

Article

Breaking Dormancy and Effects of Shade Level and NPK Fertilizer Rates on Yield of *Zingiber zerumbet* (L.) Smith (Lempoyang)

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Abstract: *Zingiber zerumbet* (L.) Smith, known as Lempoyang in Malaysia, belongs to the family Zingiberaceae. Previous studies on Lempoyang mainly focused on the chemical properties and biological activities of the rhizome extracts of this plant. Despite the tremendous demand for the rhizomes of *Z. zerumbet*, there is a lack of information on cultivation practices and a scarcity of planting materials. By using the pre-soaked technique, the challenges posed by *Z. zerumbet* dormancy can be overcome, obtaining good quality and uniform planting material throughout the year. Besides that, it is also crucial to determine the optimum shade level and NPK fertilizer rates to obtain a high yield and good quality rhizomes of Lempoyang. Six concentrations of 6-benzylaminopurine (BAP, 0, 50, 100, 150, 200, and 250 mg/L) and ethephon (0, 150, 300, 450, 600, and 750 mg/L) were tested to evaluate their effects on breaking rhizome dormancy. Three different shade levels (full sun, 30%, and 50% shade levels) and four combinations of Nitrogen (N), phosphorus (P), and potassium (K) (NPK) fertilizer at different rates were evaluated to study their effects on plant growth and yield performance. The results showed that BAP at 100 mg/L and ethephon at 300 mg/L performed better than the other compound concentrations tested in promoting the breaking of bud dormancy. A significant interaction effect was observed between shade levels and NPK fertilizer rates in all the growth parameters examined, except for the number of tillers per plant. Plants grown under 30% shade with NPK 4 produced the highest rhizome fresh weight, dry weight, and yield, but plants grown under 50% shade with NPK 4 showed the highest plant height and number of tillers per plant.

Keywords: *Zingiber zerumbet*; dormancy; shade level; fertilizer; rhizome yield; NPK

1. Introduction

Zingiber zerumbet (L.) Smith is commonly found in the lowland forest of Malaysia where it grows naturally on hill slopes or in damp and shaded areas. This native rhizomatous herb has high medicinal values and is also used as food flavoring. Previous studies on Lempoyang have mainly focused on the chemical properties and biological activities of the rhizome extracts of this plant, and it was reported that *Z. zerumbet* has many biological activities such as anti-inflammatory [1], antinociceptive [2], antitumor [3], and others. However, the agronomic practices are still not well studied, and there is insufficient knowledge of the required growing conditions. Basic information on breaking bud dormancy, light requirement, and nutrient requirement is still lacking. *Z. zerumbet* undergoes dormancy for a period of 1 to 2 months, which causes rhizomes to be susceptible to fungal diseases which also affect the quality of the plant and the cropping cycles. Breaking bud dormancy of *Z. zerumbet* rhizome

can solve the problems faced during the dormancy period and could provide good quality planting materials throughout the year.

Previous studies reported that plant growth regulators such as cytokinins and auxins play an important role in breaking dormancy. Cytokinins are known to induce bud break in many kinds of plants on both aerials and below-ground parts [4]. In tissue culture, it was reported that medium with 6-benzylaminopurine (BAP) and combinations with other phytohormones successfully induced micro-rhizomes of *Zingiber officinale* and *Zingiber cassumunar* [5,6]. Ethephon (2-chloroethyl phosphoric acid) is an ethylene-releasing compound which is widely used as a plant growth regulator. Its effect will vary depending on the plant species, its chemical concentration, and the timing and duration of the application of exogenous ethylene or the ethephon solution [7]. Endogenous cytokinins and auxins exhibited great influence on initiation and development in the rhizomes of *Z. officinale* [8], while ethephon used in pre-planting soaking and foliar treatment was able to promote shoot emergence and increased shoot numbers in *Z. officinale* [9,10]. On the basis of the previous literature, plant growth regulators like BAP and ethephon were believed to be able to break rhizome dormancy of *Z. zerumbet*. In addition, there is still no report available related to BAP and ethephon ability to break rhizome dormancy of *Z. zerumbet* using the pre-soaked technique.

Light intensity is an important factor not only in the photosynthesis process and food production but also in plant growth and development. The photosynthetic capacity can be increased by increasing the light intensity, and plant growth will be enhanced at the same time [11,12]. Therefore, the rhizome yield of *Z. officinale* was reduced when growing under a high shade level [13,14]. To increase the yield of crops, the application of fertilizers is essential, and the adequate amount of fertilizers to be applied to boost rhizome yield should be known to avoid wastage. Increasing fertilizer rates significantly increase the rhizome yield in the Zingiberaceae family, such as in turmeric (*Curcuma longa* L.) and ginger (*Z. officinale*) [15,16]. Three major nutrients which are Nitrogen (N), phosphorus (P), and potassium (K) play a very important role in plant growth, yield, and plant quality [17]. *Z. zerumbet* is a long-term exhaustive crop which requires a high amount of fertilizer. Nutrient availability is a very important factor in crop development and yield production, therefore the optimum fertilizer rate to produce the maximum yield is crucial. However, there is a lack of information on the optimum shade levels and fertilizer rates for maximum growth and rhizome yield of Lempoyang. Therefore, this study was performed to evaluate the potential and the optimum concentration of BAP and ethephon to break rhizome dormancy and to determine the effect of various shade levels and NPK fertilizer rates on the growth and production of rhizome in *Z. zerumbet*.

2. Materials and Methods

2.1. Plant Materials

The rhizomes of *Z. zerumbet* were bought from Kizaherb Enterprise, Kuala Krau in Pahang, Malaysia. Dormant rhizomes were used for the dormancy study, while some rhizomes were propagated to produce more planting materials. The plants were then sent to the Herbarium of the Institute of Bioscience, University Putra Malaysia (UPM), to be identified and kept as voucher specimens (SK 3183/17). The plants were grown in Ladang 2, UPM, for 6 months, under different growing conditions.

2.2. Breaking Dormancy

A selection of rhizomes was made on the basis of their dormant state, number of eye buds, and size. Only rhizomes that were dormant, with at least three eye buds, and with a length of 3–5 cm were used. Rhizomes were treated with the fungicide Benlate® (Benomyl) for 30 min and were air-dried before being soaked in treatment solutions. BAP at 0, 50, 100, 150, 200, and 250 mg/L and Ethephon at 0, 150, 300, 450, 600, and 750 mg/L were selected, on the basis of a literature review. The rhizomes were soaked in treatment solutions for 30 min and placed on moistened cotton in transparent containers in aseptic conditions. The containers were arranged in Completely Randomized Design (CRD) and

maintained at 25 ± 2 °C, 16 h daily illumination with 1000 lux of white fluorescent light for 30 days. Two separate experiments were conducted simultaneously with four replications. Each replication consisted of two rhizomes as an experimental unit that made up a total of 96 rhizomes. The breaking dormancy study was conducted twice at different times, using the same method and conditions. Data on the number of days to visible buds, mean number of buds, mean number of shoots, percentage of buds sprouted, and rhizome weight were collected.

2.3. Shade Levels and NPK Fertilizer Rates on Growth and Rhizome Yield

One-month-old seedlings were planted in 18 × 20 inches polybags and grown under three different shade levels (0%, 30%, and 50%). The three major nutrients (NPK) with different combination rates were evaluated 0.5, 1, 1.5, and 2 times the base recommended rate for NPK 2 (Table 1). A media mixture was used with a combination of soil, cocopeat, burnt rice husk, and topsoil at the ratio of 1:1:1:1. The experimental design used was a split-plot design laid in a randomized complete block design (RCBD). The main plots were three shade levels, and the sub-plots were four NPK fertilizer rates. Four replications with a total of 144 plants were used for this study. An irrigation system was fixed to water the plants once a day in the evening. Weeding was done manually by hand. Fertilization was divided equally with three-month intervals, and the first fertilizer was applied two weeks after planting. Growth parameters were collected before harvesting. The plants were harvested in the sixth month after planting. The harvested rhizomes were washed and air-dried for 30 min. The fresh weight of the harvested rhizomes was recorded. Freshly harvested rhizomes were then put into an oven for 4 days for drying, and the rhizomes' dry weight was recorded.

Table 1. Combination of Nitrogen (N), phosphorus (P), and potassium (K) (NPK) fertilizers rates.

Treatments	NPK (kg/ha/year)
NPK 1	(30:35:62)
NPK 2	(60:70:115)
NPK 3	(90:105:172)
NPK 4	(120:140:230)

2.4. Statistical Analysis

The collected data were subjected to analysis of variance (ANOVA) using SAS program version 9.2. The means were compared by Duncan's Multiple Range Test (DMRT) when the F-test of the ANOVA for the treatments was significant at the $p < 0.05$. All data of breaking rhizome dormancy were arcsine-transformed prior to ANOVA.

3. Results and Discussion

3.1. The Effects of BAP and Ethephon on Breaking the Rhizome Dormancy of *Z. zerumbet*

In Experiment 1, there were significant effects of different BAP concentrations on the number of days to visible buds, the mean number of buds, the mean number of shoots, and the percentage of buds sprouted at $p < 0.05$. Even though BAP at the highest concentration (250 mg/L) was able to decrease the number of days to visible buds, the mean number of buds and shoots were also reduced. As regards the number of shoots, BAP at concentration 100 mg/L or less induced a higher number of shoots than at higher concentrations and, concerning the bud sprouting percentage, BAP 100 mg/L produced the highest percentage (62.50%) which was 37.5% higher than that observed in the control. All five levels of BAP treatments did not produce any significant effects on the rhizome weight (Table 2). BAP has been successfully used to break the dormancy of various geophytic units [18]. In breaking the dormancy of *gladiolus* cv Friendship corms, 100 mg/L was reported as the optimum concentration of BAP [19]. BAP treatments were slightly more successful in breaking the dormancy of *Heliconia* compared to the absence of treatment [4]. In this study, BAP at 100 mg/L induced the highest number of shoots, which is similar to the results obtained with *Z. officinale* and turmeric plants

in tissue culture [20,21]. This study suggests that BAP at a concentration higher than 200 mg/L is not recommended as it reduced the mean number of shoots and the percentage of buds sprouted. Among all the tested concentrations of BAP, 100 mg/L showed a better performance in breaking bud dormancy, as it produced the highest percentage of bud sprouted and the highest number of shoots, even though it did not produce any significant difference in the number of days to visible buds when compared to the control. On the other hand, ethephon at different concentrations showed significant effects on all parameters, except for the rhizome weight in Experiment 2 (Table 3). Increasing concentrations of ethephon from 300 to 600 mg/L significantly reduced the number of days to visible buds at $p < 0.05$. The highest mean number of buds was achieved with ethephon 300 mg/L (2). Ethephon at 300 mg/L and 600 mg/L showed the same effect on the mean number of shoots (1.25), which was significantly different from that of other treatments. The dormant rhizomes that were treated with ethephon at less than 700 mg/L showed a higher percentage of bud sprouted when compared with untreated rhizomes. Among all the concentrations, ethephon at 300 mg/L produced the highest percentage of bud sprouted (72.50%), a value significantly different from those obtained with ethephon at 750 mg/L and in the control. Ethephon released ethylene when absorbed by the plant tissues and induced an increment in respiration, whereby glucose was broken down, and metabolic energy was synthesized. When the metabolic energy was synthesised, the rhizomes were able to break dormancy [7,22]. The effectiveness of ethylene to break dormancy was shown in the rhizomes of *Rheum rhabarbarum* L. and *Globba winitti* and in the tuber of *Helianthus tuberosus* L. [23–25]. In *Z. officinale*, ethephon at 750 mg/L was demonstrated to produce the highest number of shoot production and the lowest shoot height [10]. However, in this study, ethephon was more efficient in breaking bud dormancy at lower concentrations. Ethephon at 300 mg/L determined the lowest number of days to visible buds, the highest number of buds, and the highest percentage of bud sprouted. This might be due to the sensitivity of *Z. zerumbet* to ethephon. Higher concentrations of ethephon were associated with a higher number of days to visible buds, but this may vary depending on the plant type and the combination with other phytohormones.

Table 2. The effect of different concentrations of 6-benzylaminopurine (BAP) on the number of days to visible buds, mean number of buds, mean number of shoots, percentage of buds sprouted, and rhizome weight of *Z. zerumbet*.

Treatment (mg/L)	Days to Visible Buds	Mean Number of Buds	Mean Number of Shoots	Percentage of Buds Sprouted	Rhizome Weight (g)
Control	17.00 a	0.88 a	0.50 b	25.00 c	3.68 a
BAP 50	13.25 b	1.00 a	0.75 ab	40.63 ab	4.71 a
BAP 100	10.25 b	1.13 a	0.88 a	62.50 a	4.93 a
BAP 150	14.88 b	1.25 a	0.38 bc	16.63 b	4.89 a
BAP 200	22.63 a	1.38 a	0.25 c	18.75 b	4.43 a
BAP 250	7.13 c	0.63 b	0.38 bc	18.75 b	4.31 a

Means within the same column followed by a common letter are not significantly different based on DMRT at ($p < 0.05$) level.

Table 3. The effect of different concentrations of ethephon on the number of days to visible buds, mean number of buds, mean number of shoots, percentage of buds sprouted, and rhizome weight of *Z. zerumbet*.

Treatments (mg/L)	Days to Visible Buds	Mean Number of Buds	Mean Number of Shoots	Percentage of Buds Sprouted	Rhizome Weight (g)
Control	17.00 a	0.88 b	0.25 b	25.00 b	3.68 b
Etp150	21.00 a	0.75 b	0.13 b	62.50 a	5.41 a
Etp300	10.63 b	2.00 a	1.25 a	72.50 a	4.81 a
Etp450	11.25 b	1.13 ab	0.75 b	50.00 a	4.74 a
Etp600	12.25 b	1.38 ab	1.25 a	67.50 a	4.80 a
Etp750	24.25 a	0.63 b	0.25 b	12.50 b	5.72 a

Means within the same column followed by a common letter are not significantly different based on DMRT at ($p < 0.05$) level.

3.2. Interaction Effects of Shade Levels and NPK Fertilizer Rates on the Growth and Rhizome Yield of *Z. zerumbet*

The interaction effects of shade levels and NPK fertilizer rates were found to be significant for all parameters, except for the number of tillers per plant. Plant height was significantly affected by the interaction of shade levels and NPK fertilizer rates (Table 4). Under 50% shade level, plant height was in the following order: NPK 4 (48.78 cm) > NPK 3 (35.75 cm) > NPK 2 (31.85 cm) > NPK 1 (29.25 cm). A shade level of 30% and full sun reacted differently with NPK fertilizer rates, regarding plant height. Plants grown under 30% shade were the tallest when treated with NPK 4 (40.75 cm), but, under full sun condition, NPK 3- and NPK 4-treated plants were both taller than plants treated with other NPK fertilizer rates and showed a non-significant difference between them (Figure 1). The plants tended to grow taller when the shade level increased, as a result of the elongation of the internodes. The height of sweet pepper and turmeric was also reported to increase when the shade was increased [26,27]. Increasing the shade levels significantly increased the number of tillers. As for main effect, the highest mean number of tillers was obtained under 50% shade (5.00) while the highest NPK fertilizer rate, i.e., NPK 4 produced highest mean number of tiller at 5.17. However, there was no interaction effect on the number of tillers at $p < 0.05$. The number of leaves per tiller and the leaf area per plant dependent on the interaction effect between shade level and NPK fertilizer rates. The number of leaves in plants grown under 50% shade was the highest when the plants were treated with NPK 3 (30.31) and NPK 4 (31.63), with no significant difference between them. Furthermore, NPK 4 produced the highest number of leaves in 30% shade (30.00) and full sun (25.13), respectively. When the plants were grown under 50% shade and were treated with NPK 4, the highest leaf area per plant was obtained (132.41 cm²). In addition, the lowest leaf area per plant was obtained in plants treated with NPK 1 and planted under full sun (57.33 cm²) and 30% shade level (66.10 cm²). The significant interaction between shade and NPK fertilizer rates produced a varying effect on the leaf area per plant in Lempoyang. The growth parameters of plants grown under shade were better and improved compared to plants grown under full sun. A high light intensity causes damage on shade-lover plants, which explains the poor growth performance of *Z. zerumbet* under 0% shade level. A reduction of the leaf area was also observed in the open field, which may be due to a higher radiation level that damaged the photosynthetic pigment [28]. When a plant's leaf area reduces, photosynthesis will eventually decrease, thus leading to poor rhizome yield. The responses of *Z. zerumbet* to different shade levels will probably vary in different geographical areas, seasons, and cultivars. This study found that there were significant interaction effects on rhizome fresh weight, dry weight, and yield. When Lempoyang was exposed to 30% shade level and NPK 4, the highest rhizome fresh weight, dry weight, and yield were obtained (Table 4). On the other hand, under 50% shade level, NPK 3 performed better regarding rhizome fresh weight and dry weight, and NPK 4 induced a reduction of rhizome yield. The plants grown under full sun showed a lower rhizome yield when NPK fertilizer rates were reduced; however, NPK3 and NPK 4 showed similar effects on rhizome fresh weight in full sun condition. Our results are supported by previous studies. Increasing the shade level was reported to produce a higher biomass in turmeric and sweet pepper [26,27,29]. In contrast, the yield of *Z. officinale* was reduced when the plant was grown under high shade level [13,14]. The findings of this study confirm that *Z. zerumbet* is a true shade-type species that grows best under low light intensity, a condition similar to its native shaded habitat. Increasing the fertilizer rate increased all the growth parameters considered, in all shade levels. The increasing rates of NPK increased the nutrient uptake of the plants, and this might be the reason why higher fertilizer rates produced higher rhizome yields. The study on *Z. officinale* and *C. longa* L. also reported that increasing fertilizer rates produced higher rhizome yields [16,30]. The results of this study indicate that the growth and rhizome yield of *Z. zerumbet* depends on the shade level and the applied NPK fertilizer rates. The interaction study revealed a general trend of yield increase with the increase of NPK fertilizer rates and shade levels. The combination of 30% shade level and NPK 4 was able to produce the best plant growth and the maximum rhizome yield of *Z. zerumbet*.

Table 4. Effects of shade levels and NPK fertilizer rates on growth parameters, rhizome fresh weight, rhizome dry weight, and rhizome yield of *Z. zerumbet*.

Treatments	Plant Height (cm)	Number of Tillers per Plants	Average Number of Leaves per Tiller	Leaf Area per Plant (cm ²)	Rhizome Fresh Weight (g/plant)	Rhizome Dry Weight (g/plant)	Yield (Tonne per Hectare)
Shade levels ($\mu\text{mol m}^{-2}\text{s}^{-1}$)							
Full sun	29.47 \pm 1.80 b	4.40 \pm 0.39 b	20.25 \pm 0.91 c	67.22 \pm 1.35 c	157.04 \pm 2.05 ab	34.39 \pm 1.70 c	0.44 \pm 0.01 ab
30% Shade	30.03 \pm 1.48 b	4.72 \pm 0.77 b	26.32 \pm 0.93 b	84.56 \pm 2.33 b	159.37 \pm 6.27 a	38.46 \pm 1.37 b	0.44 \pm 0.02 a
50% Shade	36.41 \pm 1.39 a	5.79 \pm 0.71 a	29.24 \pm 1.01 a	100.71 \pm 2.74 a	154.73 \pm 2.89 b	41.41 \pm 1.78 a	0.43 \pm 0.01 b
NPK rates (kg/ha/year)							
NPK 1 (30:35:62)	24.75 \pm 4.58 d	3.83 \pm 1.40 c	21.79 \pm 3.48 d	68.25 \pm 4.58 b	117.56 \pm 14.84 d	29.13 \pm 5.21 d	0.33 \pm 0.04 d
NPK 2 (60:70:115)	28.55 \pm 3.35 c	4.70 \pm 1.39 b	24.09 \pm 1.41 c	76.11 \pm 3.35 b	141.88 \pm 8.09 c	33.85 \pm 3.48 c	0.39 \pm 0.02 c
NPK 3 (90:105:172)	33.22 \pm 9.78 b	5.06 \pm 1.99 b	26.27 \pm 1.57 b	87.16 \pm 9.78 a	176.58 \pm 6.02 b	42.99 \pm 5.49 b	0.49 \pm 0.01 b
NPK 4 (120:140:230)	41.36 \pm 7.94 a	6.29 \pm 2.73 a	28.92 \pm 4.89 a	105.13 \pm 7.94 a	192.17 \pm 15.87 a	46.36 \pm 5.23 a	0.53 \pm 0.04 a
F value							
Shade levels (S)	82.56 ***	15.49 **	296.42 ***	1074.82 ***	5.83 *	42.73 **	5.83 *
NPK rates (NPK)	217.12 ***	34.51 ***	84.49 ***	317.22 ***	568.50 ***	336.48 ***	568.50 ***
S \times NPK	14.45 ***	1.44 N.S.	4.15 *	28.11 **	62.84 ***	11.58 ***	63.24 ***
CV (%)	5.26	12.09	4.53	3.69	3.11	3.95	3.06

Values are means \pm SD. Same letter within the same column indicate no significant difference ($p < 0.05$). The means separations were determined using DMRT. F value represented with * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; N.S. = not significant.

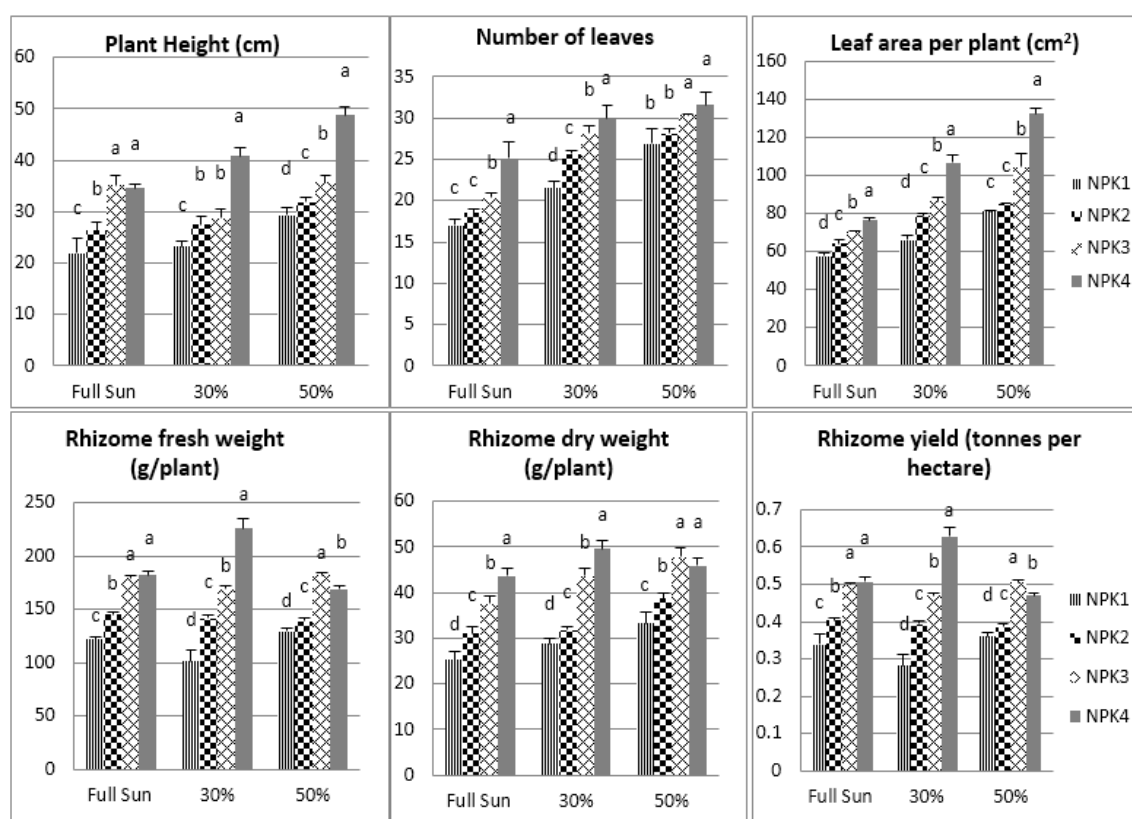


Figure 1. Effect of shade levels and NPK fertilizer rates on growth parameters, rhizome fresh weight, rhizome dry weight, and rhizome yield of *Z. zerumbet*. The means separations within each shade level were determined using DMRT at ($p < 0.05$) level.

4. Conclusions

The pre-soaked technique is an easy and practical way to break bud dormancy and can be applied in the production of *Z. zerumbet*. The use of BAP and ethephon was successful in breaking the bud dormancy of *Z. zerumbet*. BAP at 100 mg/L produced the highest percentage of bud sprouted, while ethephon at 300 mg/L was associated with the earliest time to visible eye buds, the highest number of shoot, and the highest percentage of bud sprouted. The findings of this study show that BAP at 100 mg/L and ethephon at 300 mg/L are the most effective to break the rhizome bud dormancy of Lempoyang. Shade levels and NPK fertilizers rates showed interaction effects on the growth and yield of *Z. zerumbet*. Growing *Z. zerumbet* under 30% shade in the presence of NPK 4 (120:140:230 NPK kg/ha/year) was found to be the best cultural practice to improve growth performance and rhizome yield. On the basis of these findings, cultivation of *Z. zerumbet* can be improved, and its planting in Malaysia can be encouraged to overcome the shortage of planting materials and fulfill the market demands.

Author Contributions: S.G. and T.L.A. proposed and designed the study. S.G. carried out the experiment and collected the data. S.A.H. assisted in methodology and statistical analysis. J.S. aided in interpreting the results and discussions. S.G. wrote the manuscript in consultation with T.L.A., S.A.H. and J.S. All authors discussed the results and commented on the manuscript.

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