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Effectiveness of citronella, clove, and neem essential oil mix formulas against budok disease of patchouli plant

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Abstract. *Synchytrium pogostemonis* is a fungal pathogen causes budok disease of patchouli plant. A research to control the disease was conducted at Indonesian Spice and Medicinal Crops Research Institute. The research was purposed to evaluate effectiveness of mix combination and single formulas of citronella, clove, and neem essential oils against the disease. Two synthetic fungicides (benomyl and bourdeaux mixture) were also tested, as well as, water as a control treatment. One month-old patchouli plants were sprayed with single and mix combination formulas of those three essential oils (5 ml/l), benomyl (3 g/l), bourdeaux mixture (1 g/l) and water as much as (100-200 ml/plant) every two weeks for 5 times respectively. The result indicated that the mix essential oil formula of (neem+citronella) was the most effective in reducing budok disease incidence. Its effectiveness was higher (56.51%) then benomyl (47.82%) and bourdeaux mixture (52.18%). The mix formula of (citronella+neem) could be recommended for controlling budok disease of patchouli. The use of essential oil mix formulas, hopefully could control budok disease, inhibit the resistance of target pathogen, reduce phytotoxicity and concentration of the more toxic essential oil once, as well as, reduce the uses of synthetic pesticides.

1. Introduction

S. pogostemonis Patil & Mahabale is one of the most destructive fungal pathogens of patchouli plants (*Pogostemon cablin* Benth.) [1-4]. This pathogen causes budok disease and often responsible for patchouli production decline in several plantation areas in Indonesia [2]. *S. pogostemonis* is an obligate fungal parasite that spreaded throughout seedlings, soils, and water rainfall [1, 4-6]. Therefore, control of the disease should be conducted as soon as possible before the disease outbreak in patchouli growing areas. So far, the most effective control measures against the disease is using synthetic fungicides, such as benomyl, bourdeaux mixture, and penta chloronitre benzene (PCNB) [2, 7]. However, there has been considerable pressure by consumers to eliminate the use of synthetic pesticides since the use of synthetic pesticides to control plant pathogens often leads to carcinogenicity, high and acute residual toxicity, hormonal imbalance, long degradation period, environmental pollution, and causes side effects on food and humans [8, 9]. Recently, there is an increase interest to find alternative natural substances to substitute and reduce the use of synthetic pesticides.

Essential oils (EOs) are natural substances contain complex mixtures of components that possess broad range antimicrobial properties [10-13]. Those EOs exhibit antibacterial, antinematoda, antifungal,



antiviral, and antioxidant properties [13, 14]. They also function as pesticides for a wide range of fungi [15-17]. Those EOs might be effective as well to be use to control budok disease of patchouli.

The use of combined EOs is a new approach to increase their effectiveness for the control of plant diseases, taking the advantages of their synergistic and additive effects [18, 19]. Mix between *Thymus vulgaris* and *Pimpinella anisum* EOs possessed high inhibitory activities against pathogenic bacteria as compared to the standard antibiotic maxipime in which resisted by *Bacillus cereus*, *Salmonella typhi*, *S. typhimurium*, and *Pseudomonas aeruginosa* [20]. Synergistic effects were exhibited by *Ocimum basilicum* and *O. santum* (1:1) that was more effective than each EOs alone against various gram positive and negative bacteria and fungi [21]. Antifungal properties of *Ocimum* spp. have been predominantly associated with the main components, such as linalool and methyl chlavicol [21]. Those two components demonstrated synergistic effects on *Rhizopus nigra* [21].

This research was purposed to evaluate effectiveness of mix combination formulas of citronella, clove, and neem EOs against budok disease of patchouli. The results of previous experiment showed that application of single formula of citronella, clove, and neem EOs could reduce budok disease incidence of patchouli [22]. In this research, those formulas of citronella, clove, and neem EOs were mixed and combined then evaluated their effectiveness against budok disease of patchouli. Mix formulas of those three EOs hopefully more effective against budok disease than their each single formulas.

2. Materials and Methods

Main materials used in this experiment were patchouli seeds (stem cuttings) of Sidikalang variety; three different EO formulas (citronella, clove, and neem); organic (manure) and an-organic (Urea, SP 36, and KCl) fertilizers.

2.1. Preparation of Essential Oil Formulas

Citronella (*Symbopogon nardus*) and clove (*Syzgium aromaticum*) oils were extracted from leaves using a method of hydro destillation. The leaves were allowed to stand in water in a copper still, they were then heated to a boiling point. Essential oil was evaporized and together with the steam passes into a condenser. Upon cooling, the oil accumulated on the water surface was removed and filtered [23]. While neem (*Azadirachta indica*) oil was extracted from seeds using a method of hot expression. This process involve the abrasion of neem seeds and removal of the oil in the form of an aqueous emulsion that is subsequently separated in a centrifuge [23]. Then each of the citronella, clove, and neem EOs were formulated. The last concentration of each EOs in the formula was 20% (EC 20). Mix combination formulas of those three EOs were also prepared, such as (citronella+clove), (citronella+neem) (clove+neem), and (citronella+clove+neem).

2.2. Field Experiment

Experiment was conducted in an budok disease endemic field, that previously planted by patchouli plants located in Cimanggu Research Station at Indonesian Spice and Medicinal Crops Research Institute. The field was cleaned from weeds and other vegetations and then divided into 3 longitudinal blocks (3 replicates). Each replicate was divided into 10 transversal blocks (10 treatments). Each block treatment was planted with 16 patchouli seedlings. While the planting distance was 50 x 40 cm.

2.3. Patchouli Seedlings

Seedlings were prepared from stem cuttings of healthy patchouli plants of Sidikalang variety (not infected by budok and other diseases). Stem cuttings were planted in a mix of soil and manure (1:1) in small polyethylene bags. Patchouli seedlings were incubated in a glasshouse and covered with a transparent polyethylene for 1-2 weeks. After 2 weeks the polyethylene cover was opened. One-month-old patchouli seedlings were removed from polyethylene bags and replanted in field. Organic fertilizer (2 kg manure/plant) was applied before planting. While an-organic fertilizers (20 g/plant Urea, 10 g/plant SP-36, and 18.75 g/plant KCl) were applied after planting. The manure (2 kg/plant) was reapplied at 3 months after planting.

2.4. Effectiveness of Essential Oil (EO) Formulas Test

One month-old patchouli plants were sprayed either with the single and mix combination formulas of citronella, clove, and neem EOs (5 ml/l), benomil (3 g/l), and bourdeaux mixture (1 g/l), as well as water as a control treatment every two weeks for 5 times respectively with the spray volume of 100-200 ml/plant (tabel 1). Number of patchouli plants showing budok disease symptom, and plant height were evaluated every 2 weeks. Fresh weight of plant biomass was also recorded after the plants were harvested (6 months after planting). The plant biomass were air dried for two weeks and the dry weight were also recorded. Budok disease incidence was calculated based on the persentase of the infected plants, as followed:

$$\text{Disease incidence (I)} = \frac{n}{N} \times 100\%$$

where *n* is number of infected plants and *N* is number of observed plants.

This research was conducted in a randomized completely block design, consisted of 3 replicates, 10 treatments, and 16 plants/replicate. The data recorded were statistically analyzed with Duncan's Multiple Range Test (DMRT) at 5%.

Table 1. Concentration of EOs formulas, synthetic fungicides, and water used in this experiment.

No.	Tested treatment	Concentration
1	Water (control)	0%
2	Citronella (single formula)	(5ml/liter, EC 20)
3	Clove (single formula)	(5ml/liter, EC 20)
4	Neem (single formula)	(5ml/liter, EC 20)
5	Citronella+Clove (1:1)	(5ml/liter, EC 20)
6	Citronella+Neem (1:1)	(5ml/liter, EC 20)
7	Clove+Neem (1:1)	(5ml/liter, EC 20)
8	Citronella+Clove+Neem (1:1:1)	(5ml/liter, EC 20)
9	Benomyl (synthetic pesticide)	(3 gr/l)
10	Bourdeaux mixture (synthetic pesticide)	(1 gram/l)

3. Results and Discussion

3.1. Budok Disease Incidence

Symptom of budok disease was varied and start noticed either in the control and treated patchouli plants within 2-4 months after planting of the seedling in field. Disease incidence in plants sprayed with citronella, clove, and neem EOs single formulas were 33.33, 38.33 and 25.00% respectively. Those disease incidences were less as compared to in control plants (40.00%). They were greater than in plants sprayed with benomyl (20.00%) and bourdeaux mixture (18.33%). Disease incidence in plants sprayed with EOs mix formulas of (citronella+clove) and (clove+neem) were 36.67 and 31.67% respectively. Those incidence were also less as compared to in control plants. They were also greater than in plants sprayed with benomyl (20.00%) and bourdeaux mixture (18.33%). While disease incidence in plants sprayed with mix formula of (citronella+neem) was least (15.00%). This incidence was lees as compared to the control and EOs treatment, as well as, benomyl and bourdeaux mixture. While disease incidence in plants sprayed with mix formula of (citronella+clove+neem) was 41.67%. This was highest as compared to the control and other treatments (Figure 1).

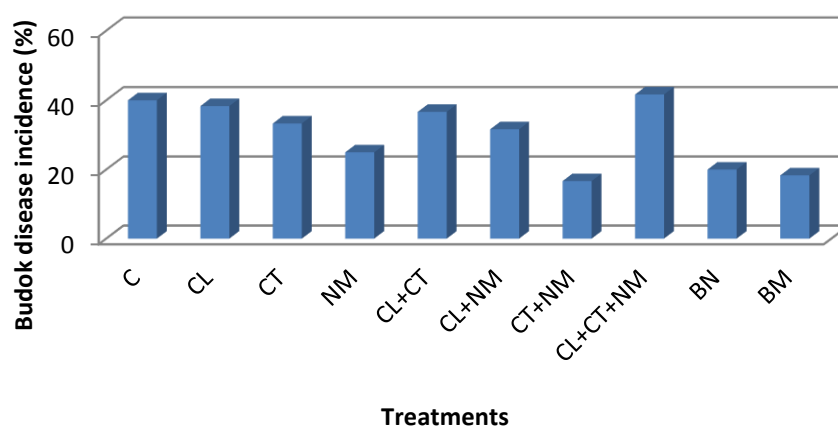


Figure 1. Budok disease incidence in patchouli plants sprayed with single and mix EOs formulas of citronella (CT), clove (CL), and neem (NM)); synthetic fungicides of benomyl (BN) and bourdeaux mixture (BM); and water (C) as a control treatment.

3.2. Effectiveness of Essential Oil Formulas.

Effectiveness of citronella, clove, and neem EOs single formulas were 16.67%, 4.17%, and 37.50% respectively. Those were less effective than benomyl (50.00%) and bourdeaux mixture (54.17%). Among those three single formulas of EOs tested, neem was the most effective against budok disease. Effectiveness of (citronella+clove), (citronella+neem), and (clove+neem) mix formulas were 8.32%, 58.32%, and 20.82% respectively. Among those three mix formulas, the mix formula of (citronella+neem) was the most effective (58.32%). This mix formula was more effective as well as compared to benomyl (50.00%) and bourdeaux mixture (54.17%). Effectiveness of (clove+neem) and (citronella+clove) mix formulas were (8.32%) and 20.82% respectively. Those were more effective as compared to the control treatment, however, they were less effective than benomyl and bourdeaux mixture. While the mix formula of three EOs (citronella+clove+neem) demonstrated comparatively not effective (-4.17%). Thus this experiment indicated that among the EO mix formulas tested, the mix of (citronella+neem) was the most effective against budok disease (Figure 2).

Effectiveness of (citronella+clove) mix formula was (8.32%). This was in between the effectiveness of clove (4.17%) and citronella (16.67%) single formulas. Thus citronella increased the effectiveness of clove and vice versa clove reduced the effectiveness of citronella. Effectiveness of (clove+neem) mix formula was 20.82%. This was in between the effectiveness of clove (4.17%) and neem (37.50%) single formulas. This indicated that neem increased the effectiveness of clove and vice versa clove reduced the effectiveness of neem. Effectiveness of (citronella+neem) mix formula was 58.32%. This effectiveness was greater than each single formulas of citronella (16.67%) and neem (37.50%). This indicated that both citronella and neem single formulas increased the effectiveness of each other. It was found that mix formula of (citronella+neem) exhibited a greater effectiveness (58.32%) than the sum effect (54.17%) of citronella (16.67%) and neem (37.50%) single formulas. This result showed that mix formula of (citronella+clove) and (clove+neem) exhibited an additive effect, while mix formula of (citronella+neem) exhibited a synergistic effect against budok disease.

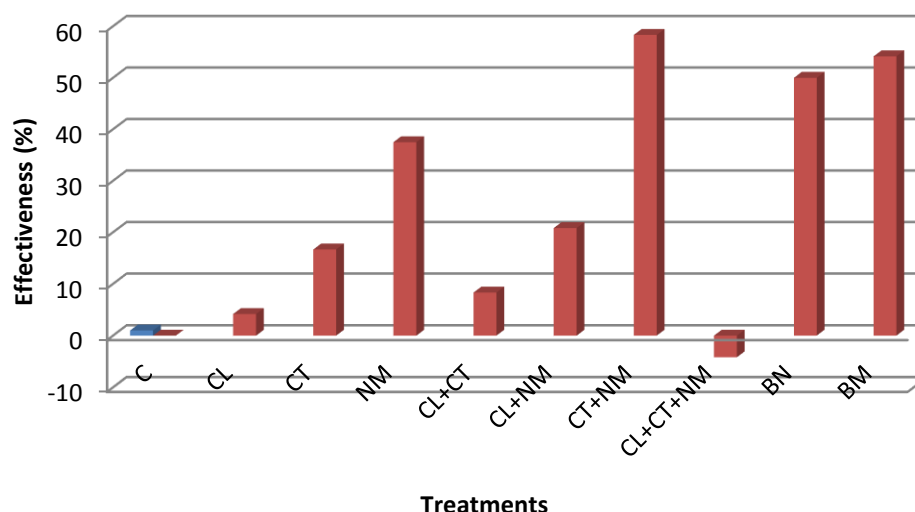


Figure 2. Effectiveness of EO single and mix formulas of citronella (CT), clove (CL), and neem (NM); synthetic fungicides of benomyl (BN) and bourdeaux mixture (BM); and water (C) against budok disease of patchouli plant.

3.3. Effects of Essential Oil Formulas of patchouli Plant Growth

All the treatments tested varied in their effects of patchouli plant growth (plant height, fresh and dry weight of plant biomass). Application of clove single formula did not affect significantly of patchouli plant height, however it was significantly reduced the fresh and dry weight of plant biomass. Citronella and neem single formulas did not affect significantly of patchouli plant height, however they significantly increased the fresh and dry weight of plant biomass. Thus those three EOs single formulas were similar in their effect of patchouli plant height, however, they differed in their effect on fresh and dry weight of patchouli plant biomass (table 2).

Table 2. Plant growth (stem height, fresh and dry weight of biomass) of patchouli applied with single and mix EOs formulas, synthetic fungicides (benomyl and bourdeaux mixture), and water.

No	Treatments	Patchouli plant growth		
		Stem height (cm)	Fresh weight of biomass (gr)	Dry weight of biomass (gr)
1	Water (control)	49.13 ab	750 bcd	2.283.3 bcd
2	Citronella (single formula)	48.47 ab	1.270.0 b	3.393.3 ab
3	Clove (single formula)	49.87 ab	370 d	1.073.3 d
4	Neem (single formula)	47.73 ab	1.033.3 bc	2.796.6 abcd
5	Citronella+Clove (1:1)	47.93 ab	553.3 cd	1.583.3 cd
6	Citronella+Neem (1:1)	52.22 a	1.090.0 bc	2.973.3 abc
7	Clove+Neem (1:1)	49.87 ab	366.6 d	1.826.6 bcd
8	Citronella+Clove+Neem(1:1:1)	51.53 ab	836.6 bcd	2.293.3 bcd
9	Benomyl (synthetic fungicide)	48.60 ab	1.943.3 a	4.600.0 a
10	Bourdeaux mixture (synthetic fungicide)	44.83 b	906.6 bcd	2.640.0 bcd

Numbers followed by the same letter in each coloumn are not significantly different at 5%.

The mix formula of (citronella+clove) and (clove+neem) did not affect significantly of patchouli plant height, however, they significantly reduced the fresh and dry weight of plant biomass. While mix formula of (citronella+neem) was significantly increased either the plant height, as well as, the fresh and dry weight of plant biomass. The mix of three EO formulas (citronella+clove+neem) did not affect the plant height, as well as the fresh and dry weight of plant biomass. This mix formula showed similar effect of patchouli plant growth as compared to the control treatment. Bourdeaux mixture significantly reduced plant height, however, did not affect significantly on fresh and dry weight of plant biomass. While benomyl did not significantly affect plant height, however it was significantly increased the fresh and dry weight of plant biomass (Table 2).

It was shown that clove single formula demonstrated the most greatest effect in inhibiting patchouli plant growth as compared to citronella and neem, especially in reducing the fresh and dry weight of plant biomass. It seems that clove single formula that mixed with citronella or neem, such as (citronella+clove) and (clove+neem) increased in inhibiting the plant growth. Thus among the treatments tested, only the mix formula of (citronella+neem) that demonstrated effect in stimulating plant growth (Table 2).

This result showed that the effect of EOs formulas in patchouli plant growth varied and did not related with their effectiveness against budok disease. It was shown that the mix formula of (citronella+neem) was the most effective against budok disease and showed the greatest effect in stimulating patchouli plant growth. While the mix formula of (clove+neem+citronella) was not effective against budok disease, however, this mix formulas demonstrated no effect of patchouli plant growth.

This experiment showed that mix formula of (citronella+clove) and (clove+neem) did not exhibit an additive or a synergistic effect on their effectiveness against budok disease. While the mix formula of (citronella+neem) showed a synergism effect on its effectiveness against budok disease (figure 1). Furthermore, the mix formula of (citronella+neem) stimulated the patchouli plant growth. This mix formula was the most potential to be developed as a botanical pesticide and could be recommended for the control of budok disease of patchouli plants.

Antimicrobial activities of EOs mostly appear from oxygenated terpenoid, particularly phenolic terpene, phenylpropanoid and alcohol components. Other components e.g. hydrocarbons that typically showed low activities can be used in mix with other components to increase their bioactivities. Thus mix between EOs might demonstrate an interaction and exhibit antagonistic, additive, or synergistic effects on their activities against microorganisms [11, 12, 24]. A synergistic interaction produced if the effect of combined essential oils or components is greater than the sum effect of the individual essential oils or components. An additive interaction observed if the effect of combined essential oils or components is equal to the sum effect of the individual essential oils or components. While an antagonistic interaction occurs if the effect of combined essential oils is less than the sum effect of the individual essential oils or components [11-13, 24, 25].

Mix of two or more EOs might demonstrate synergistic effects on their activity as pesticides. This phenomenon might due to the present of minor components in those essential oils that increased the effectiveness of the major or the other minor components [19, 26]. EOs formula mixture of *Artemisia princeps* and *Cinnamomum camphora* exhibited synergistic effects as a pest repellent if they are mixed with the EOs of thyme, anis, and saffron [19, 27]. A formula mixture of EOs that contains rosemary, sage, citrus and gliserol was effective against some food microorganism contaminants, therefore, it is often used as a food preservative. According to [19, 28], *eucalyptus* and *pyrethroids* EOs mixed with borax also showed synergistic effects on pests.

Although a synergistic effect is often demonstrated by mixtures of EOs components. However, a synergistic phenomenon among plant metabolites may result in a higher bioactivity as compared to the isolated components [18, 29]. Generally the major components are found to reflect quite well the biophysical and biological features of the EOs from which they were isolated [10]. It is possible that the activity of the main components is modulated by other minor molecules [10, 30].

There is a limited reports on the mechanism of action of mix or their purified components of EOs that involved in producing antagonistic, additive, and synergistic effects on specific microorganism.

Generally essential oil components with similar structures exhibit an additive rather than synergistic effect. The additive effect produced among EOs generally related to their main phenolic compounds e. g. carvacrol and thymol. While an antagonistic effect has been attributed to the interaction between non oxygenated and oxygenated monoterpene hydrocarbons [11].

Minor components found in low percentages may act as synergistic, enhancing the effectiveness of the major components through a variety of mechanisms. Another possibility for the synergistic effects could be that antimicrobials have different mode of actions, thereby attacking two different sites on or in the cell, in which indirectly depend on each others. While antagonistic effect occurs when combining bacteriostatic and bacteriocidal antimicrobials or the antimicrobials combined have the same site of action or interact with each others [11-13].

The mix of double components showing synergistic effects will then reduce the concentration needed to yield the same microbial effect when compared with the sum of the purified component [11]. Therefore, the use of combined EOs could be used to reduce the effect of the more phytotoxic of the other antimicrobials.

4. Conclusion

The result of this experiment showed that the mix formula of (citronella+neem) was the most effective in reducing budok disease incidence of patchouli plant as compared to the other single and mix combination of EOs formulas, as well as, benomyl and bourdeaux mixture. This mix formula of (citronella+neem) exhibited a synergism effect on its effectiveness against budok disease. This mix formula was not phytotoxic and even demonstrated a stimulating effect of patchouli plant growth. Thus this mix formula could be recommended for controlling budok disease of patchouli plants. The use of EOs mix formulas, hopefully could control budok diseases, inhibit the resistancy of target pathogen, reduce phytotoxicity and concentration of the more toxic essential oil once, as well as, the use of synthetic pesticides.

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