

# Allelopathic Effects of Aqueous Rhizome Extract of *Kaempferia galanga* on the Growth of Red Chilli (*Capsicum annuum*)

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**Abstract:** *Kaempferia galanga* L showed an allelopathic clues when intercropping planted with coconut plants, however aspects related to allelochemicals of this plant and their inhibitory or stimulatory properties against other crop plants has not been investigated in earnest. This research was conducted in order to determine whether the crude extracts of kacholam have allelopathic properties, either negative or positive, against red chillies. By using completely randomized design, 25 chilli plants were grouped into five consist of 5 plants each. Each plant was grown individually in a poly bag containing mixture of soil and compost in a ratio of 2:1. Group 1 is the chillies given 0% (v/v) kacholam extract as the control. Group 2, 3, 4 and 5 are the plants treated with kacholam rhizome extract at a concentration of 25%, 50%, 75% and 100% respectively. After being treated for one week the chillies are harvested and all the study parameters namely plant height, dry weight, and concentration of chlorophyll were assessed. The results showed, plant height of red chillies significantly suppressed by kacholam rhizome extract with a concentration of 50% or higher. The dry weight of red chillies was significantly decreased by treatment of kacholam rhizome extract in all level of concentration. However, crude water extract of kacholam plant rhizome showed no significant effects on the concentration of chlorophyll a as well as chlorophyll b. In conclusion, the crude extract kacholam plant rhizomes contains suppressive allelochemicals and, thus *Kaempferia galanga* is potential to be used as a negative, instead of positive, allelopathic crops.

**Keywords:** Kaccholam, Kencur, *Kaempferia galanga*, Allelopathy, Allelopathic Effect, Rhizome Extract, Red Chili, *Capsicum annuum*

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## 1. Introduction

Initially, allelopathy means the injurious effects of one plant upon the other. Later, the term is defined as both inhibitory and stimulatory effects of one plant on another plant. Today, the definition has been extended to as any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that, either positively or negatively, affect the growth and development of agricultural and biological systems (excluding animals) [1].

Given all plants produce secondary metabolites then each plant species has the potential to be allelopathic to each other, either negatively or positively. In modern agriculture paradigm where all dependence on synthetic herbicides and

other agricultural chemicals are encouraged to be reduced, then the integration of agriculture with allelopathy is the best choice [2]. One of the agricultural systems suitable for the sustainable agriculture paradigm where the allelopathy concept can be applied is an intercropping farming. By integrating intercropping agriculture with allelopathy, the ecological balance, utilization of resources, quantity and quality of products will increase while the damage by pests, diseases and weeds will decrease [3].

Although theoretically the integration of intercrop agriculture with allelopathy is reasonable, but in practice there are many aspects to be considered appropriately. The

scientific data about what type of plants, what type of allelochemicals that are produced, and what are their effects on certain crops for instance are still far from sufficient [4]

The allelopathic effects of an allelochemical, in fact, were not always the same at different concentrations and on different plant species. Some allelochemicals may promote growth at low concentration but suppress the growth if applied at high concentration. Allelopathic water extracts of sorghum, brassica, sunflower, rice, wheat, maize and moringa, for example, improve crop growth when applied at low concentrations [5]. Based on the facts mentioned above, the first crucial step to determine the allelopathic potential of a plant is by field observation to see if one plant shows inhibitory or stimulatory effects against another. There are several clues that can indicate that a species is negatively allelopathic, for example, plant species that are particularly aggressive in their interactions with other species [6]. The next step may be the laboratory screening of active ingredients from an allegedly allelopathic plant, followed by laboratory testing to investigate the specific effects of the allelochemicals on the growth and development of other plants [7].

*Kaempferia galanga* L., the scientific name of ekangi (Bengali's name) or kaccholam (Malayalam name), a herbaceous plant belong to Zingiberaceae family which in Indonesia is called kencur, is the best example of a putative allelopathic plant due to its allelopathic clues. This herb plant was known to be able to absorb more  $^{32}\text{P}$  when planted intercropping with coconut palm, *Vateria indica* L., *Ailanthus triphysa* (Dennst.) Alston. or *Grevillea robusta* A. Cunn. Moreover, the density of coconut palm roots on the kaccholam beds is lower than that of on other three intercropped plant beds [8].

Even though allelopathic clues of kaccholam was evident, but aspects related to allelochemicals of this plant and their inhibitory or stimulatory properties against other crop plants has not been investigated in earnest. In fact, kaccholam proven to contain a variety of phytochemicals which some of them are putatively allelopathic namely alkaloids, steroids, flavonoids, polyphenols, including ethyl-p-methoxy cinnamate, methylcinnamate, kaempferol, kaempferide, cinnamaldehyde [9], [10], [11].

This research was conducted in order to determine whether the crude extracts of kaccholam have allelopathic properties, either negative or positive, against other crops such as red chillies. Red chili (*Capsicum annuum* L) chosen as the recipient of the kaccholam allelochemicals was based on the fact that in Indonesia, both chillies and kaccholam are often planted side by side.

## 2. Materials and Methods

### 2.1. Kaccholam Plant Samples

The rhizome of *Kaempferia galanga* used in the study was obtained from kaccholam farmers in the district of Bandar Lampung.

### 2.2. Plant Extracts

Water rhizome extracts of kaccholam was prepared by grinding the fresh rhizome, about 100 grams, into powder form. The rhizome powder then soaked in 100ml distilled water for 24 hours at room temperature. The samples then filtered using Whatman No. 1 filter paper. Filtrate collected in erlenmeyer flask and is noted as a stock solution. The stock solution was diluted in accordance with the treatment concentrations designed for the experiment.

### 2.3. Plant Seedlings

The herb plant designed as the allelochemical recipient in this study is red chili (*Capsicum annuum* L). The chili seeds used are produced by East West Indonesia Ltd. The seeds were sown on a mixture media in the poly bags consisted of soil and compost in a ratio of 1:2. The compost applied in the study are the product of Trubus Mitra Swadaya Ltd. The chillies were allowed to grow for three weeks before being transferred to test media.

### 2.4. Experimental Design and Treatments

By using completely randomized design, 25 chilli seedlings were grouped into five consist of 5 plants each. Each chili seedling was grown individually in a poly bag containing freshly mixture of soil and compost in a ratio of 2:1. Group 1 is the chillies given 0% (v/v) kaccholam extract as the control. Group 2, 3, 4 and 5 are the plants treated with kaccholam rhizome extract at a concentration of 25%, 50%, 75% and 100% respectively. Application of kaccholam rhizome extract on tested plants is done by watering the growing medium with 20 ml of the extract. After being allowed to grow for one week the chillies are harvested and all the study parameters were assessed.

### 2.5. Study Parameters

The allelopathic effects of crude extract of kaccholam rhizomes on red chili plants were based on the parameters assessed one week after extract application. The study parameters are: plant height, dry weight, and concentration of chlorophyll a dan b.

- Plant height is the total length of the plant measured (using ruler meter) from soil surface level to highest peak of the crop
- Plant dry weight was determined by measuring plant weight after the samples were dried in an oven at 60°C
- Concentration of chlorophyll a dan b.

The concentration of chlorophyll a and b was determined by following Miazek [12] protocol as follows. The fresh leaves of red chilli with a weigh of 0.1g milled in a mortar and then dissolved in 95% ethanol. After filtration the chlorophyll contained in the filtrate measured spectrophotometrically using a UV-1800 UV-VIS Spectrophotometer from Shimadzu. The concentration of chlorophyll a and chlorophyll b in the ethanolic extract of chili leaves calculated using equations below:

$$\text{Chla} = 13.36.A664 - 5.19.A648 \quad (1)$$

$$\text{Chlb} = 27.43.A648 - 8.12.A664 \quad (2)$$

In the equation (1) and equation (2), A664 mean absorbance at wavelength 664 nm, while A648 mean absorbance at wavelength 648 nm. By multiplying the absorbance values by  $[v/(w \times 1000)]$ , where v is the volume of solvent and w is the weight of plant tissue, the concentration of chlorophylls (in mg/g plant tissue) are obtained.

**2.6. Statistical Analysis**

Study results were reported as mean±standard error (SE) and One-way analysis of variance (ANOVA) with LSD test was used to determine the significant differences between the means at the 5% level.

**3. Results and Discussion**

Effect of crude extract of the kaccholam plant rhizomes on

**Table 1.** Effect of crude rhizome extract of kaccholam (*Kaempferia galanga L.*) on the plant height of red chillies (*Capsicum annuum L.*)

Concentration of Extract	Plant Height (cm)					Mean ± SE
	1	2	3	4	5	
0%	10.5	11.5	10.4	10.9	11	10.86 ± 0.039 <sup>a</sup>
25%	10.5	8.5	9.5	11.1	10.6	10.04 ± 0.216 <sup>a</sup>
50%	9.3	8.5	9.5	9.7	8	9.00 ± 0.104 <sup>b</sup>
75%	8	7.5	7.4	6.9	7.5	7.46 ± 0.031 <sup>c</sup>
100%	5.4	5.1	6.2	6.5	6.3	5.90 ± 0.075 <sup>d</sup>

ANOVA of the data are as follows: F=42.899, P-value=0.000, Fcrit.= 2.886

LSD test results: The values of Mean ± SE followed by the same superscript are not significantly different at α = 5%

**Table 2.** Effect of crude rhizome extract of kaccholam (*Kaempferia galanga L.*) on the plant fresh weight of red chillies (*Capsicum annuum L.*)

Concentration of Extract	Fresh Weight (g)					Mean ± SE
	1	2	3	4	5	
0%	29,0	33.5	30,0	30.3	25.1	29.58 ± 1.82 <sup>a</sup>
25%	24,0	27.3	23.6	23.6	23.4	24.38 ± 0.54 <sup>b</sup>
50%	27.05	23.1	24,0	25.55	20.3	24.00 ± 1.31 <sup>b</sup>
75%	22.1	21.8	20.9	19.4	24.1	21.66 ± 0.59 <sup>bc</sup>
100%	23.8	21.35	18.85	12.2	14.8	18.20 ± 4.46 <sup>c</sup>

ANOVA results of the data are as follows: F=9.941, P-value =0.0001, Fcrit.= 2.886

LSD test results: The values of Mean ± SE followed by the same superscript are not significantly different at α = 5%

**Table 3.** Effect of crude rhizome extract of kaccholam (*Kaempferia galanga L.*) on the chlorophyll content of red chillies (*Capsicum annuum L.*)

Concentration of Extract	Concentration of Chlorophyll (mg/g plant tissue)		
	Chl a	Chl b	Total
0%	6.683	16.208	22.891
25%	6.539	15.932	22.471
50%	5.681	14.22	19.901
75%	5.633	13.472	19.105
100%	6.42	15.81	22.23
F	0.7949	0.9574	0.9962
P-value	0.5424	0.4521	0.4326
F-criterion	2.886	2.886	2.886

Based on the data presented in Tables 1, 2 and 3, it can be

the growth of red chillies are presented in Table 1. Based on the results of analysis of variance (ANOVA) and the LSD test at α=5% it is clear that kaccholam rhizome extract with a concentration of 50% or more very markedly suppressed the growth of red chillies. The growth parameters of red chillies after given the crude extract of kaccholam plant rhizome are presented in Table 2. Based on the results of ANOVA and the LSD test at α=5%, kaccholam rhizome extract in all level of concentrations significantly decrease the dry weight of red chili plants.

The possible effects of kaccholam plant rhizome against the chlorophyll content of red chili leaves has also determined and the results are presented in Table 3. Given all the F-values of ANOVA against chlorophyll a (0.7949), chlorophyll b (0.9574) and total chlorophyll (0.9962) are smaller than F-criterion (2.886), it can be affirmed that the content of chlorophyll in the leaves of red chilli is not affected by the crude extract of the kaccholam plant rhizome.

stated that the crude extract of the kaccholam plant rhizome significantly inhibit the growth of red chili plants, but exhibit no significant influence on its chlorophyll content. These findings seem a bit odd, because similar allelopathic studies that use the same parameters suggested that if an allelochemical compound inhibited the growth of a plant, it will also decrease the chlorophyll content [13].

The reasonable explanation to these contradictions is the aqueous extract of kaccholam rhizome do not contain a typical-chlorophyll allelochemical, or if any, the concentration is too low to affect chlorophyll metabolism. Indeed, as reported by Narasinga Rao and Kaladhar [14], rhizome extract of *Kaempferia galanga* contains numerous types of phytochemical including amino acids, protein,

carbohydrate, alkaloids, steroids, cholesterol, cardiac glycosides, flavonoids, saponins, tannins, terpenoids, phlobatannins, fatty acids, anthocyanins, leucoanthocyanins, coumarins, phenols, quinones, and emodins. However, such phytochemicals are extracted using different types solvent, namely chloroform, ethanol, ethyl acetate, and methanol. When only water is used, the phytochemicals obtained are unspecific compounds such as phenols, tannins, phlobatannins, coumarins, and cardiac glycosides. On the other hand, the findings reported by Yang et al [13] involve highly specific phenolic allelochemicals namely ferulic acid, o-hydroxyphenylacetic acid, and p-coumaric acid.

Despite the crude rhizome extracts of kaccholam plant showed no significant effect on chlorophyll content in red chillies, but the extracts evidently exhibit inhibitory effects on the plant growth. A similar study targeting red chillies as the recipient plants conducted by Sahoo et al. [15] using leaf extract of *Citrus reticulata* found germination inhibition of the recipient plants up to 55%. Previously, phytochemical screening against the leaves extract of *C. reticulata* has been done and resulted several secondary metabolites allegedly allelopathic such as flavonoids, tannins, steroids, and phenolic compound [16]. Aqueous extract of kaccholam rhizome, as indicated by Narasinga Rao and Kaladhar [14] in fact contains some of the compounds including tannins and phenols.

The inhibitory effects of the aqueous extract of kaccholam rhizome seem to assert that secondary metabolites of kaccholam plants might possess phytotoxic effects. The toxicity potential of plant extract of *K. galanga* even seen in insect, as indicated by Ghosh et al [17] that kaccholam extract was lethal to the mosquito larvae, scientifically named *Culex quinquefasciatus*, by an LC<sub>50</sub> value of 42.33 ppm. Alkaloids, coumarins, flavonoids, hydroxamic acids, phenolic acids, anthraquinone, saponins, tannin, steroids, terpenoids, glycosides, phlobatannins, carboxylic acid are among the plant secondary metabolites that known to phytotoxic [18], [19].

All the putative phytotoxic chemicals are potentially found in kaccholam, since the extract of whole plant parts known to contain ethyl-cinnamate, 1,8-cineole, delta 3 carene, (+) alpha pinene, (-) alpha pinene, camphene, borneol, cymene, alpha terpineol, alpha gurjunene, germacrene, cadinenes, beta-caryophyllen, ethyl-p-methoxycinnamate, kaempferol, kaempferide, and cinnamaldehyde [20]. Meanwhile, of the rhizomes are found phytochemicals such as 3-carene, camphene, borneol, cineol, kaempferol, kaempferide, cinnamaldehyde, p-methoxycinnamic acid, ethyl cinnamate and ethyl p-methoxycinnamate [21]. Aqueous extract of the rhizome, as indicated by Narasinga Rao and Kaladhar [14], contains amino acids, carbohydrates, alkaloids, cardiac glycosides, tannins, phlobatannins, coumarins, phenols. Phenolic acids, tannins, alkaloids and coumarins were proven to allelopathic against tomato plants [22] and maize [23].

Another assumption can be drawn from the research findings is that the water extract of kaccholam plant rhizomes is having negative properties, instead of positives, against the red chili plants. The condition may be due to the aqueous

rhizome extract of kaccholam does not contain allelochemicals that can promote the growth of the recipient plants. Or, if any, the type of the compound does not have typical-stimulatory properties and the concentration does not appropriate. As suggested by Farooq et al [5], the examples of positive allelopathic phytochemicals are sorgoleon, 5-ethoxysorgoleone, 2,5-dimethoxysorgoleone, phenolics such as vanillic acid, p-hydroxybenzoic acid, phydroxybenzaldehyde, p-coumaric acid, and ferulic acid. All these allelochemicals only exhibit growth promotive properties when used at lower concentration. To ascertain the allelopathic properties of kaccholam plants, whether positive or negative, then the in-depth investigation on the types and concentration levels of the plant secondary metabolites is very necessary.

## 4. Conclusion

Given red chili treated with aqueous extract of kaccholam showed the decrease in plant height and weight it can be concluded that the crude extract of kaccholam plant rhizomes containing suppressive allelochemicals. Thus, *Kaempferia galanga* is potential to be used as a negative, instead of positive, allelopathic crops.

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