The Effect of Concentration of Tobacco (*Nicotiana tabacum*) Extract on Growth Parameters of Rice (*Oryza sativa*) Inpari-32

D Sumardi1*, M Bahariawan1, R R Maulani1, S Suhandono1, C Novia2, A F P Harahap2, M Gozan2

1Agriculture Engineering Study Program, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia, ORCID ID: 0000-0002-4433-0592

2Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI, Depok, 16424, Indonesia

Abstract. Rice plants (*Oryza sativa*) are a significant food crop. Tobacco plants (*Nicotiana tabacum*) can be beneficial and affect growth due to allelochemical content. The study aims to determine the effect of tobacco extract (*N. tabacum*) on the growth and yield of rice (*O. sativa*) Inpari-32 variety. The study method used the RBD as an experimental design. Three treatments with different concentrations of tobacco extract and one control with three replications. Growth variables were observed: growth rate, plant height, number of tillers, number of leaves, shoot and root dry weight, flowering age, and phytohormones. Observations of the harvest stage include the weight of stove r, number of panicle seeds, the total number of tillers, weight of 1000 seeds, the ratio of the weight of filled grain, and productivity (tons/ha). The results showed that although not significant, tobacco extract treatment increased the number of tillers and leaves, the content of phytohormone IAA in the vegetative phase, the content of GA3 in the generative phase, shoot-root ratio, number of tillers, and accelerated flowering age. The implication of tobacco extract positively affects the plant growth variable and accelerated flowering age of rice variety (*O. sativa*) Inpari 32.

1. Introduction
Rice (*Oryza sativa*) is the primary source of calories and protein for most Indonesian populations. As much as 35-80% of the total calories needed by humans can be met by consuming rice [1]. Then, the efforts to increase the productivity of rice plant is essentials. Plants, including rice plants, actually have phytohormones, which carry chemical messages that can affect cell activity and growth [2]. It is necessary to add exogenous hormones such as plant growth regulators (PGR) [3]. The chemical components in tobacco extract can act as stimulants or growth inhibitors depending on the concentration of the chemical components, where low concentrations can encourage plant growth. In contrast, high concentrations are used as pesticides [4]. It is known that a-amylase enzyme activity and chlorophyll levels are directly related to the function of the gibberellin acid hormone that affects plant growth [5].

Part of tobacco plants, especially leaves, have been used as traditional insecticides [6]. Studies have also shown that tobacco extracts can be used as and fungicides [7] [8]. The extract also shows effectiveness
for killing larvae [9] [10], coffee borer [11], and potato beetle [12]. Combination with other biopesticides is also showing effective results [13] [14]. The safety of using tobacco extract has been discussed [15]. This study aims to determine the effect of giving tobacco leaf extract in different concentrations on the growth and yield of rice varieties Inpari-32. The results of this study are expected to be used as information material regarding the use of tobacco extracts in rice cultivation.

2. Method
2.1. Materials
Rice (Oryza sativa) seeds of Inpari 32 variety were purchased from the Indonesian Center for Rice Research, Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture in Sukamandi, Subang, West Java. Tobacco extract was purchased from Zeus Kimiatama Indonesia, West Java, Indonesia. Commercial fertilizers such as Urea, KCl, and SP36 were purchased from the nearest agriculture shop.

2.2. Time and Location
The research was carried out from May 2019 to January 2020 in a screen house with coordinates 6°55'38.1" LS, 107°46'04.2" east longitude, Lab. Biomass Production System Engineering ITB Jatinangor Campus.

2.3. Field Experiment
There are three treatments with different concentrations of tobacco extract and one control with specifications K0: control, K1: tobacco extract concentration 1ml/l, K2: tobacco extract concentration 2ml/l, and K3: tobacco extract concentration 3ml/l [13] in three replications. One replication consisted of 15 polybags/treatment, and five polybags control so that a total of three replications amounted to 150 samples. Tobacco extract is dissolved according to the concentration of each treatment, then add 1-2 drops of liquid soap as an emulsifier solution—a solution of tobacco extract stored in a 1 L sprayer bottle. The solution was sprayed onto the surface of the leaves of rice plants evenly as much as 18 ml/clump weekly. A large plastic bulkhead is used to avoid exposure to tobacco extract on samples of other treatment plants during application. The process of rice cultivation lasts for 120 days. Rice cultivation was carried out using polybags measuring 40x40 cm. The study used the randomized block design (RBD). The planting medium used is the soil of the ITB Jatinangor campus with manure:soil ratio of 1:2. The seedling was carried out up to 14 (Days After Planting) DAP, as many as 3 seeds in a group. Watering was carried out uniformly with the volume of water given as much as 1259 ml/polybag at each watering. Watering is done every two days in the vegetative phase, and every day, it enters the generative phase.

2.4. Observation Variable
The growth variable includes growth rate, plant height, number of tillers, number of leaves, dry weight of shoot and root, flowering age, and phytohormones. Observation of the harvest yields includes weight of stover, number of panicle seeds, the total number of tillers, weight of 1000 seeds, the weight of filled grain, and productivity (tons/ha).

2.5. Data analysis
The data obtained from the observations were analyzed using One-Way ANOVA at the 5% level. The P-value < 0.05 means a significant difference between treatments, followed by the DMRT (Duncan Multiple Range Test) tests at the 5% level.
3. Results and discussion

During the Inpari-32 rice cultivation, the environment and soil analysis showed that the soil used had suitable criteria for rice cultivation. A preliminary study shows the analysis of soil content tests conducted by the Department of Soil Science of Universitas Padjadjaran [16]. The soil has an optimal temperature for rice growth, a high value of C-organic, low C/N ratio so that the soil becomes fertile. The macronutrient content of the soil is quite good because it has moderate N, high P, and very high K. The CEC value obtained on the soil is classified as very high.

3.1. Plant Growth Rate

Table 1 shows that the growth rate has good linearity (>90%). However, based on the Anova test at the 5% level, the table indicates that tobacco extract did not significantly affect the growth rate. K2 treatment tended to give a higher rate of plant height growth (5.17 cm/week) than other treatments. Meanwhile, treatments K1 and K2 showed the same number of tillers increasing, namely eight tillers/week. Treatment K1 showed the highest rate of addition of the number of leaves compared to other treatments.

| Treatment | Cm/week | R²  | Tillers/week | R²  | Leaves/week | R²  |
|-----------|---------|-----|--------------|-----|-------------|-----|
| K0        | 7.11 ±2.18 | 0.91 | 7.00 ±3.27   | 0.99 | 19.08±8.39 | 0.99 |
| K1        | 7.11 ±1.08 | 0.90 | 7.61 ±3.67   | 0.98 | 25.19±8.20 | 0.99 |
| K2        | 7.17 ±0.88 | 0.97 | 7.89 ±3.16   | 0.96 | 24.64±11.08 | 0.99 |
| K3        | 4.13 ±1.37 | 0.94 | 6.94 ±4.37   | 0.96 | 22.97±12.01 | 0.97 |

3.2. Plant height Number of Tillers and Leaves

Plant height is one of the parameters for plant growth and development results, which occurs due to cell division and elongation. Table 2 shows that the K0 treatment (control) gives a higher value than treatment K2, K3, and K1. Phenolic substances, which are substances contained in tobacco extracts, decrease the ability to carry out photosynthesis [17]. This condition can be related to the gibberellin hormone present in rice plants. Gibberellins play a role in synthesizing a-amylase enzymes that stimulate starch hydrolysis and increase cell elongation [18].

| Treatment | Plant Height [cm] | Number of Tillers | Number of Leaves |
|-----------|-------------------|-------------------|-----------------|
| K0        | 71.2              | 42                | 128             |
| K1        | 63.2              | 49                | 164             |
| K2        | 66.7              | 48                | 163             |
| K3        | 63.2              | 47                | 158             |

Table 2 shows the number of rice tillers decreased with increasing concentration. Treatment K1 tends to have the highest number of tillers, followed by K2 and K3. However, treatment K0 (control) showed the lowest number of tillers. The high number of tillers indicated that the activity of the a-amylase enzyme by gibberellins which was influenced by tobacco extract, was going well [19]. Besides that, it is also influenced by varieties, planting levels, and nutrients contained in the soil also affect the number of tillers formed [20]. The number of leaves of rice plants in Table 3 shows that K1 tended to have a higher number of leaves than other treatments. The number of leaves obtained is directly proportional
to the number of tillers. Each rice can form tillers in stages, where the tillers will form tillers again. This increases the chance of increasing the number of leaves [21]. Previous research stated that chemical components in tobacco extracts such as nicotine affect the number of leaves. Leaves are the main organ in the continuity of the photosynthesis process, where the higher the rate of photosynthesis, the higher the number of leaves formed [22].

3.3. IAA And GA3 Phytohormones Content

Auxin (IAA) plays a role in cell elongation, phloem, xylem differentiation, root formation, inhibiting leaf, flowering, and fruit drop. IAA levels will differ according to the place where auxin is synthesized [23]. However, the K3 treatment in Figure 1 tended to have a higher concentration of IAA in the vegetative phase. The most significant decrease in IAA content from the vegetative to a generative phase which was 19.75 ppm compared to other treatments. A decrease in IAA concentration signals the transition of growth to the reproductive phase [24].

![IAA and GA3 Concentration Charts](image)

**Figure 1.** a. IAA Concentration Chart; b. GA3 Concentration Chart.

Gibberellins (GA3) are growth hormones that stimulate cell elongation, germination, flowering, fruit ripening and cause plants to grow taller [19]. Figure 1a and Figure b show that the concentration of GA3 in all treatments increased when it reached the generative phase. K3 treatment tends to give the highest
concentration of GA3 in the generative phase, which is 19.36 ppm. At the same time, the K0 treatment had the slightest change in GA3 concentration, which only increased by 3.93 ppm but had a higher average GA3 concentration than other treatments. This result contradicts the statement [24] that the treatment of giving tobacco extract can trigger the synthesis of the GA3 hormone through the autonomic pathway. Because the observations show that it is not significant, it does not rule out that other factors can affect the GA3 content, such as genetic differences in plant species and external conditions, namely the microclimate [25].

3.4. Flowering Age
Observations of flowering age variables were recorded when the flowering population was more than 50%. Based on Table 3 showed that the control treatment had a longer flowering age than the K3 treatment.

| Treatment | Flowering Age [DAP] |
|-----------|---------------------|
| K0        | 82.33 ±0.58         |
| K1        | 78.56 ±2.12         |
| K2        | 78.89 ±2.69         |
| K3        | 76.67 ±2.89         |

Flowering age tends to be shorter when given the concentration of tobacco extract. However, because the statistical results did not show a significant difference between the concentrations of the different treatments, it was not possible to determine which concentration caused the faster flowering. Flower formation is also supported by gibberellin phytohormones which are stimulated by tobacco extract [19]. Flowering age showed an acceleration in line with the increase in the concentration of tobacco extract. In this study, the generative phase, the increase in the concentration of tobacco extract is in line with the rise in GA3 levels in rice leaves in Figure 1a.

3.5. Dry Weight and Shoot-Root Ratio
Based on Figure 2a and Figure 2b, treatment K0 (control) tends to show a lower shoot-root ratio than treatment with tobacco extract. Observation showed that the root ratio at the end of the vegetative period was higher than harvest time. The ambient temperature at the vegetative's end time was higher than the harvest time in terms of transpiration. The dry weight of a plant is the result of photosynthate accumulation. Tobacco extract given in the form of a solution, when accumulated, was able to reduce the dry weight value of the related plants [26]. In addition, environmental factors such as nutrients, water, carbon dioxide, and sunlight are essential during its formation and affect growth. Because the result showed that the variable shoot-root ratio was not significantly different, external factors could influence the variable weight of shoot roots compared to the effect of tobacco extract.
3.6. Percentage of Productive Tillers
A tiller is said to be productive when grains are formed on the panicle. Based on Figure 2, the ratio of productive tillers tended to be higher in the K2 treatment. The number of productive tillers in rice can be determined by the tillers that grow before the primordia phase [20]. Nutrients N and K have an essential role in producing panicles, where the photosynthesis process is stimulated to form productive tillers.

4. Conclusion
Based on the ANOVA test at 5% level with Duncan's 5% follow-up test, the effect of concentration of application of tobacco extract (Nicotiana tabacum) on rice (Oryza sativa) Inpari-32 variety did not have a significant effect on all test parameters. Although not significant, tobacco extract treatment increased the number of tillers and leaves, the content of phytohormone IAA in the vegetative phase, the content of GA3 in the generative phase, shoot-root ratio, number of tillers, and accelerated flowering age. Acceleration of flowering age was in line with the increase in the concentration of tobacco extract and was related to the activity of the hormone gibberellins. This study with a limited sample still needs to be expanded to get a transparent effect. On the other hand, molecular observations are also needed for the presence of phytohormones.

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References
1. Maslaita, Rauf A dan Purba E 2017 Respons Pertumbuhan Dan Produksi Beberapa Varietas Padi Gogo (Oriza Sativa L.) Dengan Ketebalan Tanah Mineral Pada Lahan Gambut Jurnal Pertanian Tropik, 4(1), 40-46.
2. Su, Y, Xia S, Wang R, Xiao L 2017 Phytohormonal quantification based on biological principles In ed J Li, C Li, S M Smith Hormone Metabolism and Signaling in Plants (Academic Press) pp. 431-470.
3. Paramita W S L 2018 Aplikasi Pupuk Organik dan Zat Pengatur Tumbuh dalam Peningkatan Produktivitas Tanah dan Tanaman (Universitas Jember)

4. Mahmood K A, Z Khaliq, A Cheema and M Arshad 2013 Allelopathic activity of Pakistani wheat genotype against wild oat (Avena fatua L.) Pak. J. Agri. Sci., Vol. 50(2), 169-176,

5. Tetuka K A, S Parman and M Izzati 2015. Pengaruh Kombinasi Hormon Tumbuh Giberelin dan Auxin terhadap Perkecambahan Biji dan Pertumbuhan Tanaman Karet (Hevea brasiliensis Mull. Arg.) Jurnal Akademika Biologi, vol. 4, no. 1, pp. 61-72, Feb. [Online].

6. Harahap A F P, A Fauzantoro and M Gozan 2022 Bio-oil from the tobacco plant Chapter 21 in eds S Abd-Aziz, M Gozan, M F Ibrahim, L Y Phang, Biorefinery of Oil Producing Plants for Value-Added Products, (Weinheim, Germany: Wiley-VCH)

7. Fauzantoro A, Y Muhamad and M Gozan 2017 Improvement of Nicotine Yield by Ethanolic Heat Re reflux Extraction of Nicotiana tabacum var. Virginia Origin of Ponorogo Int. J. Applied Eng. Research 12 13891-13897

8. Gozan M, Y Yasman, P Wulan and E Dawitri 2014 Tobacco Leaves Pyrolysis for Repellent Active Compound Production Int. J. Applied Eng. Research 9 9739-9749

9. Andjani H N, Y Sentosa, K Yati, M Jufri, A Fauzantoro, A and M Gozan 2019 Determination of LC50 value of Nicotiana tabacum L. extract against Gryllus bimaculatus imago and Galleria mellonella larvae in AIP Conference Proceedings, 2193 (1): 030024,

10. Sentosa Y, H N Andjani, K Yati, M Jufri, Haryuni and M Gozan 2019 Determination of LC50 Value of Nicotiana tabacum L. Extract Against Tenebrio molitor and Zophobas morio Larvae in AIP Conference Proceedings, 2193 (1): 030021,

11. Harahap A F P, A Fauzantoro, H Haryuni, T S K Dewi, E Suprapti, M Y A Ramadhan, Y J Yo and M Gozan 2020 Field efficacy of Nicotiana tabacum L. var Virginia extract against coffee borer beetle (Hypothenemus hampei) attacking coffee berries in plantation area Int. J. Agronomy, 2020: 8898063

12. Potera C 2011 Innovative Technologies: Tobacco Bio-Oil Kills Agricultural Pests Environ Health Perspect, 119(1): A18-A18

13. Haryuni, T S K Dewi, E Suprapti, S F Rahman and M Gozan 2019 The Effect of Beauveria bassiana on the Effectiveness of Nicotiana tabacum Extract as Biopesticide Against Hypothenemus hampei to Robusta Coffee J. J. Technol. 10(1): 159-166

14. Haryuni H, A F P Harahap, A Priyatmojo and M Gozan 2020 The Effects of Biopesticide and Fusarium oxysporum f. sp. vanillae on the Nutrient Content of Binucleate Rhizoctonia-Induced Vanilla Plant International Journal of Agromony, 2020: 8898063,

15. Andjani H, Y Sentosa, K Yati, A Fauzantoro, M Gozan and Y Yoo 2019 Acute Oral Toxicity Test of Nicotiana tabacum L. Bio-Oil Against Female Winstar Rats in IOP Conference Series: Earth and Environmental Science, 353(1): 012047

16. Ruswandi A 2018 Sifat Fisik dan Kimia Biologi Tanah” (Bandung: Institut Teknologi Bandung [in Bahasa])

17. Yang C M, C N Lee and C H Chou 2002 Effects of three allelopathic phenolics on chlorophyll accumulation of rice (Oryza sativa) seedlings: I. Inhibition of supply-orientation Bot.Bull Acad. Sin, 43: 299-304

18. Utama R C and Sugiyatna 2016 Pengaruh Aplikasi Gibberelin Pada Padi Sawah (Oryza Sativa L.)Varietas Hibrida (Hipa Jatim 2) dan Varietas Unggul Baru (Ciherang) Bul Agrohorti, 4(1): 56-62.

19. Farooq M, T Hussain, A Wakeel, and Z Cheema 2014 Differential response of maize and mungbean to tobacco allelopathy Expl Agriculture 50 :611-624. 2014

20. Wang Y, T Ren, J W Lu, R Ming, P F Li, H Saddam, R H Cong and X K Li 2016 Heterogeneity in rice tillers yield associated with tillers formation and nitrogen fertilizer Agronomy Journal 108: 1717–1725
21. Wangiyana, Z Laiwan and Sanisah 2009 Pertumbuhan dan Hasil Tanaman Padi var. Ciherang dengan Teknik Budidaya “SRI (System of Rice Intensification) pada Berbagai Umur dan Jumlah Bibit per Lubang Tanam Crop Agro, 2(1): 70-78

22. Kirschbaum M U F 2011 Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO$_2$ Enrichment Studies$^{[16]}$ Plant Physiology, 155: 117-124.

23. Hidayatti, 2009 Kadar hormon auksin pada tanaman kenaf (hibiscus cannabinus l.) Bercabang dan tidak bercabang Agrovigor, 2(2): 89-96, [in Bahasa].

24. McKenzie 2018 The Effect of Plant Hormones [Online]. Available: https://www.topcropmanager.com/the-effects-of-plant-hormones-21192. [Downloaded May 26th 2020].

25. Chailakhyan M Kh 2003 Hormonal Regulation of Plant Development in the Studies In ed. I Machácková and G A Romanov Phytohormones in Plant Biotechnology and Agriculture (Proceedings of the NATO-Russia Workshop held in Moscow, 12–16 May 2002) pp :3-7.

26. Hussain I, M S Baloch, E A Khan, and A A Khan 2019 Morphological and Physiological Response Of Maize To Some Allelopathic Plant Extracts Pak. J. Weed Scie. Res. 25(2):137-145