Pak. j. sci. ind. res. Ser. B: biol. sci. 2024 **67B**(1) 49-53

Fruit Vinegars as Biological Control Strategy Against Fruit Born Fungal Pathogens

Asmara Imtiaz*, Humaira Niamat and Muhammad Amjad Khan
Department of Biology, Lahore Garrison University, Lahore, Pakistan

(received May 5, 2020; revised May 12, 2023; accepted May 17, 2023)

Abstract. The aim of the study is to use natural sources as antifungal against food born fungi to avoid chemical toxicity on fruits and vegetables. In current study, antifungal property of different vinegars have been evaluated against some fruits born fungi. Fungal isolates were identified by macroscopic and microscopic study. Antifungal activity of three types of vinegars was evaluated by agar well diffusion method. Three types of fungal isolates i.e. *Acremonium* species, *Aspergillus flavus* and *Fusarium solani* isolated form orange, guava and strawberry respectively, were identified using 40x and 100x lenses of microscope. Synthetic white vinegar and mixed fruit vinegar showed maximum activity against *Acremonium* species forming zone of inhibition of 15±0.05 mm and 12±0.05 mm. *Syzygium cumini* vinegar showed maximum antifungal activity against *A. flavus* forming zones of inhibition 10±0.05 mm.

Vinegar coatings, sprays or fumes can be better alternate of chemical fungicides to keep fungal pathogens and chemical toxicity away from food.

Keywords: antifungal, fruit born, fungal pathogens, *Aspergillus flavus*, *Fusarium solani*, *Acremonium* sp., *Syzygium cumini*

Introduction

Fungal pathogens cause worldwide reduction in economically important crops productivity and postharvest loss every year (Chen *et al*., 2023; Zhang *et al*., 2021; Ncama *et al*., 2019). A global analysis of pathogen distributions revealed that a country's ability to accurately estimate and report its pathogen load and then design pathogen control strategies and increases its per capita gross domestic product (GDP) (Bebber *et al*., 2014). According to an estimate 25% of the global fruits are lost by pathogens every year from production to postharvest field (Bano *et al*., 2023; Petrasch *et al*., 2019). Use of fungicides on fruits and vegetables makes consumers more exposed to fungicides as compared to other pesticieds. Moreover, these fungicides are making fungal pathogens resistant. This situation is turning the trend towards use of biological control management (Soylu *et al*., 2010). Various environmental intrinsic factors i.e. pH, water content, substrate composition and external factors e.g. temperature, relative humidity and other microbiota already living on fruit surface, contribute and increase the chances of fungal infection in fruits (Bano *et al*., 2023; Jiao *et al*., 2021; Ciliberti *et al*., 2016). Vinegars have long been used extensively as acidulant in pickles, tomato products, salad dressings and mustards and in food processing as an important preservative. Acetification of alcoholic solutions derived from sugary or starchy materials results in the formation of vinegars. White distilled vinegar that is made by diluting the pure alcohol with water to which certain nutrients have been added) is the most commonly used vinegar in industry (Sholberg *et al*., 2000). In ayurvedic time, the juice of ripened fruits of *Syzygium cumini* (L.) is used for preparing vinegar that has been used to treat enlarged spleen, urine retention and chronic diarrhea (Kumar *et al*., 2023). In folk culture, vinegars have had been used as a cleaning agent for the treatment of wounds by inhibiting the growth of bacteria and fungi (Mota *et al*., 2014). Physico-chemical parameters and chemical composition of vinegars are influenced by different fermentation conditions (acetification system) provided (Ozturk *et al*., 2015). Modern products derived from vinegars have revealed that fruit inclusion has a major role in final quality of vinegars. The combination of vinegars nutritional benefits and fruits antioxidant content strengthens the viability of fruit vinegar production from fruit concentrates for the enhancement of functional features and preservation (Coelho *et al*., 2017).

*Author for correspondence;
E-mail: Asmaraimtiaz@lgu.edu.pk

Materials and Methods

Fungal isolation and identification. Fungal infected strawberry, guava and orange were selected for fungal isolates. Commercially available medium potato dextrose agar of company "Bio Life" was prepared according to company's instruction. Fungal isolates from each infected fruit were picked up by separate sterile loops for each fungal isolate and placed on PDA medium plates under laboratory sterile conditions. Small amount of fungal spores were placed on each plate and plate containing fungal spores were incubated at 37 °C ±2 for 72 h. After growth, very small amount of conidia were taken to observe under light microscopes with different powers of lenses i.e. 40X and 100X.

Antifungal activity. Three vinegars i.e. distilled white vinegar, mixed fruit vinegar and *Syzygium cumini* vinegar, were selected for their antifungal properties. Antifungal activity of vinegars was observed by Kirbey Bauer's agar well diffusion method (Roy *et al*., 2015; Hudzicki, 2009). Fungal isolates were swabbed separately on PDA agar plate and 100 µL of each vinegar was poured into the well and incubated at 37 °C ±2 for 72 h. Three zones of inhibition were measured with the help of vernier caliper.

Results and Discussions

Three fungal species isolates were identified on the basis of colony morphology characteristics and microscopic details i.e. *Fusarium solani* from strawberry, *Acremonium* sp. from orange and *Aspergillus flavus* from guava (Fig. 1a, b and c).

Synthetic white vinegar showed maximum size of zone of inhibition against *Acremonium* sp. i.e. 15±0.05 mm, while 10±0.05 mm against each of *A. flavus* and *F. solani* (Fig. 2a, b and c).

Mixed fruit vinegar showed maximum size of zone of inhibition against *Acremonium* sp. i.e. 10±0.05 mm, while 8±0.05 mm and 6±0.05 mm against *A. flavus* and *F. solani* respectively (Fig. 3a, b and c).

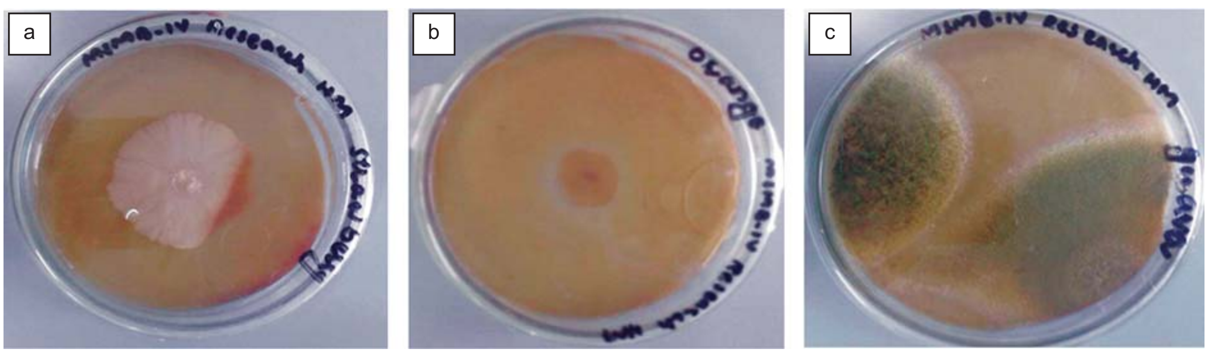

Fig. 1. Fungal isolates obtained from fruits grow on PDA medium **(a)** *Fusarium solani* **(b)** *Acremonium* sp. **(c)** *Aspergillus flavus*.

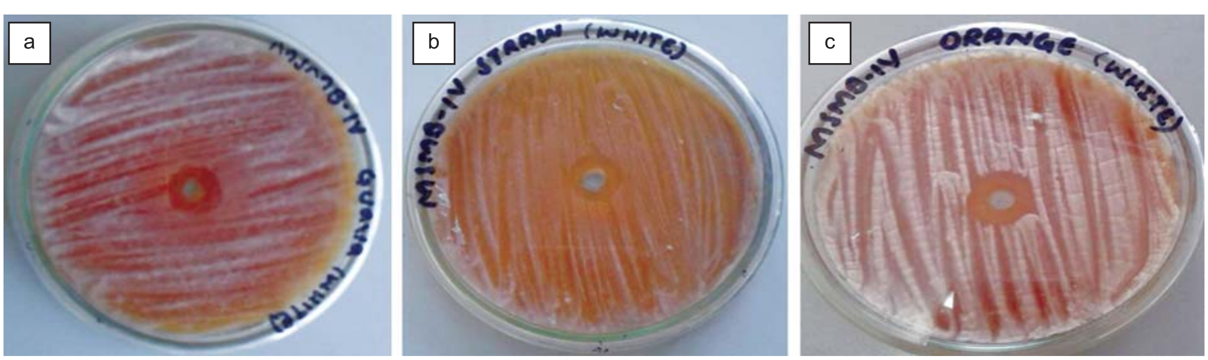

Fig. 2. Antifungal effect of synthetic white vinegar against fruit born fungal isolates **(a)** *Acremonium* sp. **(b)** *A. Flavus* **(c)** *F. solani*.

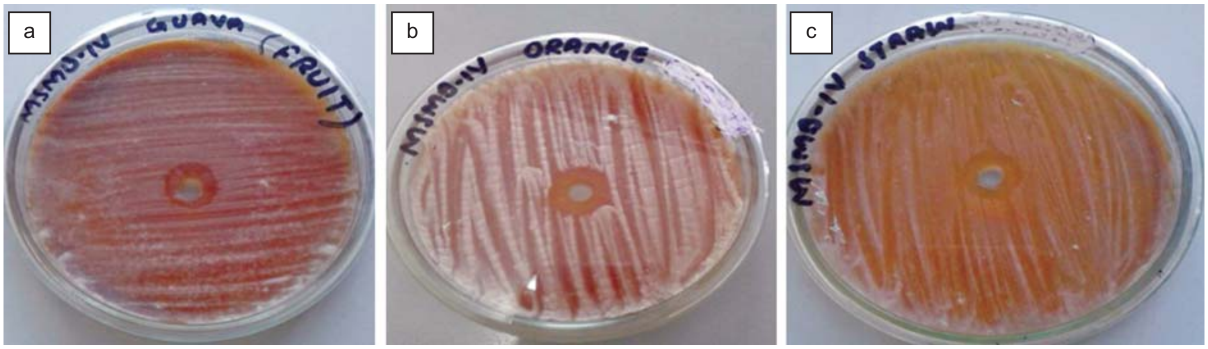

Fig. 3. Antifungal effect of synthetic white vinegar against fruit born fungal isolates **(a)** *A. flavus* **(b)** *Acremonium* sp. **(c)** *Fusarium solani.*

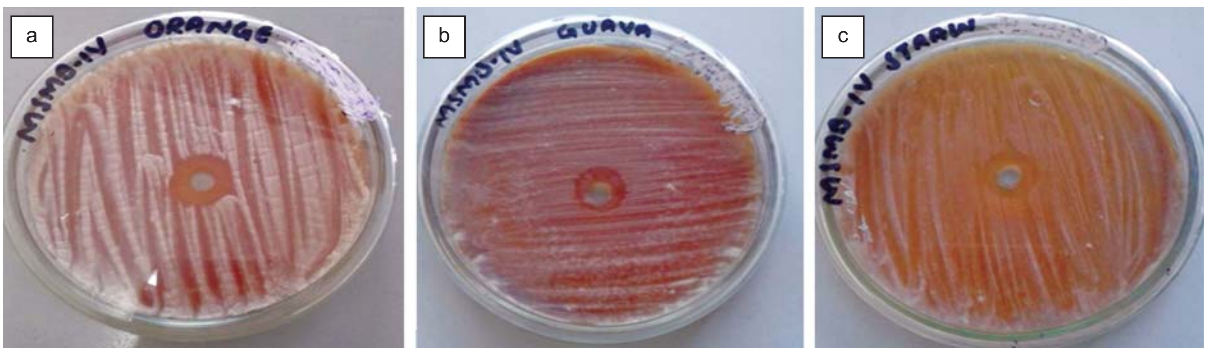

Fig. 4. Antifungal effect of synthetic white vinegar against fruit born fungal isolates **(a)** *Acremonium* sp. **(b)** *A. flavus* **(c)** *F. solani.*

Table 1. Collective response all vinegars against all fungal isolates

Vinegars 100 µL	Zone of Inhibition made by vinegars against different fungi (mm)		
	A. flavus	*Fusarium solani*	*Acremonium* sp.
Synthetic white	10±0.05	10±0.05	15±0.05
Mix fruit	8±0.3	6±0.3	10±0.05
Syzygium cumini	5±0.05	7±0.05	16±0.05

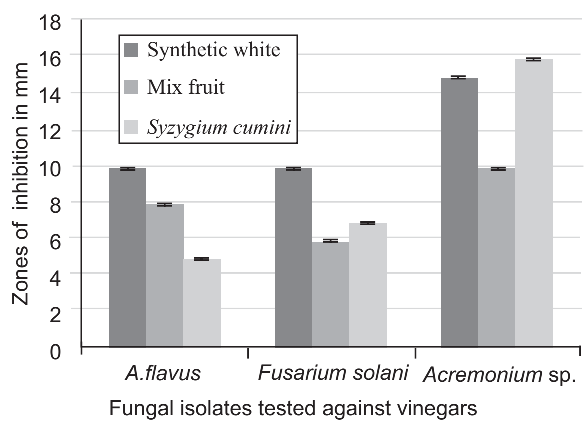

Fig. 5. Collective response all vinegars against all fungal isolates.

Syzygium cumini vinegar showed maximum size of zone of inhibition against *Acremonium* sp. i.e. 16±0.05 mm, while 7±0.05 mm against each of *A. flavus* and *F. solani* (Fig. 4a, b and c). Collective response all vinegars against all fungal isolates can be seen in Table 1 and Fig. 5.

To control post-harvest diseases of fruits and vegetables, a variety of biological control strategies are available as alternatives to the synthetic fungicides. Natural plant metabolites, micro-organisms, natural volatiles are being used as part of biological control strategies. Vinegars have long been used extensively as food preservative. The principal organic component of vinegar is acetic acid that is known for its flavouring and preservative properties. Early fumigation trials of vinegars on fruit inoculated with conidia of various postharvest fungal pathogens revealed that low quantity of pure acetic acid was required to inactivate the conidia on the fruit samples tested. Vapourizing allows acetic acid molecules to become a gas, dissociated acid that is a mixture of monomers, hydrogen bonded dimers and trimers. This un-dissociated form is responsible for its antimicrobial activity. This is because the acetic acid in the form of un-dissociated molecules can easily pass through the conidia membrane present on the fruit surface and either inactivate or kill them by lowering the pH of the cell protoplasm (Sholberg *et al*., 2000).

In current study, the commercially available vinegars have been evaluated for their antifungal activity by agar well diffusion method. Previous, studies show fumigation of these vinegars could have shown even better results than those of current study but atleast this study has shown the antifungal potential of the vinegars used. Distilled white vinegar has shown maximum antifungal properties against almost all fungal isolates as it has lowest concentration of acitic acid as compared to other vinegars used.

Fruits are highly acknowledged for having phyto-chemicals with antimicrobial and antioxidant activities. This is the reason; there is high interest on developing strategies for preservatives that add nutritive value instead of toxicity and fruit vinegars poses as a strong candidate for the design of an enhanced functional preservative (Coelho *et al*., 2017). Moreover, depending on the type of raw material used, bioactive properties of vinegars can vary in a wide range (Davalos *et al*., 2005). The study of grape vinegars showed higher bioactive properties as compared to that of apple vinegar (Ozturk *et al*., 2015). In current study, *Syzygium cumini* vinegar and mixed fruit vinegar also showed effective results against all fungal isolates and reducing concentration of acitic acid can show more improve results. *Syzygium cumini* it self has broad spectrum antimicrobial activity and vinegar of its juice in vaporized form can also be an effective preservative for food. Current study has revealed that plants metabolites in combination with various vinegars can be a better option as part of integrative biological control management. *Syzygium cumini* vinegar has been used to treat gastrointestinal problems but no antifungal property of this vinegar has been found to be reported up to best of our knowledge uptill now.

Conclusion

To avoid chemical toxicity of fungicides, mixture of vinegars with other bioactive compounds can be an effective addition in integrated biological control strategies. Fruit vinegars having fruits bioactive antimicrobial components in them can be an effective and toxin free source to avoid fruit borne pathogens.

Conflict of Interest. The authors declare that they have no conflict of interest.

References

Bano, A., Gupta, A., Prusty, M.R., Kumar, M. 2023. Elicitation of fruit fungi infection and its protective response to improve the postharvest quality of fruits. *Stresses*, **1:** 231-255.

Bebber, D.P., Holmes, T., Smith, D., Gurr, S.J. 2014. Economic and physical determinants of the global distributions of crop pests and pathogens. *New Phytologist*, **3:** 901-910.

Chen, D., Chen, T., Chen, Y., Zhang, Z., Li, B., Tian, S. 2023. Bio-source substances against postharvest diseases of fruits: mechanisms, applications and perspectives. *Postharvest Biology and Technology*, **198:** 112240.

Ciliberti, N., Fermaud, M., Roudet, J., Languasco, L., Rossi, V. 2016. Environmental effects on the production of *Botrytis cinerea* Conidia on different media, grape bunch trash and mature berries. *Australian Journal of Grapes and Wine Research*, **22:** 262-270.

Coelho, E., Genisheva, Z., Oliveira, J.M., Teixeira, J.A., Domingues, L., 2017. Vinegar production from fruit concentrates: effect on volatile composition and antioxidant activity. *Journal of Food Science and Technology*, **12:** 4112-4122.

Dávalos, A., Bartolomé, B., Gómez-Cordovés, C. 2005. Antioxidant properties of commercial grape juices and vinegars. *Food Chemistry*, **2:** 325-330.

Hudzicki, J. 2009. Kirby-Bauer disk diffusion susceptibility test protocol. *American Society for Microbiology*, **15:** 55-63.

Jiao, W., Liu, X., Li, Y., Li, B., Du, Y., Zhang, Z., Chen, Q., Fu, M. 2022. Organic acid, a virulence factor for pathogenic fungi, causing postharvest decay in fruits. *Molecular Plant Patho*logy, **23:** 304-312.

Kumar, S., Sharma, S., Kumar, V., Sharma, A., Kaur, R., Saini, R. 2023. Jamun (*Syzygium cumini* (L.) skeels): the conventional under-utilized multi-functional plant-an exotic gleam into its food and functional significance. *Industrial Crops and Products*, **191:** 115873.

Mota, A.C.L.G., de Castro, R.D., de Araújo, O.J., de Oliveira, L.E. 2015. Antifungal activity of apple cider vinegar on *Candida* species involved in denture stomatitis. *Journal of Prosthodontics*, **4:** 296-302.

Ncama, K., Mditshwa, A., Tesfay, S.Z., Mbili, N.C., Magwaza, L.S. 2019. Topical procedures adopted in testing and application of plant-based extracts as biofungicides in controlling postharvest decay of fresh produce. *Crop Protection*, **115:** 142-151.

Ozturk, I., Caliskan, O.Z.N.U.R., Tornuk, F., Ozcan, N., Yalcin, H., Baslar, M., Sagdic, O. 2015. Antioxidant, antimicrobial, mineral, volatile, physico-chemical and microbiological characteristics of traditional home-made Turkish vinegars. *LWT-Food Science and Technology*, **1:** 144-151.

Petrasch, S., Silva, C.J., Mesquida-Pesci, S.D., Gallegos, K., van den Abeele, C., Papin, V., Fernandez-Acero, F.J., Knapp, S.J., Blanco-Ulate, B. 2019. Infection strategies deployed by *Botrytis cinerea*, *Fusarium acuminatum* and *Rhizopus stolonifera* as a function of tomato fruit ripening stage. *Frontiers in Plant Science*, **10:** 223.

Roy, P., Abdulsalam, F.I., Pandey, D.K., Bhattacharjee, A., Eruvaram, N.R., Malik, T. 2015. Evaluation of antioxidant, antibacterial and antidiabetic potential of two traditional medicinal plants of India: *Swertia cordata* and *Swertia chirayita*. *Pharmacognosy Research*, **5:** 57-62.

Sholberg, P., Haag, P., Hocking, R., Bedford, K. 2000. The use of vinegar vapor to reduce postharvest decay of harvested fruit. *Hort Science*, **5:** 898-903.

Soylu, E.M., Kurt, Ş., Soylu, S. 2010. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *International Journal of Food Microbiology*, **3:** 183-189.

Zhang, Z.Q., Chen, T., Li, B.Q., Qin, G.Z., Tian, S.P. 2021. Molecular basis of pathogenesis of postharvest pathogenic fungi and control strategy in fruits: progress and prospect. *Molecular Horticulture*, **1:** 1-10.