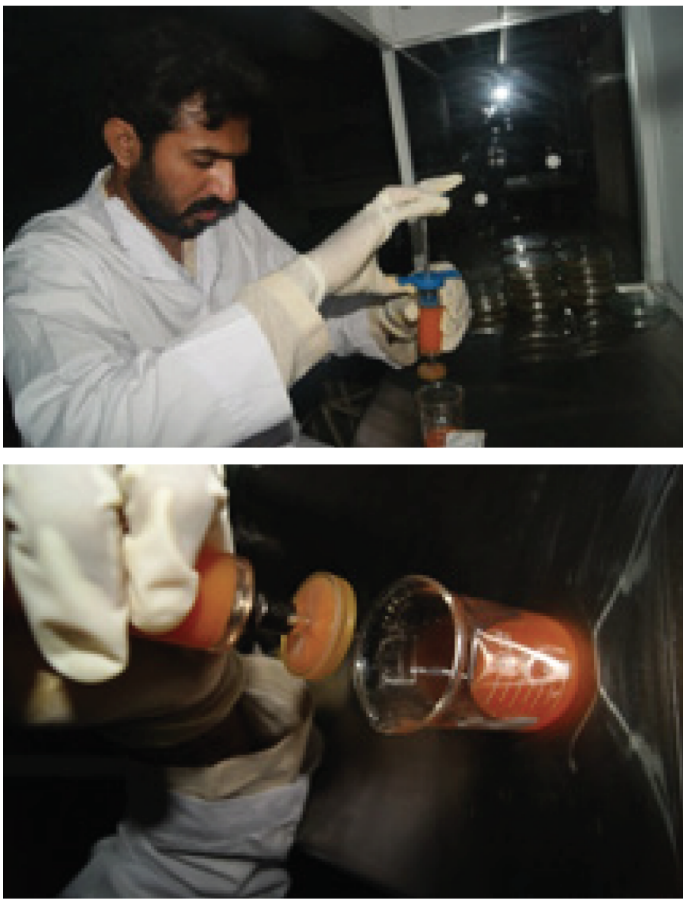
The effectiveness of different organic salts and plant extracts to inhibit the pathogen growth may vary from plant to plant (Castillo *et al.*, 2010). Hence, possible alternatives of chemical fungicides such as biologically based approaches that must be equally or more effective, economical and non-hazardous to consumers and eco-friendly have been much needed to be evaluated against postharvest diseases. Therefore, treatments with allelochemicals and chemical elicitors could be recommended to control citrus green mould (*P. digitatum)* and their use may be an effective method to improve the integrated pest management strategy.

2. Materials and Methods

The present research work was done in the laboratory of Plant Pathology, College of Agriculture, University of Sargodha during 2015-16 in order to evaluate the efficacy of chemical elicitors and different plant extracts against postharvest disease of citrus. The samples of green mould were collected from different locations of district Sargodha and pathogen was isolated from diseased fruit samples and then these fruits cut into small pieces of about 1.5-2cm. Surface of these cuttings sterilized with 0.1% bleach for approximately 2 minutes and then washed three times with distilled water and placed on petri plates containing potato dextrose agar (PDA). These petri plates were incubated for one week to check the sporulation for further studies. Pathogenicity of isolated pathogens to confirm the association with host was done according to Koch's postulates. Pure culture of pathogen was preserved at 4^0C for experiments and cultures were replaced by new one after a month.

2.1 Inoculum preparation

Morphological characterization of the pathogen, *P. digitatum* was done on the basis of conidial size and shape and colony characteristics and was confirmed as described by Mills *et al.* (2004) and conidial suspension was prepared by culturing on PDA media containing petri plates. Sterilized distilled water (10mm) was added to seven days pure old culture. The petri plates were gently shivered and suspension was filtered with 3 layers of cheesen cloth to remove debris as reported by Janisiewicz *et al.* 2000.



Preparation of inoculum

2.2 Preparation of plant extracts

Fresh leaves of different plants e.g Kurtuma (*Citrullus colocynthis)*, Chabir, Ginger (*Zingiber officinale)*, Puthkanda (*Achyranthes aspera L.)*, Garlic (*Allium sativum)*, Parthenium (*Parthenium hysterophorus)* and Pomegranate (*Punica granatum)* were collected from research area, College of Agriculture and then washed with distilled water to remove surface pollutant. Leaves of all tested plants were blended in distilled water with 1:1(w/v) to obtain its crude extract. After blending this crude extract was passing through double layer of muslin cloth and then centrifuged at 8500 rpm for 8 min and supernatant was obtained in 1000ml glass flask as reported by (Chohan et al., 2011).

2.3 Postharvest application of allelochemicals

The efficacy of six plant extracts (Table 1) on green mold of kinnow was done on 1st week of April 2015. Healthy, uniform and blemish free fruits were taken

from local market of Sargodha and their surface sterilization was done by dipping of fruits in 0.5% commercial bleach solution for 5 minutes. Five fruits for each replication were used and each treatment was replicated three times. Fifteen micro liter spore suspension of *P. digitatum* was injected with micropipette. Inoculated fruits were set in ridged boxes and maintained at 85% relative humidity and 20c° temperature. Data were taken on daily basis.

Table 1: List of Plant used for Postharvest Disease Control.

Sr.#	Common Name	Botanical Name	Family
1	Kortuma	*Citrullus colocynthis*	cucurbitaceae
2	parthenium	*Parthenium hysterophorus*	Asteraceae
3	Ginger	*Zingiber officinale*	Zingiberaceae
4	Garlic	*Allium sativum*	Amaryllidaceae
5	Anar	*Punica granatum*	Lythraceae
6	Puthkanda	*Achyranthes aspera L.*	Amaranthaceae
7	Chabir	*Melaleuca genus*	Myrtaceae

2.4 Postharvest application of allelochemicals with wax

Each fruit was coated with plant extract with 0.73ml concentration of wax and dried under shade for 30 minutes. Spore suspension (15 µ L) of *P. digitatum* was injected with micropipette and fruits were incubated at 20° temperature and 90% relative humidity in growth room.

2.5 Evaluation of salicylic acid and with wax

Effectiveness of salicylic acid was evaluated by amending 0mM (control), 3 mm, 6 mm and 9 mm into potato dextrose agar and each treatment was replicated three times. Fruits were wounded by making puncture by using sterilized borer and inoculated with 5 micro liter of conidial suspension of fungi. Inoculated fruits were arranged in rigid boxes and maintained at 90% relative humidity at 20c°. Fifteen fruits of citrus for each treatment were coated with wax and 0.73ml wax used for each fruit and then amended with different dose of elicitors to check their efficacy against green mould.

Data were recorded for lesion diameter on each fruit of each treatment with the help of scale in millimeters while experiments was repeated two times.

3. Results and Discussion

3.1 Postharvest applications of allelochemicals/plant extracts

The maximum growth inhibition percentage (35.90%) was shown by Anar followed by Chabir (33.60%) and Garlic (29.16%), while minimum growth inhibition % was recorded from Parthanium (17.43%) and Ginger (19.94%). In case of lesion diameter minimum mean lesion diameter (48.06 mm) was recorded from Anar followed by Chabir (49.78 mm). In case of lesion diameter, the maximum lesion diameter (61.90 mm and 60.02 mm) was recorded of Parthanium and Ginger extracts. Based on our experimental results, we can conclude that for obtaining maximum growth inhibition % plant extracts of Anar, Chabir and Garlic was the best as they showed highest growth inhibition percentage of *P. digitatum* (Table 2, Figure 1 and 2).

Table 2: Effect of plant extracts on colony growth of *P. digitatum*.

Treatments	Lesion diameter (mm) on Kinnow fruit							Growth Inhibition %
	Time (days)							
	1st	2nd	3rd	4th	5th	6th	Mean	
Kortuma	3.00	28.43	68.47	75.47	87.37	94.60	59.56	20.56
Ginger	3.77	37.93	65.87	77.13	85.33	90.07	60.02	19.94
Chabir	1.67	25.40	52.73	64.50	72.93	81.43	49.78	33.60
Garlic	3.53	26.33	60.33	62.00	78.77	87.67	53.11	29.16
Parthanium	13.37	41.43	65.60	74.27	85.27	91.47	61.90	17.43
Anar	4.57	36.20	43.60	56.27	67.47	80.23	48.06	35.90
Pathkanda	6.47	33.07	49.67	71.20	83.33	88.87	55.43	26.06
Fungicide	3.40	25.93	55.47	71.13	83.73	93.00	55.44	26.04
Wax	3.03	32.47	60.13	69.27	80.93	92.00	56.31	24.89
Control	20.40	65.47	78.20	90.07	97.33	98.33	74.97	

3.2 Postharvest Applications of Allelochemicals + Wax

The experiment was conducted on postharvest application of Plant Extracts/ Allelochemicals + Wax against *P. digitatum*. Results were presented Table 3 and Figure 3, 4 for growth inhibition percentage and lesion diameter on kinnow fruit after each day after treatment. Results showed that maximum growth inhibition (44.36 %) was shown by Anar+Wax followed by Chabir+Wax (42.78%) and Fungicide+Wax (37.45%), while minimum growth inhibition % was recorded from Fungicide (15.49%) and Kortuma+Wax (17.48%). In case of lesion diameter minimum mean lesion diameter (36.71 mm) was recorded from Anar+Wax followed by Chabir+Wax (37.76 mm). Maximum lesion diameter (55.76 mm and 54.44 mm) was recorded from

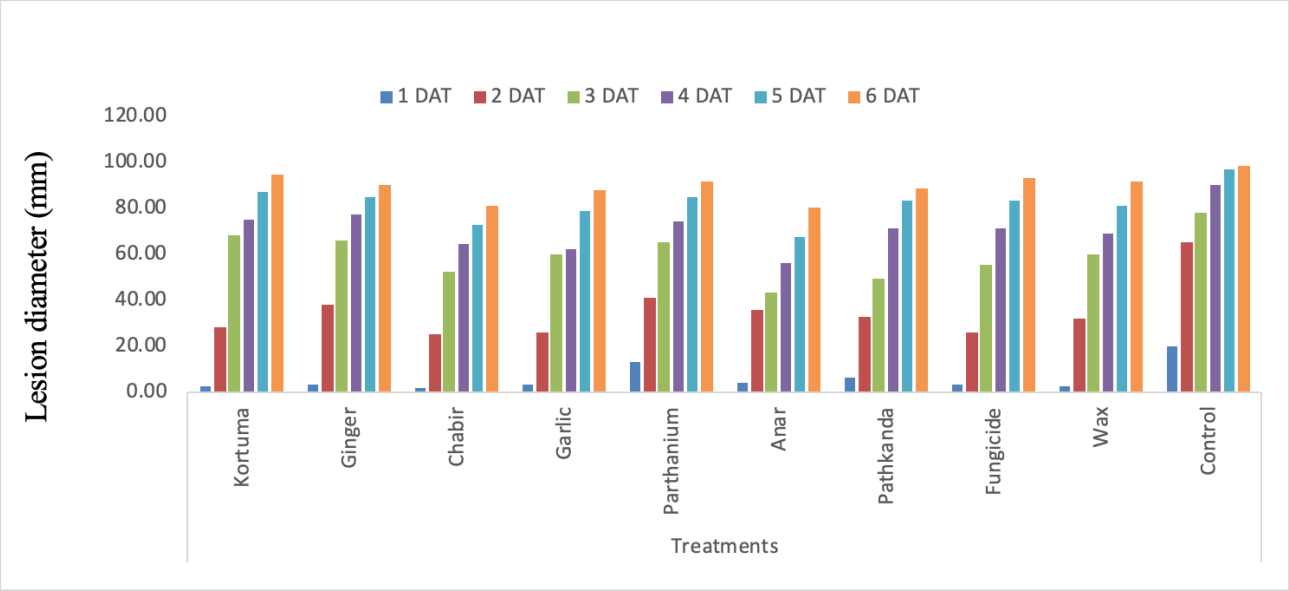


Figure 1: Effect of different plant extracts on lesion diameter (mm) on kinnow fruit.

Table 3: Effect of allelochemicals + Wax on colony growth of *P. digitatum*.

Treatments	Lesion diameter (mm) on Kinnow fruit							Growth Inhibition %
	Time (days)							
	1st	2nd	3rd	4th	5th	6th	Mean	
Kortuma+Wax	11.67	28.80	58.60	70.87	76.13	80.60	54.44	17.48
Ginger+Wax	7.53	25.27	41.10	59.27	69.40	73.33	45.98	30.30
Chabir+Wax	5.20	18.87	37.67	42.77	56.23	65.80	37.76	42.78
Garlic+Wax	11.73	28.87	39.60	60.93	69.03	76.73	47.82	27.53
Parthanium+Wax	15.40	29.00	50.27	63.27	77.93	81.20	52.84	19.91
Anar+Wax	5.27	18.07	34.00	44.83	55.70	62.40	36.71	44.36
Pathkanda+Wax	13.80	34.20	51.13	68.87	74.13	81.00	53.86	18.37
Fungicide	3.40	27.80	55.47	71.13	83.73	93.00	55.76	15.49
Fungicide+Wax	5.27	16.27	33.93	54.20	64.00	73.93	41.27	37.45
Control	23.93	46.20	65.73	79.00	86.00	95.00	65.98	

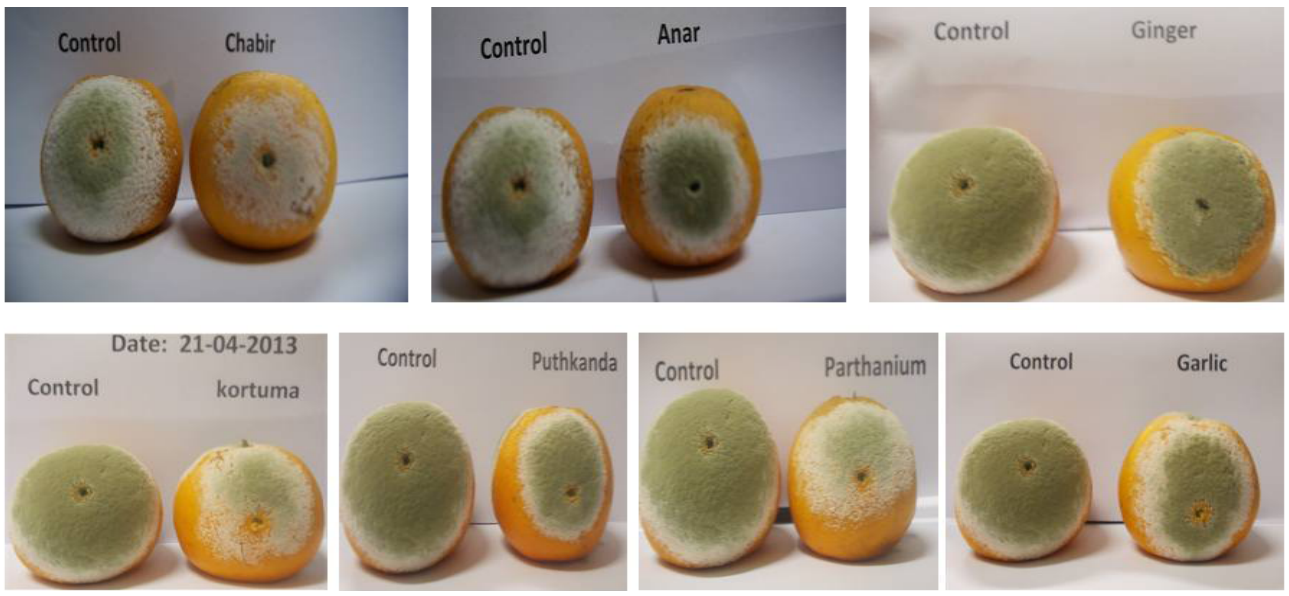


Figure 2: Comparison of application of different plant extract with control.

Table 4: Postharvest application of salicylic acid on colony growth of *P. digitatum*.

Treatments	Lesion diameter (mm) on Kinnow fruit							Growth Inhibition %
	Time (days)							
	1	2	3	4	5	6	Mean	
SA 3 mm	0.00	8.67	19.05	31.64	58.68	78.51	32.76	33.40
SA 6 mm	0.00	8.47	21.34	36.65	53.12	73.03	32.10	34.73
SA 9 mm	4.93	17.33	30.00	40.41	49.67	68.62	35.16	28.51
Fungicide10%	3.40	27.80	55.47	71.13	83.73	93.00	55.76	-13.37
Control	15.33	25.33	35.30	54.49	70.77	93.87	49.18	

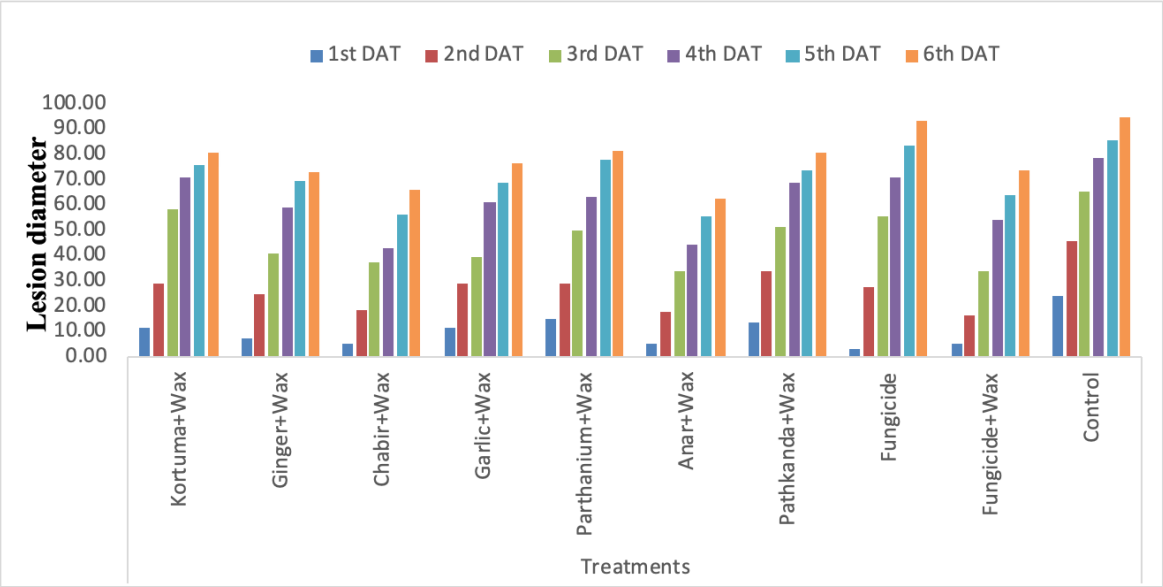


Figure 3: Effect of different allelechemicals+Wax on lesion diameter (mm) on kinnow fruit.

Fungicide and Kortuma+Wax extracts. So it was concluded that for obtaining maximum growth inhibition % plant extracts of Anar+Wax, Chabir+Wax and Fungicide+Wax was the best as they showed highest growth inhibition percentage of *P. digitatum*.

3.3 Postharvest applications of salicylic acid

The present study was conducted to evaluate the effect of different concentrations of salicylic acid against *P. digitatum*. The results revealed that (Table 4 and Figure 5, 6) the growth inhibition % and lesion diameter (mm) on kinnow fruit, respectively. It was observed that maximum growth inhibition (34.73 %) was shown by Salicylic acid concentration of 6 mm followed by Salicylic acid (33.40 %) at 3 mm concentration and Salicylic acid (28.51 %) at 9 mm concentration, while minimum growth inhibition % was recorded from Fungicide 10 % (-13.37 %). In case of lesion diameter minimum mean lesion diameter (32.10 mm) was recorded from salicylic acid at concentration of 6 mm, followed by Salicylic acid (32.76 mm) at concentration of 3 mm. Maximum lesion diameter (49.18 mm and 55.76 mm) was recorded from control and fungicide 10%. So it was concluded that for obtaining maximum growth inhibition salicylic concentration of 3 mm and 6 mm was the best as they showed highest growth inhibition percentage of pathogen.

Discussion

Postharvest pathogens significantly reduce the production of crops by lowering both fruit quality and quantity. Pathogens multiply on fruits during storage as a result of latent infection or through wound injury during handling operations. Results showed that in case of plant extracts, maximum growth inhibition (35.90 %) was shown by Anar followed by Chabir (33.60%) and Garlic (29.16%), while minimum growth inhibition was recorded from Parthanium (17.4 %) and Ginger (19.94 %). In case of plant extracts + wax maximum growth inhibition (44.36%) was shown by Anar+Wax followed by Chabir + Wax (42.78 %) and Fungicide + Wax (37.45%), while minimum growth inhibition was recorded from Fungicide (15.49 %) and Kortuma + Wax (17.48 %) while salicylic acid

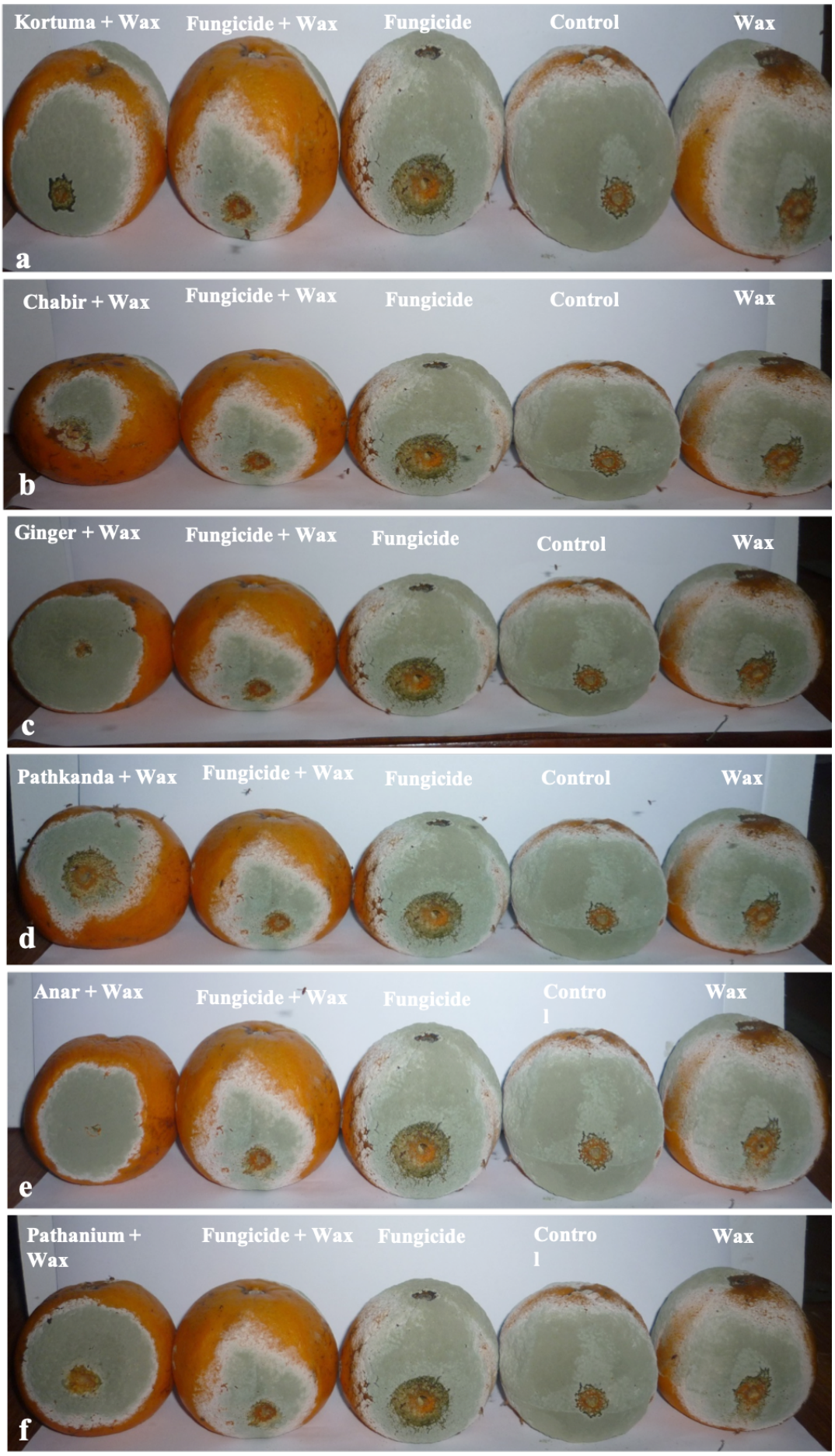


Figure 4 : Effect of plant extracts on lesion diameter: a) Kortuma+Wax; b) Chabir+Wax; c) Ginger+Wax; d) Pathkanda+Wax; e) Anar+Wax; f) Pathanium+Wax.

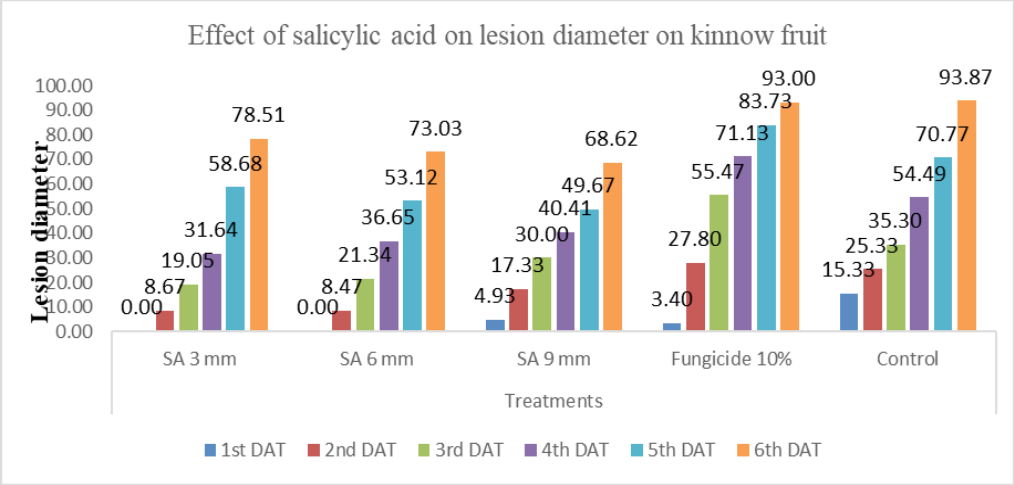


Figure 5: Effect of different concentrations of salicylic acid on lesion diameter (mm) on kinnow fruit.

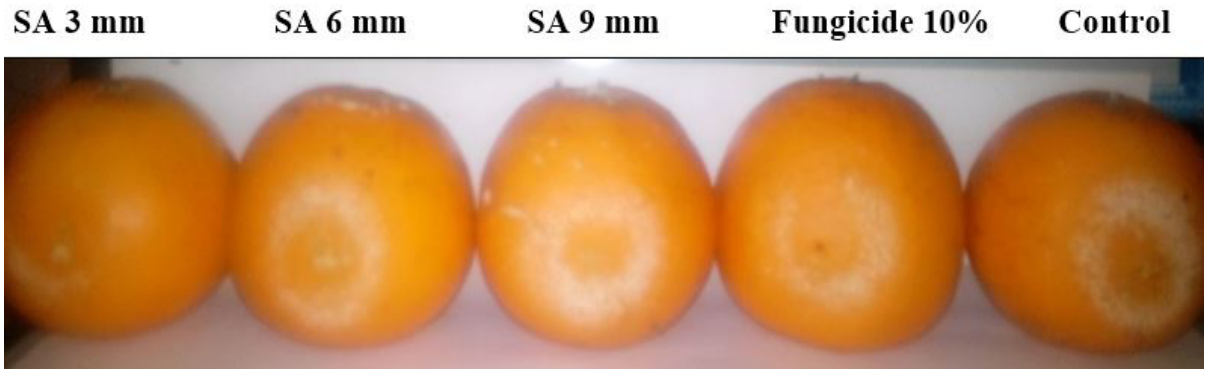


Figure 6: Effect of salicylic acid (SA) concentrations on lesion diameter on kinnow.

when used @ different concentrations, maximum growth inhibition % (34.73 %) was shown by Salicylic acid concentration of 6 mm followed by (33.40 %) at 3 mm concentration and (28.51 %) at 9 mm concentration, while minimum growth inhibition was recorded from Fungicide 10 % (-13.37 %). Almost similar results were documented in case of plant extracts when different plant extracts e.g: garlic, lemon, ginger, chillies, kortumba and pomegranate showed effective control as observed by El-Ghouth *et al.* (2000) when they performed an experiment by using different extracts of to control the fungal growth while Obagwu and Korsten (2003), Seeram *et al.* (2006), Ikeura *et al.* (2011) and Tunwari and Nahunnaro (2014) performed experiments to check the efficacy of garlic extracts on control of green moulds. They observed that all the treatments significantly reduced the both fungal growth. Contrary to our results, Al-Samarrai *et al.* (2013) observed that the application of Neem and Chili extracts at the rate of 3000 ppm resulted in complete (100%) reduction in growth of *Panicillium digitatum* in lab conditions. Abo-Elnaga (2013) studied growth inhibition percentage of *P. digitatum* on by application of garlic extract and powder and observed that application of 1% garlic resulted reduction in disease incidence and disease severity after 7 days from inoculation as compared to control. Natural resistance in fruit crops induced by the application of physical, chemical and biological elicitors have received much concentration and considered a potential strategy in postharvest disease control (Terry and Joyce, 2004). Post-harvest storage losses can be minimized by the application of SA if it will spray pre harvest as observed by Ahmad et al. (2014). Treatments of elicitor's could affect the induced systemic resistance and play a key role in natural disease control and used in IPM approach (Terry and Joyce, 2004). Similar findings also observed by El-Mougy (2002) who observed that increasing SA concentration up to 80mM caused complete inhibition to the growth of the storage pathogen such as *P. digitatum* of different fruit crops. Application of salicylic acid resulted in significantly reduction of spore formation and germination of spores of green mould as compared to control. Application of salicylic acid at the rate of

6 mM resulted in complete stoppage and reduction in growth, sporulation and conidial germination resulted by Iqbal *et al*. (2012). The results of the present study lined with all above mentioned reports that the tested plant extracts and SA significantly reduced the mycelial inhibition of the pathogen.

Conclusion

The present study suggests a potential for the control of postharvest diseases of citrus fruit by using allelochemicals and chemical elicitors. The increase in fruit resistance to fungal infection through the use of natural and low toxicity substances has become a more acceptable and an effective strategy for the management of pre- and postharvest fungal pathogens. The present findings concluded that Anar + Wax showed maximum growth inhibition (44.36 %), so it was recommended that for getting maximum growth inhibition percentage and minimum lesion diameter on fruit the combination of plant extracts with wax showed maximum growth reduction of pathogen, *P. digitatum* caused of citrus green mould.

Recommendations

The major challenge of the widespread use of alternatives to control green mould is to meet the requirement of a low production cost. Therefore, it is necessary to develop eco-friendly approaches with no or little toxicity that control post-harvest pathogens even when applied at low concentrations.

Future Prospective

More studies related to chemical elicitors and plant extracts concerning their mode of action might contribute to expand their application in the field and packinghouses.

Author's Contribution

Dr. Iqbal conceived the idea and supervised the experiment. Mr. Hafiz Abdullah Akbar executed the field visits and took the experimental data while Dr. Ghazanfar and Dr. Ahmed help to facilitate and guided the research activities in laboratory of Plant Pathology and Mr. Raza compiled the data and finalized the manuscript.

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