Seed viability and fungi infected seeds of *Leucaena* sp. at several treatments of essential oils, temperatures and storage periods

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Abstract. *Leucaena* sp. is one of fast growing species of biomass energy-producing timber. The cultivation of this species needs a good quality of seeds and free from pests and diseases. The objective of this study was to investigate the response of essential oils, temperature and storage periods on the seed germination and the percentage of fungi infected seeds of *Leucaena* sp. A complete randomized design of factorial pattern was used, i.e. essential oils (citronella grass and clove oils), storage temperatures (27°C -30°C; 15°C - 20°C and 0°C -5°C) and storage periods (0, 2 and 4 months). The measurement of infected seeds was carried out by using germination test of top of paper and incubation test. The results showed that storage periods and essential oils significantly influenced the germination capacity and the number of infected seeds at both of germination of top of paper and incubation test. Interaction of storage periods and essential oils treatments affected the germination capacity and infected seeds percentage only when the seeds were germinated on top of paper method. Before and after storing the seeds, essential oils treatments were not effective in reducing fungi infection when they were germinated on top of paper, but it was powerful at the time of the incubation test. Essential oils were able to increase the germination capacity before seeds were being stored. However, it was reduced when the seeds were stored for two months.

1. Introduction

*Leucaena* sp. is one of multipurpose species that all the parts of the plant can be used for domestic needs such as the wood is utilized for fuel sources and wood carpentry, the leaves are used for animal feed and its fruit is edible [1]. The plantation program of *Leucaena* sp. species requires high quality seeds and always be available when needed. The quality seeds will determine the survived seedlings and further growth of the plants in the field.

Some obstacles are often found in the provision of quality seeds, among other is the borne pathogen fungi carried by seeds. Seed borne pathogen can be detrimental both in the short and long term of storage. In the short time, the viability of seeds and seeds vigor will drop which then can affect to the plant in the field. In the long term of storage, it can be harmful because it will be a source of inoculum that settled in the soil after the seed is sown [2].

However, on the other hand, seed storage is very useful in the effort of providing seeds that is to ensure the availability of seeds at the time of planting program. Therefore, it is necessary to anticipate the handling of seeds during and before storage by controlling the pathogenic fungi, as well as setting the appropriate storage temperature. According to Sutopo [3], in the storage room, the activity of...
microorganisms and room storage temperature are highly influencing the storability of seeds. Govender et al. [4] mentioned that the main factors affecting seed storage are temperature, moisture, seed characteristics, micro-organisms and storage structure.

The use of essential oils in controlling pathogen is one of alternatives of ecological environment restrain. Essential oils such as citronella grass and clove oils are recognized to have anti-bacterial and anti-fungal active ingredients. Citronella grass and clove oils of 0.1% concentration are able to control pathogen caused by Xanthomonas vesicatoria [5] and dead shoots on mimba caused by Phomopsis azadiractae [6].

Mould is a major deterioration of seed at high moisture content and temperature. Although some fungi may survive at low temperature and moisture content, their activity rapidly drops between 10% moisture content and 10°C temperature [7]. Storing the seed under cold (12°C) and dry conditions can maintain the viability of Acacia tortilis seeds for 18 months [8].

In term of some problems found in seed handling, an investigation was employed to determine the influence of essential oils, storage temperature and storage periods on seed germination of Leucaena sp. and fungi-infected seeds percentage. The findings were able to suppress the growth of fungi and increase the viability of the seeds.

2. Materials and Methods
2.1 Materials and Location
Seeds of Leucaena sp. used for this trial collected from surrounding Bogor. The study was carried out in Pest and Diseases laboratory of Forest Tree Seed Technology Research & Development Centre (FTSTRD) in Bogor, from February to June 2018.

2.2 Procedure
Seeds of Leucaena sp. was washed out and then soaked with clove and citronella grass oils solution for 15 minutes, and the concentration was 0.1% for each solution. Seeds were then dried up for 2 x 24 hours and measured for its moisture content before being stored. When seed moisture content reached of less than 10%, the seeds were stored in a refrigerator (T 0-5°C), Air-conditioned (T 15-20°C) and ambient room temperature (T 27-30°C) for 0, 2 and 4 months.

The observed parameters were germination capacity and fungi infected seeds. The percentage of fungi infected seeds were observed using method of incubation and germination test of top of paper (TP). The incubation method, seeds were directly sown on a moist paper and incubated for 7 days under NUV lighting for 12 hours every day. In the germination test method, the evaluation of fungi infected seeds was carried out in accordance with the counting of seed germination capacity.

2.3 Data Analysis
The experiment used randomized experimental design with three factors (2x3x2) replicated three times and each replication consist of 50 seeds. Data were analyzed using ANOVA followed by Duncan’s significant difference test.
3. Results and Discussions

Analysis showed that storage periods and essential oils as well as the interaction significantly influenced the germination capacity and fungi infected seeds of both methods of incubation and germination test (Table 1). Temperature affected the percentage of fungi infection at the time of germination test.

**Table 1.** Analysis of variance of the effect of essential oils, storage temperatures, and storage periods on the germination capacity and fungi-infected seeds at both methods of incubation and germination tests.

| Treatments | F calc | Germination capacity | Percentage of fungi infected seeds at the germination test | Percentage of fungi infected seeds at incubation method |
|------------|--------|----------------------|---------------------------------------------------------|------------------------------------------------------|
| A (essential oils) | 83.3** | 7.98** | 10.80** |
| B (temperature) | 1.99tn | 4.53* | 0.11tn |
| C (storage periods) | 4.35 * | 10.82** | 23.75** |
| Interaction AB | 0.10tn | 1.13tn | 0.28tn |
| Interaction BC | 1.16tn | 1.52tn | 0.10tn |
| Interaction AC | 5.64** | 3.67* | 2.26tn |
| Interaction ABC | 0.11tn | 0.54tn | 0.95tn |

Remarks: tn= not significance, *= significance at 5 %, **= significance at 1 %

The effect of essential oils and periods storage on germination capacity and fungi-infected seeds are presented in Table 2. The treatment of citronella grass and clove oil were able to increase the germination capacity of the seeds and decrease the infected seeds at the method of incubation. However, increase the infected seeds at the germination test. Germination capacity of *Leucaena* sp. seed is increased by giving citronella grass oil treatment.

Storage periods of 2 and 4 months increased the viability of the seeds and decreased the infected seeds at the germination test. However, the increase was at the time of the incubation method. The longer the storage, more numbers of infected seeds were found at the incubation method.

**Table 2.** The effect of essential oils and storage periods on the germination capacity and fungi infected seeds.

| Factors | Treatments | F calc | Germination capacity | Percentage of fungi infected seeds at the germination test | Percentage of fungi infected seeds at incubation method |
|---------|------------|--------|----------------------|---------------------------------------------------------|------------------------------------------------------|
| Essential oils | Control | 74.15 b | 10.67 b | 27.41 a |
| | Citronella grass oil | 87.33 a | 16.22 ab | 15.63 b |
| | Clove oil | 79.11 b | 20.29 a | 10.29 b |
| Storage periods | 0 month | 74.67 b | 22.22 a | 6.89 b |
| | 2 months | 82.52 a | 12.22 b | 14.29 b |
| | 4 months | 83.41 a | 12.74 b | 32.15 a |

Remark: numbers followed by the same letters in the same column are not significantly difference according to Duncan’s at 5% level
The influence of storage temperatures on the percentage of fungi infected seeds at the germination test are presented in Table 3. The temperature of 15-20˚ C and 0-5˚ C were effective to suppress the fungi infected seeds at the method of germination test.

Table 3. Duncan’s test of the effect of temperature on the fungi infected seeds at germination test.

| Temperature (°C) | Percentage of infected seeds (%) |
|------------------|----------------------------------|
| 0-5              | 13.85 b                          |
| 15-20            | 13.41 b                          |
| 27-30            | 19.93 a                          |

Remark: numbers followed by the same letters in the same column are not significantly difference according to Duncan’s at 5% level

Citronella grass and clove oils were effective to inhibit the growth of fungi at the method of incubation. However, there was increase the growth of fungi at the germination test. It is suspected that citronella grass and clove oils were only effective when reducing the fungi on the surface of the seed coat, the solvents did not enter the inside the seed.

Anti-fungal compounds contained in essential oils i.e terpene and terpenoid such as neral, geranial [9], eugenol,limonene, geraniol, citronellal [10]. Citronella grass compounds among others are limonene, neral, citronellal, geraniol, citronellol, geraniol, cytrnellyl acetate, geranyl acetate, elemene, cubebene, caddidene, elemol, eusdemol meanwhile clove oils compounds consist of caryophyllene, humulene, eugenol, caryophyllene oxide [11]. The working of compounds contained in essential oils such as eugenol and citronellol in inhibiting the growth of fungi is by damaging plasma membranes and fungus cell walls [10].

Seed germination capacity of 2 and 4 months storage periods are higher than before storage, this may be due to the growth and activity of fungi (especially field fungi) which is inside the seeds were higher than those seeds before storage. This was approved by the higher of percentage of fungi infected seeds before storage compared to those were stored. The influence of interaction of essential oils and storage periods on the germination capacity and fungi infected seeds are presented at Table 4.

Table 4. Duncan’s test of interaction of essential oils and storage periods on the germination capacity and fungi infected seeds.

| Storage periods (months) | Essentials oil  | Germination capacity | Percentage of fungi infected seeds at the germination test | Percentage of fungi infected seeds at incubation method |
|--------------------------|-----------------|----------------------|----------------------------------------------------------|--------------------------------------------------------|
| 0                        | Control         | 56.67 b              | 10.67 b                                                  | 14 cde                                                 |
|                          | Citronella grass oil | 89.33 a              | 23.33 b                                                  | 0 e                                                    |
|                          | Clove oil       | 78 a                 | 32.67 a                                                  | 6.67 de                                                |
| 2                        | Control         | 84.44 a              | 10.44 b                                                  | 24.22 bc                                               |
|                          | Citronella grass oil | 82.67 a              | 14.22 b                                                  | 10.89 cde                                              |
|                          | Clove oil       | 80.44 a              | 12 b                                                     | 7.79 de                                                |
| 4                        | Control         | 81.33 a              | 10.89 b                                                  | 44 a                                                   |
|                          | Citronella grass oil | 90 a                 | 11.11 b                                                  | 36 ab                                                  |
|                          | Clove oil       | 78.89 a              | 16.22 b                                                  | 16.44 cd                                               |

Table 4 shows that essential oils are enable to increase the viability of the seeds before being stored and even reaches the highest value of germination capacity (89.33%) following treated by citronella grass oil. After being stored for 4 months, the viability of the seeds showed a high value when treated
by citronella grass oil. This combination gave a good germination capacity, and the percentage of infected seeds at the time of germination test tended to increase. However, the percentage of infected seeds at the incubation method showed a significant decrease following essential oil treatments. Clove oil tends to decrease seed viability following the storage. This might be happened when the seeds are exposed by the compound contained in a long time which causing toxic to the seeds and resulting in disruption to physiological process. Terpene compound contained in the essential oils such as sesquiterpene can affect the germination process, as well as monoterpane [12]. D’Abrosa et al. [13], reported that sesquiterpene brings about phytotoxic to lettuce. De Martino et al. [12], mentioned that monoterpane such as geraniol, limonene dan citronellol at high concentration can inhibit seed germination.

Several fungi were found in Leucaena sp. seeds at the time of germination test are Chaetomium sp., Fusarium sp., Aspergillus sp. and Penicillium sp. (Table 5). Seed fungi before being stored were dominated by field fungi among other is Fusarium sp., while in the stored seeds there were mostly found warehouse fungi of Aspergillus sp., especially when it stored at temperature of 27-30 ° C.

Table 5. The percentage of infected fungi at the time of germination test following storage at various treatments of essential oils, temperature and period storage

| Storage periods (months) | Temperature (°C) | Essential oils | Species of fungi |
|-------------------------|-----------------|----------------|------------------|
|                         |                 |                | Chaetomium sp.   | Fusarium sp.    | Aspergillus sp. | Penicillium sp. |
| 0                       | 0-5             | Control        | 0               | 9.33            | 0.67            | 0.67            |
|                         |                 | Citronella grass oil | 0        | 22              | 0              | 1.33            |
|                         |                 | Clove oil      | 0               | 32.67           | 14.67           | 0               |
| 2                       | 0-5             | Control        | 0.67            | 3.33            | 6.67            | 2               |
|                         |                 | Citronella grass oil | 1.4        | 3.33            | 9.93            | 0.67            |
|                         |                 | Clove oil      | 0.67            | 1.4             | 18              | 1.4             |
| 15-20                   |                 | Control        | 0               | 4               | 6               | 0.67            |
|                         |                 | Citronella grass oil | 0        | 6.67            | 5.33            | 0.67            |
|                         |                 | Clove oil      | 0               | 0               | 7.33            | 0               |
| 27-30                   |                 | Control        | 0               | 5.3             | 2.76            | 0               |
|                         |                 | Citronella grass oil | 0        | 2               | 3.33            | 0               |
|                         |                 | Clove oil      | 0               | 2.67            | 4               | 0.67            |
| 4                       | 0-5             | Control        | 0.67            | 4               | 29.33           | 0               |
|                         |                 | Citronella grass oil | 0.67        | 0.67            | 10.67           | 0               |
|                         |                 | Clove oil      | 0               | 4               | 24              | 0               |
| 15-20                   |                 | Control        | 0               | 1.33            | 4.67            | 0               |
|                         |                 | Citronella grass oil | 0        | 0.67            | 3.33            | 0               |
|                         |                 | Clove oil      | 0               | 0.67            | 12              | 0               |
| 27-30                   |                 | Control        | 0               | 1.33            | 3.33            | 0               |
|                         |                 | Citronella grass oil | 0        | 0               | 6.67            | 0               |
|                         |                 | Clove oil      | 0               | 0               | 14.67           | 0               |

In the incubation method, the fungi of Fusarium sp. was still found many in the storage seeds (Table 6). Meanwhile, Chaetomium sp. was found a lot at the time of incubation test, so it was suspected this fungus live on the surface of the seeds and Fusarium sp. and Aspergillus were live both on the surface and inside the seeds.
Table 6. The percentage of infected fungi at the time of incubation method following storage at various treatments of essential oils, temperature and period storage.

| Storage periods (months) | Temperature (°C) | Essential oils          | Incubation method | Chaeotomium sp. | Fusarium sp. | Aspergillus sp. | Penicillium sp. |
|-------------------------|-----------------|-------------------------|-------------------|----------------|---------------|----------------|----------------|
| 0                       | 0-5             | Control                 | Citronella grass oil | 3.33          | 9.9          | 0.67           | 0              |
|                         |                 | Clove oil               | 0                  | 0              | 0            | 0              | 0              |
| 15-20                   |                 | Control                 | Citronella grass oil | 0.67          | 6           | 0              | 0              |
|                         |                 | Clove oil               | 0                  | 0              | 0            | 0              | 0              |
| 27-30                   |                 | Control                 | Citronella grass oil | 0.67          | 6           | 0              | 0              |
|                         |                 | Clove oil               | 0                  | 0              | 0            | 0              | 0              |
| 2                       | 0-5             | Control                 | Citronella grass oil | 0.67          | 4.67         | 18             | 0              |
|                         |                 | Clove oil               | 1.4                | 2              | 4            | 4              | 0              |
| 15-20                   |                 | Control                 | Citronella grass oil | 3.33          | 19.33        | 0              | 0              |
|                         |                 | Clove oil               | 0.67               | 9.33           | 0.67         | 0              | 0              |
| 27-30                   |                 | Control                 | Citronella grass oil | 0             | 4            | 2              | 0              |
|                         |                 | Clove oil               | 0                  | 0              | 0            | 0              | 0              |
| 4                       | 0-5             | Control                 | Citronella grass oil | 0.67          | 13.33        | 8.67           | 0              |
|                         |                 | Clove oil               | 5.33               | 4.67           | 4            | 0              | 0              |
| 15-20                   |                 | Control                 | Citronella grass oil | 2             | 3.33         | 3.33           | 0              |
|                         |                 | Clove oil               | 0.67               | 17.33          | 4            | 0              | 0              |
| 27-30                   |                 | Control                 | Citronella grass oil | 7.33          | 4.67         | 6.67           | 0              |
|                         |                 | Clove oil               | 0.67               | 1.33           | 1.33         | 0              | 0              |

4. Conclusions
The use of citronella grass and clove oil were able to increase the viability of *Leucaena* sp. seeds before being stored. In addition, such essential oils were only effective in decreasing infected fungi on the surface of the seeds.

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