



Research Article

Management of Storage Decay of Strawberry Caused by *Botrytis cineria* by Using Organic Salt Coating

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Abstract

The study analyses an experimental investigation for managing the storage decay of strawberries caused by *Botrytis cineria* and extending the shelf life of strawberry using inexpensive and environmentally safe organic salts. Three organic salts were used (i) Magnesium chloride (ii) Sodium chloride (iii) Calcium chloride. Fresh fruit was coated with different salt concentrations of 10%, 20%, and 30% using a cotton swab. . One chemical TBZ was also used as fruit coating materials with same concentrations of 10%, 20%, and 30%. All these fruits were placed in polythene zipper bags and rotting symptoms were checked after 2-, 3- and 4-days intervals. Recorded data was subjected to analysis of variance (ANOVA) at 5% level of significance and for statistical analysis by using LSD test and "Statistics" software. Among all the organic salts, magnesium chloride exhibited maximum growth inhibition (5.248) followed by sodium chloride (10.619), calcium chloride (11.833), as compared to control.

Keywords: Strawberry; Post-harvest; *Botrytis cinerea*; Organic salt.

Introduction

Strawberries (*Fragaria ananassa* L.) are cultivated in many areas in the world. The U.S is generating more than 1 million tonnes of strawberries every year and declared as primary producer of strawberries. California, Oregon, and Florida are major strawberry producing countries. California accounts for 80% of total strawberry production in the United States. The estimated number of strawberries grown in New Zealand was 6,500 tonnes (Aitken and Hewette, 2012). It contains a lot of dietary value. It is consumed fresh as well as in the form of different products such as jams, jellies, juices, and squashes. Many varieties of strawberries are cultivated worldwide, but in Pakistan, Noor, Chandler, Karoz, Tuftus, Douglas, Commander, and Pajero are cultivated (Mahmood *et al.*, 2012). Strawberry is grown in many areas of Pakistan primarily in Gujrat, Charsada, Islamabad, Lahore, Mardan, Haripur, Swat, Mansehra, and Karachi. In Islamabad, mostly chandler variety is grown for edible purpose while Pajero, Douglas, and Commander are grown only for the research purpose. The required pH of soil to produce strawberries is about 4.6-6.5 (Milosevic, 1997). Strawberries are rich source of



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photochemical, minerals, and vitamins that are energetic for ideal health. Strawberries also contain anthocyanin that are very effective against various diseases such as scurvy, cancer, and ageing. Strawberry is also an outstanding source of vitamin C, which helps the body to generate a healthy immune system and display excessive antagonism against inflammation. It creates recycled free radicals from the body due to its significant antioxidant activity (Lee and Kader, 2000).

Pathogens can easily attack on the strawberry as it contains a high level of sugar and a low pH. Post-harvest diseases destroy 10-30% of the total crop production in underdeveloped countries; they often harm higher than 30% of crop yield in many perishable crops (Agrios, 2005; Lee and Kader, 2000). Grey mould and leak are causing many dangerous diseases on strawberries (Ceponis *et al.*, 1987). The pathogen that causes strawberry fruit rot after harvest is *Botrytis cinerea*, which is followed by *Rhizopus stolonifer*, *Mucor spp.*, *Colletotrichum spp.*, and *Prnicillium Spp.* The use of fungicides before harvesting is the standard method of controlling post-harvest strawberry deterioration. When strawberry is at the flowering stage, *B. cineria* attack it and cause rotting (Babalar *et al.*, 2007). *B. cinerea* is thought to be the most prevalent and significant pathogen of the strawberry during postharvest. Application of chemicals on strawberries deteriorate the quality of fruit so the alternative ways should be explored. The current study was conducted to evaluate the efficacy of different organic salts against *B. cinerea* under in vitro conditions to avoid the non-judicious use of chemicals.

Methodology

Samples were collected in polythene zipper bags from different Faisalabad markets, fruit stores, and cold storage and brought to the Seed Pathology Lab, Department of Plant Pathology, UAF. The samples were taken at various seasons of the year. The normal disease signs and symptoms of a fungal infection were considered during the collection of samples.

Preparation and pouring of PDA media

For the purpose PDA media was prepared and poured into respective petri dishes and placed in laminar flow chamber.

Isolation, identification, purification and preservation of Fungi

The fungus was isolated from affected strawberries samples and identified under microscope on the basis of morphological characters. Purification was done by using single hyphal method. For preservation, test tubes were poured with PDA media and placed on a slant position to solidify. After solidification, the PDA slants were inoculated with pure culture and labeled. Then the inoculated PDA slants were incubated at 25 ± 2 °C for 2-3 days. After that, the PDA slants were stored at -4 °C for future studies. A pathogenicity test was performed in the laboratory to determine whether the fungus was responsible for producing rots in strawberries.

Management of *Botrytis cineria* with Different Salts

In this experiment, strawberries were coated with different salts and their effects were studied in inhibiting the growth of fungus which causes decay in strawberries. Three different salts named MgCl, NaCl, CaCl, and one chemical TBZ were used as fruit coating materials with three concentrations of 10%, 20%, and 30%. For the 10% concentration, 1ml of the salt was mixed with 10ml of the ethanol. For the 20% concentration, 2ml of salt was mixed in 10 ml of the ethanol, and in the same way, for the 30% concentration, 3ml of salt was mixed in 10 ml of the ethanol. The experiment was replicated thrice.

Preparation of stock solution

To make stock solution, the active ingredients of the relevant salts with appropriate amount were weighed and dissolved in 100 ml of sterilized distilled water in glass bottles. Stock solution was prepared in conical flask of 250ml and then saved in the plastic bottles with proper labelling. Stock solution was prepared by using the formula given below (Bano *et al.*, 2021).

Statistical analysis

The data was recorded and subjected to statistical analysis by using LSD test according to Steel *et al.*, 1997.

Result and Discussion

Management by Organic Salt Coating

Different chemicals and fungicides are used extensively throughout the world, but they contain many residual effects that affect human health and destroy the environment. So, the current study was carried out by using the salts as an alternative to the fungicides. It is better than fungicides because it does not leave any residual effects and can also control the pathogen of strawberries efficiently. These salts are not so expensive; rather, they are cheaper than chemicals. Making proper spacing between the plants with proper cleanliness standards can considerably reduce the occurrence of grey mold (Legard *et al.*, 2000).

In this experiment, strawberry fruits were coated with different organic salts like sodium chloride, calcium chloride, magnesium chloride, and their effects were studied in inhibiting the growth of fungus which causes decay in strawberries. Three different salt suspensions were made: 10%, 20%, and 30%. 10ml of the water was combined with 1ml of salt to create the 10% suspension. For a 30 percent suspension concentration, the same amounts of ethanol and salt were combined to make a 30 percent suspension (10ml of water was combined with 3ml of salt). TBZ was also used as a standard. First, salt was coated on the strawberry fruit with the help of a cotton swab. Then a circle was made on the fruit and the fruit was injured with the help of a contaminated needle in the different points inside the circle. The data was then noted for the 2-, 3-, and 4-day intervals. A Complete Randomized Design (CRD) was used to evaluate the given data, and an ANNOVA was used to test the efficacy of the trial interventions. The data was collected using statistical techniques, and the significance of the results was determined by testing the mean.

Evaluation of fungicides against *Botrytis cinerea* under *In-vivo* conditions

The ANOVA indicated that all treatments (T), days (D), concentrations (C) and their interactions (T×C) and (T×D) except (C×D) and (T×C×D) showed significant results against *Botrytis cinerea* (Table 1).

Table 1. Evaluation of salts against *B. cinerea* under lab conditions.

Source	DF	SS	MS	F	P
Concentration	2	258.22	129.11	15.28	0.0000*
Days	2	656.57	328.29	38.84	0.0000*
Treatment (T)	4	7852.74	1963.18	232.27	0.0000*
Concentration*Days	4	60.34	15.09	1.78	0.1390 ^{NS}
Concentration*T	8	163.78	20.47	2.42	0.0000*
Days*Treatment	8	747.83	93.48	11.06	0.8403 ^{NS}
Concentration*Day*T	16	86.94	5.43	0.64	
Error	88	743.80	8.45		
Total	132				
LSD	1.305				

Evaluation of salts against *Botrytis cinerea* causing gray mold of Strawberry under lab conditions

Among all the treatments TBz as a Chemical (2.789) and magnesium chloride as a salt exhibited maximum growth inhibition (5.248) followed by Sodium chloride (10.619), Calcium chloride (11.833), as compared to control (Table 2, Figure 1).

Interaction between treatment and day (T×D) showed that TBz (1.1004, 2.7009, 5.6674) & MgCl₂ exhibited maximum growth inhibition (5.6111, 3.200, 7.525) after 1st, 2nd and 3rd day respectively followed by NaCl₂ (9.644, 11.867, 13.989), CaCl₂ (7.488, 10.578, 13.220) and K₂CO₃ (15.522, 18.244, 20.656), after 1st, 2nd and 3rd day respectively as compared to control (25.000, 25.000, 25.000) (Table 3, Figure 2).

Table 2. Interaction between treatments and their concentrations (T×C) against *Botrytis cinerea* under lab conditions.

Sr #.	Treatment		Growth		
	Salts	Concentration (%)	10	20	30
T1	MgCl	5.6111efg	3.2000fg	7.5233be	13.022b
T2	CaCl	7.4889de	11.444bc	13.989b	5.667ef
T3	NaCl	9.644cd	1.40000h	2.7000gh	25.000a
T4	TBz	1.40000h	25.000a		
T5	Control	25.000a			
LSD		1.256			

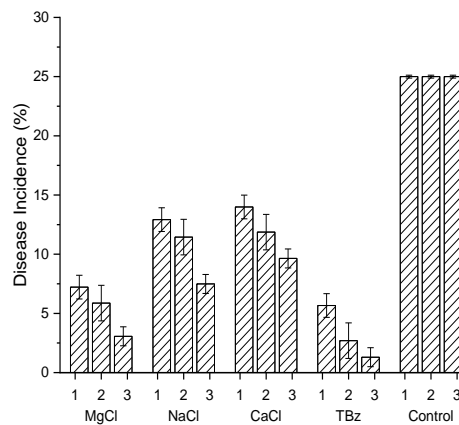


Figure 1. Impact of interaction between treatments and Days (T×D) against *Botrytis cinerea* causing gray mold of strawberry under lab conditions.

Table 3. Interaction between treatments and Days (T×D) against *Botrytis cinerea* causing gray mold of Strawberry under lab conditions

Sr.#	Treatment		Growth		
	Salts	Concentration (%)	10	20	30
T1	MgCl	5.6111efg	3.2000fg	7.5233be	13.022b
T2	CaCl	7.4889de	11.444bc	13.989b	5.667ef
T3	NaCl	9.644cd	1.1000h	2.7000gh	25.000a
T4	TBz	1.1000h	25.000a		
T5	Control	25.000a			
LSD		1.023			

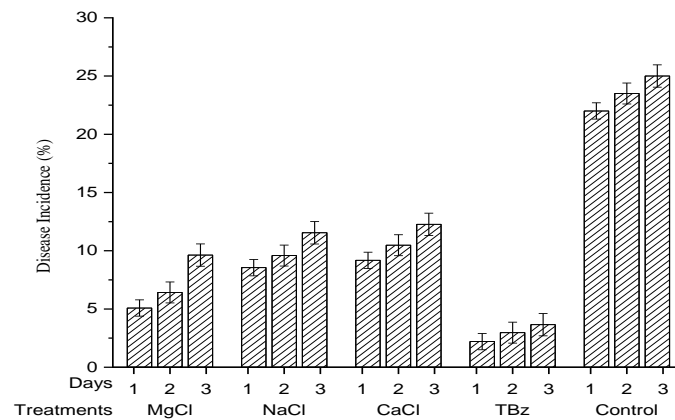


Figure 2. Evaluation of interaction between treatments and Days (T×D) against *Botrytis cinerea* under lab conditions

Conclusion

Among all the treatments TBz as a Chemical (2.789) and magnesium chloride as a salt exhibited maximum growth inhibition (5.248) followed by Sodium chloride (10.619), Calcium chloride (11.833), as compared to control

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

References

- Anwar, S. 1989. Investigations on nematodes associated with field, vegetable and fruit crops. Technical Bulletin. Barani Agricultural College, Rawalpindi, Pakistan.
- Anwar, S., S. Gorski, M. Anwar-ul-Haq and P. Yousuf. 1991. Plant parasitic nematodes of some field, vegetable, fruit and ornamental crops. Journal of Agricultural Research, 29: 233-49.
- Anwar, S. and M. Khan. 1992. Evaluation of four vegetables against *Meloidogyne incognita*. Journal of Agricultural Research, 30: 415-21.
- Anwar, S. A. and M. McKenry. 2012. Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses in Punjab, Pakistan. Pakistan Journal of Zoology, 44: 327-33.
- Anwar, S. A., A. Zia, M. Hussain and M. Kamran. 2007. Host suitability of selected plants to *Meloidogyne incognita* in the Punjab, Pakistan. International Journal of Nematology, 17: 144-50.
- Barker, K. R. and S. R. Koenning. 1998. Developing sustainable systems for nematode management. Annual Review of Phytopathology, 36: 165-205.
- Brand, D., C. Socol, A. Sabu and S. Roussos. 2010. Production of fungal biological control agents through solid state fermentation: A case study on *Paecilomyces lilacinus* against root-knot nematodes. Micologia Aplicada International, 22: 31-48.
- Eisenback, J., H. Hirschmann, J. Sasser and A. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes [*Meloidogyne* species] with a

pictorial key.

- Fourie, H. and A. McDonald. 2000. Nematodes. ARCLNR leaflet. Crop Prot. Ser, 18.
- Hunt, D. J. and Z. A. Handoo. 2009. Taxonomy, identification and principal species. Root-knot nematodes, 1: 55-88.
- Hussain, M., M. Zouhar and P. Rysanek. 2017. Comparison between biological and chemical management of root knot nematode, *Meloidogyne hapla*. Pakistan Journal of Zoology, 49: 205-10.
- Hussain, M. A., T. Mukhtar and M. Z. Kayani. 2014. Characterization of susceptibility and resistance responses to root-knot nematode (*Meloidogyne incognita*) infection in okra germplasm. Pakistan Journal of Agricultural Sciences, 51: 319-24.
- Jairajpuri, M., M. Alam and I. Ahmad. 1990. Nematode biocontrol. Aspects and prospects. CBS Pub. and dist. Pvt. Ltd. Dehli, India: 152.
- Javed, N. 2000. Evaluation of neem products against root knot nematode *M. javanica* and their possible integration with other biocontrol agents *Pasteuria penetrans*, Ph. D. dissertation, University of Reading, U. K.
- Kayani, M. Z., T. Mukhtar, M. A. Hussain and M. I. Ul-Haque. 2013. Infestation assessment of root-knot nematodes (*Meloidogyne* spp.) associated with cucumber in the Pothowar region of Pakistan. Crop Protection, 47: 49-54.
- Maqbool, M., S. Hashmi and A. Ghaffar. 1988. Problems of root-knot nematode in Pakistan and strategy for their control. US-Pakistan International Workshop on Plant Nematology, Karachi (Pakistan), 6-8 Apr 1986.
- Maqbool, M. A. 1986. Classification and distribution of plant parasitic nematodes in Pakistan: 58.
- Mateille, T., B. Thio, Y. Konate, A. Sawadogo and M. Diop. 2000. Incidence de quelques facteurs agronomiques sur les populations de *Meloidogyne* spp. et leurs principaux organismes parasites en culture maraichère sahélienne. Nematology, 2: 895-906.
- Menjivar, R., M. Hagemann, J. Kranz, J. Cabrera, A. Dababat and R. Sikora. 2011. Biological control of *Meloidogyne incognita* on cucurbitaceous crops by the non-pathogenic endophytic fungus *Fusarium oxysporum* strain 162. International Journal of Pest Management, 57: 249-53.
- Moens, M., R. N. Perry and J. L. Starr. 2009. *Meloidogyne* species—a diverse group of novel and important plant parasites. Root-knot nematodes, 1: 483.
- Mukhtar, T., M. Arooj, M. Ashfaq and A. Gulzar. 2017a. Resistance evaluation and host status of selected green gram germplasm against *Meloidogyne incognita*. Crop Protection, 92: 198-202.
- Mukhtar, T., M. Arshad H. and Zameer K. 2013a. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. Phytopathologia Mediterranea, 52: 66-76.
- Mukhtar, T., I. Arshad, M. Z. Kayani, M. A. Hussain, S. B. Kayani, A. M. Rahoo and M. Ashfaq. 2013b. Estimation of damage to okra (*Abelmoschus esculentus*) by root-knot disease incited by *Meloidogyne incognita*. Pak. J. Bot, 45: 1023-27.
- Mukhtar, T., M. A. Hussain and M. Z. Kayani. 2017b. Yield responses of 12 okra cultivars to southern root-knot nematode (*Meloidogyne incognita*). Bragantia, 76: 108-12.
- Mukhtar, T., M. A. Hussain, M. Z. Kayani and M. N. Aslam. 2014. Evaluation of resistance to root-knot nematode (*Meloidogyne incognita*) in okra cultivars. Crop Protection, 56: 25-30.
- Mukhtar, T., M. Z. Kayani and M. A. Hussain. 2013c. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Industrial Crops and Products, 42: 447-53.

- Mukhtar, T., M. Z. Kayani and M. A. Hussain. 2013d. Response of selected cucumber cultivars to *Meloidogyne incognita*. *Crop Protection*, 44: 13-17.
- Nico, A. I., R. M. Jiménez-Díaz and P. Castillo. 2004. Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. *Crop protection*, 23: 581-87.
- Sasser, J. 1979. Economic importance of *Meloidogyne* in tropical countries. *Root-knot nematodes*: 359-74.
- Sasser, J. 1980. Root-knot nematodes: A global menace to crop production. *Plant Disease*, 64: 36-41.
- Sasser, J. and D. Freckman. 1987. A world perspective on nematology: The role of the society. In: *Vistas on nematology*. Society of Nematologists, Hyatsville, MD: 7-14.
- Shahbaz, M. U., T. Mukhtar and N. Begum. 2015. Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. *International Journal of Agriculture and Biology*, 17: 31-40.
- Siddiqui, I. A. and S. S. Shaukat. 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* CHA0 in tomato: Importance of bacterial secondary metabolite, 2, 4-diacetylphloroglucinol. *Soil Biology and Biochemistry*, 35: 1615-23.
- Sikora, R. and E. Fernandez. 2005. Nematode parasites of vegetables, Luc M., Sikora RA, and Bridge J.(Eds.), *Plant parasitic Nematodes in Subtropical and Tropical Agriculture*. Wallingford, UK: CABI Publishing: 39 – 319.
- Tariq-Khan, M., A. Munir, T. Mukhtar, J. Hallmann and H. Heuer. 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. *Journal of Plant Diseases and Protection*, 124: 201-12.
- Veremis, J. and P. Roberts. 1996. Relationships between *Meloidogyne incognita* resistance genes in *Lycopersicon peruvianum* differentiated by heat sensitivity and nematode virulence. *Theoretical and Applied Genetics*, 93: 950-59.
- Whitehead, A. 1998. *Plant nematode control*. CAB International. Received, 31: 2009.