Concentration Effect of Ethanol Extract *Pinus merkusii* Leaves Litter on *Zea mays* L. Seed Germination

Mita Dewi Retnoningrum1*, Mohammad Arfi Setiawan2
1Biology, Universitas Billfath, Lamongan, Indonesia
2Chemical Engineering, Universitas PGRI Madiun, Madiun, Indonesia

*deewitha.mdr@gmail.com

Abstract. Human resources is one of the main factor in forest management, so it is important to develop a model of community-based forest management through agroforestry system. The agroforestry system in Trenggalek was implemented by combining a pine plant with Bisi-2 corn varieties. However, the presence of dry pine leaves litter suspected to contain allelopathy, thus make the percentage of germination of seed corn in a low. This study aimed to determine the concentration effect of ethanol extract of pine leaves litter (*Pinus merkusii*) on the germination of seed corn (*Zea mays* L.) Bisi-2 varieties. The research design that used Completely Randomized Design and four replications with concentration variations of 0% (control), 10%, 15%, 20% and 25%. Based on ANOVA and Duncan Multiple Range Test with significant level of 5% showed that there was the influence of the ethanol extract of pine leaves litter on the germination of seed corn Bisi-2 varieties. The higher concentration, make the germination of corn seeds more inhibited.

1. Introduction

Forest is a renewable resources, so management and utilization must be kept wisely to maintain ecosystem balance. Human resources is one of the main factor in forest management, so it is important to apply a model of forest management through an agroforestry system. In the Sumurup village, Bendungan, Trenggalek, citizens combine pine with corn Bisi-2 varieties. However, the percentage of germination corn seeds became low due to the litter of pine leaf that allegedly contained allelopathy from the decomposition process.

Allelopathy is a chemical compound produced by plants that potentially inhibit seed germination [1]. Pine plants have a resin channel that can produce a secondary metabolite. Allelopaths in the resin belong to terpenoid group of compounds, namely α-pinene and β-pinene monoterpenes [2]. According to Brearley et al [3], at plant seedling level, litter create different micro environments with release of nutrients or phytotoxic mixtures during decomposition. It causes higher moisture in the litter layer that can grow the pathogenic fungi to attack the seeds.

Desai and Gaikwad [4] explained that the concentration of mangrove leaf extract (*Excoecaria agallocha* L.) 5%, 10%, 25%, 50%, 75% and 100% showed the decrease germination in three rice
cultivars, Jaya, Ratna 4 and Ratna 24. Concentrations of 75% and 100% resulted the highest inhibition for germination process that compared with other concentrations. Another study by Padhy et al [4] explained that at all concentrations of *Eucalyptus* leaf litter extract at concentrations of 5%, 10%, 15% and 20% inhibited germination of the seeds *Eleusine coracana* Gaertn cv AKP-2. More over, decrease respiration rate, catalase enzyme activity, α-amylase and peroxidase. From both studies it can be seen that giving extract has inhibited physiological germination of rice seed and *Eleusine coracana* caused by allelopathy extract litter of mangrove leaf and *Eucalyptus*. However, Wusono et al [4] explained that the extract of mahogany leaf litter (*Swietenia mahogani* L.) on corn and green beans did not affect the germination process as seen from the percentage and the rate of germination. Thus, it is necessary to conduct a research to find out the effect of pine leaf litter extract concentration (*Pinus merkusii*) to germination of corn seed (*Zea mays* L.) Bisi-2 varieties.

2. Methodology

2.1 Extraction

Pine leaves litter were obtained from agroforestry area in Sumurup Village, Bendungan, Trenggalek. Extraction was done in Chemical Laboratory of Chemistry Department Universitas Islam Negeri Malang. Dried litter of pine leaves crushed into powder using a blender. Then weighted as much as 100 grams and soaked with 200 ml 70% ethanol in a closed container and allowed to stand for 24 hours. Next, filtered and the result was dissolved again with ethanol 70% with 3 times repetition. The result of extraction was concentrated with rotary evaporator, and then the concentrated extract was used for germination test with dilution. The dilution was done by taking the extract according to its concentration then adding with aquades up to 100 ml.

2.2 Treatment with Pine Leaf Litter Extract

The seeds of Bisi-2 varieties were obtained from UPT Development of Palawija Seeds Malang. The process of seed planting and treatment with was done in Physiology Laboratory of Biology Department Universitas Islam Negeri Malang. The corn seeds (*Zea mays* L.) of Bisi-2 varieties are grown on petri dishes with 3 pieces of paper sheets. Each petri dish was planted with 10 corn seeds. Each petri dish was dampened with aquades first, then added the extract that has been made each of 10 mL. Giving extracts only applied at the beginning of planting seeds, and maintained the moisture with watering every 2 days.

2.3 Variable

Data were obtained at 12 days after planting sprouts (HST). After 12 HST, the sprouts are removed from the substrate and calculated:

2.3.1 Percentage of Germination

Percentage of Germination indicates the amount of normal sprouts that seed produce in environmental conditions within a given time period. According Sutopo [5], to calculate the percentage of germination power used formula as follows:

\[
\% \ DB = \frac{\sum \text{normal sprouts}}{\Sigma \text{seed planted}} \times 100\%
\]

2.3.2 Rate of Germination

Rate of germination is the ability of sprouts to appear at any given time. Measurements are performed daily until day-12. According to Sutopo [5], the rate of germination can be measured by counting the number of days required for the appearance of radicles. The rate of germination is calculated using the following formula:

\[
RG = \frac{N1.T1 + N2.T2 + N3.T3 + \cdots + Nx.Tx}{\text{Total of seed germination}}
\]

RG: Rate of Germination

N : Sprouts that appear at any given time

T : Time between the beginning of the test to the end of observation
2.3.3 Hypocotyl Length
The measurement of hypocotyl length ranges from hypocotyl border and root to epicotyl boundary.

2.3.4 Root Length
Measurement of root length is from the root tip to the hypocotyl limit

2.3.5 Dry Sprouts Weight
Performed by sprouts is inserted into envelopes that have been labeled treatment, then put into the oven. According to Salisbury [6], to know the dry weight of the plant must be ovened for 48 hours with a temperature of 80 °C. After that weighed using an analytical scale.

2.4 Data Analysis
To know the effect of treatment is done by single variance analysis (ANOVA). If the treatment had real effect then continued with DMRT test with 5% significance level.

3 Result and Discussion
The results of variance analysis (ANOVA) showed that $F > F_{\text{table}} (\alpha = 0.05)$, it showed that there is influence of pine leaf litter extract concentration on all variable. Furthermore, the results of 5% DMRT test are presented in Table 1.

Table 1. Results of DMRT Test

| Conc. | Percentage of Germination (%) | Rate of Germination (days) | Hypocotyl Length (cm) | Root Length (cm) | Dry Sprouts Weight (gram) |
|-------|------------------------------|----------------------------|-----------------------|------------------|---------------------------|
| 25%   | 40a ± 11.55                  | 1.69a ± 0.0096             | 0.86a ± 0.173         | 0.75a ± 0.397    | 0.40a ± 0.112             |
| 20%   | 62.5b ± 9.57                 | 1.71a ± 0.015              | 1.28ab ±0.266         | 0.80a ± 0.212    | 0.60ab ± 0.101            |
| 15%   | 67.5b ± 9.57                 | 1.73b ± 0.016              | 1.65b ±0.324          | 0.99a ± 0.359    | 0.76abc ± 0.409           |
| 10%   | 75bc ± 5.77                  | 1.82c ± 0.0096             | 1.74b ±0.533          | 1.24ab± 0.388    | 0.96bc ± 0.50             |
| 0%    | 85c ± 5.77                   | 1.84d ±0.0058              | 2.65c ±0.394          | 1.64b ± 0.287    | 1.17c ± 0.37              |

(control)

The number followed by the same letter are not significantly different based on the 5% DMRT test.

Based on Table 1, it was known that the extract of pine leaf litter (*Pinus merkusii*) with different concentration gives significantly different result to the growth of corn seed. Percentage germination of seed without treatment (control), was not significantly different from the percentage germination of seed with 10% extract treatment. But, the control was significantly different from percentage germination of seed with extract 15%, 20% and 25%. The rate of seed germination with no treatment (control) was significantly different from all concentration treatments with ethanol extract of pine leaf litter, while the 20% was not significantly different with the 25%. The hypocotyl length of corn seeds with no treatment (control) was significantly different from all treatments of pine leaf litter concentration, whereas at the 10% concentration treatment did not significantly different with the 15%. The root length of corn seeds with no treatment (control) was significantly different from all treatments of pine leaf litter concentration, whereas at the 10% concentration treatment did not significantly different with the 15%. The dry sprouts weight with no treatment (control), was significantly different with concentration treatments 20% and 25% concentration treatments. Based on Figure 1, it can be seen that percentage of germination, rate of germination, hypocotyl length, root length and dry sprouts weight of corn seed become decrease caused by increasing concentration of ethanol extract of pine leaf litter.
Concentration of pine leaf litter extract affects the growth of corn seed. This is in accordance with research Cahyanti et al [7] that higher concentration of allelopathy gave the more inhibit the growth of seeds and the rate of germination. In addition, according to Suseelamma and Venkataraju [8] the inhibition of plant germination process depends on the concentration of litter, because of dissolved alelochemical compounds into the seeds. It can inhibit the induction of growth hormone such as gibberellin (GA) and indolacetic acid (IAA). According to Rice [9], inhibition of gibberellin synthesis there will be no enzyme α-amylase, as a result the process of hydrolysis starch into glucose in the

**Figure 1.** Effect of Pine Leaf Litter Extract Concentration for Corn Germination
endosperm or cotyledon is reduced and amount of glucose that can be sent to the growing points becomes less. The occurrence of a decrease in the length of hypocotyl and root of corn is possible due to the presence of alelochemical compounds in pine leaf litter extracts one of them tannin compounds. According to Marisa [10], tannin compounds can inhibit the growth of hypocotyl, eliminate a respiratory control in mitochondria and disturb the transport of \( \text{Ca}^{2+} \) and \( \text{PO}_4^{3-} \).

The results of this study are in accordance with several studies, namely research of Li et al [11], explained that root growth and dry weight of *Eremochloa ophiuroides* become inhibited along with addition leaf litter of *Eucalyptus grandis*; Padhy et al [12] explained that the higher concentration of leaf litter Eucalyptus extract will cause decreased of respiratory rate and enzyme activities such as catalase enzyme, \( \alpha \)-amylase enzyme and peroxidase enzyme. Furthermore, the rate of photosynthesis became decreased and inhibited chlorophyll synthesis in the leaves that will decrease in the growth of hypocotyl and root *Eleusine coracana*; Desai and Gaikwad [4] explained that the concentration of mangrove leaf litter (*Excoecaria agallocha* L.) of 75% and 100% will decrease significantly the germination, hypocotyl length and dry weight of 3 rice seed varieties if compared with concentration 5%, 10%, 25% and 50%.

Pine leaf litter ethanol extract can interfere water absorption and nutrients in a plant. The result is plasmolysis of plant cells, because plant cells are in hypertonic solutions, so the plant cells become wrinkled and the plastic membrane is pull away from the cell wall. This is in accordance with Sutopo [5], that the process of water imbibition by seed will be slower when the seed is placed in a solution, because of water diffusion. Higher concentration of a solution causes the pressure of water diffusion will be lower. As a result the germination process will inhibited. In conclusion, concentration of ethanol extract of pine leaf litter (*Pinus merkusii*) influences the growth of corn seed (*Zea mays* L.) Bisi-2 varieties. The higher concentration of pine leaf litter extract will further inhibit the process of seed germination of varieties of Bisi-2. The extract concentration of 25% yields the highest percentage of germination decline in all observation parameters.

4 Conclusion

Concentration of pine leaf litter ethanol extract (*Pinus merkusii*) influenced the growth of Bisi-2 varieties of corn (*Zea mays* L.) seeds. The higher concentration of pine leaf litter extract, will inhibit the germination process of Bisi-2 seeds. 25% extract concentration give the highest percentage of reduction in germination in all observed parameters.

References

[1] B. Kristanto, “PERUBAHAN KARAKTER TANAMAN JAGUNG (Zea mays L.) AKIBAT ALELOPATI DAN PERSAINGAN TEKI (Cyperus rotundus L.) [The Changing of Corn (Zea mays L.) Character Caused by Allelopathy and Competition with Purple Nutsedge (Cyperus rotundus L.)],” *J. Pengemb. Peternak. Trop.*, vol. 3, no. 31, pp. 189–194, 2006.

[2] Y. A. Senjaya and W. Surakusumah, “POTENSI EKSTRAK DAUN PINUS (Pinus merkusii Jungh. et de Vriese) SEBAGAI BIOHERBISIDA PENGHAMBAT PERKECAMBAHAN Echinochloa colonum L. DAN Amaranthus viridis,” *J. Perenn.*, vol. 4, no. 1, pp. 1–5, 2008, doi: 10.24259/perennial.v4i1.175.

[3] F. Q. Brearley, M. C. Press, and J. D. Scholes, “NUTRIENTS OBTAINED FROM LEAF LITTER CAN IMPROVE THE GROWTH OF DIPTEROCARP SEEDLINGS,” *New Phytol.*, vol. 160, pp. 101–110, 2003, doi: 10.1046/j.0028-646x.2003.00851.x.

[4] N. Desai and D. K. Gaikwad, “Allelopathic effects of leaf litter leachates of mangrove Excoecaria agallocha L. on rice seedlings,” *Allelopath. J.*, vol. 36, no. 2, pp. 293–302, 2015.

[5] L. Sutopo, *Teknologi Benih*. Jakarta: Raja Grafindo Persada, 2004.

[6] F. B. Salisbury and C. W. Ross, *Fisiologi Tumbuhan Jilid 3*. Bandung: ITB, 1995.

[7] L. D. Cahyanti, T. Sumarni, and E. Widaryanto, “POTENSI ALELOPAT DAUN PINUS (Pinus spp.) SEBAGAI BIOHERBISIDA PRA TUMBUH PADA GULMA KROKOT (Portulaca oleracea),” *Gontor AGROTECH Sci. J.*, vol. 1, no. 2, p. 21, 2015, doi: 10.21111/agrotech.v1i2.262.
[8] M. Suseelamma and V. R. R. R, “Effects of Digera muricata (L) Mart. Extracts on the germination and seedling growth of groundnut,” *Allelopath. J.*, vol. 1, pp. 53–57, 1994.

[9] E. L. Rice, *Biological control of weeds and plant diseases: advances in applied allelopathy*. Oklahoma: University of Oklahoma Press, 1995.

[10] H. Marisa, “Pengaruh Ekstrak Daun Pinus (Pinus merkusii) terhadap Perkecambahan Dan Pertumbuhan Vegetatif Tanaman Kedelai (Glycine max (L.) Merr.),” ITB, 1990.

[11] Y. Li, T. Hu, F. Zeng, H. Chen, and X. Wu, “Effects of Eucalyptus grandis Leaf Litter Decomposition on the Growth and Resistance Physiology Traits of Eremochloa ophiuroides,” *J. Plant Stud.*, vol. 2, no. 1, pp. 158–165, 2013, doi: 10.5539/jps.v2n1p158.

[12] B. Padhy, P. K. Patnaik, and A. K. Tripathy, “Allelopathic potential of Eucalyptus leaf litter leachates on germination and seedling growth of fingermillet,” *Allelopath. J.*, vol. 7, no. 1, pp. 69–78, 2000.