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BIOLOGY

# Impact of composted guava leaves and neem seeds on the growth and curcuminoid- and xanthorrhizol-yields of *Curcuma zanthorrhiza* RoxB

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**ABSTRACT**: *The compost from the waste of pharmaceutical industries, such as guava leaves (GL) and neem seeds (NS) is used in organic agriculture. Curcuma zanthorrhiza RoxB. is a widely recognized herbal medicine that grows natively in Indonesia. Curcuminoids and xanthorrhizol are the primary bioactive components of C. zanthorrhiza. In this study, we aimed to evaluate the impact of GL and NS compost on the growth and bioactive yields of C. zanthorrhiza. Treatments consisted of cow manure, GL compost, NS compost, GL+NS compost, or a no compost control, at 2 and 4 kg per plant. Results demonstrated that the NS and GL+NS compost applied with 4 kg per plant had increased fresh rhizome yield compared with the other treatments. Composted NS at 2 kg per plant increased the plant height and pseudo stem diameter traits compared with the control treatment. The compost application of GL+NS at 2 and 4 kg per plant significantly increased the leaf length and number of shoots. All treatments showed unchanged the leaf width and number of leaves. The compost application of GL and NS (2 kg per plant) showed higher production of curcuminoidsthan the control. The compost consisting of GL (2 kg per plant), NS (4 kg per plant), and GL+NS also increased the production of xanthorrhizol compared with the control treatment. Results illustrated the practical application of GL and NS composts from industrial pharmaceutical extraction waste for the organic farming cultivation of C. zanthorrhiza.*
**Key words**: *agricultural biochemistry, Curcuma zanthorrhiza, guava leaves, neem seeds, extraction waste, compost.*

## Impacto de folhas de goiabeira compostada e sementes de nim no crescimento e r endimento de curcuminóides e xanthorrizil de *Curcuma zanthorrhiz*a RoxB

**RESUMO**: *O composto dos resíduos das indústrias farmacêuticas, como folhas de goiaba (GL) e sementes de nim (NS), é usado na agricultura orgânica. Curcuma zanthorrhiza RoxB. é um medicamento fitoterápico amplamente reconhecido que cresce de forma nativa na Indonésia. Os curcuminóides e o xanthorrizol são os principais componentes bioativos de C. zanthorrhiza. Neste trabalho, objetivou-se avaliar o impacto do composto GL e NS no crescimento e produtividade bioativa de C. zanthorrhiza. Os tratamentos consistiram em esterco de vaca, composto GL, composto NS, composto GL + NS ou controle sem composto, em 2 e 4 kg por planta. Os resultados demonstraram que o composto NS e GL + NS aplicado com 4 kg por planta aumentou a produção de rizoma fresco, em comparação com os outros tratamentos. A NS compostada a 2 kg por planta aumentou as características de altura da planta e diâmetro do pseudoestêmico em comparação com o tratamento controle. A aplicação de composto de GL + NS em 2 e 4 kg por planta aumentou significativamente o comprimento das folhas e o número de brotações. Todos os tratamentos apresentaram alteração na largura e número de folhas. A aplicação de composto de GL e NS (2 kg por planta) apresentou maior produção de curcuminóide do que o controle. Os compostos constituídos por GL (2 kg por planta), NS (4 kg por planta) e GL + NS também aumentaram a produção de xanthorrizol em comparação com o tratamento controle. Os resultados ilustraram a aplicação prática de compostos GL e NS de resíduos de extração farmacêutica industrial para o cultivo orgânico de C. zanthorrhiza.*
**Palavras-chave**: *bioquímica agrícola, Curcuma zanthorrhiza, folhas de goiabeira, sementes de nim, resíduos de extração, composto.*

## INTRODUCTION

*Curcuma zanthorrhiza* RoxB. (Java turmeric) is a native medicinal plant in Indonesia that belongs to the Zingiberaceae family. The rhizome contains curcuminoids and xanthorrhizol as the major secondary compounds in this plant (AWIN et al., 2019; JANTAN et al., 2012; OON et al., 2015). Recently, several pharmacological activities for curcuminoids and xanthorrhizol have been reported, including anti-dengue (BALASUBRAMANIAN et al., 2019), anti-inflammatory(LI, et al., 2019), antioxidant (MARTINS et al., 2013), anticancer (NURCHOLIS et al., 2016a; NURCHOLIS et al., 2018), and antimicrobial (HWANG et al., 2000; RUKAYADI et al., 2013) activities. Due to the biological activities of these compounds, it is important to cultivate *C. zanthorrhiza* with high levels of curcuminoid and xanthorrhizol production.

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Compost use is popular in organic farming because it replaces fertilizers and improves soil fertility (PANE et al., 2013). In addition, consumers tend to choose organic plants compared with others because they are perceived as safer and more nutritious (REEVE et al., 2016). Several reports have demonstrated that the application of compost improves yield, nutritional and metabolite quality, and plant health (GIMÉNEZ et al., 2019; SARWAR et al., 2019; ZHANG, X. et al., 2019). Industrial organic waste of extracted plant herbs being used as composted green waste has a number of advantages, including materials that have biological activity (MORALES-CORTS et al.,2018) and low toxicity of heavy metals, hormones, aromatic hydrocarbons, pollutants, pharmaceuticals, salmonella, fecal coliforms, and viruses (MORETTI et al., 2015). However, to date, there is no information on the application of composted guava leaves (GL) and neem seeds (NS) from organic waste for growth and yield improvement of crops.

As the benefits of organic cultivating and the need to use composted organic waste are becoming more apparent, the purpose of this research was to evaluate *C. zanthorrhiza* growth parameters and bioactive yields when applying different organic composts. To this end, we used the *C. zanthorrhiza* plant to evaluate whether GL and NS composts or the combination of both can increase plant growth and bioactive productivities compared with manure and an unfertilized control. Our results showed that the compost of GL and NS enhances growth parameters and the bioactive yields of *C. zanthorrhiza*.

## MATERIALS AND METHODS

The experiment was conducted in the SOHO Center of Excellence in Herbal Research during September 2017–May 2018 in Cihanjawar Village, Nagrak, Sukabumi, West Java, Indonesia, at a 1,697 m altitude (6°49′55.49″ S, 106°49′3.09″ E). This study used *C. zanthorrhiza* of the Cursina 2 variety, which was cultivated by local farmers using common farming equipment. Organic waste from industrial pharmaceutical extraction processes, including GL and NS, were obtained from PT SOHO Industri Pharmasi and Eco Learning Camp Foundation. Cow manure was also used in the compost. At a 1:1 volumetric ratio, 10 g of organic waste were mixed with cow manure before the composting process. Raw material mixtures (20 g) were added with 100 ml cellulolytic microorganisms (*Trichoderma* sp. and *Bacillus* spp.) and 1 L of water. The composting was turned over every seven days for 60 days. The experimental design used a one-factor randomized block design. The treatment groups included the various compost compositions, 1) control, 2) cow manure (2 and 4 kg per plant), 3) composted GL (2 and 4 kg per plant), 4) composted NS (2 and 4 kg per plant), and 5) composted GL+NS (2 and 4 kg per plant). Each experiment unit consisted of 10 plants with three replicates.

Plant height, number of leaves, pseudo stem diameter, leaf length, leaf width, and number of shoots were recorded manually on three randomly selected representative plants from each experiment five months after the planting of *C. zanthorrhiza*. Plants were harvested nine months after planting, and the fresh rhizome weight and bioactive yield were determined for each plant. Curcuminoid and xanthorrhizol levels were analyzed using high-performance liquid chromatography (HPLC), as described previously (NURCHOLIS et al., 2016a) and (NURCHOLIS et al., 2018). All solvents used were HPLC grade. The standards for curcumin, demethoxycurcumin, and bisdemethoxycurcumin were purchased from ChromaDex Inc., and xanthorrhizol was obtained from Merck. The curcuminoid- and xanthorrhizol-yields were determined by comparing the measurements to each respective standard and were expressed as a percentage (w/w) to weight basis.

For statistical analysis, SPSS 25 was used to calculate ANOVA, and Prism 7 was used to calculate the means ± SE using the Duncan test and to plot graphs. A $p$-value≤0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

In this research, we aimed to determine whether the use of GL and NS from industrial waste as organic fertilizer in *C. zanthorrhiza* cultivation would enhance growth and levels of valuable secondary metabolites, such as curcuminoids and xanthorrhizol compounds, in *C. zanthorrhiza*.

The effect of GL and NS composts on the growth performance of *C. zanthorrhiza* is presented in Figure 1. Applications of the NS compost at 4 kg per plant increased the fresh rhizome yield of *C. zanthorrhiza* (2.58 kg per plant) compared with the control, cow manure, and other treatments. No significant differences were observed between the control, cow manure, and compost treatment of GL, NS, and GL+NS. Several studies have used different

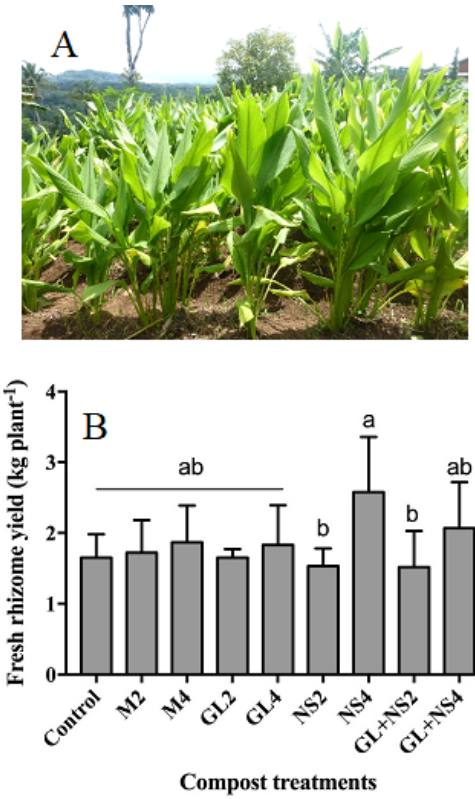


Figure 1 - Effects of compost treatments on the growth of *C. zanthorrhiza.*(A) At five months after planting and fresh rhizome yield and (B) at nine months after planting. M = manure; GL = guava leaves; NS = neem seeds. 2 = dose of 2 kg per plant; 4 = dose of 4 kg per plant. Values are mean ± SE. Different letters indicate significant differences with the Duncan test at $P<0.05$.

fertilizers for improving rhizome production in *C. zanthorrhiza*. NIHAYATI et al., (2013) reported that fertilization with an NK combination increases the fresh rhizome weight (0.33 kg per plant) of *C. zanthorrhiza* at six months after planting. Other studies have reported that the use of manure as an organic compost increases the fresh rhizome weight (0.55–0.65 kg per plant) of *C. zanthorrhiza* at six months after planting (KARTIKASARI et al., 2018). The present research suggested that the use of GL and NS from industrial pharmaceutical extraction waste in compost may be effective in improving the rhizome production in the organic agriculture of *C. zanthorrhiza*.

Figure 2 display the effects of the compost treatments on the plant height, number of leaves, pseudo stem diameter, leaf length, leaf width, and number of shoots of *C. zanthorrhiza*. The number of leaves and leaf width of *C. zanthhorriza* were not significantly affected by all treatments. The highest plant height (182.20 cm) was recorded with the NS (2 kg per plant) treatment, and the lowest plant height (127.13 cm) was observed in the control treatment. The greatest pseudo stem diameter was recorded with the NS compost treatment (2 kg per plant) with a value of 4.57 cm, whereas the control treatment had the lowest (2.71 cm). *C. zanthorrhiza* treated with cow manure and GL and NS compost displayed significant differences in the leaf length compared with the control. The greatest leaf length (94.28 cm) of *C. zanthorrhiza* was observed with the GL+NS (2 kg per plant) treatment, and the smallest leaf length (69.03 cm) was recorded in the control treatment. Compared

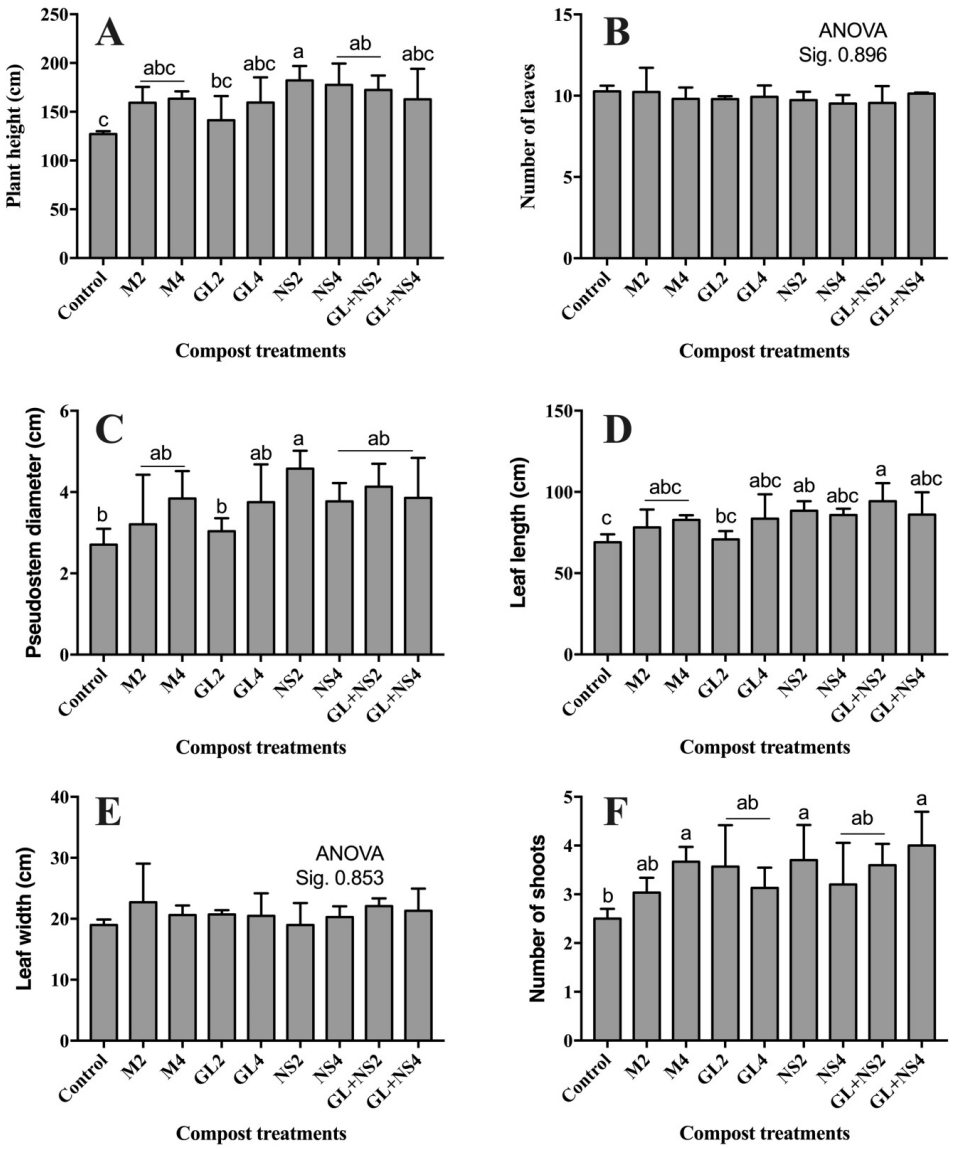


Figure 2 - Effects of compost treatments on (A) plant height, (B) number of leaves, (C) pseudo stem diameter, (D) leaf length, (E) leaf width, and (F) number of shoots of *C. zanthorrhiza* at five months after planting. M = manure; GL = guava leaves; NS = neem seeds. 2 = dose of 2 kg per plant; 4 = dose of 4 kg per plant. Values are mean ± SE. Different letters indicate significant differences with the Duncan test at $P<0.05$.

with the control, the GL+NS (4 kg per plant), NS (2 kg per plant), and cow manure (4 kg per plant) compost had a significantly higher number of shoots. The plant height, pseudo stem diameter, leaf length, and the number of shoots are growth parameters that provide information about nutritional plant status (CARBALLAR-HERNÁNDEZ et al., 2018; LI, et al., 2017). This research suggested that using composted GL and NS in *C. zanthorrhiza* cultivation leads to the stimulation of growth parameters and rhizome production.

Curcuminoids and xanthorrhizol are valuable components of the *C. zanthorrhiza* rhizome in that they have several pharmacological activities (NURCHOLIS et al., 2016b; NURCHOLIS et al., 2018). Therefore, we aimed to identify a

cultivation parameter that leads to the greatest yield of these compounds in *C. zanthorrhiza*. Figure 3 displays the effects of the compost treatments on the curcuminoid- and xanthorrhizol-yields of *C. zanthorrhiza*. There were no statistically significant differences in curcuminoid content among any of the treatments; however, NS compost treatment at 2 kg per plant had the highest curcuminoid content at 3.24% (w/w). Fertilization with cow manure (2 kg per plant) had significantly greater xanthorrhizol yield,3.82% (w/w), than the other treatments. There were no statistically significant differences in the xanthorrhizol yield with the treatment of composted NS (4 kg per plant) or GL+NS (2 and 4 kg per plant); although, the xanthorrhizol content was greater with these treatments than that of the control, with 3.00% (w/w) for the control, 3.31% (w/w) for NS (4 kg per plant), 3.35% (w/w) for GL+NS at 2 kg, and 3.15% (w/w) for GL+NS at 4 kg. These findings indicate that GL and NS composts can potentially be used to improve curcuminoid and xanthorrhizol production in *C. zanthorrhiza* through organic farming. Phenol and carboxylic acid are essential components of the compost and play a role in plant growth and bioactive yield enhancement (GUO et al. 2019). The present research demonstrated that composted GL and NS from industrial waste may be effective in the stimulation of growth and bioactive productivity of *C. zanthorrhiza*. Therefore, these results encourage the practical use of composted GL and NS in organic

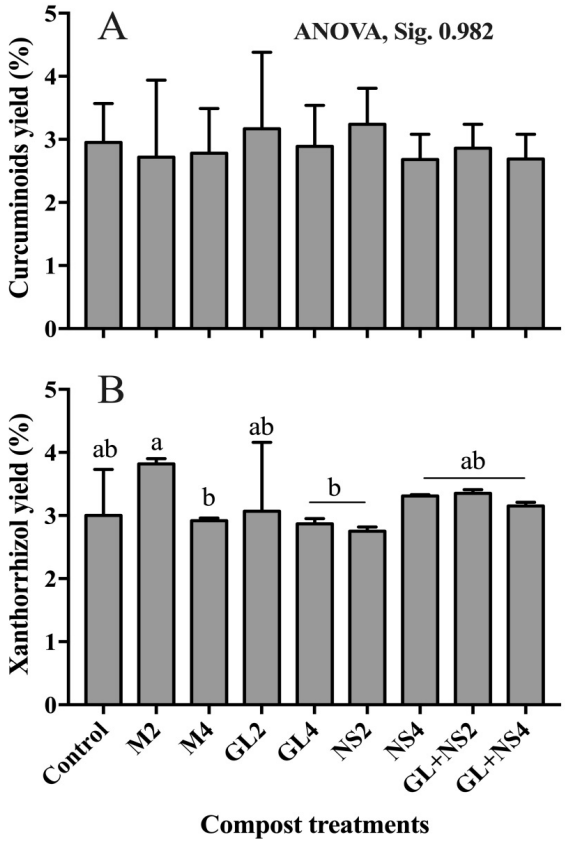


Figure 3 - Effects of compost treatments on the curcuminoid levels (A) and xanthorrhizol levels. (B) Yields in *C. zanthorrhiza* at nine months after planting. M = manure; GL = guava leaves; NS = neem seeds. 2 = dose of 2 kg per plant; 4 = dose of 4 kg per plant. Values are mean ± SE. Different letters indicate significant differences with the Duncan test at $P<0.05$.

farming of *C. zanthorrhiza*. However, future studies should further investigate the mechanisms of action of composted GL and NS in plant metabolism and the metabolism of soil microorganisms.

## CONCLUSION

GL and NS composted from industrial pharmaceutical extraction waste showed stimulatory effects on plant growth and bioactive productivity in *C. zanthorrhiza*. This indicated that GL and NS compost can potentially be used for sustainable organic agricultural practices for *C. zanthorrhiza* cultivation. Further research is needed to identify the mechanism of action of this compost and the effect it may have on plant metabolism and the metabolism of soil microorganisms.

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## DECLARATION OF CONFLICT OF INTERESTS

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## AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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