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RESEARCH ARTICLE

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Antifungal activity of some essential oils against the post-harvest fungal pathogens of guava (*Psidium guajava* L.)

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ABSTRACT: Guava (*Psidium guajava* L.) is a nutritionally and medicinally valuable fruit, but postharvest losses are primarily caused by fungal pathogens at various stages of harvesting. Chemical fungicides, while effective, pose risks to human health and the environment, prompting the search for natural alternatives. Essential oils from aromatic plants have demonstrated significant antifungal properties, making them potential substitutes for synthetic fungicides. This study evaluated the antifungal efficacy of essential oils against postharvest fungal pathogens isolated from guava fruit. A total of eight fungal species were identified: *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor*, *Colletotrichum gloeosporioides*, *Monilia fruticola*, *Penicillium* sp, and *Pestalotia psidii*. Essential oils from *Acorus calamus*, *Callistemon citrinus*, and *Juniperus indica* were tested against *A. alternata*, *C. gloeosporioides*, and *P. psidii*. Essential oils were extracted through hydrodistillation using Cleavinger's apparatus, followed by GC-MS analysis to characterize their physicochemical properties relevant to antifungal activity. All tested oils significantly inhibited mycelial growth, with *A. calamus* exhibiting complete inhibition at 20 µl/ml and 40 µl/ml concentrations. These findings highlight the potential of *A. calamus* essential oil as a natural fungicide to control postharvest fungal pathogens. However, further in vivo research is necessary to assess its efficacy under storage conditions, along with evaluations of toxicity, sensory impact, and economic feasibility for commercial application.

KEYWORDS: Aromatic Plant, Chemical Fungicides, Fungitoxicity, Growth rate, Medicinal Value

INTRODUCTION

Postharvest loss means to affect the fruits, vegetables and seeds that has been harvested from the plants at various stages (including storage, packaging, and marketing) infection by pathogens, under moist conditions or high humidity. Pre harvest and postharvest initiation are the two forms of postharvest infection initiation that are noticed [1]. After harvesting, degradation in both quantity and quality of fruits and vegetables. The post-harvest loss of guava ranged between 20-25% according to studied conducted in India [2] and similar stances can be found in Nepal. Post harvest loss is most common in guava fruit due to its highly perishable nature [3].

Guava (*Psidium guajava* L.) form Myrtaceae family is the most significant fruit. In both Tropical and sub-tropical regions, guava is one of the most widely produced fruits in Nepal. It has expanded from a height of 155 meters above sea level in Bara district, to 1600 meters above sea level in Terhathum district. Guava can be categorized as the second most important fruit crop in Nepal, following oranges and are cultivated in 23 districts in the hills and Terai regions of Nepal [4]. Its fruit is a very healthy source of several nutrients, such as dietary fiber, pectin), vitamin A, phosphorus, vitamin C, calcium, iron, thiamine, niacin, riboflavin, and carotene [5]. High dietary fibre content such as crude fibre content is 2.8%–5.5% and moisture content is77%–86% in guava fruit which helps to decrease the hight blood pressure [6]. Numerous bacteria, fungi, and other organisms attack guava fruits, resulting in a variety of illness signs. Fungal infections invade the damaged guavas as they are being transported and stored. Fungal infections during storage and transit account for more than 25 to 30 percent of fruit loss [4]. After harvest of guava fruit, the rate of loss is huge due to various factors, among the biological factors is one of the major causes of guava fruit loss, virus bacteria, fungi are mainly responsible for the guava fruit disease; among them the fungi that infect guava after harvesting include *Alternaria* sp., *Aspergillus niger*, *Penicillium*, *Pestalotia psidii*, *Rhizopus stolonifera*, *Colletotrichum*

gloeosporides, etc. [7]. The use use of chemical fungicides has dangerous effects on the environment, human health [8]. Bioactive essential oils are a rich source of promising chemicals for alternative and possibly more ecofriendly and cost effective of disease management. Essential oils have generally antifungal properties due to presence of aroma [9, 10].

This study evaluates the antifungal efficacy of essential oils from *Acorus calamus*, *Callistemon citrinus*, and *Juniperus indica* against postharvest fungal pathogens of guava (*Psidium guajava* L.). By reducing postharvest losses, it supports economic benefits such as lower production costs, affordable consumer prices, and increased farmer revenue. Conducted in Nepal, the research provides region-specific insights into fungal contamination, addressing environmental factors influencing pathogen prevalence. Unlike previous studies, it focuses on guava-specific pathogens while promoting sustainable, eco-friendly alternatives to synthetic fungicides for effective postharvest disease management. to identify postharvest fungal pathogens affecting guava and assess the in vitro antifungal efficacy of essential oils against the isolated postharvest fungal pathogens.

MATERIALS AND METHODS

Collection of samples

The samples of guava fruit were collected from the major vegetables market of Kathmandu valley (Kalimati Fruits and vegetables Market, Balkhu vegetables Markets, and retailers). A total of 20 fruit samples were collected in sterile paper bags kept in sterile zip lock bags and brought to Plant Pathology Laboratory, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu for laboratory experimentation.

Isolation and identification of postharvest fungal pathogens

Infected fruit guavas were taken and surface sterilized by 70% ethyl alcohol for one minute and then washed with distilled water. The contaminated surface layer of guava samples was cut into pieces of roughly 3mm using a sterile razor blade. Pieces were inoculated on sterilized glass petri plate containing potato dextrose agar (PDA) medium at three points and incubated at 25±2°c temperature for one week. After that, pure culture of that fungal colony was done by inoculating the small part of colony on Petri plate containing PDA media. This all the process was done in laminar air flow. Lastly the fungi were identified through the microscope image and their characteristics compared using standard literatures [11, 12]. The pure culture of identified fungi was further sub cultured in sterilized petri plates containing PDA media and incubated at 25±2°c temperature.

The fungi pathogens frequency was calculated on the basis of present or absent of pathogens in each petri plates count and noted and the formula is

$$\text{Pathogens frequency} = \frac{\text{No. of presence of pathogens}}{\text{Total no. of plates}} \times 100\%$$

Extraction of plant essential oils

Leaves samples of (*Acorus calamus*, *Callistemon citrinus* and *Juniperus indica*) were collected from Kathmandu, Nepal and the collected plant leaves were allowed to air dry in the shade for a full day. 100g of shade-dried leaves were taken and surface-sterilized them. Then a leaf sample was collected and placed in a Cleavenger apparatus with 1,000 mL of water for 6–8 hours of hydro-distillation. Two uppermost aromatic layers were collected, dried on sodium sulphate, and stored between 4 and 10°C [13]. The yield percentage of essential oil was calculated by using a formula.

$$\text{Yield percentage} = \frac{\text{Volume of essential oil extracted in ml}}{\text{Weight in gm}} \times 100\%$$

First, five distinct concentrations of 40µl/ml, 20µl/ml, 10µl/ml, 5µl/ml, and 2.5µl/ml were prepared by diluting the stock solution of essential oils with 60% acetone [13] using following formula: $V_1S_1=V_2S_2$

Where, V_1, S_1, V_2 and S_2 are volume of stock solution, concentration of stock solution, volume of final solution and concentration of final solution respectively. Then, these five distinct concentrations of each essential oils used for the evaluation of fungitoxicity.

Fungitoxicity of essential oils

The antifungal assay of essential oils to the choose test fungal contaminants of guava was evaluated using the poison food approach. PDA on petri plates that were plated with varying quantities of essential oils was used to investigate the antifungal activity. In order to ensure an equal distribution of essential oils and PDA, this procedure involved first pouring 0.5ml of each essential oils concentration into sterilize petri plates, then adding 9.5ml of PDA to it and carefully rotating it in both clockwise and anticlockwise directions. No essential oil was utilized in the negative control sets; 60% acetone was used in positive control sets instead of essential oils. After that, actively developing mycelium block of test fungi was inoculated in these PDA mediated plates. The plates were sealed with cello tape or para-film and incubated at 25±2°c temperature. Each treatment had three replicates. Finally, on the 7th day, a mean mycelial growth at all concentration treatment was calculated [14]. The antifungal

activity of three different essential oils concentration by measuring the percentage suppression of the test fungus's mycelial growth [13]. The growth inhibition mycelial percentage was calculated by using formula [15].

Mycelial growth inhibition percentage = [(Gc- Gt)/Gc] × 100%

Where, Gc= colony diameter in control sets (mm), Gt = colony diameter in treatment sets (mm).

GC-MS (Chromatography-Mass Spectrometry) analysis of essential oils Gas

GC-MS was used to analysed the chemical composition of essential oils. As the carrier gas, helium gas was employed at a steady flow of 1.03ml/min and an injection volume of 1µl at 220°C. One milligram of essential oil was used diluted with one milliliter of dichloromethane to create a stock solution. The stock solution was further diluted Dichloromethane was added to 100µl of the stock to make 1 milliliter, and the mixture was then examined on Gas Chromatography that was connected to Mass Spectrometry. The components of essential oils were determined using their relative intensive (RI) and a quasi-linear equation put out by Van Den Dool and Kratz. Different mass fragmentation patterns were used to identify the distinct components of essential oils. Using a calibration curve of the dose-peak area of pure compound with the relative quantity of each individual composition of essential oils were made. The proportion of the peak area to the overall peak area is how it is stated.

Statistical analysis

The obtained data were analyzed using SPSS software (version 25). Frequency ranked curve was used to find the occurrence frequency of fungal pathogens. One-way ANOVA (analysis of variance) was performed to compare mycelial growth between control and treatment sets following post-hoc Tukey's HSD (honestly significant difference) test with $p<0.05$. Graphs, charts, and percentage frequency curves were plotted using Microsoft Excel for data visualization.

RESULTS AND DISCUSSION

Frequency of fungal pathogens

Altogether eight species of pathogens were detected from the collected samples viz. *Pestalotia psidii, Colletotrichum gloeosporioides, Alternaria alternata. Aspergillus neiger, Aspergillus flavuus, Aspergillus versicola, Penicillium* sp., *Monilia fruticola* (Table 1). Among them, *P. psidii, C. gloeosporioides, A. alternata* were dominant pathogens that causes post-harvest disease of guava fruit (Figure 1). According to Fatima [4] reported that a large majority (25-30%) fungal disease caused by various pathogens (*Alternaria alternata, Colletotrichum, Curvularia, Fusarium, Monilia, Penicilium, Pestalotia, Phytopthora* and *Rhizopus*) from which five pathogens were common with compared with present research. The morphological characteristics of fungal isolates observed during this investigation agreed with standard literature [16-21].

Table 1. Descriptions of pathogens on the basis of microscopic and morphological characterization

S.N.	Name of Pathogens	Morphological Characteristics
1	*Pestalotia psidii* Speg.	Colony cottony, white to milk creamy and backside light yellow in colour. Hyphae branched, Conidia five-celled, hyaline, spindle-shaped, and darkly pigmented in bulk
2	*Colletotrichum gloeosporioides* Penz.	Colony cottony, initially white and later turned grey. Conidia with hyaline, one-celled, ovoid to oblong, slightly curved.
3	*Alternaria alternata* (Fr.) Keissel	colony white at initial later it changed to grey, dark grey and black colour with regular or irregular white colour margins. Hyphae highly branched, septate and light brown to dark brown in colour. Conidia are singly or acropetal chain formed at the tip.
4	*Monilia fruticola* (G. Winter)	Colony white to milk cream in colour growing in an irregular pattern. Hyphae highly branched and hyaline. Conidia lemon or ellipsoid shaped formed in long chain
5	*Aspergillus niger* Tiegh.	Colony appears black with the conidial production, powdery. Conidial head globose, Metula and phialides entirely covered vesicles.
6	*Aspergillus flavus* Link.	Colony is flat in the middle and has ridges, a white border, and a yellowish to grey green color, septate hyphae have long, translucent conidiophores with spiny or rough surfaces.
7	*Aspergillus versicolor* (Vuill.) Tirab	Colony white floccose to green or light yellow. Conidia spherical, green, loosely arrange in chain form.
8	*Penicillium* sp. Link	Colony fast growing turned from white to yellow at first, then matured into a bright green color. Conidia ellipsoidal or fusiform, globose, hyaline, divergent or arranged in columns, and developed in long chains.

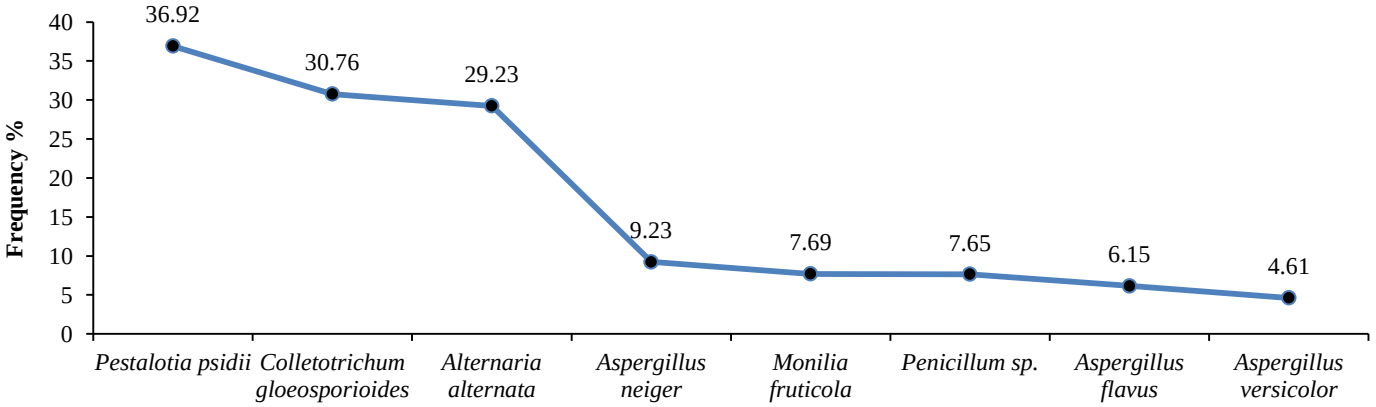

Fig. 1. Frequency rank curve of postharvest fungal pathogens in Psidium guajava (guava fruit).

Yield percentage of essential oil and their GC-MS analysis

The three plants species namely *Juniperus indica*, *Calistemon citrinus* and *Acorus calamus* whose percentage yield of essential oils were determined to be 0.4%, o.9% and 0.3% respectively (Figure 2). The essential oils content of *J. indica* varied with in (0.27% to 0.70%) at 200 g weight [22] *C. citrinus* varied 0.8% to 1% at 100g weight [23, 24]. *A. calamus* oil yield percent was 1.20 at 300g weight [25].

The GC-MS of *J. indica* showed that alpha-pienene (25.5%), cederol-epi (20.87%), carene<delta>3(11.26%), caryophyllene-E-(4.73%), limonene (3.43%), pienene-beta (1.58%) and sabinene (2.63%) were the major components (Figure 3 and Table 2). Various authors [22, 26] also reported alpha-pienene, limonene, terpine-4-ol, sabienene as major components.

In this research, *Callistemon citrinus* had eucalyptol (68.8%), alpha-pienene (17.44%), terpienene-4-ol (6.45%), beta-pienene (1,53%) were major components (Figure 4, Table 2). Bhandari et al. [24] also observed eucalyptol, alpha-pienene and d-limonene as major components. The result of *A. calamus* indicate that asarone-Z (51.92%), longifolol (6.42%), asorene-E (3.12%), isocalamendiol-dihydroxy (3.9%), linalool (2.93%) were major compounds with other minor compounds (Figure 5, Table 2). Shukla et al. [27] and Radusiene et al. [28] also reported that the *A. calamus* essential oils include Z- asoerene, Z-methyl isoeugenol as the main component supporting the present study.

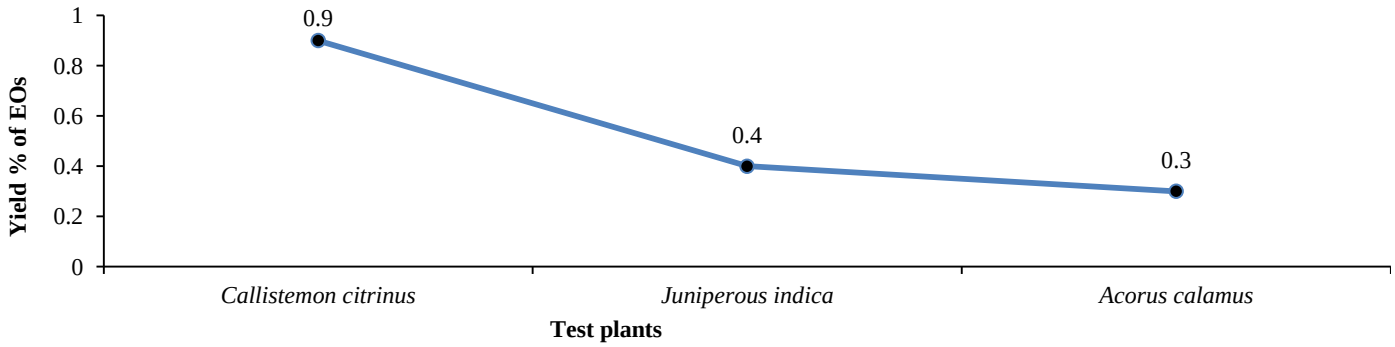

Fig. 2. Yield percentage of essential oil from test plants.

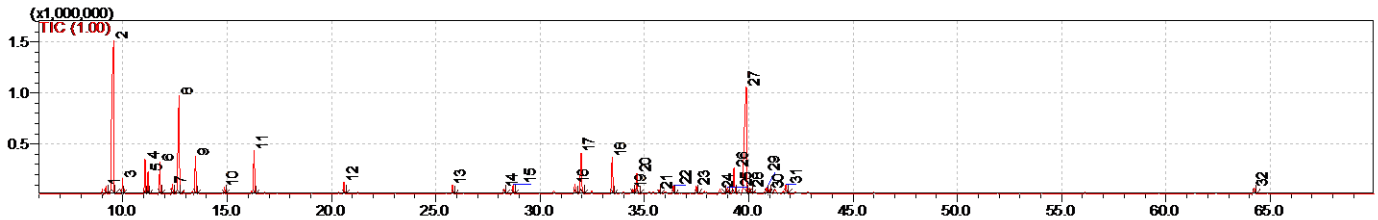

Fig. 3. Chromatogram of the *Juniperus indica* essential oil used in this research.

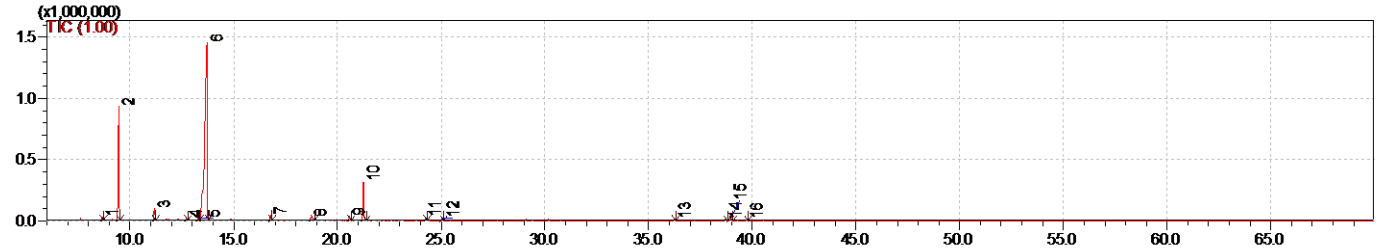

Fig. 4. Chromatogram of the *Callistemon citrinus* essential oil used in this research.

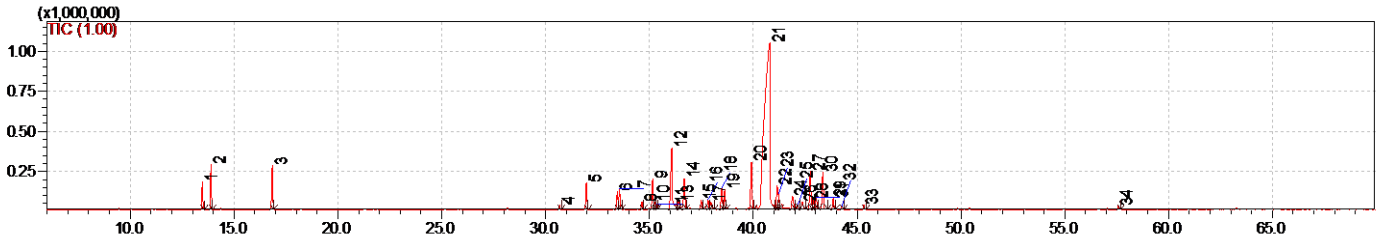

Fig. 5. Chromatogram of the *Acorus calamus* essential oil used in this research.

Table 2. The chemical components of essential oil of test plants determined by GC-MS.

Peak	*Acorus calamus*			*Juniperus indica*			*Callistemon citrinus*		
	Retention Time	**Area %**	**Name of components**	**Retention Time**	**Area %**	**Name of components**	**Retention Time**	**Area %**	**Name of components**
1	13.474	1.49	Limonene	9.188	0.63	Thujene-alpha	8.663	0.34	Isobutyrate - isobutyl
2	13.892	2.5	Pinene-alpha	9.559	25.34	Pinene-alpha	9.459	17.44	Pinene-alpha
3	16.847	2.93	Linalool	9.984	1.08	Fenchene-alpha	11.173	1.53	Pinene-beta
4	30.674	0.28	Elemene-beta	11.06	2.63	Sabinene	12.712	0.28	Isobutyrate-isopentyl
5	31.964	1.84	Caryophyllene-E	11.202	1.58	Pinene-beta	13.282	0.53	Cymene-ortho
6	33.459	1.25	Humulene -alpha	11.777	2.43	Myrcene	13.713	68.85	Eucalyptol
7	33.563	1.35	Isoeugenol -methyl-Z	12.375	0.73	Phellandrene-alpha	16.784	0.74	Linalyl anthranilate
8	34.644	0.51	Cubebene-beta	12.693	11.26	Carene-delta-3	18.724	0.5	Pinocarveol-trans
9	35.158	2.25	Drim-8(12)-ene	13.48	3.43	Limonene	20.581	0.74	Terpinen-4-ol
10	35.259	0.56	Viridiflorene	14.864	0.55	Terpinene-gamma	21.253	6.45	Terpineol-alpha
11	35.325	0.31	Bicyclogermacrene	16.288	3.86	Terpinolene	24.245	0.54	Nerol
12	36.075	6.42	Longifolol	20.6	0.99	Terpinen-4-ol	25.044	0.43	Crotonate-(E)-, ethyl
13	36.414	0.67	Cadinene -delta	25.794	0.85	Bornyl acetate	36.259	0.32	Vanillin methyl ether
14	36.683	2.36	Kessane	28.267	0.51	Terpinyl acetate-alpha	38.71	0.44	Spathulenol
15	37.493	0.62	Elemol-alpha	28.706	0.88	Terpinyl acetate-gamma	38.966	0.58	Aromadendrene

16	37.816	0.68	Nootkatone -1,10-dihydro	31.666	1.26	Cedrene-beta	39.731	0.29	Cedrol-epi
17	37.959	0.62	Nerolidol-Z	31.962	4.73	Caryophyllene-E			
18	38.481	1.58	Isoelemicin-E	33.456	3.68	Humulene-alpha			
19	38.629	1.38	Naphth-1-ol<1,2,3,4,4a,7,8, 8a-octahydro-, 4-isopropyl-, 1,6-dimethyl	34.447	0.58	Curcumene-gamma			
20	39.914	3.9	Isocalamendiol-dehydroxy	34.636	2.17	Cubebene-beta			
21	40.799	51.92	Asarone-Z	35.692	0.54	Bisabolene-beta			
22	41.151	2.12	Tetradeca-(9Z,12E)-dien-1-ol	36.388	0.93	Cadinene-delta			
23	41.215	0.75	Agarofuran-alpha	37.488	0.75	Elemol-alpha			
24	41.893	1.29	Cadin-4-en-10-ol	38.621	0.55	Dauca-5,8-diene			
25	42.145	0.36	Agarofuran-alpha	38.981	0.72	Caryophyllene oxide			
26	42.365	0.7	Longiborneol	39.294	2.82	Cedrol-allo			
27	42.753	3.12	Asarone-E	39.885	20.87	Cedrol-epi			
28	42.883	0.69	Bisabolol-epi-alpha	40.056	0.64	Humulene epoxide II			
29	43.029	0.87	Bulnesol	40.85	0.71	Acorenol-beta			
30	43.352	2.85	Shyobunol	41.006	0.44	Acorenol-alpha			
31	43.849	0.85	Khusinol acetate	41.778	1.04	Cadin-4-en-10-ol			
32	44.252	0.28	Benzaldehyde-3,4,5-trimethoxy	64.227	0.79	Sempervirol			
33	45.316	0.4	Brahmanol						
34	57.578	0.31	Phytol acetate-E						

Fungitoxic effect of essential oils in mycelial growth of test fungi

The mycelial growth of *P. psidii, C. gloeosporioides and A. alternata* were significantly different ($p<0.05$) among different concentration of *Acorus calamus* oil (Table 3). It showed the marked antifungal activity against all tested fungi. The mycelium growth of all tested fungi were decreased by increasing the concentration of EOs.

Essential oils of all selected plants (*Juniperus indica, Callistemon citrinus, Acorus calamus*) have shown a somewhat partial or complete antifungal inhibitory effect on the mycelial growth of selected test fungi (*P. psidii, C. gloeosporioides, A. alternata*) at different concentration. But the antifungal activities were different according to plant essential oils and their concentration. Among the essential oils used, *A. calamus* has shown a complete inhibitory effect on all test fungi at 20 µl/ml and 40 µl/ml where the *P. psidii* complete inhibited in 10 µl/ml concentration.

According to the result of this research, EOs of *Callistemon citrinus* shown the inhibitory effects against the all three tested pathogens. The mycelial growth of *P. psidii*, C. *gloeosporioides and A. alternata* were significantly different among different concentration of *Callistemon citrinus* oil ($p<0.05$). It showed the marked antifungal activity against all tested fungi. Among all three test fungi the best significant antifungal activity of *C. citrinus* against the *A. alternata* followed by *C. gloeosporiodes* and *P. psidii* at concentration of 40 µl/ml (Figure 6). The positive control was comparatively less effective for all three fungi than oil at 2.5 µl/ml concentration. This result of present study is supported by Sameza et al. [29] who reported that *Callistemon citrinus* EOs contain 1,8-cineole, alpha-pinene, limonene were major chemical compounds which have

potential to inhibit the mycelial growth of *Aspergillus flavus* on stored fish (*Ethmolosa fimbriata)* and also could be used on dry fish. Also, Shukla et al. [27] reported that *Callistemon rigidus* and *Callistemon citrinus* EOs contain 1,8-cineole, terpinene-4-ol, alpha terpineol were major chemical compounds that have antifungal properties against the *Phaeoramularia angolensis* could constitute an alternative to synthetic fungicides. In this study, the essential of *Callistemon citrinus* contains alpha-pinene, beta-pinene, terpinene-4-ol, eucalyptol and other chemical compounds which may be reason for their effective antifungal activity.

Juniperus indica oil showed significant ($p<0.05$) antifungal activity over all the test fungi. The mycelium growth of three test fungi were decreased by increasing the concentration of essential oils. The *J. indica* oil showed best antifungal activity against the *P. psidii* at concentration of 40 µl/ml followed by *A. alternata* and then *C. gloeosporioides*. The essential oil of *J. indica* did not completely inhibit the test fungi in present study while Wu et al. [30] found that *Juniperus* essential oils inhibited the growth of gray mold (*Botrytis cinerea*) oils contains the limonene, terpinene, linalool were considered as major compounds. Silimarly, Angioni et al. [31] reported that *Juniperus oxycedrus* L. EOs contained alpha-pienene, beta-pienene, sabinene, limonene, these chemical compounds have potential to inhibit the different fungi. Also, Aljaiyash et al. [31] investigated that *Juniperus phoencea EOs* found beta-pienene, alpha-pinene were major chemical compounds that inhibit the growth of *Penicillium expansium* and all molds. The current study also shown that essential oils of *Juniperus indica* contained alpha-pienene, beta-pienene, sabinene, delta-carene, caryophllene chemical compounds that shows the inhibiting mycelial growth of tested pathogen (Figure 7).

Table 3. One way ANOVA (effect of different concentration of essential oils of Callistemon citrinus, Juniperus indica and Acorus calamus oils on test fungi).

Name of Test plants	Name of test Pathogens	Sum of Squares	Df	Mean Square	F	Sig.
Callistemon citrinus	*Pestalotia psidii*	23.186	5	4.637	43.989	P<0.000
	Colletotrichum gloeosporioides	22.037	5	4.407	114.463	P<0.000
	Alternaria alternata	11.666	5	2.333	63.631	P<0.000
Acorus calamus	*Pestalotia psidii*	24.045	6	4.007	35.065	P<0.000
	Colletotrichum gloeosporioides	26.077	6	4.346	90.986	P<0.000
	Alternaria alternata	14.223	6	2.370	48.330	P<0.000
Juniperus indica	*Pestalotia psidii*	58.217	6	9.703	95.833	P<0.000
	Colletotrichum gloeosporioides	74.944	6	12.491	330.983	P<0.000
	Alternaria alternata	21.043	6	3.507	64.749	P<0.000

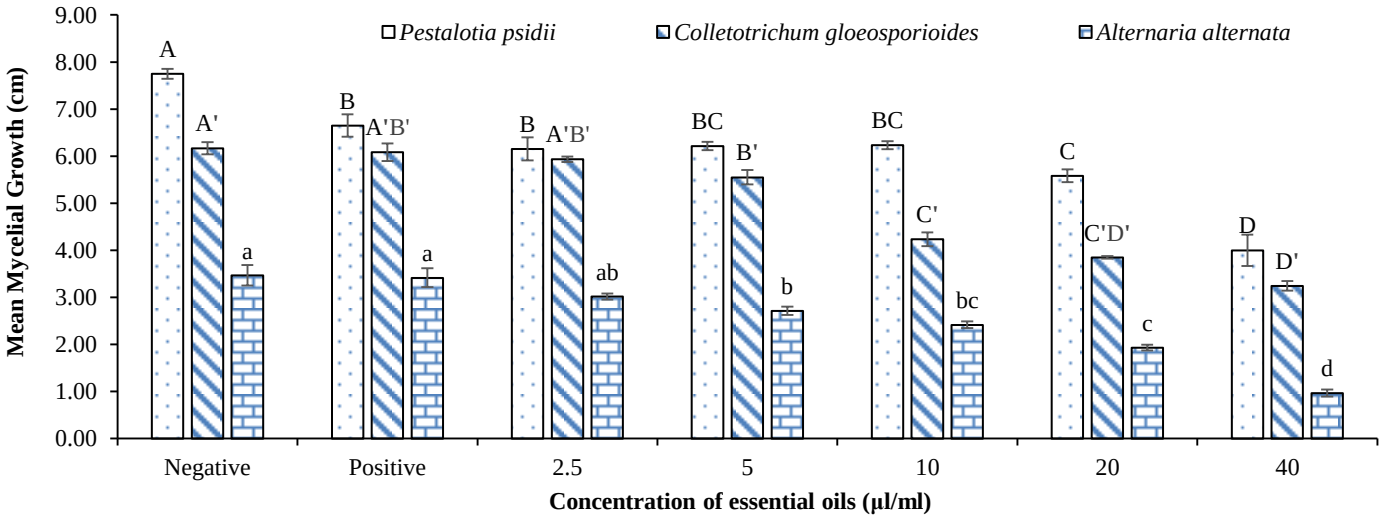

Fig. 1. Antifungal properties of *Callistemon citrinus* against the *Pestalotia psidii, Colletotrichum gloeosporides* and *Alternaria alternata*. Note: The mean value with the same alphabet (capital letter for *Pestalotia psidii*, capital letter with single prime for *Colletotrichum gloeosporioides* and small letter for *Alternaria alternata*) were not significantly differ from eachother. The error bar represents the standard error of mean mycelial growth on the effect of EOs.

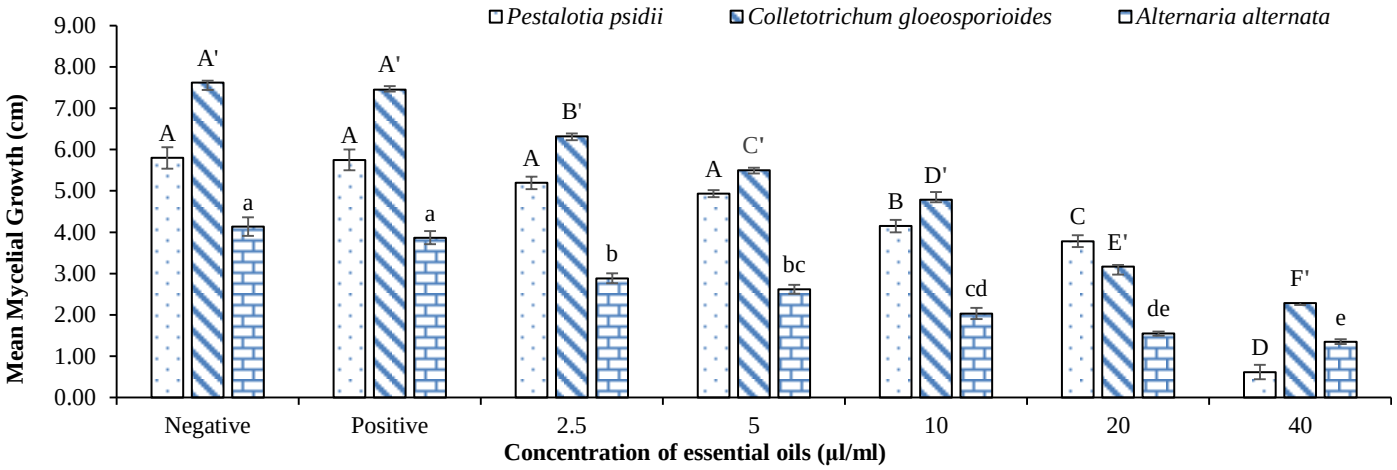

Fig. 2. Antifungal properties of *Juniperus indica* against the *Pestalotia psidii, Colletotrichum gloeosporides* and *Alternaria alternata*. Note: The mean value with the same alphabet (capital letter for *Pestalotia psidii*, capital letter with single prime for *Colletotrichum gloeosporioides* and small letter for *Alternaria alternata*) were not significantly differ from eachother. The error bar represents the standard error of mean mycelial growth on the effect of EOs.

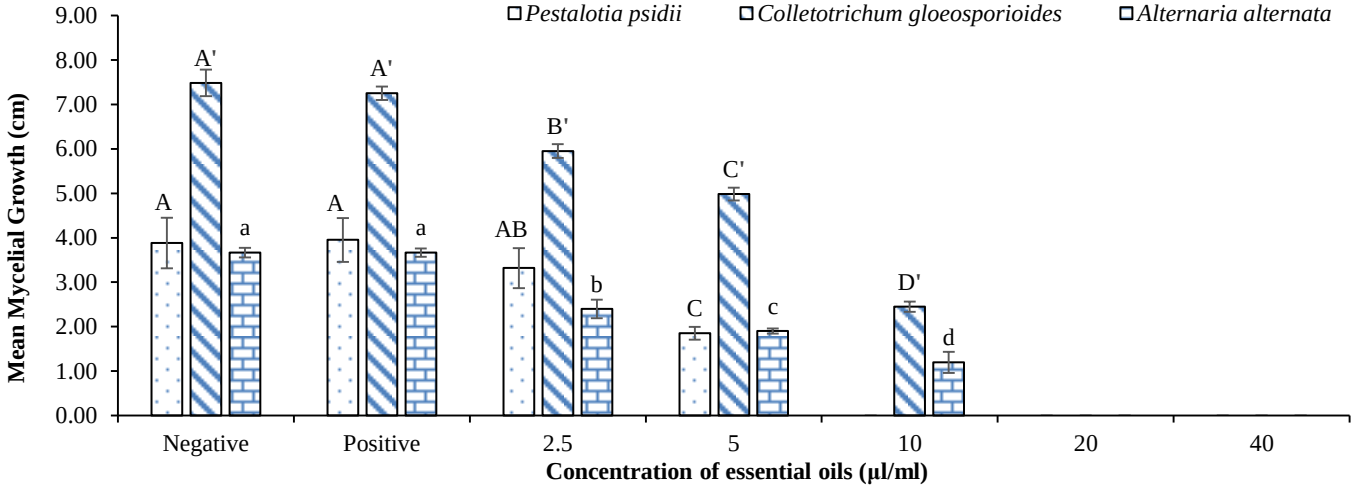

Fig. 3. Antifungal properties of *Acorus calamus* against the *Pestalotia psidii, Colletotrichum gloeosporioides* and *Alternaria alternata*. Note: The mean value with the same alphabet (capital letter for *Pestalotia psidii*, capital letter with single prime for *Colletotrichum gloeosporioides* and small letter for *Alternaria alternata*) were not significantly differ from each other. The error bar represents the standard error of mean mycelial growth on the effect of EOs.

The mycelial growth of *P. psidii, C. gloeosporioides* and *A. alternata* were significantly different ($p<0.05$) among different concentration of *Acorus calamus* oil (Figure 8). It showed the marked antifungal activity against all tested fungi. The mycelium growth of all tested fungi were decreased by increasing the concentration of EOs. At 40 µl/ml and 20 µl/ml concentration the mycelial growth of all three test fungi were completely inhibited. At 10 µl/ml the mycelial growth of *P. psidii* was completely inhibited while the *C. gloeosporioides* and *A. alternata* were not completely inhibited. The mycelial growth of *C. gloeosporioides* was high compare to *P. psidii* and *Alternaria alternata* at concentration of 2.5 µl/ml, 5 µl/ml and 10 µl/ml. Previous literatures [27, 28, 33] reported that *A. calamus* oil exhibited excellent antifungal activity. Yami and Shukla [34] found that *A. calamus* essential oils was an effective inhibitor of biodegrading and storage of contaminating fungi. As a mycelial growth of *Alternaria alternata*, *Fusarium oxysporium* was completely inhibited using by poisoned food technique method. Dethoup et al. [35] also found complete inhibition fungitoxic activity of *Acorus calamus* against growth of fungi *Alternaria* sp., *Colletotrichum* sp., *Bipolaris oryza*, *Phytophthora* and other plant pathogenic fungi.

In the present research, the oil of *Acorus calamus* was most effective against all test pathogen. Complete inhibitory effect on mycelial growth of test pathogen may be due to

presence of higher concentration of active chemical compounds present on the essential oils which could supress the physiological development of the fungus. In this study *Callistemon citrinus* EOs show the less inhibition effect on mycelial growth of test pathogen this might be less presence of phytochemical compounds. Nowadays, peoples mostly used synthetic chemicals fungicides to control post-harvest fungal disease and impact of fungicides on human health and environment [36]. Instead of essential oils used as alternatives to control the post-harvest pathogens [10, 37]. Essential oils contain antifungal properties and safe to use for human and environment [38].

CONCLUSION

This study identified eight postharvest fungal pathogens affecting guava, with *Pestalotia psidii*, *Colletotrichum gloeosporioides*, and *Alternaria alternata* being the most prevalent in Kathmandu Valley. Essential oils from *Acorus calamus*, *Callistemon citrinus*, and *Juniperus indica* exhibited significant antifungal activity, with *A. calamus* showing complete inhibition at 20 µl/ml and 40 µl/ml concentrations, highlighting its potential as a natural alternative to synthetic fungicides. However, all experiments were conducted in vitro, necessitating further in vivo trials to assess effectiveness under storage conditions. Additionally, toxicity and sensory effects on guava fruit remain unexplored, and the study does not address potential fungal resistance development, which is critical for long-term application. Economic feasibility and commercial scalability must also be considered before large-scale implementation. Future research should focus on these aspects to establish the practicality and sustainability of essential oil-based postharvest disease management.

DECLARATION

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Authorship Contributions

Concept and Design: Priyanka Khadka and Dr. Sanjay Kumar Jha; Methodology & Data Collection: Priyanka Khadka; Analysis & Interpretation: Priyanka Khadka and Asst. Prof. Hari Sharan Adhikari; Writing: Priyanka Khadka; Review and Editing: Asst. Prof. Hari Sharan Adhikari; Research Supervision: Dr. Sanjay Kumar Jha.

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Ethics approval and consent to participate

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Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Porat, R., Lichter, A., Terry, L. A., Harker, R., Buzby, J. (2018): Postharvest losses of fruit and vegetables during retail and in consumers' homes: Quantifications, causes, and means of prevention. Postharvest Biology and Technology 139: 135–149. https://doi.org/10.1016/j.postharvbio.2017.11.019

[2] Droby, S. (2006): Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticulturae 709: 45–52. https://doi.org/10.17660/ActaHortic.2006.709.5

[3] Kumar, A., Singh, J. P., Singh, P., Javeria, S. (2021): Postharvest Diseases of Papaya and Their Management. In: Singh, D., Sharma, R.R., Devappa, V., & Kamil, D. (Eds.) Postharvest Handling and Diseases of Horticultural Produce, CRC Press, Boca Raton, Florida, USA, pp. 239-248. https://doi.org/10.1201/9781003045502-20

[4] Shrestha, A. K. (2005): Critical appraisal of management practices in Nepalese guava orchards. Journal of the Institute of Agriculture and Animal Science 26: 127-133. https://doi.org/10.3126/jiaas.v26i0.665

[5] Fatima, S. (2019): Introduction to major post-harvest diseases of guava. Journal of Drug Delivery and Therapeutics 9: 591–593. https://doi.org/10.22270/jddt.v9i4.3592

[6] Liu, H., Wei, S., Shi, L., Tan, H. (2023): Preparation, structural characterization, and bioactivities of polysaccharides from *Psidium guajava*: A review. Food Chemistry 411: 135-423. https://doi.org/10.1016/j.foodchem.2023.135423

[7] Sharma, R. R., Singh, D., Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control 50: 205–221. https://doi.org/10.1016/j.biocontrol.2009.05.001

[8] Weisenburger, D. D. (1993): Human health effects of agricultural use. Human Pathology 24: 571-576. https://doi.org/10.1016/0046-8177(93)90234-8

[9] Pitarokili, D., Tzakou, O., Couladis, M., Verykokidou, E. (1999): Composition and Antifungal Activity of the Essential Oil of *Salvia pomiferasub* sp. *calycina* Growing Wild in Greece. Journal of Essential Oil Research 11: 655–659. https://doi.org/10.1080/10412905.1999.9701233

[10] Adhikari, H. S., Jha, S. K. (2023): Postharvest mycobial contaminants of white button mushroom (*Agaricus bisporus*) and their management using plant essential oils. International Journal of Agriculture Environment and Food Sciences 7(3): 500-507. https://doi.org/10.31015/jaefs.2023.3.4

[11] Barnett, H. L., Hunter, B. B. (1972): Illustrated genera of imperfect fungi. Burgess Publishing Co., Broken Arrow, USA. Pp. 1-185. https://www.academia.edu/31570962/Book_Illustrated_Genera_of_Imperfect_Fungi

[12] Watanabe, T. (2010): Pictorial atlas of soil and seed fungi: morphology of cultured fungi and key to species, CRC press, Boca Raton, Florida, USA. https://archive.org/details/pictorialatlasof0000wata

[13] Rao, G. P. and Srivastava, A. K. (1994): Toxicity of essential oils of higher plants against fungal pathogens of sugarcane. Current Trend in Sugarcane Pathology 15(2): 347-365

[14] Grove, R. K., Moore, J. D. (1962): Toximetric studies of fungicides against brown rot organism Sclerotina fruticola. Phytopathology 52:

867-880. https://www.cabidigitallibrary.org/doi/full/10.5555/19631101156

[15] Vincent, J. M. (1947). Distortion of Fungal Hypha in the Presence of Certain Inhibitors. Nature, 159: 850–850. https://doi.org/10.1038/159850b0

[16] Lal, B., Rai, R.N., Arya, A., Tewari, D.K. (1980). A new rot of *Psidium guajava* L. National Academy of Science Letters 3: 361-362. https://www.cabidigitallibrary.org/doi/full/10.5555/19821381187

[17] Nongmaithem, N. (2014): Control of post-harvest fungal diseases of guava by essential oil of *Azadirachta indica*. Indian Journal of Hill Farming 2(1): 238-246. https://epubs.icar.org.in/index.php/IJHF/article/view/46890

[18] Sharma, M. (2021). Postharvest Diseases of Guava (Psidium guajava L.) and Their Management. In: Singh, D., Sharma, R.R., Devappa, V., & Kamil, D. (Eds.) Postharvest Handling and Diseases of Horticultural Produce, CRC Press, Boca Raton, Florida, USA, pp. 219-230. https://doi.org/10.1201/9781003045502

[19] Kaushik, C. D., Thakur, D. P., Chand, J. N. (1972): Parasitism and control of *Pestalotia psidii* causing cankerous disease of ripe guava fruits. Indian Phytopathology 25: 61-64. https://www.cabidigitallibrary.org/doi/full/10.5555/19731303412

[20] Misra, A.K. (2006). Wilt of guava - a disease of national importance. Indian Phytopathology 59: 269-280. https://epubs.icar.org.in/index.php/IPPJ/article/view/17362

[21] Utikar, P. G., Shinde, P. A., Soawane, C. S. (1986): Influence of temperature and incubation period on fruit for initiation and development by postharvest fungi of guava. Current Research Reporter, Mahatma Phule Agricultural University 2: 209–211. https://www.cabidigitallibrary.org/doi/full/10.5555/19891129407

[22] Adams, R. P. (2011): Junipers of the World: The Genus *Juniperus*. Trafford Publishing, Victoria BC, Canada.

[23] Zandi-Sohani, N., Hojjati, M., Carbonell-Barrachina, A. A. (2012): Volatile Composition of the Essential Oil of *Callistemon citrinus* Leaves from Iran. Journal of Essential Oil-Bearing Plants 15: 703–707. https://doi.org/10.1080/0972060X.2012.10644109

[24] Bhandari, N. L., Khadka, S., Dhungana, B. R., Bhandari, G., Bhatt, T. D., Pandey, D. P. P. (2021): Study of phyto-and physicochemical analysis, antimicrobial and antioxidant activities of essential oil extract of *Callistemon citrinus* (curtis) skeels leaves. Advanced Journal of Chemistry-Section B 3(2): 109-119. https://doi.org/10.22034/ajcb.2021.254741.1067

[25] Lal, M., Borah, A., Pandey, S. K. (2019): Identification of a New High Essential Oil Yielding Variety "Jor Lab AC-1" of *Acorus calamus* L. Journal of Essential Oil Bearing Plants 22: 695–703. https://doi.org/10.1080/0972060x.2019.1653797

[26] Lohani H., Haider, S. Z., Chauhan, N. K., Mohan, M. (2010): Essential oil composition of leaves and berries of *Juniperus communis* and *Juniperus indica* from Uttarakhand Himalaya. Journal of Medicinal and Aromatic Plant Sciences 32: 199–201. https://jmaps.in/

[27] Shukla, R., Singh, P., Prakash, B., Dubey, N. K. (2016): Assessment of Essential Oil of Acorus calamus L. and its Major Constituent β-Asarone in Post Harvest Management of *Callosobruchus chinensis* L. Journal of Essential Oil Bearing Plants 19: 542–552. https://doi.org/10.1080/0972060X.2014.901627

[28] Radusienė, J., Judzentiene, A., Peciulyte, D., Janulis, V. (2007): Essential oil composition and antimicrobial assay of Acorus calamus leaves from different wild populations. Plant Genetic Resources: Characterization and Utilization 5: 37–44. https://doi.org/10.1017/S1479262107390928

[29] Sameza, M. L., Tchameni, S. N., Ekoue, J. D. A., Jazet, P. M. D., Tchoumbougnang, F. (2016): Growth inhibition of the stored fish (*Ethmalosa fimbriata*) fungus *Aspergillus flavus*, exposed to extracted essential oils from *Callistemon citrinus* and *Ocimum canum*. African Journal of Microbiology Research 10(30): 1164-1172. https://doi.org/10.5897/AJMR2016.8028

[30] Wu, Y.-X., Zhang, Y. D., Li, N., Wu, D. D., Li, Q. M., Chen, Y. Z., Zhang, G. C., Yang, J. (2022): Inhibitory effect and mechanism of action of juniper essential oil on gray mold in cherry tomatoes. Frontiers in Microbiology 13: 1000526. https://doi.org/10.3389/fmicb.2022.1000526

[31] Angioni, A., Barra, A., Russo, M. T., Coroneo, V., Dessí, S., Cabras, P. (2003): Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. Journal of agricultural and food chemistry 51(10): 3073-3078. https://doi.org/10.1021/jf026203j

[32] Aljaiyash, A. Ghami, M., Satrani, B., Labiad, H., Echchelh, A., Chaouch, A. (2016): Chemical Composition of Essential Oils of Ripe and Unripe Berries and Leaves of *Juniperus phoenicea* L. and Determination of their Antimicrobial Activities. International journal of Emerging Engineering Research and Technology 4 (10): 7-14. https://ijeert.ijrsset.org/papers/v4-i10/2.pdf

[33] Sintawarak, P., Uthairatsamee, S., Keawgrajang, T. (2020): In Vitro and In Vivo Inhibition of *Cylindrocladium reteaudii* by Essential Oils of Acorus calamus Rhizomes. Environment and Natural Resources Journal 19: 34–42. https://doi.org/10.32526/ennrj/19/2020076

[34] Yami, H., Shukla. (2016): Antifungal activity of essential oils derived from some plants against phytopathogenic fungi. Annals of Plant Sciences 5: 1374. https://doi.org/10.21746/aps.2016.07.002

[35] Dethoup, T., Songkumarn, P., Sirirak, T., Kijjoa, A. (2019): Fungicidal activity of *Acorus calamus* L. extracts against plant pathogenic fungi. Agriculture and Natural Resources 53(5): 527-532. https://li01.tci-thaijo.org/index.php/anres/article/view/226217

[36] Oliveira Filho, J. G., Silva, G. da C., Cipriano, L., Gomes, M., Egea, M. B. (2021): Control of postharvest fungal diseases in fruits using external application of RNAi. Journal of Food Science 86: 3341–3348. https://doi.org/10.1111/1750-3841.15816

[37] Adhikari, H. S., Jha, S. K. (2017): Postharvest microbial contamination in oyster mushroom and their management using plant Essential oils. Bio Bulletin 3(1): 104-108. https://www.biobulletin.com/articles/postharvest-microbial-contamination-in-oyster-mushroom-and-their-management-using-plant-essential-oils.pdf

[38] Jobling, J. (2000): Essential oils: A new idea for postharvest disease control. Good fruit and vegetables magazine 11(3): 50-54. https://web.archive.org/web/20180721194224id_/http://www.postharvest.com.au/GFV_oils.PDF