Application of neem (Azadirachta indica) as biological pesticides in cocoa seed (Theobroma cacao) storage using various local adsorbent media

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Abstract. Cocoa seeds are recalcitrant (the water content is more than 40%) that require special handling. The use of adsorbent media to reduce the decrease in the quality of cocoa seeds and extend their shelf life in this storage has not been widely done. Local adsorbent media such as sawdust, sand and ash have the potential to maintain the viability of cocoa seeds. The objective of this research was to determine the interaction of the application of neem (Azadirachta indica) as biological pesticides and the use of various natural adsorbent media in the storage of cocoa seeds (Theobroma cacao). It was an experimental study with a factorial design composed of three factors. The first factor was the medium adsorbent type for the storage of cocoa seed, which consists of three levels (river sand, ash, and sawdust). The second factor was the concentration of neem leaves for pre-storage treatment with three levels (10, 20, and 30%). The third factor was the storage time (10 and 20 days). The results of the study indicated that the combination of the three factors showed a significant interaction in the height of the plant and the diameter of the stem of the seedling at 28 days after sowing. The fresh weight of the seedlings of the seeds that were stored in ash media gave a better result than the seedlings of seeds that had been stored in the river sand and the sawdust as adsorbent media. The application of 20% extract of neem leaves gave the best influence for the seeds that were stored for 20 days.

1. Introduction
Cocoa beans (Theobroma cacao) is one of the most important agricultural export products in Indonesia. Along with the growing demand for the product, the production of cocoa beans has increased enormously due to the rapid expansion of the participation of small farmers. Indonesia is the third world exporter of cocoa beans [1,2]. The production of cocoa beans depends on many factors, including the quality of the seeds. It is one of the important production factors that determine the success of the expansion of the plantation area. The problem is that cocoa seeds are recalcitrant (they have more than 40% water content) that require special handling during storage [3]. Calistrut et al. [4] said that recalcitrant hydrated seeds can be stored only for days or months. However, the condition of recalcitrant seeds (high in humidity) makes them easier to deteriorate and also lead to the proliferation of fungi and bacteria. Storing recalcitrant seeds in moist media will cause them to weaken rapidly, losing vigor and viability sooner. At that time, the seeds become susceptible to attack by...
microorganisms, especially fungi. Therefore, it needs special treatments to reduce the decrease in the quality of the cocoa seed during the entire storage time. The combination of the appropriate insecticide and media storage will maintain the moisture content of the seeds and reduce the deterioration of the seeds throughout the storage time.

The use of natural adsorbent media in the storage of cocoa seeds to extend shelf life is still limited. Natural adsorbent media such as sawdust, sand and ash have the potential to maintain the viability of cocoa seeds. It will delay germination of the seed and reduce the attack of the disease. The combination of natural adsorbent media with the application of neem as natural antifungal at several doses will provide a significant benefit to maintain the quality of the cocoa seed in a period of storage time. This research has carried out a combination of several natural local adsorbent media combined with the biological treatment of pesticides using neem leaves extract to maintain the quality of the cocoa seeds during storage. This study was aimed to determine the interaction of the application of neem leaves (*Azadirachta indica*) in several doses as organic pesticides and the use of various local adsorbent media in the storage of cocoa to increase performance of cocoa seedlings.

2. **Material and Methods**

2.1. **Material**

The research was carried out in the Agricultural Laboratory of UNISMA and in the greenhouse of the Faculty of Agriculture, University of Islam Malang. The materials used were cocoa sp seeds Creole f2. The seeds were selected based on the uniform size and weight. They must be free from disease and free from mechanical damage. And for the germination and seedling process, we use the mixture of soil, sand, and manure with a ratio of 2:1:1 as a medium. The tools used in this research were polyethylene bags, a digital scale, beaker glass, and polybags.

The experimental design used was a random block design, organized in factorial composed of three factors. The first factor was the use of natural adsorbent media (river sand, ash, and sawdust). The second factor was a concentration of neem leaves extract that consisted of 3 levels: 10%, 20%, and 30%. The third factor was days of seeds storage in the media (10 days and 20 days).

2.2. **Research Implementation**

The seeds were selected, washed and dried under the sun. The dried seeds were then dissolved in distilled water at a concentration of 10%, 20% and 30% neem extract for 10 minutes. The storage media used in this investigation (river sand, ash, and sawdust) were sifted with the same particle size and then pasteurized at a temperature of 70°C. The seeds were stored in each medium according to the treatments for 10 and 20 days. Then, the seeds were planted to be seedlings.

The variables measured in this study were seed percentage weight loss, moisture content and seed germination after according to the storage time; height, stem diameter, total leaves, the total length of the root and total dry weight of the seedlings. The seedling growth was observed at 7, 14, 21, 28 days after sowing. The data from three time replications for each treatment were analyzed using Analysis of Variance continued with Duncan Multiple Range Test (DMRT) 5% to see the difference between the treatments for the significant ones.

3. **Results and Discussion**

The result of the research showed that the seeds that were stored in sawdust media had the lowest weight decrease which was significantly different with the seeds that were kept in river sand and ash media (Table 1). Karthikeyan *et al.* [5] stated that ash is capable adsorbent media in keeping the seed moisture content and prolonging the storage time.

The result also showed that seeds stored for ten days had better germination percentage than seeds stored for 20 days. It showed that the quality of the seeds stored for 20 days had decreased. Table 2 shows that the combination of 20% leaves of neem extract and 10 days of storage gave the best germination compared to the other combination. Bewley *et al.* [6] stated that the viability of the seeds showed a close relationship with the storage period.
Table 1. Percentage weight loss of cocoa seed based on media treatment.

| Media Treatment | Percentage weight loss (%) |
|-----------------|----------------------------|
| River Sand      | 24.32±1.28 a               |
| Ash             | 36.42±0.82 b               |
| Sawdust         | 18.80±1.04 a               |

significantly different in ANOVA Test (5%)

Table 2. Seed germination after 7 days planting based on neem extract treatment and time storage.

| Treatments | Germination (%) |
|------------|-----------------|
| % neem extract | Storage time |
| 10% | 10 days | 84.44±2.68 bc |
| | 20 days | 70.00±2.64 ab |
| 20% | 10 days | 92.22±3.85 c |
| | 20 days | 60.00±2.29 a |
| 30% | 10 days | 82.22±2.54 bc |
| | 20 days | 74.44±2.21 b |

Sig (P:0.05)

The percentage of germination of the seeds treated with 20% extract of neem leaves and kept for 10 days has the highest value. It showed that the application of 20% neem leaves extract was more effective in preventing fungal attack during storage compared to the other concentrations. According to Bansod and Rai [7] neem leaves extract has an excellent potential to produce antifungal compounds against the fungus Candida albicans and Aspergillus niger. It was also affirmed that the extracts of neem leaves can be used as an alternative to natural insecticides to control insect pests [8].

Table 3. Seedling height and fresh weight after 28 days of planting.

| Treatments | Height (cm) | Fresh Weight (g) |
|------------|-------------|------------------|
| % of neem extract | Time storage | |
| 10% (P1) | 10 days (H1) | 18.52±1.36 cd |
| | 20 days (H2) | 12.58±0.52 bcd |
| 20% (P2) | 10 days (H1) | 18.26±0.65 cd |
| | 20 days (H2) | 6.65±0.51 bc |
| 30% (P3) | 10 days (H1) | 18.42±0.52 cd |
| | 20 days (H2) | 9.77±0.67 bc |
| Ash | 10% (P1) | 18.99±1.31 d |
| | 20 days (H2) | 14.43±1.07 bcd |
| 20% (P2) | 10 days (H1) | 17.09±0.80 cd |
| | 20 days (H2) | 15.66±0.77 cd |
| 30% (P3) | 10 days (H1) | 18.25±0.75 cd |
| | 20 days (H2) | 14.67±1.52 cd |
| Sawdust | 10% (P1) | 18.56±0.51 cd |
| | 20 days (H2) | 16.12±0.78 c |
| 20% (P2) | 10 days (H1) | 9.06±0.41 bc |
| | 20 days (H2) | 15.36±0.36 c |
| 30% (P3) | 10 days (H1) | 8.03±0.96 c |

Sig(P:0.05) Sig(P:0.05)
The statistical analysis showed an interaction between the 3 factors for the height of the stem and the fresh weight of the seedling 28 days after sowing. Table 3 shows that seeds that had been stored in ash media with 10% treatment with neem extract for ten days resulted in the highest and significantly different seedling with some other treatments. The seedlings of the seeds that had been stored in ash media with 10% neem extract for ten days had the highest result. It was the best combination for the height of the seedlings compared to the other combinations. It also showed that seeds stored in ash media treated with 10% biological pesticides for ten days had the highest fresh weight. Karthikeyan et al., [3] affirmed that the use of ash and neem oil in the storage of seeds has been widely used to prevent the deterioration of seeds caused by insects and attacks of microorganisms. The application of ashes to the seed storage media can control weight seed losses in sorghum, considerably up to 80%. The use of neem oil is known as popular post-harvest procedure in India. Neem oil acted as the repellent of several insects. The result also showed that the seeds that had been stored for 20 days had a worse vigor and viability compared to the seeds that had been stored for 10 days. Olaiya (2016) [9] said that the longer retention period of seed cocoa after harvest will result in worse performance of the seedlings.

4. Conclusion
The combination of the media, the concentration of the neem leaf extract and the storage time showed a significant interaction in the height of the seedlings and the fresh weight of the seedlings after 28 days after sowing. The yield of seedlings from seed that had been stored in ash media was better than seedlings stored in sand and sawdust. The application of neem extract had a positive effect on the performance of the seedlings.

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