



# Effects Of IAA-Containing Bacterial Application and Dolomite Dosage Reduction on the Growth of Emprit Ginger (*Zingiber officinale* Var. *Amarum*) on Ultisol Soils

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**Abstract.** Java ginger, locally known as emprit ginger, is an herb in the rhizome category with many medicinal uses. Indonesia is one of the largest ginger producing countries in the world, however its production in 2019-2022 tends to be unstable. Marginal soil, such as ultisol, can be utilized to increase the Java ginger production. Dolomite and IAA-producing bacteria are some soil ameliorants that can be applied to improve the soil quality. This study aimed to determine the effects of IAA-producing bacterial application and dolomite dosage reduction as well as identify the bacterial isolates and the optimal dose of dolomite in white ginger cultivation on ultisol. The study employed a completely randomized two-factorial design. The observed parameters include plant height (cm), leaf area (cm<sup>2</sup>), total leaf area (cm<sup>2</sup>), number of leaves, number of buds, and leaf greenness. Results show that S3 bacterial isolate had significant effects on plant height, leaf area, number of leaves, and leaf greenness. The dolomite dose of 100% showed the best effects on plant height, number of leaves, and number of buds. The interactions between IAA-producing bacteria and dolomite dosage affected plant height, number of leaves, and number of buds.

**Keywords:** emprit, java ginger, bakteria, IAA, dolomite, ultisol.

## 1. Introduction

Ginger is a significant commodity in Indonesia as it is one of the largest ginger producing and exporting country in 2010-2020 (FAO, 2020). Ginger is also a popular spice as well as medicinal herbs with known uses for anti-inflammatory, antipyretic, gastroprotective, cardiotoxic, antihepatotoxic, antioxidant, anticancer, antiangiogenesis,

and antiatherosclerotic. Those benefits generally are the results of the bioactive compounds contained in ginger, such as phenols, flavonoids, and terpenoids (Kusnadi & Tivani, 2017). Ginger is the most common ingredient in traditional medicine in Indonesia (Yuliana *et al.*, 2015). BPS (Central Statistics Bureau, 2022) reported that ginger production in the country tended to fluctuate in 2019 – 2022, recorded as 174 thousand tons (2019), 183 thousand tons (2020), 307 thousand tons (2021), and 247 thousand tons (2022). The challenges in pharmacological products containing ginger include decreased and unstable productions. Low productivity is also a significant problem in ginger cultivation (Septiana, 2021). The productivity of a commodity can be measured by the ratio of production and land area used in the cultivation (Kanaya & Firdaus, 2014).

There are three main kinds of ginger in Indonesia, which are gajah ginger (white ginger/*Zingiber officinale* Rosc. var. *Officinale*), emprit ginger (Java ginger/*Zingiber officinale* Rosc. var. *Amarum*), and merah ginger (red ginger/*Zingiber officinale* Rosc. var. *Rubrum*) (Iskandar *et al.*, 2016). The hot and bitter tastes of ginger come from the oleoresin content, and ginger essential oil gives off a distinct aroma.

Expansion of ginger cultivation area can be a method to increase production. Marginal land has the potential for this purposes, and ultisol soil is an example of that. There is 45.794.000 ha of ultisol in Indonesia, which translates to 25 % of the landmass in the country (Syahputra *et al.*, 2015). One of the locations of this soil type is in Banyumas regency, Central Jawa, spread throughout 12 sub-districts (Rokhminarsi *et al.*, 2012).

The characteristics of ultisol include unstable aggregates, as well as low permeability, pH (4.2-4.8), and organic matter. Additionally, ultisol has the clay texture, and contains the secondary mineral kaolinite with a small mixture of gibbsite and montmorillonite (Sujana & Pura, 2015). To improve ultisol quality, applications of fertilizers as organic sources would not be sufficient because they would not be effective in soil with pH lower than 4.5. Applications of soil ameliorants to improve the efficacy of organic addition can be one of the strategies to overcome this challenge as well as sustainably increase productions.

Advancements in biotechnology and increased awareness of environmental health have inspired the utilization of natural products such as microorganisms that produce secondary metabolites that promote plant growth. Indole acetic acid (IAA) is one such metabolites that promotes plant growth and produced by bacteria (Istiqomah *et al.*, 2017). Employing IAA-producing bacteria as soil ameliorants therefore has the potential to improve ginger cultivation in ultisol. It has been shown that bacteria with higher IAA production were able to increase the root length of wheat (Mohite, 2013).

Previous study demonstrated that bacterial isolates from the GI tract of black soldier fly (BSF) larvae had the capacity to produce IAA (Leana *et al.*, 2021). The bacterial isolates designated as S3, N15, and N19 were able to produce IAA at 77.06 mg/L, 13.71 mg/L, and 10.43 mg/L, respectively. These isolates have become a part of collection of the Agronomy and Horticulture Laboratorium, Agriculture Faculty, Jenderal Soedirman University.

The biggest challenge in the agricultural utilization of ultisol is the low content of phosphorus (P), which necessitates the use of soil ameliorants. In such acidic soil, P is found to be tightly bound to Aluminium (Al) and Iron (Fe) ions (cation, oxide, and hydroxide) and trapped in clay (Prasetyo *et al.*, 2022). To free phosphorus, dolomite is often used to increase pH and add Calcium (Ca) and Magnesium (Mg), which stimulate microbial growth and activities in soil decomposition (Yuniar *et al.*, 2021). The objectives of this study were therefore determining the optimal bacterial isolate, dolomite dosage, and combination of the two variables in improving the production of emprit ginger on ultisol soil.

## **2. Methodology**

### **2.1 Location**

The study was conducted in the Laboratorium (Screen House) of Agriculture Faculty, Jenderal Soedirman University, Karangwangkal, Purwokerto. The site is located at the elevation of 110 Meter above sea level (MASL) with the daily temperature average of 23 °C – 32°C. Bacterial isolates were grown in and obtained from the Agronomy and Horticulture Laboratorium, Agriculture Faculty, Jenderal Soedirman University.

### **2.2 Materials**

Rhizomes of emprit ginger were grown in ultisol soil with addition of dolomite, composted manure, supplemented with Urea, TSP, and KCl fertilizers. Polybags were used to contain the plants and soil. Bacterial isolates N15, N19, and S3 were grown in media containing yeast, peptone, glucose, K<sub>2</sub>HPO<sub>4</sub>, MGSO<sub>4</sub>, glycerol, beef extract, dextrose, and 70% alcohol. Soil, light, and weather conditions were monitored using pH meter, lux meter, and thermohygrometer.

## 2.3 Study design

The study was as a randomized block design with two factorials, which were dolomite dosage and isolates of IAA producing bacteria. Dolomite dosage consisted of D0: no dolomite, D1: 50% dolomite (21.32 gram polybag<sup>-1</sup>), and D2: 100% dolomite (42.64 gram polybag<sup>-1</sup>). The applications of isolates were divided into I0: no isolate, I1: N15 isolate, I2: N19 isolate, and I3: S3 isolate. Bacterial isolates were applied every three weeks at the density of 10<sup>8</sup> cfu ml<sup>-1</sup>.

## 2.4 Analysis Data

The variables measured in this study comprised of plant height (cm), number of leaves (unit), number of buds, leaf greenness (unit), leaf area (cm<sup>2</sup>), and total leaf area (cm<sup>2</sup>). Observation was conducted until the plants reached the age of five months. Data were analyzed with Analysis of Variance (ANOVA) at  $\alpha$ -level = 5% to determine the treatment effects. Further analyses, employing Duncan Multiple Range Test (DMRT) for variables that showed significant difference in response to the treatments (F statistics > F table 5 %) at  $\alpha$ -level = 5%.

## 3. Results

The results of ANOVA are presented in Table 1 that lead to further analyses on treatments that produced significantly different results using DMRT, with results presented in the subsequent tables. Table 1. ANOVA results on the effects of treatments on emprit ginger cultivation using the variables of dolomite dosage and isolates of IAA-producing bacteria.

**Table 1.** ANOVA results

No	Observed variable	Treatment		
		Dolomite (D)	Bacterial isolate (I)	Interaction (D x I)
1.	Plant height	o	o	o
2.	Number of leaves	o	o	o
3.	Number of buds	o	u	o
4.	Leaf area	u	o	u
5.	Total leaf area	o	o	o
6.	Leaf greenness	u	o	u

Note: o = observable ( $P < 0.05$ ) and u = unobservable ( $P > 0.05$ )

Dolomite dosage was shown to significantly affect plant height, number of leaves, number of buds and total leaf area. Meanwhile the isolates of IAA-producing bacteria influenced the plant height, number of leaves, leaf area, total leaf area, and leaf greenness.

### 3.1 The effects of dolomite application at different doses on the growth variables of emprit ginger

DMRT analyses showed that full application of dolomite (P2) at 42.64 grams per polybag produced the highest value of plant height at 217.17 cm. This value is significantly greater than those of plants getting reduced (P1) or no dolomite (P0), which further supports the previous finding by Ilham *et al.*, (2019). Plant height is improved by the application of dolomite because it increases the soil pH, which in turn makes macro- and micronutrients and cations available to the plants. Dolomite application can also prevent aluminium poisoning and increase phosphorus availability by freeing it from AlP and FeP. Table 2 shows DMRT results on the effects of dolomite dosage on growth of emprit ginger.

**Table 2.** DMRT results on the effects of dolomite dosage on growth of emprit ginger.

Dolomite Dosage	Observed variable			
	PH	NL	NB	TLA
D0 (no dolomite)	152.67 c	52.08 c	8.00 b	744.86 b
D1 (50%; 21.32 gr)	186.33 b	65.67 b	9.42 a	1035.74 a
D2 (100%; 42.64 gr)	217.17 a	72.25 a	9.58 a	1138.73 a

Note: values followed by the same letters indicate that those results are not significantly different based on DMRT at  $\alpha$ -level = 5%. PH = Plant height, NL = Number of leaves, NB = Number of buds, TLA = Total leaf area.

### 3.2 The effects of application of IAA-producing bacterial isolates on the growth variables of emprit ginger

DMRT analyses produced significant differences on the growth variables of emprit ginger treated with different isolates of IAA-producing bacteria. Table 3 shows DMRT results on the effects of IAA-producing bacterial isolates on growth of emprit ginger.

**Table 3.** DMRT results on the effects of IAA-producing bacterial isolates on growth of emprit ginger.

Bacterial Isolate	Observed variable				
	PH	NL	LA	TLA	LG
I0 (no isolate)	169.56 c	56.33 c	12.82 c	727.70 c	16.94 c
I1 (N15)	187.33 b	64.44 ab	12.53 c	793.45 c	22.79 b
I2 (N19)	183.44 bc	62.33 bc	16.32 b	1005.38 b	29.42 a
I3 (S3)	201.22 a	70.22 a	19.51 a	1365.93 a	29.74 a

Note: values followed by the same letters indicate that those results are not significantly different based on DMRT at  $\alpha$ -level = 5%. PH = Plant height, NL = Number of leaves, LA = leaf area, TLA = Total leaf area, LG = leaf greenness.

Bacterial isolate S3 produced significantly taller plants than the other treatments at 201.22 cm. Plant height of ginger treated with N15 (187.33 cm) was significantly different from that of no bacterial treatment (169.56 cm) but not from that of N19 (183.44 cm). Meanwhile, ginger treated with N19 was taller than that with no treatment, but the difference was not significant. As previously mentioned, S3 produces the highest IAA, compared to N15 and N19. This finding shows that plant height is positively correlated to the IAA contents that the bacteria produce. IAA, which is also known as auxin, is a plant hormone that functions in cell growth, inhibition of side branch formation, stimulation of abscission, development of xylem and phloem, as well as root development and elongation (Herlina *et al.*, 2016).

The numbers of leaves are also affected by the differences in bacterial applications. S3 yielded significantly higher number, at 70.22 leaves, than no isolate and N19 but not different from N15. The numbers of leaves between N15 and N19 treated ginger were not significantly different at 64.44 and 62.33 respectively. Untreated plants produced the smallest number of leaves at 56.33, but it is not significantly different from those treated with N19. This finding support that of a previous study by Puspita *et al.*, (2018), in which increased number of leaves was associated with the ability of bacterial isolates in producing IAA. Rini & Wibowo (2021) posited that the production IAA is one of the bacterial mechanisms to increase the growth of plant roots that impacts the developments of plant shoots. IAA plays an important role in cell division and growth that produce developing leaf primordia (Tetuka, 2015). High number of leaves would increase the area for and, thus, optimize photosynthesis.

### 3.3 The effects of interactions between dolomite dosage and application of IAA-producing bacteria on the growth variables of emprit ginger

Table 4 shows the results of the DMRT test combined with administering IAA-producing bacterial isolates and reducing the dose of dolomite on the growth of ginger plants.

**Table 4.** The results of the DMRT test

Interaction (D X I)	Observed variable						
	PH		NL		NB		TLA
D0I0	131.67	f	47.67	ef	7.33	c	509.7299 f
D0I1	148.00	ef	45.00	f	7.67	c	567.501 ef
D0I2	164.33	cde	56.00	def	8.33	bc	883.2697 cd
D0I3	166.67	cde	59.67	cd	8.67	bc	1018.961 bc
D1I0	168.00	cde	62.67	cd	9.33	bc	848.6232 cd
D1I1	178.00	cd	63.67	cd	8.67	bc	777.6617 de
D1I2	152.00	def	54.00	def	8.67	bc	904.5894 cd
D1I3	247.33	a	82.33	a	11.00	ab	1612.087 a
D2I0	209.00	b	58.67	cde	9.33	bc	824.7541 cd
D2I1	236.00	a	84.67	a	12.00	a	1035.173 bc
D2I2	234.00	a	77.00	ab	8.33	bc	1228.275 b
D2I3	189.67	bc	68.67	bc	8.67	bc	1466.733 a

Note: values followed by the same letters indicate that those results are not significantly different based on DMRT at  $\alpha$ -level = 5%. PH = Plant height, NL = Number of leaves, NB = number of buds, TLA = Total leaf area.

There were significant interactions between dolomite dosage and application of IAA-producing bacteria. Those interactions can be observed on the plant height, number of leaves, and number of buds. Treatment of 50% dolomite and S3 isolate (D1I3) yielded the highest plant height at 247.33 cm. Ginger plants treated with 100% dolomite and N15 isolate (D2I1) produced an average leaf number of 84.67 units. This treatment also generated the highest number of buds at an average of 12 shoots.

#### 4. Discussion

The results of the interactions between these two variables on emprit ginger cultivation reflect those of dolomite dosage, as shown in Table 1. Additionally bacterial applications also affect the weights of whole plants (fresh and dry) and rhizome (fresh and dry).

Reducing the amount of dolomite by 50% was also shown to decrease the number of leaves, however was not sufficient to reduce the number of buds of emprit ginger compared to 100% dolomite application. The significantly low numbers of leaves in reduced and without dolomite treatments might have been caused by the correlation between plant height and number of leaves. According to Ariyanti *et al.* (2018), the development of leaves as photosynthetic organs positively correlates with the developments of other plant organs.

The analyses on the number of buds showed that dolomite dosage also had a significant effect. While there is no difference between 100% and 50% applications, at 9.58 and 9.42 respectively, the absence of dolomite significantly reduced the number of buds to 8.00. This difference might be caused by the low level of Mg content, which is necessary for the formation of chlorophyll. The addition of dolomite increases the amounts of Ca and Mg that not only improves the photosynthesis and respiration but also develops better root systems (Sudianto *et al.*, 2018).

The significance of dolomite in plant growth is further shown by the total leaf area, in which zero application (744.68 cm<sup>2</sup>) was significantly smaller than those of 50% (1035.74 cm<sup>2</sup>) and 100% (1138.73 cm<sup>2</sup>). This phenomenon could be the results of low N and K contents, two elements needed in leaf developments. Furthermore, according to Arini (2011), Ca in dolomite can neutralize the deleterious effects of Al as well as soil acidity. Ca compounds in plants are closely associated with pH by influencing the availability of N and P (Riyani *et al.*, 2020). Insufficient nutrients cause inhibition of leaf development marked by narrowness and yellow or red coloration (Putra, 2019).

S3 isolate, again, generated significantly larger leaf area than other treatments at 19.51 cm<sup>2</sup>, followed by N19 (16.32cm<sup>2</sup>). N15 produced the smallest leaf area at 12.53 cm<sup>2</sup>, however, this value is not significantly different from that of untreated plants at 12.82 cm<sup>2</sup>. This phenomenon could be explained by some evidence that IAA has the capacity to increase the division of meristematic cells and inhibit plant compression. Puspita *et al.* (2018) reported that the application of IAA-producing bacteria affected the leaf areas of cacao saplings. This can be attributed to the photosynthetic process supporting the functions of plant cells during differentiation. The increased rate of differentiation, in turn, stimulates development of leaves, branches, and roots of the plants. The increased

leaf elongation is the result from an increased rate of cell division and stimulates cell differentiation. Application of auxin can affect the leaf development especially on the length of its vascular tissues. Widiastoety (2014) stated that the length and width of leaves are closely associated with the directions of division, development, number, and cell distribution.

The differences in total leaf area among treatments show similar a similar pattern to the individual leaf area, in which S3 produced the largest total leaf area at 1365.93 cm<sup>2</sup>, followed by N19 at 1005.38 cm<sup>2</sup>. Ginger treated with N15 isolate resulted in larger TLA at 1005.38 cm<sup>2</sup> than ginger without bacterial application at 727.70 cm<sup>2</sup>, but this difference was not statistically significant. Total leaf area is defined as the number leaves multiplied by leaf area. This phenomenon can be explained the same way as the leaf area in previous paragraph.

There is significant difference between the untreated and treated ginger plants on the greenness of leaves. There is no significant difference between S3 and N19 groups at 29.74 unit and 29.42 unit, respectively. These values are significantly greater than those of N15-treated (22.79 unit) and untreated (16.94 unit) plants. The latter groups were also significantly different from each other in terms of leaf greenness. Leaves are plants organs where photosynthesis takes place. Photosynthesis is a process by which solar energy is used to convert CO<sub>2</sub> and water into sugar and O<sub>2</sub> catalyzed by chlorophyll. In addition to being a catalyst in photosynthesis, chlorophyll is also responsible for the green coloration of leaves. This finding shows that isolates of IAA-producing bacteria increase the presence of chlorophyll in the leaves. This process might be caused by the capacity of IAA to dissolve phosphates and bind to N, which is used by plants as nutrition in photosynthesis. Rokhmah (2020) stated that when plant nutrients are sufficient, physiological processes can take place properly and stimulate plant growth. Ariyanti *et al.* (2020) demonstrated that large and flat surface areas allow maximum photon capture per volume unit of leaves. Leaf area may be related to leaf greenness in determining the rate of photosynthesis in plants.

ANOVA results (Table 1) showed that the numbers of buds were not affected by the applications of bacterial isolates. DMRT further confirmed this finding, in which the numbers of buds (9.44 for S3, 8.44 for N19, 9.44 for N15, and 8.67 for untreated) were not significantly different. This might be caused by the different conditions of the ginger rhizomes at planting. The quality of a rhizome is determined by its maturity. Rusmin *et al.* (2015) described the accumulation of starch and quantity of fiber during the formation and development of rhizomes. High starch and fiber contents ensure the survival of rhizomes.

Widawati (2011) demonstrated that IAA-producing bacteria had the capacity to release P that was bound to Al, Ca, and Fe and make it available for plants. *Azospirillum* sp. is one of the bacteria that have the ability to provide phosphate involved in the mineralization of organophosphate by producing acid and basic phosphomonoesterase enzymes. These enzymes can hydrolyze an ester bond of organophosphate and converting it into inorganic phosphate (H<sub>2</sub>PO<sub>4</sub> and HPO<sub>4</sub><sup>2-</sup>) that become available to plants (Widiawati & Muharam, 2012).

Tuhuteru (2019) reported that isolates of *Burkholderia* sp. and *Bacillus* sp. showed the capacity to increase nutrients in ultisol-type soil by producing IAA/auxin hormone and phosphomonoesterase (PMEase). These substances increase the soil phosphoric contents by dissolving phosphate that are bound to the surfaces of iron and aluminium oxides in the forms of Fe-P and Al-P compounds. These processes result in increased availability of soil nutrients that are beneficial for plant growth (Widiawati & Saefudin, 2015).

## 5. Conclusions

Cultivation of emprit ginger on marginal soil, like ultisol, could be optimally achieved by using less dolomite and IAA-producing bacterial isolates. There are observable positive effects of IAA-producing bacterium applications with S3 isolate producing the best result as indicated by the growth variables of plant height 201.22 cm, number of leaves 70.22 units, and leaf area 19.51 cm<sup>2</sup>. Reducing the amount of dolomite by 50% yielded an observable result on the number of buds at an average of 9.42 sprouts. There are significant interactions between dolomite dosage and bacterial isolates manifested on the plant height, number of leaves, and number of buds.

## Author Contributions

Contributions: Conceptualization, N.W.A.L. and Z.A.R.; methodology, P.S.; software, K.K.; validation, N.W.A.L., Z.A.R., and I.B.G.P.; formal analysis, P.S.; investigation, K.K.; resources, I.B.G.P.; data curation, Z.A.R.; writing—original draft preparation, N.W.A.L.; writing—review and editing, K.K.; visualization, Z.A.R.; supervision, P.S.; project administration, I.B.G.P.; funding acquisition, N.W.A.L. All authors have read and agreed to the published version of the manuscript.

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Not applicable

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## Conflicts of Interest

The authors declare there no conflict of interest

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