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Antifungal Activities of some Essential oil against selected Post-harvest Pathogens

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ABSTRACT

Plant pathogens are causing serious destruction of post harvest vegetables during storage and transport. Though, easy availability, immediate effects and cost-effectiveness are the reasons for which the synthetic pesticides and preservative chemicals are being frequently used world wide but reports of food poisoning and environmental degradation are also being associated with such acts. Therefore, scientists are searching for some alternatives that should be cheap, effective and environment friendly. In the present investigation, antimicrobial activity of 12 aromatic plant's essential oils was examined against two post harvest plant pathogens namely *Geotrichum sp.* and *Mucor sp.* by following agar cup diffusion method. The investigation reveals that *Elettaria cardamomum, Murraya koenigii, Curcuma zedoaria* and *Pandanus amaryllifolius* were most effective essential oil and their killing time revealed that they can kill the pathogens effectively within 24 hours. Therefore, these essential oils can be used to preserve vegetables after harvesting effectively.

Key words : Moulds, Post-harvest, Preservatives, Terpenoids, Volatile.

INTRODUCTION

Horticultural crops and vegetables faces trouble during post-harvest storage and in post-harvest decay, microorganisms play a major role for rotting of horticultural crops and vegetables (Kumar and Iqbal, 2020). These microorganisms mainly include bacteria, yeast and moulds. Fungal and bacterial infections cause around 50% losses to the post-harvest crops (Magro *et al.*, 2006), again moulds colonizes foods and synthesizes a number of enzymes causing economic losses. But fungi causes the most destruction of vegetable and fruit crops by causing numerous plant diseases and economic losses due to favorable environmental conditions like water activity, PH, temperature, atmosphere, time etc. Magan and Aldred (2007) documented *Aspergillus sp.*, *Fusarium sp. Alternaria sp.* and *Penicillium sp.* as the most prevalent fungal genera which causes loss in quantity and quality of fruit and vegetable production. As microorganisms are omni present in our environment and they can easily reach and grow in foods anytime during their harvesting from agricultural field to packaging and transporting to commercial market areas. As there is increasing concerns about the harmful side effects of pesticides and increasing cost of development of new synthetic preservatives, there is a need for alternative methods to control disease occurrence. There is also an increasing case of fungicide resistance (Bradshaw *et al.*, 2021). Therefore scientists are constantly searching for safer alternatives to effectively control the post harvest pathogens which should be eco-friendly and also increase the shelf life of vegetables and food products (Sadgrove *et al.*, 2013). Many researchers have been investigating the utilization of plant products, plant extracts, essential oils etc. with antifungal land anti-bacterial, anti-viral activity as effective natural preservatives (Gigantra and Darmayasa, 2019). Crude extracts of leaf, stem, flower, fruit, bark, root etc are used for treatment of various human diseases from a long period of time. Similarly, essential oils have recently got a great interest as a natural antimicrobial agent against

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human and plant pathogenic bacteria (Herman *et al.*, 2019). Essential oils have antimicrobial property because they consist monoterpenes, sesquiterpenes as primary terpenoids and secondary terpenoids like diterpenes which can effectively kill or slow growth of yeasts, moulds and bacteria (Chorianopoulos *et al.*, 2008). They are volatile metabolites which are extracted from stem, leaf and roots of plants through steam or hydro distillation method which produces a fragrant mixture of compounds (Thierry *et al.*, 2012). Assam is regarded as a great zone for medicinal plants which are used extensively by local peoples in treating various diseases. The local people use there plants in the form of decoction, extracts etc in a regular basis to cure their diseases. As the medicinal plants are regularly being studied for their antimicrobial activity against human pathogens but there still few studies have been done to explore their effectivity against post harvest plant pathogens. Therefore, in this study we have collected some medicinal plants used by local people of Assam and tried to find the antimicrobial activity of the essential oil extracted from the collected plant specimens against some post harvest plant pathogens.

MATERIAL AND METHODS

The present investigation was conducted in the Mycology and Plant Pathology laboratory, Department of Botany, Guwahati University, Gopinath Bordoloi Nagar, Guwahati, Assam.

1. COLLECTION OF PLANT MATERIAL

The samples were collected from Grahampur area of Kokrajhar district of Assam and Garigaon area of Kamrup district of Assam by a field visit. The plants were identified by consulting Herbaria of Botany Department of Gauhati University. The plants with fresh leaves were collected and packed in separate polythene bags and were carried to the laboratory. A total of 12 plant specimens were collected and the plants were identified as *Curcuma zedoaria, Glycosmis pentaphylla,Pandanus amaryllifolius, Paederia foetida, Murraya koenigii, Ageratum conyzoides, Elettaria cardamomum, Mansoa alliacea, Murraya paniculata, Zanthoxylum rhetsa, Hibiscus acetosella* and *Eryngium foetidum.*

2. EXTRACTION OF CRUDE OIL

The leaves of the selected plants were separated and chopped into small pieces. The materials were then put in Clevenger's apparatus and 300ml of distilled water were added to it. The apparatus was kept in heating mantle and temperature was set at 50°C and the solution was heated for 4 hours. After heating for specific time period, the oil was obtained through hydro-distillation process and collected in air tight container. The oil extract was concentrated by passing through magnesium chloride to obtain essential oil in pure form (100)

The essential oils so obtained were kept in refrigerator at 4°C for further study.

3. COLLECTION OF POST HARVEST PLANT PATHOGEN

Infected vegetables were collected from Pamohi market of Garchuk, Guwahati and they were brought to the laboratory in polythene bags. Then pathogens were isolated from the infected vegetable parts by inoculating the surface sterilized disease part into Potato Dextrose Agar medium and incubated for 3-5 days at 35°C. Fungal hyphae growing out of the inoculated fragments were transferred to freshly prepared PDA slants. Identification of the pathogens was carried out by morphological traits and microscopic observation of the spores.

4. ANTIMICROBIAL EVALUATION

The oil extracts were evaluated for antimicrobial activity by following agar cup diffusion method. The fungal pathogen was cultured in freshly prepared Potato Dextrose Broth. The preparations of media were done as per manufacturer's instruction. The fungal pathogens were inoculated on PDA plates by the help of sterile cotton swabs. Agar cups were prepared by scooping out the medium with a sterile cork borer (7mm in diameter). Each cup was then loaded with 0.5ml of crude oil extracts. The fungal pathogen was incubated at 35°C for 24 hours. The plates were observed for zone of inhibition. The inhibition zones so formed were measured in millimeter with the help of an SI 100mm ruler.

5. STATISTICAL ANALYSIS

Three replicas were taken for standard deviation and followed the standard deviation method to get the accurate measurement.

6. KILLING TIME DETERMINATION

Killing time was determined for the essential oil which shows highest activity against a particular pathogen. For this experiment 5 ml of broth culture was prepared and the pathogen inoculated into it and incubated for 24 hours. Generally the experiment was carried out for 12 hours duration. 12 sets of test tubes were taken where 1µl of broth culture was added. Then 6 test tubes were taken as control and in another 6 test tubes 1µl of essential oil

was added. Then the test tubes were incubated and after 2 hours a set of test tube having a control and a test tube containing essential oil were taken. With the help of cotton swab the broth cultures were inoculated on two Petri plates containing PDA media by striking method and it was incubated for 24 hours.

Table 1: List of diseased plant materials and types of plant pathogens isolated

Sl. no.	Name of vegetables	Name of pathogen isolated
1.	Squash	*Geotrichum candidum*
2.	Pointed guard	*Geotrichum candidum*
3.	Tomato	*Geotrichum candidum*
4.	Bitter gourd	*Geotrichum candidum, Aspergillus sp.*
5.	Sponge gourd	*Geotrichum candidum, Mucor sp.*
6.	Teasle gourd	*Geotrichum candidum, Aspergillus sp.*
7.	Brinjal (eggplant)	*Aspergillus sp.*
8.	Capsicum	*Aspergillus sp.*
9.	Cucumber	*Geotrichum candidum*
10.	Cabbage	*Geotrichum candidum, Mucor sp.*
11.	Pumpkin	*Geotrichum candidum*

Similarly one set of control and essential oil containing broth cultures were inoculated on Petri plates after every 2 hours and incubated. Then 24 hours of incubation of all the sets of Petri plates were evaluated. The set of Petri plates where the essential oil inhibited the growth of the pathogen by reducing the CFU at maximum amount in comparison to the control plate was determined as the killing time of the essential oil against the particular pathogen.

RESULTS

The 12 medicinal plants collected for the study were listed below in Table-2 along with their uses.

Table 2: List of essential oil bearing plants selected for antimicrobial study

Sl. no.	Plant name	Family	Activity	Sources
1.	*Ageratum conyzoides* L.	Asteraceae	Antifungal, antibacterial activity	Kambooj and Saluja, 2008
2.	*Curcuma zedoaria* Rosc.	Zingiberaceae	Antimicrobial activity	Kaushik and Jalalpur, 2011
3.	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Antimicrobial activity	Souissi et al., 2020
4.	*Eryngium foetidum* L.	Apiaceae	Food preservatives	Homer et al., 2007
5.	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae	Antimicrobial activity	Taib et al., 2022
6.	*Hibiscus acetosella* Welw. ex Hiern	Malvaceae	Antimicrobial activity	Srivastava et al., 2019
7.	*Murraya koenigii* (L.) Spreng	Rutaceae	Antimicrobial activity	Gahlawat et al., 2014
8.	*Mansoa alliaceae* (Lam) A.H. Gentry	Bignoniaceae	Fungicidal activity	Rajanna and Vinod, 2021
9.	*Pandanus amaryllifolius* Roxb.	Pandanaceae	Food preservatives	Bhuyan and Sonowal, 2021
10.	*Murraya paniculata* (L.) Jacq.	Rutaceae	Fungicidal activity	Khatoon et al., 2017
11.	*Paederia foetida* L.	Rubiacea	Can kill food borne pathogens	Bardhan et al., 2010
12.	*Zanthoxylum rhetsa* (Roxb.) DC.	Rutaceae	Antimicrobial activity	Aziz et al., 2022

Among the 12 medicinal plants, from preliminary assay it is seen that essential oil of all the medicinal plants except *Paederia foetida* and *Ageratum conyzoides* showed antimicrobial activity against both the pathogen or inhibited at least one pathogen (Table 3).

Table 3 : Preliminary assay of essential oils against plant pathogens by agar cup diffusion method

Name of Essential oil	Plant Pathogens	
	Mucor sp.	G. candidum
Pandanus amaryllifolius	+	+
Paederia foetida	--	--
Elettaria cardamomum	+	+
Murraya koenigii	+	+
Ageratum conyzoides	--	--
Monsoa alliacea	+	+
Curcuma zedoaria	+	+
Glycosmis pentaphylla	+	--
Murraya paniculata	+	--
Zanthozylum rhetsa	+	+
Eryngium foetidum	+	+
Hibiscus acetocella	+	--

"+" sign indicates presence of activity, "--"sign indicates absence of activity

In secondary screening, both *Murraya koenigii* and *Elettaria cardamomum* showed highest zone of activity of 24± 0.76mm and 24± 1.00mm respectively against *G. candidum*. Similarly, *Curcuma zedoaria* showed 20± 0.50mm of zone of inhibition against *Mucor sp.*, *Pandanus amaryllifolius* showed 19± 0.24mm of zone of inhibition against *G. candidum* and *Glycosmis pentaphylla* showed 19± 0.51mm of zone of inhibition against *Mucor sp.* (Table 4). After the secondary screening, most effective essential oils were selected for determining the exact time of killing of the particular pathogen.

TABLE 4 : Zone of inhibition of Essential oils against pathogens by agar cup diffusion Method

Name of Essential oil	Plant Pathogens	
	Zone of inhibition (mm)	
	Mucor sp.	G. candidum
Pandanus amaryllifolius	12± 1.00	19± 0.24
Elettaria cardamomum	12± 1.52	24± 1.00
Murraya koenigii	13± 1.32	24± 0.76
Monsoa alliacea	18± 1.00	--
Curcuma zedoaria	20± 0.50	10± 1.00
Glycosmis pentaphylla	19± 0.51	--
Murraya paniculata	18± 1.00	--
Zanthozylum rhetsa	18± 0.52	--
Hibiscus acetocella	12± 0.25	--
Eryngium foetidum	17± 0.65	10± 0.93

n = 3; ± = Standard deviation;--Sign indicates no significant zone of inhibition (less than 10mm)

The killing time was determined for the essential oil of *Murraya koenigii* against *Geotrichum candidum* and 6 hours was noted as the killing time of essential oil of *Murraya koenigii* against *Geotrichum candidum* (Fig.1). The killing for the essential oil of *Pandanus amaryllifolius* against *Geotrichum candidum* was 6 hours. Similarly, the killing time for essential oil of *Glycosmis pentaphylla* against *Mucor sp.* was 6 hours, the killing time of essential oil of *Curcuma zedoaria* against *Mucor sp.* was 10 hours and the killing time for the essential oil of *Elettaria cardamomum* against *Geotrichum candidum* was observed as 10 hours.

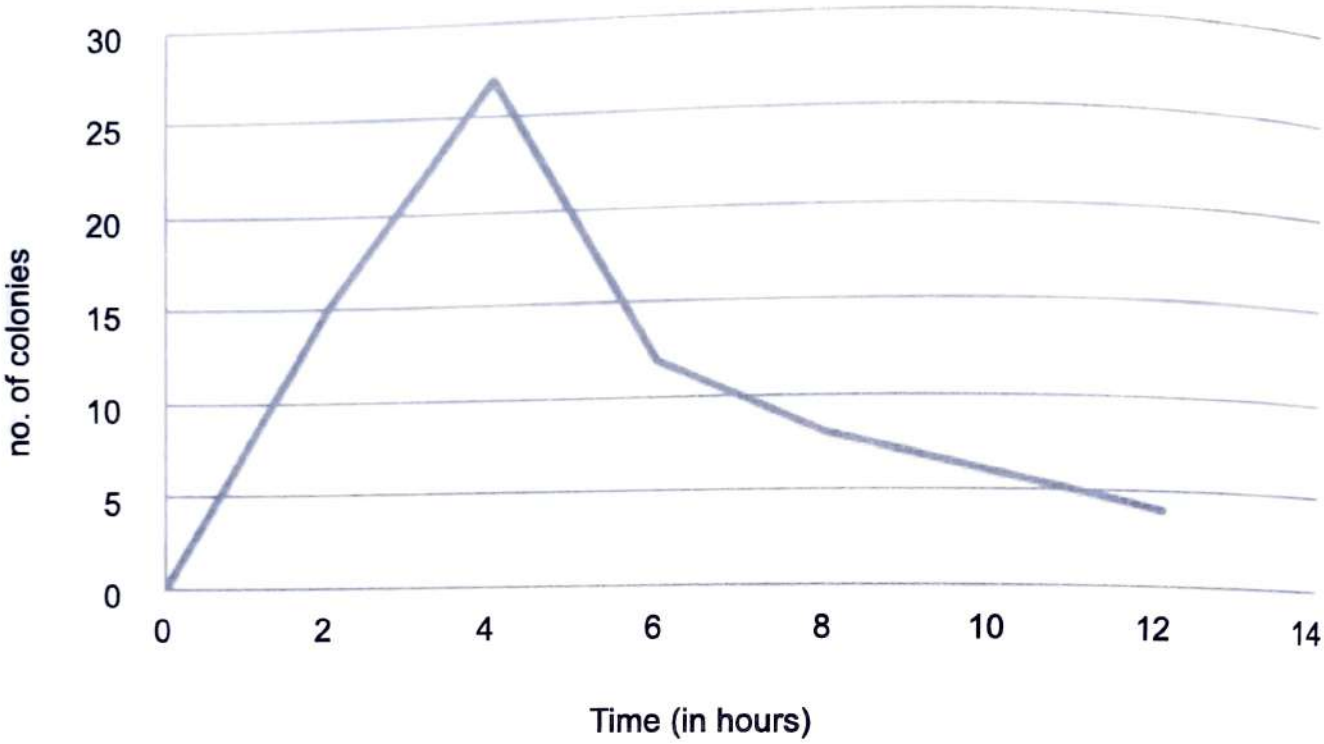

Figure 1: Graph depicting killing time of *Pandanus amaryllifolius* against *Geotrichum candidum*.

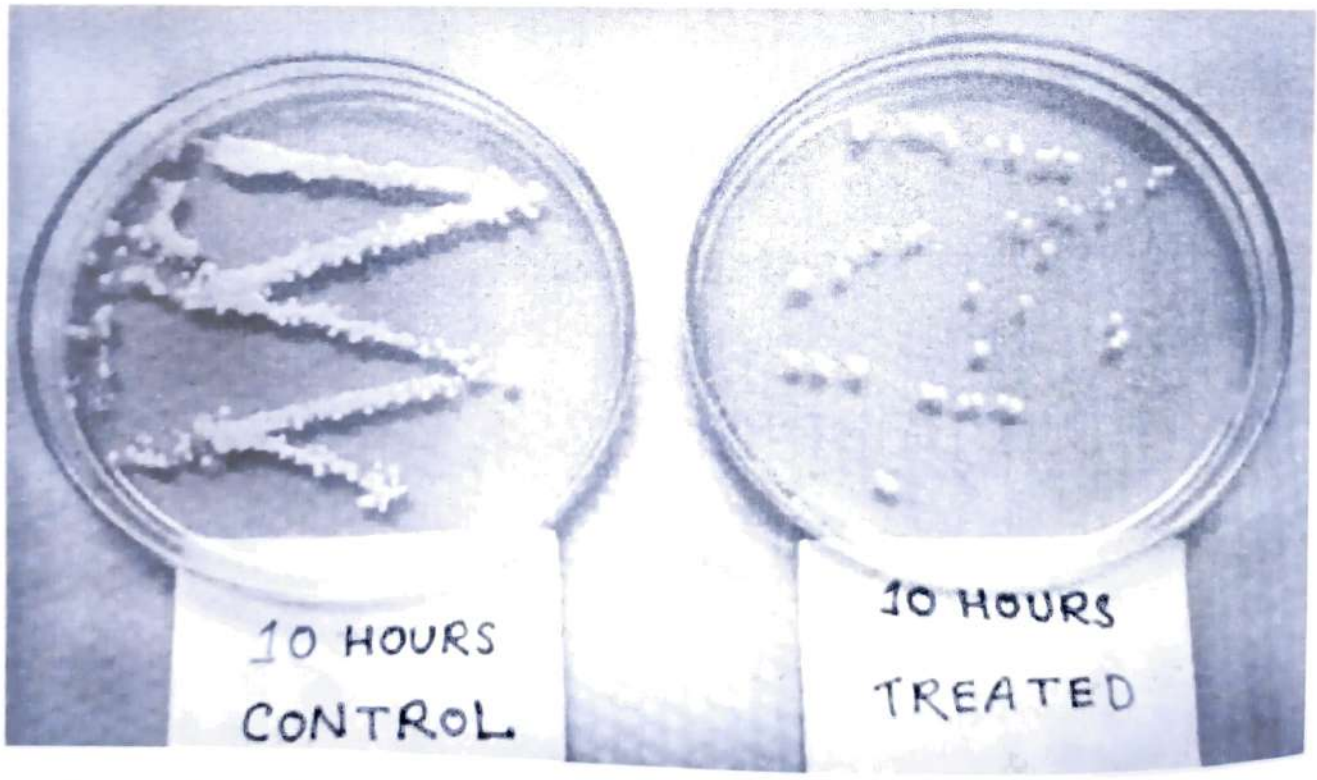

Photo plate: Photograph showing killing time of essential oil of *Curcuma zedoaria* against *Mucor sp.*

DISCUSSION

The post harvest plant pathogens are becoming an alarming factor in most of the developing countries because most of the food scarcity in those countries occurs due to post harvest food spoilage as reported by Kana *et al.*, 2012. The substantial economic losses of vegetables and fruits due to pest infestation and diseases occurred during storage and marketing crosses 50% of total productions in the developing countries. Vegetables and fresh fruits are generally get destroyed by fungal pathogens which cause a great economic loss of the farmers and suppliers (Sommer, 1985).Some fungal pathogens like *Mucor sp.*and *Geotrichum candidum* were collected for our study which causes various fruit and vegetable diseases. *Mucor sp.* causes mucor rot of pear and apple; mucor rot of strawberry; soft rot of tomato etc. (Udhave, 2011). *G. candidum* were reported causing sour rot of tomato (Sajad *et al.*, 2017); creamy rot of *Musa paradisiacal*; black spot of *Citrus reticulate*, brown spots of *Carica papaya*; milky rot of vegetables like cabbage, bottle gourd etc. Fungi produce mycotoxins which causes serious health problems to consumers. Food product industries use various synthetic chemical preservatives to prevent the growth of unwanted pathogens which produces toxic and carcinogenic compounds in food materials (Farag *et al.*, 1989). Some fungicides that are used for controlling post-harvest diseases have recently come under threat of posing a potential oncogenic risk (Barnard *et al.*, 1997). Essential oils show a wide range of antifungal activity against various plant pathogens. Moreover, they are also recognized as safe substances by Human health services to be used as antibacterial additives in food substances and they can be applied in rational quantities to improve food quality and safety and also not causing any nutritional losses to vegetable and food products (Stefanaskis *et al.*, 2013, Kumar and Iqbal, 2020). In our current study, essential oils were extracted from twelve aromatic medicinal plants used as ethno-medicine by ethnic communities which are namely *Curcuma zedoaria, Mansoa alliacea, Elettaria cardamomum, Murraya koenigii, Pandanus amaryllifolius, Paederia foetida, Ageratum conyzoides, Glycosmis pentaphylla, Murraya paniculata, Zanthoxylum rhetsa, Hibiscus acetosella* and *Eryngium foetidum.* The essential oils of all the collected plants were obtained by using hydro-distillation method and agar cup diffusion assay was done to check their antimicrobial activity against the post-harvest plant pathogens. The assay showed that essential oil of *Pandanus amaryllifolius* was effective against plant pathogens like *Mucor sp.* and *G. candidum*. Likewise, the essential oil of *Eryngium foetidum* was effective against *Mucor sp., G. candidum* and similar results were obtained by Lingaraju *et al* (2016) against plant pathogens. The essential oil of *Mansoa alliaceae, Glycosmis pentaphylla* and *Hibiscus acetocella* showed activity against *Mucor sp.* The essential oil of *Elettaria cardamomum, Curcuma zedoaria* and *Murraya koenigii* showed activity against *Mucor sp.* and *G. candidum.* From the study we can conclude that the selected essential oil showed 100% activity against *Mucor sp.* and 50% activity against *Geotrichum candidum.* It has been seen that various species of *Hibiscus* showed antimicrobial activity against fungal pathogens. But no significant work has been documented for *Hibiscus acetocella*, so this essential oil can be studied further for its detailed activity.

REFERENCES

Aziz F., Rahman F.I., Huque S., Ether S.A., Hasan C.M. and Ahsan M., 2022. *Zanthoxylum rhetsa* (Roxb.) DC: A systematic review on traditional uses, phytochemistry, and pharmacology. *Journal of Pharmacy & Pharmacognosy Research*, 10 (1): 52-72.

Barnard M., Padgitt M. and Uri N.D., 1997. Pesticides use and its measurement. *International Journal Pest Control*, 39:161–164.

Bardhan P., Faruque A.S.G., Naheed A. and David A.S., 2010. Decreasing Shigellosis-related Deaths without *Shigella spp.*-specific Interventions, Asia. *Emerging Infectious Disease*, 16: 1718–1723.

Bhuyan B. and Sonowal R., 2021. An overview of *Pandanus maryllifolius* Roxb. ex Lindl. and its potential impact on health. *Current Trends in Pharmaceutical Research*, 8(1): 138-157.

Bradshaw M.J., Bartholomew H.P., Hendricks D., Maust A. and Jurick W.M., 2021. An analysis of post harvest fungal pathogens revealed temporal-spatial and host pathogen associations with fungicide resistant-related mutations. *Phytopathology*, 111:1942-1951.

Chorianopoulos N.G., Giaouris E.D., Skandamis P.N., Haroutounian S.A. and Nychas G.J.E,

2008. Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: Bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid-base sanitizers. *Journal of Applied Microbiology*, 104:1586–1599.

Farag R.S., Daw Z.Y., Hewedi F.M. and El-Baroty G.S.A., 1989. Antimicrobial Activity of Some Egyptian Spice Essential Oils. *Journal of Food Protection*, 52(9): 665-667.

Gahlawat D.K., Jakhar S. and Dahiya P., 2014. *Murraya koenigii* (L.)Spreng: an ethnobotanical, phytochemical and pharmacological review. *Journal of Pharmacognosy and Phytochemistry*, 3(3): 109-119.

Sudigra S.K., Ginantra K. and Darmayasa I.B.G., 2019. Antifungal activity of leaf extracts of *Mansoa alliacea* against *Colletotrichum acutatum* the cause of anthracnose disease on chili pepper. 6th International Conference on Sustainable Agriculture, Food and Energy, *IOP Conf. Series: Earth and Environmental Science*, 347(1): 012058.

Hatab S., Althanaiso R., Holley R., Rodas-Gonzalez A. and Narvaez-Bravo C., 2016. Survival and reduction of shiga toxin-producing *Escherechia coli* in a fresh cold-pressed juice treated with antimicrobial plant extracts. *Journal of Food Science*, 81: 1987-1995.

Herman R.A., Ayepa E., Shittu S., Fometu S.S. and Wang J., 2019. Essential oils and their applications -A mini review. *Advances in Nutrition & Food Science*, 4(4): 1-13.

Homer S., Baccus-Taylor G. and Akingbala J., 2007. Antibacterial Efficacy of *Eryngium foetidum* (Culantro) against Selected Food-borne Pathogens, 27th West Indies Agricultural Economics Conference, Belize, 179-192.

Kana H.A., Aliyu I.A. and Chammang H.B., 2012. Review on neglected and underutilized root and tuber crops as food security in achieving the millennium development goals in Nigeria. *Journal of Agriculture and Veterinary Science*, 4: 27-33.

Khatoon A., Mohapatra S. and Satapathy K.B., 2017. Botanicals to Control Post-harvest Decay of Potato (*Solanum tuberosum* L.) Tubers in Odisha. India. *International Journal of Pharmaceutical Sciences Review and Research*, 42(1): 117-121.

Kamboj A. and Saluja A.K., 2008. *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy*, 59-68.

Kumar V. and Iqbal N., 2020. Post-harvest and disease management of horticultural crop: A brief review. *Plant Archives*, 20 (2): 2054-2058.

Kaushik M.L. and Jalalpure S.S., 2011. Effect of *Curcuma zedoaria* Rosc root extracts on behavioral and radiology changes in arthritic rats. *Journalof Advanced Pharmaceutical Technology& Research*, 2(3):170-176.

Lingaraju D.P., Sudarshana M.S., Mahendra C., and Rao P., 2016. Phytochemical screening and Antimicrobial activity of leaf extracts of *Eryngium foetidum* L. (Apiaceae). *Indo American Journal of Pharmaceutical Research*, 6(2): 4340-4344.

Magan N. and Aldred D., 2007. Post-harvest control strategies: minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, 119: 131–139.

Magro A., Carolino M., Bastos M. and Mexia A., 2006. Efficacy of plant extracts against stored products fungi. *Revistal beroamericana de Micología*, 23, 176–178.

Rajanna L. and Vinod N., 2021. Antimicrobial activity of leaf extracts of *Mansoa alliacea* (Lam.)A. H. Gentry. *Bulletin of Environment, Pharmacology and Life Sciences*, 10(12): 106-113.

Sadgrove N. and Jones G.L., 2013. Antimicrobial activity of essential oils and solvent extracts from *Zieria* species (Rutaceae). *Natural Product Communications*, 8:741–745.

Sajad U.N., Raja W.H., Kumawat K.L., Mir J.I., Sharma O.C., Singh D.B. and Sheikh M.A., 2017. Post Harvest Diseases of Temperate Fruits and their Management Strategies-A Review. *International Journal of Pure and Applied Bioscience*, 5(3): 885-898.

Sommer N.F., 1985. Strategies for control of post-harvest disease of selected commodities. In: Post-harvest Technology of Horticultural Crops. University of California Press: 83-98.

Souissi M., Azelmat J., Chaieb K. and Grenier D., 2020. Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts: Potential therapeutic

benefits for periodontal infections. *Anaerobe*, 61: 102089.

Stefanakis M.K., Touloupakis E., Anastasopoulos E., Ghanotakis D.,Katerinopoulos H.E., and Makridis P., 2013. Antibacterial activity of essential oils from plants of the genus *Origanum*. *Food Control*, 34: 539–546.

Taib M., Hassan N., Mohdkamal N.M. and KhinSoe M., 2022. Bioautography, combination effects and photo-activated enzymatic restriction inhibition activity of antimicrobial alkaloids from *Glycosmis Pentaphylla* (Retz.) DC. *Malaysian Journal of Science*, 41(3): 1-9.

Thierry R., Sandra C. and Wilma D.P., 2012. Essential Oils and Other Plant Extracts as Food Preservatives, in Progress in Food Preservation. John Wiley & Sons, Ltd: New York, USA: 539-579.

Udhave B.N., 2011. Survey of market storage diseases of some important fruits of Osmannabad District (M.S.) India. *Science Research Reporter*, 1(2): 88 – 91.