Publiscience Vol. 5 Issue 1

Allelopathic effects of *Zingiber officinale* (ginger) leaf extracts on the germination of *Vigna radiata* (mung beans)

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Article Info

Submitted: June 02, 2022
Approved: Aug 30, 2022
Published: Sept 12, 2022

Keywords:
Zingiber officinale
leaf extracts
allelopathic effect
Vigna radiata
germination

Abstract

Allelopathy is a biological mechanism in which allelochemicals from one organism may influence the growth and development of other plants. It has been found that *Zingiber officinale* allelochemicals such as flavonoids, tannins, and phenols may influence the growth of *Vigna radiata*. This research investigated the allelopathic effects of *Zingiber officinale* leaf extracts on the seed growth of *Vigna radiata*. The *Zingiber officinale* leaf extracts 0.10 g/mL, 0.15 g/mL, and 0.20 g/mL were obtained by aqueous maceration of the sun-dried *Zingiber officinale* leaves and were applied to the seeds. Germination parameters were recorded every day for seven days. A significant difference was recorded between the germination percentage, germination rate, and inhibitory rate of the control and treatment groups. Results show that *Zingiber officinale* leaf extracts at 0.10-0.20 g/mL ratio inhibit the growth of *Vigna radiata* seeds.

Introduction. - The response of a plant to the presence of other plants and the factors that may influence a behavioral change in a plant is one of the topics of interest when learning plants' behaviors. This particular phenomenon is referred to as allelopathy. It is a biological mechanism in which biochemicals from an organism may influence the germination, growth, survival, and reproduction of other organisms [1].

Allelopathic effects of selected plants can be attributed to the presence of phenolic compounds such as flavonoids, tannins, and phenols, which can interfere with the activities of respiratory enzymes in seed germination and cause an inhibitory effect on its germination. This causes the alteration in the activities of the growth hormone Gibberellic acid which is responsible for the stimulation of seed germination [2]. Allelochemicals escape from plants in different ways such as leaching, volatilization, decomposition, and exudation [3].

Vigna radiata legume crops are prevalent in Southeast Asia and have varying uses, ranging from industrial to agricultural benefits. It is widely used as a food crop due to its high protein content [5]. The proteins and lipids are found to be high in the embryo, while the starch and crude fiber are concentrated in cotyledons and seed coats, respectively [6]. Moreover, *Vigna radiata* are reasonably priced in the market due to their relative ease of cultivation which may be caused by factors such as early maturity and resilience to drought, among others [7].

Allelopathic effects of some plants on *Vigna radiata* have already been conducted. These include the allelopathic effects of sorghum water extract which stimulated the growth of *Vigna radiata* due to the presence of phenolic compounds [8]. Another study was conducted by Hossain et al. [9], that utilized *Moringa oleifera* extracts that suppressed the growth yield parameters of *Vigna radiata*. On the other hand, *Vigna radiata* are also known to have allelopathic potential on other plants as explained by a study conducted by Nwoagu and Muogbu [4] in 2015.

Zingiber officinale is an important horticultural crop in tropical Southeast Asia. However, the main problem with *Zingiber officinale* culture is that *Zingiber officinale* is not suitable for continuous cropping and *Zingiber officinale* yields are low when this species is cultivated consecutively for years on the same land [10]. It is a heavy nutrient-demanding crop. Growing it with other crops is bound to exert some pressure on the nutrient pool of the soil.

Zingiber officinale leaf extracts exhibit inhibitory effects on the growth of other plants [11]. Stems and leaves of *Zingiber officinale* are known to exhibit stronger phytotoxicity, which adversely affects different growth parameters of soybean and chive [12]. Meanwhile, for other species such as sunflower and wild barley, it has been reported that allelochemicals from sunflower which inhibit the growth of wild barley at some concentrations might stimulate the growth of the wild barley at different concentrations [13]. Even if *Vigna radiata* have stimulatory effects on the growth of *Zingiber officinale* as reported by Nwaogu and Muogbo [4], the effect of *Zingiber officinale* on the growth of *Vigna radiata* has not yet been determined. With previous studies suggesting inhibitory effects of *Zingiber officinale* leaf extracts on other plants, the study hypothesize that *Zingiber officinale* leaf extracts will inhibit the growth of *Vigna radiata* .

How to cite this article:
CSE: Gemoto, JR, Inefable R, Nacionales, RH, Sinco, RA. 2022. The pH indicator capability of *Basella rubra* (alugbati) stem peels aqueous extract. Publiscience. *5*(1): 2–7.
APA: Gemoto, JR, Inefable R, Nacionales, RH, Sinco, RA. (2022). The pH indicator capability of *Basella rubra* (alugbati) stem peels aqueous extract. *Publiscience*, *5*(1), 2–7.

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The established notion that *Vigna radiata* was able to stimulate the growth of *Zingiber officinale* plants prompted the conduct of the study by determining the effects of *Zingiber officinale* to *Vigna radiata*. The results of this research would be able to help in assessing whether *Vigna radiata* and *Zingiber officinale* could be planted together. The allelopathic activity between the two plants would serve as a great research endeavor for future studies. Also, the study aimed to raise awareness on how to efficiently utilize crop wastes by learning their implications for the environment, particularly on other plants.

This research aimed to investigate the allelopathic effects of *Zingiber officinale* leaf extracts on the germination of *Vigna radiata* seeds. It specifically aimed to:

(i) determine the germination percentage, germination rate, mean germination time, and inhibitory rate of *Vigna radiata* seeds treated with different ratios of *Zingiber officinale* leaf extract (0.10 g/mL, 0.15 g/mL and 0.20 g/mL) and distilled water (negative control);

(ii) determine the optimal ratio of the *Zingiber officinale* extract that could either enhance or inhibit the growth of *Vigna radiata* seeds during germination;

(iii) identify whether *Zingiber officinale* leaf extract has a positive or negative allelopathic effect on *Vigna radiata* seeds; and

(iv) determine significant differences between the germination percentage, germination rate, mean germination time, and inhibitory rate of the different treatment and control groups.

Methods. *Zingiber officinale* leaf extract was prepared by drying, cutting, grinding, screening, and maceration of the leaves. Meanwhile, the *Vigna radiata* seeds were subjected to a float test to identify viable seeds and were then distributed to the Petri dishes. Distilled water and the *Zingiber officinale* leaf extracts were applied to the control and treatment groups, respectively. The germination parameters of the *Vigna radiata* seeds were then recorded daily for seven days. The gathered results were then subjected to data analysis using One-way ANOVA and Tukey-Kramer Test. Personal protective equipment was also utilized throughout the conduct of their study. The data gathering procedures lasted for approximately two to three weeks.

Obtaining and verifying plant samples. The two main components of the study namely, *Zingiber officinale* leaves and *Vigna radiata* seeds were acquired from a single farm and from the local market in Jaro, Iloilo respectively, and were verified by the Department of Agriculture.

Drying, grinding, and screening of Zingiber officinale leaves. The acquired *Zingiber officinale* leaves were washed properly with distilled water to remove the impurities. Afterwards, the washed leaves were sun-dried on a threshing floor or any flat land surface for seven days. Drying techniques make the structural cell more fragile and facilitate subsequent grinding [14]. The dried leaves were chopped into 1 cm long pieces. The chopped leaves were converted into powder form with the help of an electric grinder. The next step was the screening of the *Zingiber officinale* leaves using a 0.5 mm screen mesh to yield more homogenized particles for efficient extraction to occur because the solvent must make contact with the target analytes [15].

Maceration and filtration. For the making of the aqueous leaf extracts, the screened *Zingiber officinale* leaves were weighed using a digital weighing scale. Then, 21.50 grams, 32.25 grams, and 43.00 grams of *Zingiber officinale* leaves in glass bottles were soaked in 215 mL of distilled water each forming extract ratios of 0.10 g/mL, 0.15 g/mL and 0.20 g/mL respectively. Then, the glass bottles were sealed and kept in a refrigerator (4℃) for 24 hours. After 24 hours, the extracts were filtered using a funnel with two layers of cheesecloth followed by Whatman No. 1 filter paper.

Storage. The filtered leaf extracts were stored inside dark, glass bottles before it was applied to the *Vigna radiata* seeds and kept in the refrigerator at a low temperature to prevent bacteria from growing. During transport, it was stored in an icebox to maintain the low temperature.

Preparation of research set-up. Prior to the start of the experiment, *Vigna radiata* were subjected to a seed viability test via the seed float test. A total of 120 viable *Vigna radiata* seeds and 12 Petri dishes (9 cm diameter) were surface sterilized with water: bleach (10:1) solution. The surface-sterilized seeds were evenly distributed into 12 Petri dishes that each contain 10 *Vigna radiata* seeds.

An improvised germination chamber was created by using a box container made of ¼ plywood with dimensions 90 cm by 45 cm by 60 cm. The lid of the box was drilled with holes at the top side. A 20 watts-fluorescent lamp (60 cm length) was attached at the top and was used as the source of diffused light and heat for the *Vigna radiata* seeds. Additionally, a lux meter was placed inside to monitor the temperature and light inside the growth chamber.

Maintenance and application of Zingiber officinale leaf extract and distilled water. The germination test was carried out in sterile Petri dishes with a kitchen towel acting as a soil substitute for the *Vigna radiata*. Then, 10 mL of *Zingiber officinale* leaf extracts was administered only during the first day on each Petri dish. The *Vigna radiata* seeds were immersed in the *Zingiber officinale* leaf extracts for seven days. Then, 90 of the 120 seeds were treated with *Zingiber officinale* leaf extract; 30 seeds for each of the three treatment groups (0.1 g/mL, 0.15 g/mL, and 0.20 g/mL) of the leaf extracts. The remaining 30 seeds were treated with 10 mL of distilled water. The seeds were watered at around 7:00 in the morning to allow the water to run down into the roots without too much water loss due to evaporation [16]. Approximately 10 mL of distilled water was added everyday to each replicate of all control groups to moisten the sterilized seeds [2]. Then, the Petri dishes were kept in a growth chamber, maintaining an average temperature of 27.5℃ for 12 hours for seven days.

Determination of seed growth parameters. The morphological parameters of *Vigna radiata* were

determined every day for a span of seven days. These parameters include the following: germination percentage, germination rate, mean germination time, and inhibitory rate of *Vigna radiata* seeds [17].

$$Germination\ Percentage = \frac{total\ number\ of\ seeds\ germinated}{total\ number\ of\ seeds\ in\ the\ test} \times 100$$

$$Mean\ Germination\ Time = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

$$Germination\ Rate = \frac{1}{MGT}$$

$$Inhibitory\ Rate = 100 - \frac{E_2 \times 100}{E_1}$$

where:
E_2=germinated treatment plants
E_1=germinated controlled plants
t_i=time from the start of the experiment to the ith day
n_i=number of seeds germinated in the ith time
k: last time of germination

Data Analysis. The data collected were analyzed using Microsoft Excel's Data Analysis function. The parameters were obtained by tabulating the mean of the replicates of the control and experimental groups and analyzing the means of these four groups. To further examine the results of the study, the gathered parameters in the three treatment groups were compared with the parameters obtained in the control group. Thus, one-way ANOVA with a significance level of $p \leq 0.05$ was used to examine the data. The computation for these values was done in Microsoft Excel 2019. For the post-hoc analysis tool, the Tukey-Kramer Test from Real Statistics add-in in Microsoft Excel 2019 was used to take into account unequal plant samples because some seeds died along the process.

Safety Procedure. The personal protective equipment (PPE) were worn which includes the following: laboratory gown, eye shield, surgical mask, gloves, and other protective equipment deemed necessary in the conduct of the research study. Long sleeves and long pants were also worn for additional protection. Disinfectant spray was done before and after working on the experimental area to prevent contamination.

Results and Discussion. - During the seven days of germination, there was a significant difference in terms of the germination percentage, germination rate, and inhibitory rate of the control and treatment groups. However, no significant difference in the mean germination time of the treatment and control group was found.

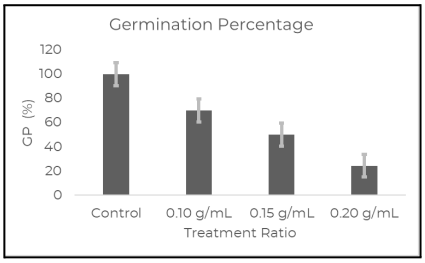
The 0.1 g/mL extract exhibited the least negative allelopathic effect of the leaf extract at 70% germination percentage while the highest negative allelopathic effect was observed at 0.20 g/mL extract with a 24.4% germination percentage.

Table 1. The Germination Percentage (GP), Mean Germination Time (MGT), Germination Rate (GR), and Inhibitory Rate (IR) of *Vigna radiata* seeds in the control and treatment groups.

Parameters	Different Concentrations of *Zingiber officinale* Leaf extract (g/mL)			
	Control	0.10	0.15	0.20
GP (%)	100.00 ±9.51	70.00 ±9.51	50.00 ±9.51	24.44 ±9.51
MGT (days)	1.79 ±0.26	1.88 ±0.26	1.93 ±0.26	1.37 ±0.40
GR (seeds/day)	0.61 ±0.08	0.68 ±0.08	0.58 ±0.08	0.15 ±0.11
IR (%)	0.00 ±8.20	31.11 ±9.30	48.89 ±9.30	75.56 ±8.20

Notes. Sample size = 30 seeds

A.1. Germination parameters from control and treatment groups. Of the three treatment groups, the 0.1 g/mL leaf extract showed 70% germination percentage. The lowest germination percentage among the treatment groups was recorded in the 0.20 g/mL extract with 24.4% germination percentage. Meanwhile, the control group had a germination percentage of 100%. As for its mean germination time, the 0.15 g/mL extract exhibited the highest mean germination time among all treatment groups with a value of 1.93 days. Meanwhile, the 0.20 g/mL extract has the lowest mean germination time value of 1.37 days. Additionally, the control group recorded a mean germination time value of 1.79 days. Following this, the 0.1 g/mL extract showed the highest germination rate among the three concentrations, with a value of 0.68. While the 0.20 g/mL extract showed the least germination rate with a value of 0.15. Meanwhile, the control group had a germination rate of 0.61. For the inhibitory rate, treatment 0.20 g/mL extract showed the highest inhibitory rate among all treatment groups with a value of 75.56 while treatment 0.1 g/mL extract had the lowest inhibitory rate with a value of 31.11. The results show a decreasing trend for the germination percentage and germination rate as the amount of the *Zingiber officinale* leaf extracts increased. While the trend of the inhibitory rate increased as the amount of the *Zingiber officinale* leaf extracts also increased.



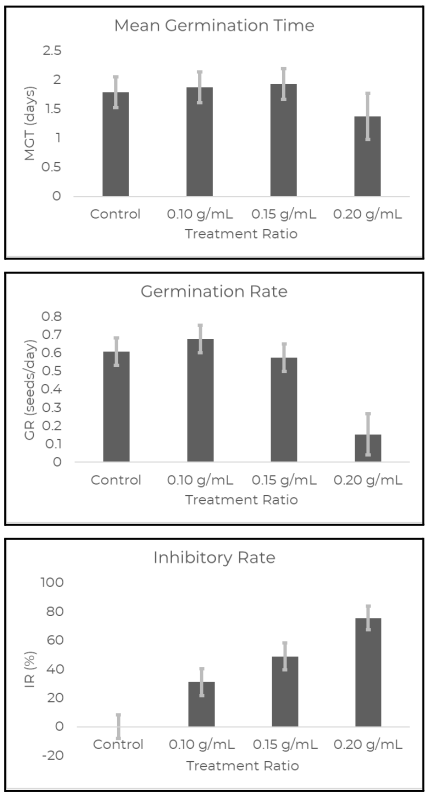


Figure 1. Germination percentage, mean germination time, germination rate, and inhibitory rate of *Vigna radiata* seeds (n=30) in different extract ratios 0.10 g/ml, 0.15 g/ml and 0.20 g/ml with significance level of $p \leq 0.05$.

These results are in line with the principle that allelochemicals that inhibit the growth of some plant species at certain concentrations might stimulate the growth of the same or different plant species at different concentrations [13]. For instance, the shoot extracts of cannabis in high concentrations was reported to have an inhibitory effect on the germination indices while root extracts had no statistically significant effect on the germination of lettuce seeds [18]. Additionally, phenolic compounds, one of the allelochemicals found in *Zingiber officinale* leaves, are reported to have stimulatory effects on seed germination and seedling growth of plants at low concentrations but resulted in a decrease in germination parameters at higher concentrations [18]. Also, some *Vigna radiata* seeds in the treatment with 0.10 g/mL extract (21.5 g/215 mL) were able to germinate for a few days and slowly disintegrated. This might be due to the direct contact of the root with the inhibitory chemicals as described with various crops and weeds [2]. Hence, roots are generally the first point of contact with chemical compounds which explains that any abnormal growth in the root is an obvious sign of chemical toxicity by allelopathic plants [12]. Similar observations were reported by [19] wherein it was discovered that the effect of allelochemicals in *E. coccinea* may have led to the metabolic impairment of *Vigna radiata*. Thus, the formation of the natural root was significantly affected.

A.2. Optimal ratio of the Zingiber officinale leaf extract. The least negative allelopathic effect of the seed extract was observed in the 0.1 g/mL extract while the highest negative allelopathic effect was observed in the 0.20 g/mL extract. As such, the 0.20 g/mL extract is the optimal ratio of the extract among the three treatment groups which would inhibit the growth of *Vigna radiata* seeds because it has the highest inhibitory rate. Given as well as there exists a significant difference between the control and treatment groups in the germination percentage as well as the inhibitory rate.

Allelopathy is often due to the synergistic activity of allelochemicals rather than single compounds. Thus, the response of the *Vigna radiata* seeds to the *Zingiber officinale* leaf extracts may be attributed to the reaction of the various allelochemicals in the leaf extracts among each other and their implications to the growth of the *Vigna radiata* [18]. The increase in the amount of these allelochemicals increased the allelopathic activity in the *Vigna radiata*. As such, treatment 0.20 g/mL extract (43. 00 g/ 215 mL), which contained more *Zingiber officinale*-leaf-powder: water ratio, caused more negative allelopathic effects on the *Vigna radiata* seeds.

A.3. The allelopathic effect of the Zingiber officinale leaf extract. The application of *Zingiber officinale* leaf extracts 0.1 g/mL, 0.15 g/mL and 0.2 g/mL on *Vigna radiata* caused a significant decrease in the germination percentage, and germination rate of the *Vigna radiata* seeds. These results indicated a negative allelopathic effect on the growth of *Vigna radiata* seeds. The *Zingiber officinale* leaf extracts in all three treatment groups showed negative allelopathic effects on the germination of *Vigna radiata* seeds in contrast to the control group, and no extracts have stimulatory effects on the growth of the seeds.

Zingiber officinale leaf extracts have previously shown inhibitory effects on the growth of other plants [11]. Stems and leaves of *Zingiber officinale* are known to exhibit stronger phytotoxicity, which adversely affects seed germination, seedling growth, water uptake, and lipase activity of soybean and chive [12]. Moreover, *Vigna radiata* in the study were at germination stage supporting its sensitive response to the concentration of bioactive compounds in their surroundings [19].

A.4. Significance of the growth parameters. The p-values of the germination percentage, germination rate, and inhibitory rate are 3.28×10^{-5}, 2.31×10^{-5}, and 3.07×10^{-6}, respectively, which are less than or equal to 0.05 showing a significant difference among the treatment groups. Meanwhile, the mean germination time does not have a significant difference present as it has a p-value of 6.96×10^{-1}.

By conducting the Tukey-Kramer test, the inhibitory rate showed a significant difference between the control group and all the treatment groups. For the germination percentage, significant results are found between the control and treatment 0.15 g/mL extract results, as well as between the control and treatment 0.20 g/mL extract results. Finally, for the germination rate, there are significant results between the control and treatment 0.20 g/mL extract groups as well as with the 0.1 g/mL extract and 0.20 g/mL extract .

Limitations. The study is limited to observing whether *Zingiber officinale* leaf extracts inhibited or

promoted the growth of *Vigna radiata* through the observance of germination parameters namely inhibitory rate, germination percentage, mean germination time, and germination rate.

Additionally, the results of the data analysis may be subject to error due to uneven sample size caused by the death of some *Vigna radiata* seeds while some ceased to grow after the fifth day of the data gathering. This brings several concerns that were addressed, such as making a germination chamber to cater to the growth of *Vigna radiata*. However, the effects of other *Zingiber officinale* extract ratios on the growth of *Vigna radiata* seeds could not be determined. Thus, the gathered result about the optimal ratio of treatment provides limited knowledge because the extract ratios used in the study were limited to 0.1 g/mL, 0.15 g/mL and 0.20 g/mL *Zingiber officinale* leaf extracts. Due to the unavailability of laboratory equipment, other parameters were not controlled, specifically, the moisture content of *Vigna radiata*. Furthermore, the study was not able to conduct phytochemical analysis. Thus, the specific allelochemicals found on the *Zingiber officinale* leaves were not determined.

Conclusion. - The results showed that the growth of *Vigna radiata* seeds during germination revealed that germination rate, germination percentage, and mean germination time were suppressed in treatments containing different ratios of *Zingiber officinale* leaf extracts. Germination percentage, germination rate, and inhibitory rate of *Vigna radiata* seeds between and among the control groups and treatment groups showed significant differences. The 0.20 g/mL extract showed the highest inhibitory rate. Thus, treatment 0.20 g/mL extract is considered the optimal ratio of extract that could inhibit the growth of *Vigna radiata* seeds. It can be concluded that *Zingiber officinale* leaf extracts have a negative allelopathic effect because it was able to inhibit the growth of *Vigna radiata* seeds. Thus, *Zingiber officinale* should not be co-planted with *Vigna radiata* seeds.

Recommendations. - It is recommended that a larger sample size is also recommended to obtain an accurate and discrete significance difference. The study could also be extended throughout the life cycle of *Vigna radiata* to gather data about other parameters such as morphological features. It is also recommended that future studies explore other treatment ratios for the study. The experiment may also be replicated in laboratory and field conditions that are ideal for the growth of *Vigna radiata* seeds in contrast to the improvised germination chamber used in the study. Also, further tests for the *Zingiber officinale leaf* extracts are recommended such as the conduct of phytochemical screening to determine the relative abundance of allelochemicals in *Zingiber officinale*.

Acknowledgment. - Gratitude is extended to the Department of Agriculture of Janiuay, Iloilo for assisting in the authentication of the *Vigna radiata* and *Zingiber officinale* variety used in the study. This gratitude is also extended to the local farmers of Miagao, Iloilo for providing the *Zingiber officinale* leaves and Jaro, Iloilo for providing the *Vigna radiata* seeds used in the study and our statistician for assisting in the data analysis process.

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