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Original article

ANALYSIS OF PHYTOHORMONE CONTENT IN LOCAL MICROORGANISMS AND THEIR RESPONSE TO TRUE SHALLOT SEED (TSS) PRODUCTION OF LOCAL EBAN SHALLOTS IN THE MIDDLE PLAINS OF NORTH CENTRAL TIMOR REGENCY

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Abstract

The technology of shallot seed production through botanical seeds (true shallot seed) is one of the alternatives that need to be developed to overcome the shortage of seeds in North Central Timor Regency (TTU). This study aims to determine the response of local microorganisms to the production of botanical seeds of local shallot cultivar Eban in the middle plains of North Central Timor Regency, as well as to determine the phytohormone content in local microorganisms of banana corms and bamboo shoots. The research design used was a factorial randomized block design. The first factor is the type of local microorganisms consisting of two factors, namely banana corm moles (M1) and bamboo shoot moles (M2). The second factor is the concentration of moles consisting of 4 levels, namely the provision of 1 liter mole: 6 liters of water (K1), the provision of 1 liter mole: 5 liters of water (K2), the provision of 1 liter mole: 4 liters of water (K3), the provision of 1 liter mole: 3 liters of water (K4) and control (without the provision of moles). The results of the study showed that the treatment of mole type and mole concentration had an interactive effect on the variables of plant height, number of leaves and tuber diameter, but the phytohormone content in local microorganisms was unable to produce flowers and seeds in the local Eban cultivar planted in the middle plains of North Central Timor Regency.

Keywords: Local Microorganisms; Phytohormone; True shallot seed

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Introduction

The production of shallots (Allium ascalonicum, L) in North Central Timor Regency (TTU) in 2023 was 4.1 tons [4]. Low production resulted in the community's needs not being met, so that many shallots were imported from neighboring regencies such as Kupang and Rote Ndao Regencies. The obstacle in shallot cultivation is that farmers still use seed bulbs, resulting in many bulbs being attacked by disease. An alternative to replacing seed bulbs is to use botanical seeds (true shallot seeds) because they have several advantages, namely preventing virus attacks from vegetative tissue, the cost of purchasing seeds is cheaper than seed bulbs, and producing healthier plants [25]. The production of true shallot seeds (TSS) for shallots has not been carried out optimally due to the lack of induction of shallot flowering, due to unfavorable environmental conditions. Genetically, local Eban shallots can produce flowers and seeds, but the development of true shallot seeds (TSS) has not been carried out at all. According to [10], tropical areas such as Indonesia are very suitable for producing TSS.

The Eban area is a center for shallot planting in North Central Timor Regency and is a plateau with an altitude of ± 1000 m above sea level. The development of shallots in the highlands is constrained by high rainfall every year, resulting in many bulbs rotting due to fungal attacks. One alternative to increase production is planting in the middle plains or lowlands. The soil conditions in the middle plains in TTU Regency are dry land so that shallot cultivation is still not optimal. Limited water, low nutrient content and poor soil structure are the main problems that can inhibit the growth and development of shallot plants in dry land [18]. The development of environmentally friendly agriculture by utilizing local microorganisms in an area is one strategy to overcome problems in dry land.

Local microorganisms have potential that can be utilized because they contain phytohormones that can stimulate flowering. According to [14] local microorganisms are a solution of fermented natural materials from various local resources in an area that are used as organic fertilizers to increase soil fertility. Local raw materials that are widely used are bamboo shoots and banana stems, because they contain macro and micronutrients and microorganisms that have the potential to be organic material decomposers such as growth stimulants and as plant pest and disease controllers so that they are good for use as biofertilizer decomposers and organic pesticides.

According to [23] local microorganisms contain growth regulators such as auxin and gibberellin. In addition, bamboo shoot and banana stem molasses have high organic C, Fe, ZN and Cu content so that they can stimulate the growth of shallot bulbs. According to [3], shallot growing media treated with banana stem extract and bamboo shoot extract can increase the wet weight of shallot bulbs by 226.4 and 249.7 grams. The results of the study by [19], the provision of 20 ml/L local microorganisms' bamboo shoot and the addition of 90 g of goat manure increased the number of bulbs by 3.19 and the dry weight of shallot bulbs by 2.65 g. The phytohormone content in each type of mol has a different composition, so it is necessary to analyze the phytohormone content to determine with certainty according to plant needs. The study aims to determine the response of local microorganisms to vegetative, generative growth and TSS production and to determine the phytohormone content in local microorganisms of banana stumps and bamboo shoots.

Materials and methods

The study was conducted at the Experimental Garden of the Faculty of Agriculture, Science and Health, Timor University from June to October 2024, at an altitude of ±500 meters above sea level. Analysis of phytohormone content in banana stump moles and bamboo shoot moles was carried out by PT Starlab Analytic, Bogor Indonesia. Sections can be divided into subsections in a sensible way so that the text will not be fragmented into many small paragraphs having a few lines. The example below illustrates two levels of subsections.

Materials

The tools used in this study were a thermohydrometer, germinator, caliper, soil meter, oven and analytical scales. The materials used are bamboo shoots, banana stems, brown sugar, red onion seeds and plastic mulch.

Experimental design

The experiment used a factorial randomized block design (RBD). The first factor is the type of local microorganisms (M) consisting of 2 treatments, namely local microorganisms' bamboo shoot (M1) and local microorganism's banana stump mole (M2). The second factor is the concentration of local microorganisms (K) consisting of 4 treatments, namely the provision of local microorganisms concentration of 1 liter mole: 6 liters of water (K1), the provision of local microorganisms concentration of 1 liter local microorganisms: 5 liters of water (K2), the provision of local microorganisms concentration of 1 liter local microorganisms: 4 liters of water (K3) and the provision of local microorganisms concentration of 1 local microorganisms: 3 liters of water (K4), as a comparison, control plants were planted (without mole). All treatments were repeated 3 times so that there were 30 experimental units in total.

Making local microorganisms

The making of local microorganisms is carried out with the following stages: bamboo shoots and banana stumps, each as much as 5 kg, are chopped separately and then ground with a mortar (mashing tool). 2 kg of brown sugar is dissolved using 20 liters of rice washing water, then divided into 2 parts for banana stem mol and bamboo shoots. The mashed banana stem and bamboo shoots are soaked in the solution separately, using a plastic drum, then tightly closed and given air holes by inserting a water pass hose connected to a bottle filled with water. The solution is left anaerobically for 3 weeks or 21 days. The cooked solution is filtered and put into a storage container, then diluted according to the treatment.

Phytohormone analysis method

Analysis of phytohormone content using the Linsken and Jackson method [5]. A 5 ml liquid sample of local microorganisms is extracted using 20 ml of 65% MeOH solvent, centrifuged at 4000 rpm for 30 minutes or filtered using Whatman 42 paper. The centrifuged supernatant is filtered using a milliphore, then injected 5-10 µl into HPLC. Flow rate 0.5 ml/min, column temperature 40^oC, stationary phase (Column) C18 and detector 254 UV.

Seed preparation

The tuber seeds used have several criteria, namely weighing >5g/tuber, free from pests and diseases, not rotting and the tubers are hard. Before planting, the seeds are wrapped in ice plastic and vernalized using a refrigerator for 1 week at a temperature of 10^oC. After the tuber seeds are prepared, a mole application is carried out by soaking for 20 minutes according to the treatment. Planting is carried out on beds with a planting distance of 20 cm x 20 cm.

Observation variables

The variables observed consist of 4 components, namely analysis of phytohormone content, namely Indole Acetic Acid (IAA), gibberellin, K2O-total, P2O5-total and N-total, Fe-Total, Mn-Total, Zn-Total and Cu-total. Vegetative growth variables are plant height, number of leaves, tuber diameter. Environmental variables are temperature, flowering variables or generative growth are the percentage of flowering plants (%), number of umbels per plant, number of full capsules per umbel, number of seeds per umbel. The components of seed quality are maximum growth potential (%), germination power (%), vigor index (%) and normal dry weight of seedlings (g).

Results and discussion

Phytohormone content analysis

The results of the analysis show that banana stump moles and bamboo shoot moles contain macro and micronutrients as well as auxin (Indole Acetic Acid) and gibberellin hormones (Table 1). According to [1], banana stumps contain microbes that decompose organic matter such as Bacillus sp, Aeromonas sp, and Aspergillus Niger, so they can be used as a source of microorganisms in the manufacture of local microorganisms. Furthermore, according to [8], the bacteria found in bamboo shoots are Lactobacillus, Streptococcus, Azotobacter and Ascosporous which play a role in accelerating decomposition to produce the best quality fertilizer. Bamboo shoots contain high levels of organic carbon and gibberellin which can stimulate and spur plant growth [29].

Table 1.

Results of phytohormone content analysis in local microorganisms of banana stems and bamboo shoots

Local microorganisms	IAA	Gibber-ellin	K2O	P2O5	N	Fe	Mn	Zn	Cu
Banana Stump	4.359 mg/Kg	2.159 mg/Kg	2.50%	0.02%	0.12%	36.02 mg/L	9.40 mg/L	77.95 mg/L	345.14 mg/L
Bamboo Shoots	10.863 mg/Kg	7.824 mg/Kg	1.20%	0.05%	0.27%	222.72 mg/L	7.21 mg/L	60.11 mg/L	161.14 mg/L

Vegetative growth variable

The results of the analysis of variance (anova) showed that there was an interaction between the types of local microorganisms and the concentration of local microorganisms on the height of shallot plants. The treatment of banana stump mole with a concentration of 1-liter local microorganisms: 3 liters of water produced the highest plant height of 44.54 cm, significantly different from other treatments. The plant height obtained ranged from 38.75-44.54 cm (Table 2).

The concentration of 1-liter local microorganisms with 3 liters of water in banana stump mole shows that the higher the concentration of local microorganisms in a solution, the more effectively it will be absorbed by plants for their growth activities. According to [15] the types of essential micronutrients needed by plants for plant growth on critical soils such as dry land are Mn, Cu and Zn which play a role in plant metabolism.

Table 2.

Interaction of local microorganisms and concentration of local microorganisms on the height of shallot plants (cm)

Local Microorganisms		Concentration of local microorganisms K1 (1L/6L water)	K2 (1L/5L water)	K3 (1L/4L water)	K4 (1L/3L water)
M1 (Banana Stump)		39.56ab	40.84ab	41.26b	44.54a
M2 (Bamboo Shoots)		38.57b	42.37ab	41.27ab	43.17ab
Control	31.99 ab				

Description: numbers followed by the same letter indicate no significant difference according to the DMRT 5% test.

The effect of interaction between the type of local microorganisms and the concentration of local microorganisms gave the highest number of leaves in the local microorganisms' bamboo shoot treatment at a concentration of 1-liter local microorganisms: 4 liters of water, namely 35.60 strands, significantly different from other treatments (Table 3). A high number of leaves will have an impact on photosynthesis activity. Organic matter contained in local microorganisms can improve soil structure so that it can increase absorption [20]. The high N-Total content in bamboo shoots, namely 0.27%, indicates that the N element applied to seeds treated with bamboo shoot local microorganisms at a concentration of 1-liter local microorganisms: 4 liters of water shows that its availability is optimal so that it can be absorbed by plants for leaf growth. The number of leaves is greatly influenced by N content because it is one of the main components of chlorophyll [9].

The treatment of local microorganisms' type and local microorganisms' concentration had an interactive effect on tuber diameter. The highest tuber diameter was found in the banana stump mole treatment with a concentration of 1-liter local microorganisms: 3 liters of water, which was 2.43 mm, not significantly different from the bamboo shoot mole treatment at a concentration of 1 liter mole: 6 liters of water (Table 4). The high tuber diameter at a banana stump local microorganisms concentration of 1: 3 was because the amount of IAA and gibberellin hormones was less so that the dilution was lower, while the bamboo shoot mole concentration of 1: 6 showed that the availability of growth hormones such as Indole Acetic Acid (IAA) and gibberellin in bamboo shoots was greater, so a higher dilution was required. The mechanism of action of auxin or IAA is to influence the flexibility of the cell wall so that water can enter by osmosis and stimulate cell elongation [16]. Furthermore, according to [6], gibberellin plays a role in cell growth and division so that it can increase tuber diameter.

Table 3.

Interaction of local microorganisms and concentration of local microorganisms on the number of red onion leaves (strands)

Lokal Microorganisms	Concentration of local microorganisms			
	K1 (1L/6L water)	K2 (1L/5L water)	K3 (1L/4L water	K4 (1L/3L water)
M1(Banana Stump)	28.87ab	29.80ab	26.67b	31.00ab
M2(Bamboo Shoots)	27.40ab	27.67ab	35.60a	31.00ab
Control	29.93ab			

Description: numbers followed by the same letter indicate no significant difference according to the DMRT 5% test.

Table 4.

Interaction of local microorganisms and local microorganism concentration on tuber diameter (mm)

Lokal Microorganisms	Concentration of local microorganisms			
	K1 (1L/6L water)	K2 (1L/5L water)	K3 (1L/4L water)	K4 (1L/3L water)
M1(Banana Stump)	2.20abc	20.00abc	2.03abc	2.43a
M2(Bamboo Shoots)	2.43a	1.80bc	2.00abc	1.97bc
Control	2.30ab			

Description: numbers followed by the same letter indicate no significant difference according to the DMRT 5% test.

The mole treatment was significantly able to provide an interactive effect on vegetative growth, namely plant height, number of leaves and tuber diameter. The phytohormone content in bamboo shoot moles and banana tubers was able to increase vegetative growth, although at a low concentration of 1:3 in bamboo shoot local microorganisms, it was able to increase plant height by 44.54 cm, the number of leaves in banana tuber moles with a concentration of 1:4, tuber diameter at a concentration of 1:6 and in banana tuber local microorganisms and 1:3 in bamboo shoot local microorganisms. The mole level with a low concentration significantly affected the vegetative growth of plants. This is suspected when the study took place, the condition of the land on the east side was covered by vegetation from several trees that grew so that the environmental temperature was lower, compared to the western land that was exposed to sunlight. Exposure to sunlight causes the temperature to increase so that evaporation occurs faster and the microbes in the local microorganisms do not grow well. The results of [17] showed that shallot plants treated with banana stem molasses at the highest concentration, namely 40 ml, did not have a significant effect on vegetative growth.

Flowering or generative growth variables

Table 5.

Average temperature during shallot plant growth (°C)

Month	Average temperature
July	24.5
August	27.3
September	33.2

The results of the analysis showed that generative growth or flowering did not differ significantly in all observation variables. However, of all the shallot plants treated with banana stem mole and bamboo shoots, there were plants that flowered, namely in the bamboo shoot local microorganisms treatment with a concentration of 1 liter mole: 6 liters of water and with the highest percentage of 0.95%, the highest number of umbels per plant 1.00, the highest number of pithy capsules per plant 6.0, and the highest number of seeds per umbel 8.3 (Table 6).

According to [26], the average air temperature for shallot flower initiation is 15.8°C to 16.2°C, while during this study, the air temperature in June was 24.5°C, July 27.3°C and August 33.2°C (Table 5). According to [21], shallots require cold temperatures for flowering. Environmental factors such as duration of exposure, temperature and humidity significantly affect the flowering phases of shallots [22]. [7] stated that Bima variety shallots planted in the highlands and lowlands were able to produce flowers, but the percentage of flowers in the lowlands was lower than in the highlands. Shallot flowering, in addition to being influenced by genetic factors, is also influenced by temperature and humidity. The results of a study by [24], the Biru Lancor variety shallots planted at an altitude of ±89 meters above sea level did not produce flowers at all.

Table 6.

Flowering or generative growth data

Observation variables	Treatment	
	M2K1	M2K2
Percentage of flowering plants	0.95 a	0.95 a
Number of umbels per planting	0.33 a	1.00 a
Number of fleshy capsules per planting	6.0 a	1.00 a
Number of seeds per umbel	8.3 a	1.33 a

Note: numbers followed by the same letter indicate no significant difference according to the DMRT test 5%. M2: Bamboo shoot local microorganisms, K1: concentration local microorganisms 1: 6, K2: consentration local microorganisms 1: 5

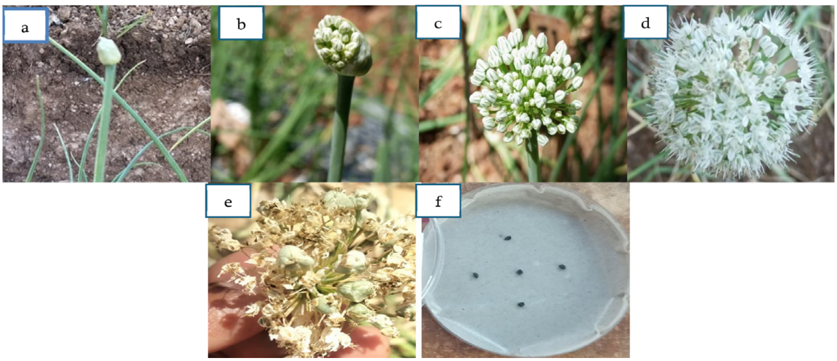

Fig. 1. The process of formation of flowers and shallot seeds

The formation of shallot flowers begins with the formation of umbels (a), the umbels are covered by a thin membrane that will break along with the development of the umbels (b and c), the flower umbels then bloom (d) and if pollination is successful, a capsule will be formed containing seeds (e) and the seeds formed (f) (Figure 1). The formation of flower umbels occurs 38 days after planting (DAP).

The local Eban cultivar shallot is thought to be unable to adapt to the environment in the midlands so that its genetic ability to produce flowers naturally does not occur. According to [30], environmental factors such as temperature are considered the most important environmental factors affecting plant phenology and some plants can only grow and develop in certain temperature environments and require certain cumulative temperatures to complete their life cycle. Furthermore, according to [13], high temperatures result in decreased flowering and seed production in the allium genus. Environmental factors are important factors that affect plant growth and development, as well as being the basis for physiological and biochemical changes.

The gibberellin content found in banana stem moles and bamboo shoot has not been able to produce TSS. [27] stated that gibberellin with low concentrations is unable to induce shallot flowering. The gibberellin content in banana stem 2,159 mg/Kg and bamboo shoots 7,824 mg/Kg (Table 5) is very low so that flowering initiation does not occur. According to [11], the higher the concentration of gibberellin, the higher the flowering percentage. The results of [28] study, the Biru Lancor variety of red onion treated with 200 ppm gibberellin and planted in the lowlands was unable to produce flowers. The number of seeds formed indicates that pollination has occurred in the red onion flowers.

Seed Quality

The seeds formed were analyzed or tested in the Laboratory. The variables of maximum growth potential, germination power, vigor index and normal dry weight of seedlings showed no significant differences (Table 7). The TSS seeds produced showed normal germination (Figure 2), after being planted using the test method on paper.

Fig. 2. Germinated shallot trus shallot seed (TSS) seeds.

Table 7.

Testing the quality of true shallot seeds

Observation Variables	Treatment	
	M2K1	M2K2
Maximum growth potential (%)	33.33 a	22.22 a
Germination Power (%)	11.00 a	22.00 a
Vigor Index (%)	5.33 a	22.00 a
Dry weight of normal sprouts (gram)	0.0028 a	0.0021 a

Note: numbers followed by the same letter indicate no significant difference according to the DMRT test 5%. M2: local bamboo shoot microorganisms, K 1: 1: 6, K2 1: 5

The quality of a seed depends on the vigor and viability of the seed. The benchmark for vigor is the vigor index while the benchmark for seed viability is the maximum growth potential, germination power and normal dry weight of the sprout. Seeds that have high vigor and viability are indicated by their ability to germinate normally above 80% [2]. Based on these criteria, the seeds tested cannot be said to have good vigor and viability because the seed testing has not met the International Seed Testing Association (ISTA) standards, because the seed production is very small. According to the [12], the number of seeds used for testing each replication is 25 seeds, while in this test the number is only 5 seeds per replication.

Conclusions

Banana stumps and bamboo shoots can be used as local microorganisms because they contain phytohormones such as IAA, gibberellin, K_2O, P_2O_5, N, Fe, Mn, Zn and Cu. The provision of local microorganisms of banana stumps and bamboo shoots has an interactive effect on the vegetative growth of the local shallot cultivar Eban, namely on the variables of plant height, number of leaves and bulb diameter. Generative growth in shallots treated with local microorganisms did not have a significant effect, but from the treatments tested, there was a treatment that could produce flowers, namely bamboo shoot microorganisms with concentrations of 6:1 (M2K1) and 5:1 (M2K2). The production of true shallot seeds did not show significant results, but the seeds tested showed normal growth.

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AUTHOR CONTRIBUTIONS

Anna Tefa: initiated the research idea on the local Eban shallot that can produce flowers and seeds, design the research method, and draft the initial draft of the manuscript.

Nikolas Nik: helped manage plant pests and diseases and conducted critical review, revision, and improvement of the manuscript to ensure clarity and good structure.

Aloysius Rusae: contributed to the development of local microorganisms that can be applied to stimulate the formation of flowers and seeds in the local Eban shallot.

Alberikus Arsenius Neonnub: collected data in the field and in the laboratory.

Iwanry Savio Manek: managed and analyzed the data using software.

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