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The Effectiveness of Botanical Pesticide as Antifungal on Chili (*Capsicum annum* L) Disease

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ABSTRACT

Colletotrichum sp. and *Phytophthora capsici* are a causative diseases in chili, they are causing damage up to 50%. Inorganic pesticides are commonly used to treat the diseases, but there are many impacts to plant and consumers of chili. As an alternative, botanical pesticide such as *Colletotrichum* sp. and *Phytophthora capsici* are developed massively, since they are good for consumer health. The aim of this research was to evaluate the effectiveness of various botanical extract of *Jatropha curcas*, *Toona sureni*, *Pangium edule*, *Syzygium aromaticum* and *Cymbopogon citratus* L as natural antifungal against the *Colletotrichum* sp. and *Phytophthora capsici*. The results showed that the inhibition on colony development of *Colletotrichum* sp. and *Phytophthora capsici* tested was significant. Extract of *P. edule* inhibited of *Colletotrichum* sp. growth at 6 DAI up to 74.06% and *P.capsici* up to 24.03%. At the same times observation (6 DAI) *C. citratus* L extract inhibited up to 44.38% colony growth of *Colletotrichum* sp. and 86.82% colony of *P. capsici.* Extract of *Syzygium aromaticum* showed the perfect inhibition (100%) on mycelial growth for *P. capsici* and 91.71% for *Colletotrichum* sp. Whereas, *J. Curcas* and *T. sureni* extract showed insignificant effect for all fungal pathogen. We are presumed that it because the bioactive compound of the extract. In this research we found that the main compounds of *S. aromaticum* is eugenol up to 70.97% that we know as antimicrobial.

1. INTRODUCTION

Chilli (*Capsicum annum* L) is a horticulture commodity with a large scale of consumption in the world. In Mexico, chili is usually used in many traditional cuisines such as "Oaxacan black mole" and become international recognition as a culinary spice [1]. Unfortunately, the chili disease caused by some of the pathogen species reduces the quality and quantity of chili production significantly [2]. Some of the main chili diseases include anthracnose, geminivirus, leaf spot, and Fusarium wilt. Chili disease can inhibit plant growth and reduce production ranging between 20-80%. *Colletotrichum* sp. and *Phytophthora capsici* are the some of the fungal pathogen in chili disease. *Colletotrichum* sp. is causative agents of anthracnose diseases and caused yield losses of up to 50% [3, 4] and *P. capsici* caused leaf blight in chili [5].

In the field, farmers still used synthetic pesticides to control chili diseases. Synthetic pesticides are known have a negative effect to environment, human health, and natural enemies [6, 7]. It has encouraged the researcher to developed of alternative non-synthetic pesticide to control chili diseases [8, 9]. One of the alternative methods is developed a botanical pesticide [10, 11].

Botanical pesticide is an important part of the concept of organic and sustainable agriculture because it is environmental friendly and safe for the human health [12]. It is derived from plants which have naturally occurring defensive properties. Many plants have been reported used as a botanical pesticide and showed promising results [13].

Anacardium occidentale, *Chromolaena odorata*, *Cassia alata* and *Albizia saponaria* are reported to inhibit the *Phytophthora palmivora* on cocoa [14]. Sarkhosh et al. [15] reported that essential oil from mint (*Mentha piperita* Willd.), savory (*Satureja khuzistanica* Jamzad.), thyme (*Thymus daenensis* Celak.), cinnamon (*Cinnamomum zeylanicum* Blume.) can inhibit mycelial growth of Colletotrichum, Botryosphaeria, Fusarium, and Phytophthora. Other reports show that the essential oils of lemongrass (*C. citratus* L), citronella (*Cymbopogon nardus* (L.)), and peppermint (*Mentha piperita* L.) effectively suppress the growth of *Helminthosporium* sp. [10]. Orange peel essential oil contains 96.6% limonene and perfectly inhibit *Aspergillus flavus* growth [16].

There are many plants that can be used as sources of botanical pesticide, included *Jatropha curcas, Toona sureni*, and *Pangium edule* and *S. aromaticum* and *C. citratus* L. However, to our knowledge, no studies have identified these plants in controlling chili disease. Therefore, research related to this topic is essential, since it will provide environmentally friendly pesticides that positively affect human health.

While in Indonesia itself, chili is used as a spice in various dishes that are consumed daily. In the rainy season in tropical areas such as Indonesia, plant diseases become the biggest obstacle to yield gain because, if not handled properly, the loss of yield quantity can reach 100%. At the same time, chemical

control causes health problems and chemical pollution. It drives the enthusiasm to get a chili vegetable fungicide that effectively controls pathogenic chili fungi.

The aim of this research was to evaluate the effectiveness of antifungal extract of *Jatropha curcas, Toona sureni,* and *Pangium edule* and *S. aromaticum* and *C. citratus* L to againts the causative agent in chili disease so it will be known which one is the most effective as an anti-fungal. The results showed that the inhibition on colony development of *Colletotrichum* sp. and *Phytophthora capsici* tested was significant.

2. MATERIAL AND METHODS

2.1 Source of isolates

The pathogen isolates (*Colletotrichum* sp. and *Phytophthora capsici*) that used in this trial were the collection isolate from Indonesian Agency for Agricultural Research and Development: BALITSA Lembang, Bandung, Indonesia. Isolated growth in potato dextrose agar and stored in refrigerator (4℃) before used.

2.2 Botanical extracts preparation

The treatment was conducted in BBPOPT Karawang, Indonesia. Plants that are used as plant-based pesticides with maceration methods were *J. curcas*, far *T. sureni* leaves, and *P.edule* leaves. The material is cut first and then cut into small pieces and dried for 5-7 days. After drying, the ingredients are put into the oven at 40℃ until the weight of the material is stable. The material is ground using a blender to powder. A total of 100 g of powder material was put into the blender, then methanol was added to the *J. curcas* leaves and *T. sureni* leaves, and *P.edule* leaves. The solvent used is 500 ml, and the two mixes are blended until mixed. The extract solution obtained was filtered using Whatman no. paper. 42, then concentrated using a vacuum drying oven at 50℃ until the extract weight is stable. The extract was stored in a refrigerator at 4 ± 1℃ until it was used for testing [17].

2.3 Essential oils preparation

The essential oil derived from *S. aromaticum* and *C. citratus* L. were obtained from the home industry in Cilacap, Indonesia. The essential oil is extracted by hydrodistillation.

2.4 GC-MS analysis

The GC-MS analyses was conducted at Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia. GC/MS analyses were performed using a Shimadzu GCMS-QP with a stationary phase of Rtx-5MS (Crossbond® 5% diphenyl / 95% dimethyl polysiloxane) column length of 30 m and diameter of 0.25 mm. For botanical extracts using the following settings, the carrier gas was helium, a pressure of 30.6 kPa, injection volume of 1 µL, injector temperature of 150℃, ion source temperature of 200℃, interface temperature of 230℃, and split mode 75 ratio. The column was programmed of 35℃ in 1 minute hold and raised to 200℃ with a rate of increase of 10℃ / minute maintained for 10 minutes.

For essential oils using the following settings, ultrahigh purity carrier gas with a pressure of 37.1 kPa, Flow 0.72 ml/ min injection volume of 5 µL, injector temperature of 250℃, ion source temperature of 230℃, interface temperature of 230℃ split 50. Column temperature programmed from 75℃ then increased at a rate of 10℃ / min to 230℃ for 230℃ during ion source temperature 230°C, interface temperature 230°C split 50. 3 minutes. Kolo final temperature, 270℃ for 3 minutes with a rate of increase of 5℃ / minute [18].

2.5 Effect of botanical pesticide on pathogen development in vitro

The research was conducted in Plant Protection Laboratory, Faculty of Agriculture, Universitas Siliwangi. Antifungal activity on fungal colony development was obtained by media contamination method. The botanical pesticide were dissolved in 5% Tween 20 and added to the 10 ml of PDA, with concentration 0.05%. One disc (0.5 cm diameter) of mycelial plug was placed into the Petri dish. The containers were then transferred to storage at room temperature and incubated for six days. Controls consisted with PDA only. The efficacy of treatments was evaluated by measuring fungal colony [19].

3. RESULTS AND DISCUSSION

3.1 Results

Enrichment of essential oils and botanical extracts to PDA media showed resulted in significant inhibition on colony development of *Colletotrichum* sp. and *P. capsici*. *P. edulis* extract inhibited *Colletotrichum* sp. growth consistently until 6 days after inoculation (DAI) (74.06%), the same result was also aimed at *P.capsici* (24.03%) as shown in Tables 1 and 2. However, *J. curcas* and *T. sureni* extract showed insignificant effect for all fungal pathogen.

The results shown at the essential oil treatment, both *S. aromaticum* and *C. citratus* L. *C. citratus* L. essential oil showed a reduction in colony development consistently until 6 DAI, 44.38% for *Colletotrichum* sp. and 86.82 for *P. capsici. S. aromaticum* essential oils showed perfect inhibition (100%) on fungal colony development for *P. capsici* and 91.71% for *Colletotrichum* sp.

Table 1. Antifungal activity of biopesticide against *Colletotrichum* sp. (cm)

Treatment	2 DAI	4 DAI	6 DAI
Control	0.66 a	2.37 a	3.74 a
T. sureni	0.73 a	2.44 a	3.56 a
J. curcas	0.77 a	2.33 a	3.98 a
P. edule	0.34 b	0.81 b	0.97 c
S. *aromaticum*	0.1 c	0.71 b	2.08 b
C. citratus L	0 c	0 c	0.31 d

Numbers followed by the same letter in the same column are not significantly different at the 95% confidence level, with Fisher's test.

Table 2. Antifungal activity of biopesticide against *Phytophthora capsici* (cm)

Treatment	2 DAI	4 DAI	6 DAI
Control	1.11 a	3.21 a	5.16 a
T. sureni	1.16 a	3.09 a	5.02 a
J. curcas	1.16 a	2.09 a	4.68 a
P. edule	0.64 b	1.4 b	3.92 b
S. *aromaticum*	0 c	0.1 c	0.68 c
C. citratus	0 c	0 c	0 c

Numbers followed by the same letter in the same column are not significantly different at the 95% confidence level, with Fisher's test.

Table 3. The chemical composition of the botanical pesticide analyzed by GC-MS

Botanical Pesticide	Name of compound	Ret. Time	% Area
	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	28.103	81.87
	n-Hexadecanoic acid	21.655	8.62
J. Curcas	Phenol, 4-[[(dimethylamino)sulfonyl]methylamino]-	19.250	3.34
	.beta.-Elemenone	16.865	3.11
	Larixone	26.704	3.07
	9,12-Octadecadienoic acid (Z,Z)-	26.555	32.15
	Phytol, acetate	13.369	30.56
T. sureni	Phenol, 2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-	28.091	15.43
	Cedren-13-ol, 8-	24.594	11.60
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.494	10.26
	Squalene	16.764	37.08
	Farnesol isomer a	20.190	29.55
P. edule	3-Allyl-6-methoxyphenol	9.924	14.60
	Citronellal	6.861	11.50
	Geraniol	8.331	7.28
	Citronellal	11.319	44.23
	Geraniol	12.957	23.84
C. citratus	2-Octen-1-ol, 3,7-dimethyl-	12.494	16.37
	Geranyl acetate	14.785	8.94
	Caryophyllene	15.580	6.63
	Eugenol	14.728	70.97
	Caryophyllene	15.626	22.24
S. *aromaticum*	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	16.071	3.71
	Copaene	14.905	1.58
	Caryophyllene oxide	17.700	1.50

The growth of *P. capsici* was faster than that of *Colletotrichum sp*. At 6 DSI, control treatment showed *P. capsici* reached 5.16 cm on colony diameter and 3.74 cm for *Colletotrichum sp*. *Colletotrichum sp.* tends to be stronger against the provision of botanical pesticides. *P. capsici* did not show growth of mycelium until the end of observation, whereas *Colletotrichum sp.* began to show there was hyphal development in 6 DSI.

The major chemical composition of the essential oil extracted was different depending on botanical pesticide material. The dominant compounds in *J. curcas* extract was phenol (81.87%), suren contained a lot of 9,12-Octadecadienoic acid (Z, Z) - and Phytol acetate, and Squalene (37.08%) and Farnesol isomer (29.55%) were the dominant compound in *P. edule*. The major compounds in the two essential oils were very specific, such as eugenol in *S. aromaticum* and citronellal in lemongrass. The composition of eugenol in *S. aromaticum* was 70.97%, and citronellal (44.23%) and geraniol (23.84%) in lemongrass.

3.2 Discussion

The present investigation showed that two essential oils tested, had high in vitro antifungal activity against *Colletotrichum* sp. dan *P. capsici.* The previous studies showed that these oils have been shown to inhibit several plant pathogenic fungi growth. Sharma et al. [20] reported that *S. aromaticum* oil exhibited complete inhibition *Fusarium oxysporum* f. sp. *lycopersici* growth and spore germination at 125 ppm. Costa et al. [21] evaluating the *S. aromaticum* essential oil effect on the hyphae of *Rizoctonia solani, F. Solni*, and *F. oxysporum*, found different morphological changes and inhibition of hyphal development. *S. aromaticum* oil also can suppress the growth of bacterial wilt in tomatoes [22]. *C. citratus* L essential oil impacts on colony growth and spore production of *C. coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger* [23]. The application of citronella oil on post-harvest fruits shows promising results [24]. In vitro test show that citronella oil can suppress post-harvest pathogen such as *Rhizopus stolonifera, Botrytis cinereal, Colletotrichum coccodes, Aspergillus niger*, and *Cladosporium herbarum* [23].

Only *P. edule* extract that was effective significantly as antifungal from tree botanical extract tested. Previous study informed that *P. edule* extract had ability to inhibit the growth of *A. flavus* [25], *C. capsici* [19], and *C. gloeosporioides* [26]. The major chemical composition of were Squalene and Farnesol isomer (Table 3). Squalene reported effected to ergosterol biosynthesis, inhibition of the terminal oxidase, and transport electron [27]. Georgopapadaku and Bertasso [28] reported that effect of squalene were biosynthesis inhibition, membrane integrity, and cell growth inhibition in *Candida albicans*.

Based on the results of the GCMS analysis of the extracts

of the four vegetable pesticides studied, several secondary metabolites were found, the main compounds being phenol, octadecadienoic acid, phytol acetate, farnesol, citronellal, geraniol, eugenol.

Jatropha leaf phenol extract obtained seven peaks, indicating the presence of 7 secondary metabolites, which showed mass spectrum or rest area greater than 3%. The main compounds identified were Phenol (range area 81.87%). The other research reported that the presence of saponins, steroids, tannins, glycosides, alkaloids, and flavonoids was discovered during the phytochemical screening of *J. curcas* stem bark extracts [29]. *J. curcas* Linn. leaf and latex extracts, contained appreciable amounts of phenolic and saponin compounds [30].

The content contained in jatropha leaves includes alkaloids, saponins, tannins, phenolics, and flavonoids [31]. The chemical compound used as a producer of biofuels, jatropha is also used in other forms, such as for lubricating oil and raw materials in making soap. High-quality raw materials in the insecticide, fungicide, and molluscide industries [32]. These chemicals are known to be physiologically active and so help *J. curcas* antibacterial activity. These secondary metabolites have antibacterial action via various methods [29]. These extracts also showed good antioxidant activity towards DPPH and NO radical scavenging activity [30].

Suren plant extract contains 32.15% octadecadienoic and 30.56% phytol acetate; both compounds have the potential as antifungal agents [33]. The kluwek plant contains farnesol which has the potential to be a plant-based fungicide. Citronellal and geniol in citronella extract reached 44.23% and 23.84%, respectively. Citronellal, geraniol, and citronellol are the main ingredients in citronella oil that can be used as antibacterial [34, 35]. The repellency of *T. sureni* seed extracts against the red flour beetle increased proportionally with the concentration of the extract [36].

Clove oil, according to GCMS, contains 70.97% eugenol, which is used in the perfume industry and can also be used as an antiseptic. The other research reported the main constituents of clove essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde [37]. According to research conducted by Campaniello et al. [38], eugenol is an effective antifungal compound. The presence of eugenol activity that can inhibit the growth of *Aspergillus niger, A. terreus, Emericella nidulan expansum*, *P. glabrum*, *P. italicum, Fusarium oxysporum*, and *F. avenaceum* with different concentrations.

Farnesol has the capability as an antifungal associated with the inhibition of fungal dimorphism. Fermesol can damage the cell membrane of pathogenic fungal hyphae and can interfere with the process of biofilm formation. Fermesol has widly used on farmacical industry as alternatif antifungal.

The antifungal activity of essential oils related to the presence of bioactive terpenes, phenolic acids, alcohols, hydrocarbons and aldehydes. *S. aromaticum* essential oil contains eugenol which include on phenolic compound. Eugenol was known to have antimicrobial ability in some pathogenic fungi, yeast and bacteria [39-41].

Mode of actions of eugenol in controlling fungal pathogen were disintegrating mycelia, inhibition of germ tube formation, inhibition of ergosterol synthesis, and disturbance of membrane permeability. The research conducted by de Oliveira Pereira et al. [42] shows that giving eugenol to the fungus growing media caused weight loss of dry mycelium. Pinto et al. [40] evaluated the effect of eugenol on the content of ergosterol. Ergosterol is the major sterol component of the fungal cell membrane. The results showed a threefold decrease in sterols in fungi treated with eugenol compared with controls [10].

The effect of eugenol on the permeability of the outer membrane has been proven by [39] by using violet crystal dye. Crystal violet penetrates the outer membrane easily enters when the membrane is defective, inversely proportional to normal conditions. The experiments were carried out on *Salmonella typhi* treated with eugenol and compared with control cells. The result showed that eugenol alters membrane permeability and makes the cells hyperpermeable to solutes, which are generally less permeable. The other research reported that *S. aromaticum* has better antimicrobial efficacy than *O. sanctum* to against planktonic and biofilm forms of *E. faecalis* [43].

Citronellal and geraniol are the two highest compounds contained in *C. citratus* L oil, based on the results of GC-MS analysis. The antifungal activity of *C. citratus* L is strongly suspected to involve the role of the compound. Previous study showed that two monoterpenoids (citronellol and geraniol) showed outstanding antifungal activity against *Cladosporium carrionii, C. cladosporioides*, and *C. oxysporum* [44]. Pereira et al. [45] reported that citronellol and geraniol had an effect on the mycelial dry weight, conidia germination, and conidiogenesis of *Trichophyton rubrum*. It's also inhibited the essential compound on cell membrane (ergosterol) biosynthesis.

Their antifungal mode of action involves destructing plasma membrane lipid bilayer. Disruption of the membrane enzymes activity, and interrupt the process of solute transport, metabolism regulation, ergosterol synthesis, cell wall formation and morphogenesis [46]. However, the cultivation of red chilies has its own challenges, especially diseases caused by fungi [47]. Therefore, the development of this botanical pesticide is important for red chili production.

The results of this study will be the basis for the development of botanical pesticides in the future. *C. citratus* L was the plant that gave the best inhibitory effect against the two tested pathogens. After knowing this information, farmers can try using oil or *C. citratus L* extract as an alternative to using synthetic pesticides.

4. CONCLUSION

This research showed that *C. citratus*, *S. aromaticum*, and *P. edule* extracts effective to inhibition of *Colletotrichum* sp. dan *P. capsici* colonies. The highest inhibition occurred in the *C. citratus* L extract treatment. Inhibitory activity occurs due to the active compounds contained in the extract. The main compounds in *C. citratus*, *S. aromaticum*, and *P. edule* extract respectively are eugenol, citronellal, and squalene.

This study provides information about the use of several sources of botanical pesticides against chili diseases, which have not been previously reported. From the results of this study, we get an overview of the types of plants that are suitable to be developed as botanical pesticides in the future.

The results of this study can also be helpful for farmers engaged in organic farming to use vegetable pesticides that are safe for human health and environmental pollution. Each farmer can produce the pesticides needed to make safe and healthy agricultural products with simple processing technology.

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REFERENCES

[1] García-Gaytán, V., Gómez-Merino, F.C., Trejo-Téllez, L.I., Baca-Castillo, G.A., García-Morales, S. (2017). The chilhuacle chili (Capsicum annuum L.) in Mexico: Description of the variety, its cultivation, and uses. International Journal of Agronomy, 2017. https://doi.org/10.1155/2017/5641680

[2] Rai, P., Kashyap, P.L., Kumar, S., Srivastava, A.K., Trivedi, M. (2018). Ecology, population biology and management of chilli anthracnose. Sustainable Agriculture Reviews 31: Biocontrol, 361-388. https://doi.org/10.1007/978-3-319-94232-2_6

[3] Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W., Hyde, K.D. (2008). Chilli anthracnose disease caused by Colletotrichum species. Journal of Zhejiang University Science B, 9: 764-778. https://doi.org/10.1631/jzus.B0860007

[4] Banya, M., Garg, S., Meena, N.L. (2020). A review: Chilli anthracnose, its spread and management. Journal of Pharmacognosy and Phytochemistry, 9(4): 1432-1438.

[5] Majid, M.U., Awan, M.F., Fatima, K., Tahir, M.S., Ali, Q., Rashid, B., Husnain, T. (2016). Phytophthora capsici on chilli pepper (Capsicum annuum L.) and its management through genetic and bio-control: A review. Zemdirbyste-Agriculture, 103(4). https://doi.org/10.13080/z-a.2016.103.054

[6] Johnsen, K., Jacobsen, C.S., Torsvik, V., Sørensen, J. (2001). Pesticide effects on bacterial diversity in agricultural soils–a review. Biology and Fertility of Soils, 33: 443-453. https://doi.org/10.1007/s003740100351

[7] Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L. (2016). Chemical pesticides and human health: the urgent need for a new concept in agriculture. Frontiers in Public Health, 4: 148. https://doi.org/10.3389/fpubh.2016.00148

[8] SArkhoSh, A., SchAffer, B., VArgAS, A.I., PAlmAteer, A.J., Lopez, P., Soleymani, A. (2018). In vitro evaluation of eight plant essential oils for controlling Colletotrichum, Botryosphaeria, Fusarium and Phytophthora fruit rots of avocado, mango and papaya. Plant Protection Science, 54(3): 153-162. https://doi.org/10.17221/49/2017-PPS

[9] LAVKOR, İ., Arioglu, H., İsıl, V.A.R., Oztemiz, S. (2019). Biological control of aflatoxigenic fungi on peanut: For the pre-harvest approach. Turkish Journal of Field Crops, 24(1): 21-27. https://doi.org/10.17557/tjfc.562634

[10] Santos, G.R.D., Brum, R.B.C.S., Castro, H.G.D., Gonçalves, C.G., Fidelis, R.R. (2013). Effect of essential oils of medicinal plants on leaf blotch in Tanzania grass. Revista Ciência Agronômica, 44: 587-593. https://doi.org/10.1590/S1806-66902013000300022

[11] Al Yousef, A.S. (2013). Antifungal Activity of Volatiles from C. citratus L (Cymbopogon citratus) and Peppermint (Mentha piperita) Oils Against Some Respiratory Pathogenic Species of Aspergillus. Int. J. Curr. Microbiol. App. Sci., 2(6): 261-272.

[12] Miresmailli, S., Isman, M.B. (2014). Botanical insecticides inspired by plant–herbivore chemical interactions. Trends in Plant Science, 19(1): 29-35. https://doi.org/10.1016/j.tplants.2013.10.002

[13] Shafaullah, K.M. (2016). Management of Pyricularia grisea, the rice blast pathogen through botanical pesticides. Int J Sci Res, 5: 973-976.

[14] Bande, L.O., Gusnawaty, H.S., Munardin, B. (2018). Effectiveness of botanical pesticides composite to decrease of Phytophthora palmivora caused black pod rot on cocoa. Journal of Agronomy, 17(3): 154-160. https://doi.org/10.3923/ja.2018.154.160

[15] Sarkhosh, A., Vargas, A.I., Schaffer, B., Palmateer, A.J., Lopez, P., Soleymani, A., Farzaneh, M. (2017). Postharvest management of anthracnose in avocado (Persea americana Mill.) fruit with plant-extracted oils. Food Packaging and Shelf Life, 12: 16-22. https://doi.org/10.1016/j.fpsl.2017.02.001

[16] Velázquez-Nuñez, M.J., Avila-Sosa, R., Palou, E., López-Malo, A. (2013). Antifungal activity of orange (Citrus sinensis var. Valencia) peel essential oil applied by direct addition or vapor contact. Food Control, 31(1): 1-4. https://doi.org/10.1016/j.foodcont.2012.09.029

[17] Zhang, Q.W., Lin, L.G., Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese medicine, 13: 1-26. https://doi.org/10.1186/s13020-018-0177-x

[18] McMaster, M.C. (2008). GC/MS: A Practical User's Guide. USA. Wily Interscience.

[19] Johnny, L., Yusuf, U.K., Nulit, R. (2011). Antifungal activity of selected plant leaves crude extracts against a pepper anthracnose fungus, Colletotrichum capsici (Sydow) butler and bisby (Ascomycota: Phyllachorales). African Journal of Biotechnology, 10(20): 4157-4165.

[20] Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., Kundu, B. (2017). Antifungal activities of selected essential oils against Fusarium oxysporum f. sp. lycopersici 1322, with emphasis on Syzygium aromaticum essential oil. Journal of Bioscience and Bioengineering, 123(3): 308-313. https://doi.org/10.1016/j.jbiosc.2016.09.011

[21] Costa, A.R.T., Amaral, M.F.Z.J., Martins, P.M., et al. (2011). Ação do óleo essencial de Syzygium aromaticum (L.) Merr. & LM Perry sobre as hifas de alguns fungos fitopatogênicos. Revista Brasileira de Plantas Medicinais, 13(2): 240-245. https://doi.org/10.1590/S1516-05722011000200018

[22] Huang, Q., Lakshman, D.K. (2010). Effect of clove oil on plant pathogenic bacteria and bacterial wilt of tomato and geranium. Journal of Plant Pathology, 701-707.

[23] Tzortzakis, N.G., Economakis, C.D. (2007). Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens. Innovative Food Science & Emerging Technologies, 8(2), 253-258.

[24] Hidangmayum, B., Singh, N.I. (2017). Efficacy of plant extracts on fruit rot pathogen of pineapple (Ananas comosus Merr.). International Journal of Engineering Development and Research, 5(4): 1353-1355.

[25] Membalik, V., George, Y.W., Rahman, A., Asman, A.

(2018). Antifungal activities of Pangium edule reinhw seed extracts inhibts the growth of Aspergillus flavus, producer of aflatoxinx, through the in-vitro test. International Journal of Pharmaceutical Sciences and Research, 10(6): 2718-2722. https://doi.org/10.13040/IJPSR.0975-8232.10(6).2718-22

[26] Johnny, L., Yusuf, U.K., Nulit, R. (2010). The effect of herbal plant extracts on the growth and sporulation of Colletotrichum gloeosporioides. Journal of Applied Biosciences, 34: 2218-2224.

[27] Ryder, N.S. (1987). Squalene epoxidase as the target of antifungal allylamines. Pesticide Science, 21(4): 281-288. https://doi.org/10.1002/ps.2780210405

[28] Georgopapadakou, N.H., Bertasso, A. (1992). Effects of squalene epoxidase inhibitors on Candida albicans. Antimicrobial Agents and Chemotherapy, 36(8): 1779-1781. https://doi.org/10.1128/AAC.36.8.1779

[29] Igbinosa, O.O., Igbinosa, E.O., Aiyegoro, O.A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African Journal of Pharmacy and Pharmacology, 3(2): 058-062.

[30] Oskoueian, E., Abdullah, N., Saad, W.Z., et al. (2011). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from Jatropha curcas Linn. J. Med. Plants Res, 5(1): 49-57.

[31] Adinata, I.P.K., Anam, K., Kusrini, D. (2013). Identifikasi senyawa metabolit sekunder fraksi aktif daun jarak pagar (Jatropha curcas L.) dan uji aktivitas larvasida terhadap larva nyamuk Aedes aegypti. Jurnal Kimia Sains dan Aplikasi, 16(2): 42-45. https://doi.org/10.14710/jksa.16.2.42-45

[32] Dahham, S.S., Tabana, Y.M., Iqbal, M.A., Ahamed, M.B., Ezzat, M.O., Majid, A.S., Majid, A.M. (2015). The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules, 20(7): 11808-11829. https://doi.org/10.3390/molecules200711808

[33] Restu, M., Larekeng, S.H., Bachtiar, B., Akzad, M.B. (2020). Utilization of leaf litter as growth media for Suren (Toona sureni Merr.) Rhizosphere Fungi Isolates. Journal of Sylva Indonesiana, 3(01): 17-27. https://doi.org/10.32734/jsi.v3i01.1986

[34] Bota, W., Martosupono, M., Rondonuwu, F.S. (2015). Potensi senyawa minyak sereh wangi (Citronella oil) dari tumbuhan Cymbopogon nardus L. sebagai agen antibakteri. Prosiding Semnastek. Corpus ID: 131657017

[35] Miftakhurohmah. (2008). Potensi Serai Wangi Sebagai Pestisida Nabati. Dalam Warta Penelitian dan Pengembangan Tanaman Industri, 14(3).

[36] Parvin, S., Zeng, X.N., Islam, T. (2012). Bioactivity of Indonesian mahogany, Toona sureni (Blume)(Meliaceae), against the red flour beetle, Tribolium castaneum (Coleoptera, Tenebrionidae). Revista Brasileira de Entomologia, 56(3): 354-358. https://doi.org/10.1590/S0085-56262012005000047

[37] Mittal, M., Gupta, N., Parashar, P., Mehra, V., Khatri, M. (2014). Phytochemical evaluation and pharmacological activity of Syzygium aromaticum: A comprehensive review. Int J Pharm Pharm Sci, 6(8): 67-72.

[38] Campaniello, D., Corbo, M.R., Sinigaglia, M. (2010). Antifungal activity of eugenol against Penicillium, Aspergillus, and Fusarium species. Journal of Food Protection, 73(6): 1124-1128. https://doi.org/10.4315/0362-028x-73.6.1124

[39] Devi, Pandima, K., Nisha, S.A., Sakthivel, R., Pandian, S.K. (2010). Eugenol (an Essential Oil of S. aromaticum) acts as an antibacterial agent against salmonella typhi by disrupting the cellular membrane. Journal of Ethnopharmacology, 130(1): 107-15. https://doi.org/10.1099/jmm.0.010538-0

[40] Pinto, E., Vale-Silva, L., Cavaleiro, C., Salgueiro, L. (2009). Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. Journal of Medical Microbiology, 58(11): 1454-1462. https://doi.org/10.1099/jmm.0.010538-0

[41] Hodiyah, I., Hartini, E., Manik, V.T., Salma, A., Meylani, V. (2022). Inhibitory Effect of Syzygium aromaticum L. Essential Oil Against the Fungal Pathogens of Capsicum annuum L. International Journal of Design & Nature and Ecodynamics, 7(3): 463-467. https://doi.org/10.18280/ijdne.170318

[42] de Oliveira Pereira, F., Mendes, J.M., de Oliveira Lima, E. (2013). Investigation on mechanism of antifungal activity of eugenol against Trichophyton rubrum. Medical Mycology, 51(5), 507-513. https://doi.org/10.3109/13693786.2012.742966

[43] Gupta, A., Duhan, J., Tewari, S., Sangwan, P., Yadav, A., Singh, G., Saini, H. (2013). Comparative evaluation of antimicrobial efficacy of S yzygium aromaticum, O cimum sanctum and C innamomum zeylanicum plant extracts against E nterococcus faecalis: a preliminary study. International Endodontic Journal, 46(8): 775-783. https://doi.org/10.1111/iej.12058

[44] dos Santos, A.S., Silva, G.S., Silva, K.V.S., de Oliveira Lima, M.I., Arrua, J.M.M., de Oliveira Lima, E., de Oliveira Pereira, F. (2017). Antifungal activity of geraniol and citronellol against food-relevant dematiaceous fungi Cladosporium spp. Revista Do Instituto Adolfo Lutz, 76: 1-8. https://doi.org/10.53393/rial.2017.v76.33544

[45] Pereira, F.D.O., Mendes, J.M., Lima, I.O., Mota, K.S.D.L., Oliveira, W.A.D., Lima, E.D.O. (2015). Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against Trichophyton rubrum involves inhibition of ergosterol biosynthesis. Pharmaceutical Biology, 53(2): 228-234. https://doi.org/10.3109/13880209.2014.913299

[46] Hyldgaard, M., Mygind, T., Meyer, R.L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, 3: 12. https://doi.org/10.3389/fmicb.2012.00012

[47] Priyadi, R., Juhaeni, A.H., Meylani, V., Fudholi, A. (2022). The development of inorganic fertilizer and bio-fertilizer combination and the effectiveness of application on the growth and production of red chili. International Journal of Design & Nature and Ecodynamics, 17(1): 87-93. https://doi.org/10.18280/ijdne.170111