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Antifungal activity of *Citronella* essential oil against stem-end rot of mango

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Abstract

Stem-end rot caused by *Diplodia natalensis* is one of the significant postharvest diseases causing setbacks in the mango industry. Essential oil shows excellent potential as an alternative method in controlling postharvest diseases, which are considered safe and biodegradable with no residual effect. Hence, the study was conducted to determine the antifungal activity of *Citronella* essential oil against *Diplodia natalensis* (Pole Evans), to identify the effective concentration of *Citronella* essential oil to control *D. natalensis In vitro* and to evaluate the potential of *Citronella* oil as treatment against stem-end rot disease of mango fruit. Results showed that *Citronella* oil at 30% - 80% concentration exhibits fungistatic activity. At the same time, *Citronella* at 90% concentration showed fungicidal activity, which was most effective, showing complete inhibition of mycelial growth in the *In vitro* experiment. Furthermore, a significant reduction in fruit decay and percent fruit decay was noted with the 90% concentration of *Citronella* essential oil compared with the control in the *In vivo* experiment. However, no significant differences were observed between treatments regarding the length of exposure at 90% concentration of the essential oil. These results suggest that *Citronella* essential oil can potentially control stem-end rot in mango.

Key words: *Citronella* essential oil, antifungal activity, stem-end rot, *Diplodia natalensis*

Introduction

Mango (*Mangifera indica* L.) is famous for its excellent flavor, attractive fragrance and nutritional value, which plays an important role in the human diet. It contains macronutrients and micronutrients (Maldonado-Celis *et al.,* 2019). As one of the top ten fruit crops in the world, the annual production is estimated to be at approximately 54.83 million tons in 2020 (Statista, 2021). In 2021, the volume of mangoes produced in the Philippines amounted to approximately 741.7 thousand metric tons (Statista, 2022).

Despite these facts, the productivity of mango in the Philippines is affected by various postharvest diseases which reduce the fruit quality and cause severe losses. Among these postharvest diseases, stem-end rot caused by *Diplodia natalensis* (P. Evans) is one of mango's most important and serious problems. The most common method to control postharvest diseases is through fungicide application. However, using synthetic chemicals to control postharvest diseases can cause side effects on human health (Unnikrishnan and Nath, 2002). Moreover, continuous synthetic fungicide use may induce resistance development on postharvest pathogens (Dianz *et al.,* 2002). These suggest the need to use alternative control measures to reduce crop losses incurred by safe postharvest pathogens that pose no risk to humans and the environment.

One of the alternatives that can be utilized is essential oils. These are a complex mixture of plant volatile compounds such as terpenoids and phenolic compounds (Fokou *et al.,* 2020). These compounds are biodegradable, non-pollutant and possess no residual properties. Several studies are well documented on the effect of essential oil in postharvest diseases. For instance, Bosquez-Molina *et al.* (2010) reported that essential oil from thyme showed fungicidal effect against both *C. gloeosporioides* and *R. stolonifer* in papaya fruit. Pérez-Alfonso *et al.* (2012) also reported that thymol, carvacrol and the mixture opure essential oils have been proved effective against *Penicillium digitatum* and *Penicillium italicum*, in lemons. Essential oils from Rosewood (RO), thyme red (TR) and fennel (FO) exhibits inhibitory effect against *B. cinerea, M. nidicola and M. piriformis, P. expansum and Penicillium sp.*, and *R. stolonifer* in peach (Lin *et al.,* 2022). Essential oil from *Cymbopogon nardus* (L.) suppressed the growth of several species of *Aspergillus, Penicillium* and *Eurotium* (Nakahara *et al.,* 2013). Sangeetha *et al.* (2010) reported that *Citronella* oil extracted from *Cymbopogan citratus, C. martinii, and C. nardus* completely arrested the mycelial growth of *Lasiodiplodia theobromae and Colletotrichum musae* (crown rot disease). These reports suggest that essential oils such as *Citronella* has the potential to be an alternative control method against postharvest diseases like *Diplodia* stem-end rot. Hence, this study aimed to determine the antifungal activity of *Citronella* essential oil against *Diplodia natalensis* (Pole Evans), to identify the effective concentration of *Citronella* essential oil to control *D. natalensis In vitro*, and to evaluate the potential of *Citronella* oil as a treatment on mango fruits against *Diplodia* stem-end rot disease *In vivo*.

Materials and methods

Isolation and culture of *Diplodia natalensis*: Infected mango was collected and brought to the laboratory in the College of Engineering, ESSU, Salcedo, Eastern Samar for pathogen isolation. Several 3 mm tissue sections of the infected fruits showing typical symptoms of stem-end rot were cut and

disinfected with 1% sodium hypochlorite for one minute to remove the surface contaminants and rinsed three times with sterile water. It was blotted dry using sterile tissue paper. Dried tissue was placed aseptically in the surface of previously plated solidified potato dextrose agar (PDA) using flamed-sterilized forceps. The plates were incubated at room temperature for 24-48 hours in an inverted position. Typical growth of *D. natalensis* was transferred to a PDA slant to have a pure culture. The culture was allowed to grow and placed inside the refrigerator.

Procurement and preparation of *Citronella* essential oil: *Citronella* essential oil was purchased online. The oil was considered as 100% pure concentration. The concentrations of *Citronella* essential oil used are 20, 30, 40, 50, 60, 70, 80 and 90%. Tween 80 was added drop by drop and was mixed thoroughly with sterile stirrer until separate layers are no longer visible.

Standard control checks such as sterile distilled water (negative control check) and 40% Acetic acid (positive control check) was also provide.

***In vitro* vapor agar technique**: The effect of *Citronella* essential oil on fungal growth was determined by cutting several agar discs from the edge of an actively growing fungal culture with 5mm cork borer. One agar disc was placed at the center of each plated PDA and was allowed to stay for 6 hours under normal position before adding treatment. Two pieces of sterile filter paper were layered inside each dish's lid. One mL of *Citronella* oil at different concentrations was pipetted into the center of the filter paper. The lid was inverted and fitted into the lid at the bottom of the dish. The petri dish was placed upside down with the mycelium plug above the filter paper. Dishes were sealed with parafilm to reduce the vaporization of essential oil from the plate.

The petri dish was arranged in a completely randomized design (CRD) with ten (10) treatments: T1 = Negative control check (sterile distilled water), T2 = 20% *Citronella* essential oil, T3 = 30% *Citronella* essential oil, T4 = 40% *Citronella* essential oil, T5 = 50% *Citronella* essential oil, T6 = 60% *Citronella* essential oil, T7 = 70% *Citronella* essential oil, T8 = 80% *Citronella* essential oil, T9 = 90% *Citronella* essential oil, and T10= 40% Acetic acid (positive control check) and were replicated three (3) times. Cultures were then incubated at room temperature for 5 days and diameter of each mycelial colony was measured in mm using a ruler. Growth inhibition percentage was determined according to the formula (Fang *et al.,* 1994).

$$\text{Growth inhibition (\%)} = 100 - [[\ \frac{a}{b}\ ] \times 100]$$

Where: a = Mycelial diameter of the treatment, b = Mycelial diameter of negative control

***In vivo* fruit fumigation technique**: Mango fruits were inoculated at least two hours before the fumigation treatment with the mycelial disc of about 3mm in diameter. The most effective concentration of *Citronella* essential oil in the *In vitro* assay was used. The fumigation chamber was designed using a plastic container with secure cover. The effective concentration in the *In vitro* experiment was used. The beaker containing the 10 mL *Citronella* oil was placed in the other side of the chamber to avoid direct contact with the fruit. The lid was closed and sealed with impermeable tape to create airtight conditions. Moist cotton was placed inside the chamber to create humid conditions for ideal infection. Three mango fruits were placed inside per chamber. The fumigation chambers were arranged in a completely randomized design (CRD) with ten (10) treatments: T1 = water treatment (negative control check), T2 = 90% *Citronella* essential oil (1 hour exposure), T3 = 90% *Citronella* essential oil (2-hour exposure), T4 = 90% *Citronella* essential oil (4 hours exposure), T5= 90% *Citronella* essential oil (6 hours exposure), T6 = 90% *Citronella* essential oil (12 hours exposure), T7 = 90% *Citronella* essential oil (24 hours exposure), T8= 90% *Citronella* essential oil (48 hours exposure), T9= 40% Acetic acid (24hours exposure), and T10 = 40% Acetic acid (48hours exposure) and replicated three (3) times.

After the incubation, the fruits were taken out of the chamber and was further incubated inside a plastic bag with moist cotton for another 24hrs; then, it was exposed at room temperature. The daily observation was taken until 60% fruit decay was noted in control. The volume of the fruit was measured and recorded upon set-up termination. The fruit volume was determined using a water displacement method described by Curran (2004). The one-liter beaker was filled with water and the volume of the water was recorded. The entire mango fruit was carefully dipped inside the beaker and the volume of the water was recorded. The volume of the mango fruit was calculated by subtracting the volume of water alone from the volume of water with the fruit. Fruit portions that showed decay were cut out and removed, then the remaining part of the fruit was dipped inside the beaker and the volume of the water was recorded. Percent fruit decay was calculated as follows:

$$\text{Percent fruit decay (\%)} = [\frac{a - b}{a}]\ \times 100$$

Where: a = Total fruit volume, b = volume of the remaining fruit part

Statistical analysis: The collected data were analyzed using the Statistical Tool for Agricultural Research (STAR) software version 2.0.1. One-way analysis of variance (ANOVA) was used to determine the significant effects of the treatments. Tukey's Honest test was used to compare means at 5% level of significance.

Results and discussion

***In vitro* vapor agar technique**: Different concentrations of *Citronella* oil are tested for its vapor effect as a fumigant. It is clearly shown that essential oil has an inhibitory property compared with the control (sterile water) that reached its maximum growth at 5th day. On the other hand, it was observed that at the 5th day there was an increase in mycelial growth starting from the lowest concentration of *Citronella* oil (20%), followed by 30% at the 6th day, 40% at the 7th day and 50, 60 70 and 80% at the 8th day (Fig. 1). The mycelial growth keept increasing until the 12th day, however, no mycelial growth was noticed for 90% *Citronella* oil and 40% acetic acid up until the 12th day (Fig. 2). This suggests that concentration of *Citronella* at 90% exhibits full inhibitory effect while concentrations below 90% exhibit partial inhibitory effect.

The results revealed that after 5 days of fumigation, the highest mycelial diameter was observed in the treatments with sterile water, followed by 20% *Citronella* oil. Treatments with 30% to 90% *Citronella* oil had the lowest mycelial growth, highly comparable to the 40% acetic acid. However, seven days after the removal of *Citronella* oil, treatments were treated with sterile

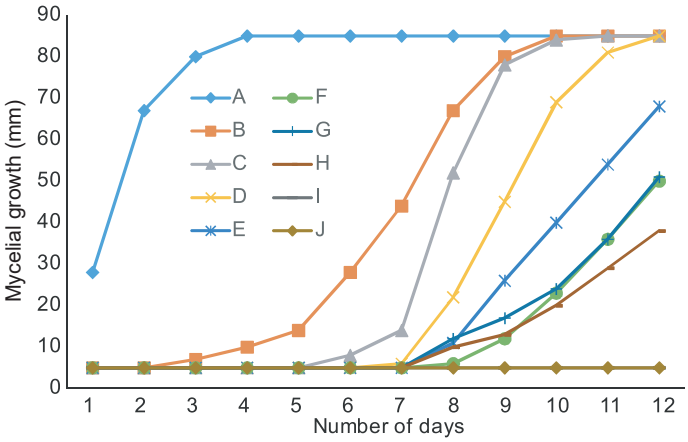

Fig 1. Mycelial growth (mm) of *D. natalensis* on vapor agar set-up as affected by the indicated treatments: A) Sterile water B) 20% *Citronella* oil, C) 30% *Citronella* oil, D) 40% *Citronella* oil, E) 50% *Citronella* oil, F) 60% *Citronella* oil, G) 70% *Citronella* oil, H) 80% *Citronella* oil, I) 90% *Citronella* oil, and J) 40% acetic acid.

water, 20, 30, 40 and 50% had the highest mycelial diameter, followed by 60%, 70% and 80% *Citronella* oil. Meanwhile, treatments treated with 90% *Citronella* oil and 40% acetic acid had the lowest mycelial diameter (Table 1).

Table 2 showed that *Citronella* oil at 30, 40, 50, 60, 70, 80 and 90% concentration and 40% acetic acid had the highest

Table 1. Mycelial mean diameter* (mm) of *Diplodia natalensis* as affected by different concentrations of *Citronella* essential oil after 5 days of fumigation and 7 days after the removal of essential oil in vapor agar set-up

Treatments	After 5 days of fumigation	7 days after removal of fumigant
Sterile water	85.00^{a}	85.00^{a}
20% *Citronella* oil	13.67^{b}	85.00^{a}
30% *Citronella* oil	5.00^{c}	85.00^{a}
40% *Citronella* oil	5.00^{c}	85.00^{a}
50% *Citronella* oil	5.00^{c}	67.67^{a}
60% *Citronella* oil	5.00^{c}	50.33^{ab}
70% *Citronella* oil	5.00^{c}	51.00^{ab}
80% *Citronella* oil	5.00^{c}	38.33^{ab}
90% *Citronella* oil	5.00^{c}	5.00^{b}
40% acetic acid	5.00^{c}	5.00^{b}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

Table 2. Growth inhibition* (%) of *Diplodia natalensis* as affected by different concentrations of *Citronella* essential oil after 5 days of fumigation and 7 days after the removal of essential oil in vapor agar set-up

Treatments	After 5 days of fumigation	7 days after the removal of fumigant
Sterile water	0.00^{c}	0.00^{b}
20% *Citronella* oil	83.92^{b}	0.00^{b}
30% *Citronella* oil	94.12^{a}	0.00^{b}
40% *Citronella* oil	94.12^{a}	0.00^{b}
50% *Citronella* oil	94.12^{a}	20.39^{b}
60% *Citronella* oil	94.12^{a}	40.78^{ab}
70% *Citronella* oil	94.12^{a}	40.00^{ab}
80% *Citronella* oil	94.12^{a}	54.90^{ab}
90% *Citronella* oil	94.12^{a}	94.12^{a}
40% acetic acid	94.12^{a}	94.12^{a}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

percent growth inhibition compared with sterile water and 20% *Citronella* oil that had the lowest percent growth inhibition after 5 days of fumigation. On the other hand, 7 days after removing the fumigant, it was observed that 90% *Citronella* oil and 40% acetic acid had the highest percent growth inhibition. The lowest percent growth inhibition was noted in treatments treated with sterile water, 20% to 40% *Citronella* oil. Results indicate that 20-80% *Citronella* oil only exhibits a fungistatic effect due to the increase in mycelial growth upon its removal after five days of fumigation. This result was in agreement with the result of Inouye *et al.* (2000) wherein there is a regrowth of the hyphae after removal of the vapor. This result suggests that essential oil's inhibitory property is highly influenced by the dosage and length of exposure (Banihashemi and Abiyardi, 2011).

On the other hand, *Citronella* oil at 90% showed fungicidal effect, same as the 40% acetic acid, showing complete inhibition of mycelial growth even at seven days after the removal of the essential oil (Fig. 2). According to Hu *et al.* (2017) fungal growth inhibition is related to the disruption of fungal cell endomembrane system including the plasma membrane and mitochondria, specifically *i.e.*, the inhibition of ergosterol synthesis, mitochondrial ATPase, malate dehydrogenase, and succinate dehydrogenase activities due to its exposure to essential oil. Tyagi and Malik (2010) also added that the volatility of the

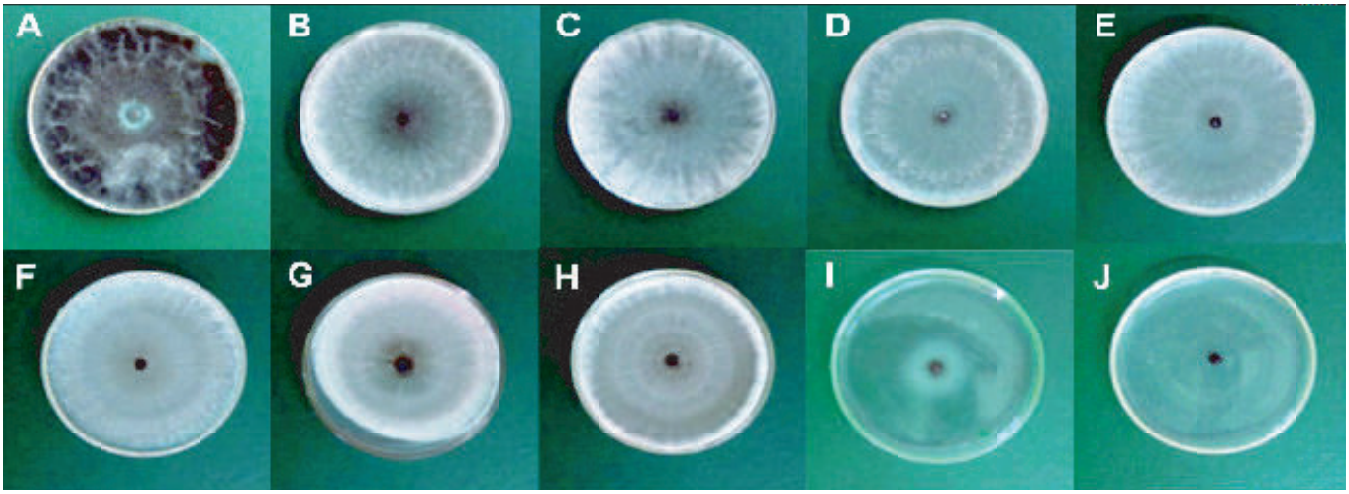

Fig 2. Mycelial growth (mm) of *Diplodia natalensis* as affected by different treatments as follows: A) Sterile water B) 20% *Citronella* oil, C) 30% *Citronella* oil, D) 40% *Citronella* oil, E) 50% *Citronella* oil, F) 60% *Citronella* oil, G) 70% *Citronella* oil, H) 80% *Citronella* oil, I) 90% *Citronella* oil, and J) 40% acetic acid.

essential oil is dominant in vapor phase assay. Furthermore, it is also reported that vapor action of essential oil inhibited filamentous fungi life-cycle through disruption on conidial germination and mycelium growth sporulation (Reyes-Jurado *et al.,* 2020).

***In vivo* fruit fumigation technique**: The inhibitory activity of *Citronella* oil at 90% concentration was assessed as a fumigant against mango stem-end rot. The fruit was inoculated with mycelial disc and were fumigated at different durations. Significant differences were observed between the treatments. Table 3 shows treatments treated with sterile water (control) had the highest fruit decay and percent fruit decay. The lowest fruit decay and percent fruit decay was observed in mango treated with 40% acetic acid at 24 hrs and 48 hrs of exposure. Meanwhile, mango treated with *Citronella* at 90% concentration had lower volume of fruit decay and percent fruit decay than mango treated with sterile water (control). On the other hand, no significant differences were observed between mangoes treated with 90% *Citronella* oil at the different duration of exposure based on the volume of fruit decay and percent fruit decay.

Table 3. Volume of Fruit decay and percent fruit decay as affected by the length of exposure to the treatments at 5th day observation

Treatments	Fruit decay* (cm^3)	Percent fruit decay (%)*
Sterile water	86.59^{a}	54.58^{a}
90% *Citronella* oil (1 hr)	37.52^{bc}	27.64^{bc}
90% *Citronella* oil (2 hrs)	43.30^{b}	32.64^{b}
90% *Citronella* oil (4 hrs)	43.30^{b}	34.76^{b}
90% *Citronella* oil (6 hrs)	40.41^{bc}	29.17^{bc}
90% *Citronella* oil (12 hrs)	37.52^{bc}	26.59^{bc}
90% *Citronella* oil (24 hrs)	43.30^{b}	30.44^{b}
90% *Citronella* oil (48 hrs)	28.86^{bc}	19.61^{bc}
40% acetic acid (24 hrs)	17.32^{cd}	13.06^{cd}
40% acetic acid (48 hrs)	0.00^{d}	0.00^{d}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

Furthermore, it is also observed that exposure to acetic acid (40%) significantly reduced fungal growth compared with other treatments *In vivo*. However, too much exposure of the mango to acetic acid (40%) at 48 hrs resulted to phytotoxicity compared with mango at 24 hrs of exposure (Fig. 3). These results suggest that the fumigation of *Citronella* essential oil is effective in reducing the fungal growth of *D. natalensis* compared with control (sterile water) in the treated mangoes *In vivo*. Other reports also indicated that essential oil as fumigants *In vivo* significantly reduced the growth of postharvest pathogens (Elshafie *et al.,* 2015; Santoro *et al.,* 2018). Throughout this study, it was observed that the efficacy of 90% *Citronella* essential oil was reduced in the *In vivo* experiment compared with *In vitro* experiment with complete mycelial growth inhibition. Hu *et al.* (2017) stated that fungal growth inhibition was related directly to the interaction of the fungal mycelia and essential oil. This is true in the *In vitro* set-up, wherein the mycelium is directly exposed to the essential oil vapor. Inouye *et al.* (2000) found that the predominant absorption of essential oil was observed in the fungal mycelia compared with in agar medium. This is highly due to mycelia's lipophilic nature concerning essential oil's volatile properties. On the other hand, the mycelial growth of *D. natalensis* in the mango fruit was not directly exposed to the essential oil under the fumigation technique since mycelial growth was predominant underneath the mango peel rather than the surface. Thus, this may suggest why stem-end rot of mango was not completely inhibited in the *In vivo* experiment compared with the *In vitro* experiment.

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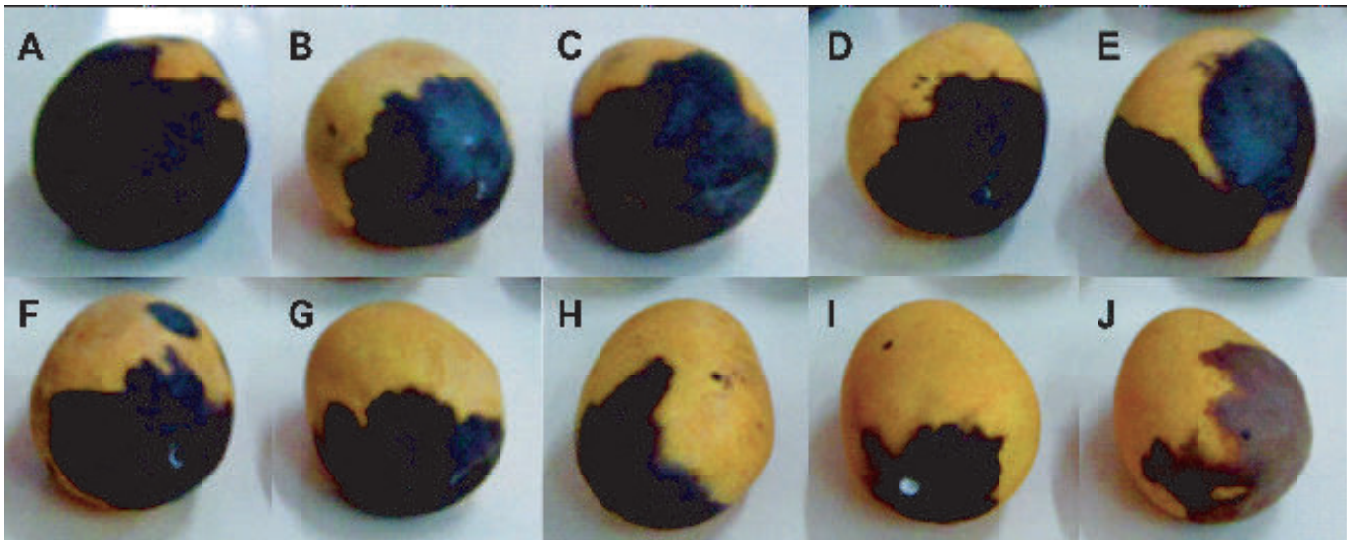

Fig 3. Stem-end rot infection on mango fruit as affected different treatments with varying time of exposure of *Citronella* essential oil as follows: A) sterile water, B) 90% *Citronella* oil (1 hr.), C) 90% *Citronella* oil (2 hrs.), D) 90% *Citronella* oil (4 hrs.), E) 90% *Citronella* oil (6 hrs.), F) 90% *Citronella* oil (12 hrs.), G) 90% *Citronella* oil (24 hrs.), H) 90% *Citronella* oil (48 hrs.), I) 40% acetic acid (24 hrs.) and J)

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