Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

BIO MANAGEMENT OF BOTRYTS ROT OF POST-HARVEST GUAVA FRUITS

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A B S T R A C T

Guava is one of the delicious fruits and has a remarkable nutritional value. *Botrytis cinerea* is an airborne phytopathogen with a necrotrophic life attacking more than 200 crops globally and hard to control because of its diversity of ways of attack. Chemical fungicides cast harmful effects on the environment as well. Therefore, bio-management is a very useful and important strategy. In this research, disease samples were collected from different areas of Rawalpindi and Islamabad, disease incidence was calculated to be 38.9%, 23.0% and 28.8% from Sabzi Mandi, Dhok Kala Khan and Raja Bazar markets, respectively. Four plant extracts*, i.e.*, Garlic, turmeric, ginger and Eucalyptus and an antagonist *Trichoderma harzianum* were used against *B. cinerea*. After treatment of plant extracts as well as *B. cinerea,* fruits were kept in sterilized boxes under plastic chamber. Temperatures and moisture level were maintained 65% at 18˚ to 23°C for optimum growth of pathogen. After 4 days, fruits were noticed for the effect of botanicals as well as biological agent *in vivo* and *in vitro* conditions. Garlic was found to be the most effective control and eucalyptus was the least effective with their specificity in antagonistic effect against *B. cinerea.* Biological control agent was found to be the least effective control measure against *B. cinerea*. This research shows the efficacy of garlic and Turmeric at 10% concentration and suggests that these may be used at domestic levels to prevent the gray mould.

Keywords: Guava, Biological control, Botrytis, *Trichoderma*.

INTRODUCTION

Guava (*Psidium guajava* L.) is a common fruit cultivated in various tropical and subtropical regions of the earth. Common guava is a tree in the Myrtaceae family. *Botrytis cinerea* is the causal agent for gray mould, show serious food and ornamental crop losses, mainly postharvest (Elad *et al*., 1997). *B. cinerea*, causes gray mold rot on guava fruits, which results in enormous losses in fruit output. *B. cinerea* attack is hard to control due to its diverse ways of attack, wide hosts as sources of inoculums and its persistence ability (Choquer *et al*., 2007). Postharvest diseases may lower the quality, taste and market value of fruit during storage and transportation. (Mehmood *et al*., 2018). *B. cinerea* caused frequent loss in quality due to onset rots (Williamson *et al*., 2007). *B. cinerea* produces a range of cell wall-degrading enzymes, toxins and other low-molecular-weight compounds such as oxalic acid (Lorito *et al*., 1994). New evidence suggests that the pathogen triggers the host to induce programmed cell death as an attack strategy. *B. cinerea* is responsible for a very wide range of symptoms. Soft rots, accompanied by collapse and water soaking of parenchyma tissues, appearance of grey masses of conidia on leaves and soft fruits (Liu *et al*., 2022).0020 In thick-skinned fruits, the dark water-soaking symptom is evident only after cutting.

Use biological agents to control plant pathogens are the most widely studied and utilized microorganism as biocontrol such as *Trichoderma spp.* has been widely used as a biocontrol agent because it affects a large variety of phytopathogenic fungi that are responsible for major crop diseases (Elad and Chet, 1995). Various plant extracts have been recognized as a natural bio-control agent and have particular substances against phyto pathogen.

Current study was conducted to examine the disease

Submitted: May 30, 2023
Revised: June 08, 2023
Accepted for Publication: June 12, 2023
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incidence of *Botrytis rot* by conducting survey and Bio management of diseases using biocontrol agents. This report will help other researchers to develop integrated strategic method to manage the disease in future to avoid post -harvest losses.

MATERIALS AND METHODS

Field survey was conducted of local markets *viz*., Sabzi mandi, Raja Bazar and Dhoke Kala Khan Markets (Islamabad/Rawalpindi) to estimate the disease incidence. Ten shops were randomly selected and collected fruit samples were checked. Disease incidence was calculated using the following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No. of Infected samples}}{\text{Total No. of samples}} \times 100$$

Laboratory work, including isolation, morphological characterization, pathogenicity tests, *in vitro* and *in-vivo* bio-control through plant extracts and antagonistic fungi were performed at the Fungal Plant Pathology Laboratory, Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.

Isolation, Purification and Identification: Infected samples of guava were rinsed under tap water dried completely over sterile filter paper. The symptomatic portions of fruits were cut into 5 to 10 mm piece and placed on Petri dish containing 9 mL potato dextrose agar (PDA). Then these media plates were placed in incubator at temperature 25+2°C. After 4-5 days, fungus culture was established and able to observe with naked eye. The cultures were purified by taking agar plug from actively growing colony edge and placed on PDA plates. Morphological identification of *B. cinerea* was on the basis of colony, colour, spores, stripe length, and septation.

***In Vitro* and *In Vivo* Management Through Plant Extracts And Biological Control Agent: Preparation of Plant Extract:** Four plant extracts i.e., ginger, garlic, eucalyptus and turmeric were used against *B. cinerea* post-harvest attack. Methanol extract technique was followed for the formation of plant extracts. In this technique, plant parts were washed with water and dried under shade and then ground it into make their powder. The methanol and plant parts (powder) were taken in 1:3 *i.e.*, 30 grams of plant powder were put in 90 ml of methanol. After continuously mixing of methanol and plant powders, these solutions were kept in glass bottles for 48 hours and homogenized solution were kept for better extraction. After 48 hours, the extracts were filtered through Whatman filter paper and kept in glass pan for the complete evaporation of methanol. After 24 hours of continuous evaporation of methanol, these extracts were harvested and applied by making 2.5%, 5%, and 10% concentrations in distilled water (Sattar *et al*., 2014).

Cultures: A previously isolated culture of *B. cinerea* was maintained at 25 °C for 7-14 days on PDA medium. Conidial suspension from this fungal culture was prepared by washing the conidial mass from pure colony in to 10mL of sterilized distilled water. Then 1mL from the suspension was poured into the test tube, which contains 9 mL of sterilized distilled water

***In-Vitro* and *In- Vivo* Application Of Plant Extracts:** *In vitro* conditions, the plant extracts were made in three different concentrations *i.e.,* 2.5%, 5% and 10% concentrations in PDA media. Each plant extract concentration was further examined by three replicates. The control was also kept as a treatment of pathogen in media plates without addition of any plant extract. Then this media was examined against the fungal pathogen by poisoned food technique. The mycelial growth of *B. cinerea* was noticed after 24 hours to seven days for the confirmation of effect of plant extract.

The fruits of guava were also coated with 2.5%, 5% and 10% solution of plant extracts *in vivo* condition. Then these coated fruits were treated with the *B. cinerea* spore suspension @ 10^5 mL^{-1}. Then these treated guava fruits were observed daily for noticing the actual result and anti-fungal property of plant extract and also their effect on the shelf life of post-harvest guava.

The impact of plant extract application was confirmed by the following formula.

$$\text{Growth inhibition(\%)} = \frac{DC - DT}{DC} \times 100$$

Where Dc is mean of colony diameter of control set and Dt is mean of colony diameter of treatment sets. This formula gave us the zone of inhibition according to each plant extract treatment.

In Vitro* and *In Vivo* Application of *Trichoderma harzianum: For *in-vitro* application of *T. harzianum*, both the pathogenic fungus (*B. cinerea*) as well as bio-control agent (*T. harzianum*) was placed in a same media plate by following the dual culture technique. After 24 hours, both fungi were grown in media plate and data were taken for seven days. The impacts of fungal growth were noticed against one another for the evaluation of bio-control agent against the mould.

For *in-vivo* application of *T. harzianum,* Guava fruits were washed with detergent first to clean the surface. Then these fruits were treated with the spore suspension of bio-control agent and after six hours these fruits were retreated with pathogenic fungus. These fruits were kept in

disposable sterilized boxes and maintained humidity in plastic chamber. After 48 hours, these fruits were noticed for the impact of bio-control agent against the fungus.

STATISTICAL ANALYSIS

Analysis for variance (ANOVA) was done for *in vitro* and *in vivo* experiments, three- factor-factorial and two-factor-factorial design were applied for *in-vitro* and *in-vivo* experiments respectively. For the confirmation of effect of different plant extracts against *B. cinerea,* means were differentiated using LSD test. Software (Statistix 8.1) was used for the purpose.

RESULTS AND DISCUSSION

According to the survey, Sabzi Mundi Islamabad was the biggest market and the total disease incidence was found to be 38.88%, Raja bazaar was the second with 28.81%and Dhoke kala khan was the smallest market surveyed with 23% disease incidence. (Figure 1). The probable reason for more incidence of gray mould at Sabzi mundi was the presence of huge bulks of many fruit consignments coming from various parts of the country, some of them letting unattended, and becoming rotten. Furthermore, sanitary conditions were not found up to the mark most of the times.

Four plant extracts *i.e.*, ginger, garlic, eucalyptus and turmeric extracts were examined against growth of *B. cinerea* in *in-vitro* conditions. Each of the plant extracts was made into three different concentrations *i.e.,* 2.5%, 5.0% and 10%. Each plant extract concentration was further examined by three replications.

In *in-vitro* conditions, garlic was the most effective plant extract in all concentrations (2.5%, 5.0% and 10%) growth of fungus was fully inhibited against 10%, 0.99 cm against 5% while just 1.8 cm against 2.5 % concentration. Turmeric was the second most effective control measure. Fungus growth was examined 2.1 cm against 2.5%, 1.39 cm against 5% while that was fully inhibited against 10% concentration of turmeric extract. Ginger was the effective control after Turmeric, growth was examined 2.4 cm against 2.5%, 1.79 cm against 5% and 0.11 against 10% concentration. Eucalyptus was the least effective control against *B. cinerea* growth in vitro. Growth was recorded 2.7 cm against 2.5%, 1.8 cm against 5% and 0.24 cm against 10 % concentration of eucalyptus extract (Table 1) after 7 days of incubation.

Application of biological agent was determined by the using of dual culture technique in which *T. harzianum* and *B. cinerea* were examined. Total 4.2 cm mean growth of pathogen was seen in dual culture against *T. harzianum* after 7 days of incubation. While 6.2 cm mean growth was examined in control replications. Many plant extracts contain phytochemicals that exhibit antimicrobial and cytotoxic effects on various pathogens (Feldberg *et al*., 1998). Allyl isothiocyanate from mustard was able to inhibit the growth of *Penicillium expansum*.

Under in vivo conditions, all plant extracts and *T. harzianum* were sprayed on fruits and then spore suspension of *B. cinerea* was sprayed. Among plant extracts and *T. harzianum* the lowest disease incidence (23%) was calculated in 10% concentration in garlic while the highest disease incidence (70%) was calculated in 2.5% concentration of eucalyptus plant extract and overall the highest disease incidence (77%) was calculated in control treatment. (Table 2) Plants were inoculated with 105 spore suspension of *B. cinerea*. These plants were incubated for 24 h. The control plants were sprayed uniformly with 10 ml of sterilized distilled water.

Plant extracts are also gaining importance as a biological control. They have a specific significance in the integrated disease management because of their no side residual effects on fruits, easy availability and environment friendly and non-toxic nature (Aqil *et al*., 2010).

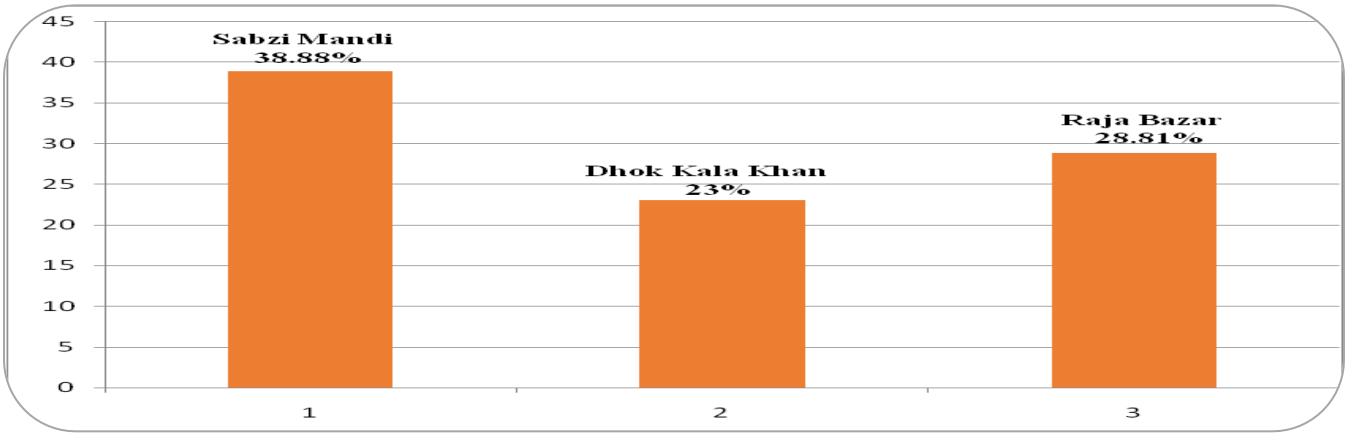

Figure 1. Mean disease incidence of Gray mold on different samples collected from various markets.

Table 1. Mean growth (cm) of *B. cinerea* on PDA media after treating with different concentrations of Plant extracts and *T. harzianum*as positive control

	Turmeric						Ginger						
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I	2.5%	M.G.I	5%	M.G.I	10%	M.G.I	Control
3	0.74	70%	0.1	95%	0	100%	0.6	75%	0	100%	0	100%	2.4
5	1.46	59%	1.17	95%	0	100%	1.48	58%	0.31	91%	0	100%	3.6
7	2.1	66%	1.39	77%	0	100%	2.4	61%	1.79	71%	0.11	98%	6.2
	Eucalyptus						Garlic						*T.harzi-anum*	
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I	2.5%	M.G.I	5%	M.G.I	10%	M.G.I	Mean	M.G.I
3	1.2	50%	0	100%	0	100%	0	100%	0	100%	0	100%	2.1	12%
5	2.3	36%	0.9	75%	0	100%	1.21	66%	0.7	80%	0	100%	3.2	11%
7	2.7	56%	1.8	70%	0.24	96%	1.8	70%	0.99	84%	0	100%	4.2	32%

Table 2. Mean disease incidence (M.D.I, %) and mean growth inhibition (M.G.I, %) over control after4 days of treatment under *in-vivo* condition

Treatment	Infected	Total	M.D.I	M.G.I over Control
Control	23	30	77%	
T. harzianum	14	30	47%	39%
Eucalyptus (2.5%)	21	30	70%	9%
Eucalyptus (5%)	18	30	60%	22%
Eucalyptus (10%)	13	30	43%	44%
Ginger (2.5%)	20	30	67%	13%
Ginger (5%)	17	30	57%	26%
Ginger (10%)	12	30	40%	48%
Turmeric (2.5%)	19	30	63%	18%
Turmeric (5%)	16	30	53%	31%
Turmeric (10%)	11	30	37%	52%
Garlic (2.5%)	18	30	60%	22%
Garlic (5%)	15	30	50%	35%
Garlic (10%)	7	30	23%	70%

An effective postharvest technology for maintaining quality of fruit is application of an edible coating to modify the internal atmosphere of the fruit (Germano *et al.,* 2019). Different edible coatings have been created using lipids, proteins and carbohydrates (Etemadipoor *et al.,* 2019).

Use of garlic as an essential oil in combination with Aloe vera gel coating on banana postharvest quality and incidence of anthracnose disease has also been reported (Khaliq *et al*., 2016). Garlic has prophylactic and therapeutic activity, also contains sulfur and polyphenol. It has good antibacterial, antifungal and antioxidant activity (Yara-Queiroz *et al*., 2009). Garlic and ginger extracts are used to preserve the quality of many fruit and vegetable crops during postharvest period. Extract of ginger is also useful for mango (Adams *et al*., 2016), papaya (Ali et al., 2016) and plantain (Banjoko *et al*., 2019) preservation.

Similar results were reported about the antifungal potential of indigenous plants of Pakistan which showed maximum inhibition of growth of *B. cinerea in vitro* and *in vivo* experiments. High contents of phenolics and flavones had major antifungal activity against *B. cinerea* related with strawberry fruit.(Zobia *et a*l., 2022)

REFERENCES

Adams, A., J. Chonga and B. Thomas. 2016. Effect of Ginger extracts and storage temperature on shelf life of 'Kent'mango fruits. International Journal of Postharvest Technology and Innovation, 4: 75-82.

Ali, A., G. Hei and Y. Keat. 2016. Efficacy of ginger oil and extract combined with gum arabic on anthracnose and quality of papaya fruit during cold storage. Journal of Food Science and Technology, 53: 1435-1444.

Aqil, F., M. Zahin, I. Ahmad, M. Owais, M.S.A. Khan, S.S. Bansal and S. Farooq. 2010. Antifungal activity of medicinal plant extracts and phytocompounds: a review. Combating Fungal Infections, 449-484.

Banjoko, I. O., O. Olatidoye, A.O. Oyelola, O.A. Ajibosin and S. Adebayo. 2019. Influence of aqueous extract of ginger and garlic on shelf life studies of plantain fingers (*Musa paradisiacal*) at ambient

and refrigerated conditions. EC Nutrition, 14: 301-308.

Choquer, M., E. Fournier, C. Kunz, C. Levis, J.M. Pradier, A. Simon and M. Viaud. 2007. *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. FEMS microbiology letters, 277: 1-10.

Elad, Y. and I. Chet. 1995. Practical approaches for biocontrol implementation. In: Reuveni R (ed) Novel Approaches to Integrated Pest Management. Lewis Publishers, CRC Press, Boca Raton, Florida, USA. pp 323–338.

Elad, Y. 1997. Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. Biological Reviews, 72: 381-422.

Etemadipoor, R., A. Ramezanian, A.M. Dastjerdi and M. Shamili. 2019. The potential of gum arabic enriched with cinnamon essential oil for improving the qualitative characteristics and storability of guava (*Psidium guajava* L.) fruit. Scientia Horticulturae, 251: 101-107.

Feldberg, L., H. Wiener and Y. Sasson. 1998. Copper-catalyzed homolytic and heterolytic benzylic and allylic oxidation using tert-butyl hydroperoxide. Journal of the Chemical Society, Perkin Transactions, 2: 2429-2434.

Germano, T. A., R.P. Aguiar, M.S.R. Bastos, R.A. Moreira, J.F. Ayala-Zavala and M.R.A. de Miranda. 2019. Galactomannan-carnauba wax coating improves the antioxidant status and reduces chilling injury of '*Paluma'guava*. Postharvest Biology and Technology, 149: 9-17.

Jabeen, Z., Riaz, A., Naz, F., Ahmed, M. S., & Raheel, A. (2022). Evaluation of Antifungal Potential of Indigenous Plant Extracts against Grey Mould and HPLC And LC-MS Based Identification of Phytochemical Compounds in *Polygonum amplexicaule* D. Don Extracts. International Journal of Phytopathology, 11(3), 287-299.

Khaliq, G., M.T.M. Mohamed, H.M. Ghazali, P. Ding and A. Ali. 2016. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biology and Technology, 111: 362-369.

Liu, Y., M. Sun, Q. Yu, R. Li, X. Hu, P. Zhang and T. Zhou. 2022. Identification and Analysis of miRNAs and siRNAs in *Botrytis cinerea*. American Journal of Plant Sciences, 13: 623-649.

Lorito, M., C. Peterbauer, C.K. Hayes and G.E. Harman. 1994. Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. Microbiology, 140: 623-629.

Mehmood, N., A. Riaz, F. Naz, I. Hassan, S. Ghuffar, A. Sattar, S. Zafar and S. Anwaar. 2018. First report of preharvest fruit rot of strawberry caused by *Botrytis cinerea* in Khyber Pakhtunkhwa province and Islamabad (Pakistan). Plant Disease, 102: 450-50.

Sattar, A., A. Riaz, S. Ahmed and I. Hassan. 2014. Efficacy of selected plant extracts for inhibition of *Penicillium expansum* growth on apple fruits. Pakistan Journal of Phytopathology, 26: 63-66.

Williamson, B., B. Tudzynski, P. Tudzynski and J.A. Van Kan. 2007. *Botrytis cinerea*: the cause of grey mould disease. Molecular plant pathology, 8: 561-580.

Yara-Queiroz, S., Y. Emília-Ishimoto, H.M. Deborah Bastos, R. Geni-Sampaio and A.F.S. ElizabethTorres. 2009. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: in vitro antioxidant activity. Food Chemistry, 115: 371–374.

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