



## Allelopathic Effect of Aqueous Papaya Seed Solutions on Seed Germination and Seedling Growth of Mung Bean and Wheat

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### Abstract

The widespread use of synthetic herbicides in agriculture has raised significant environmental and health concerns causing the urgent need for sustainable alternatives. Papaya (*Carica papaya* L.) seeds, typically regarded as agricultural industry waste, are a rich source of bioactive compounds with potential allelopathic properties. This study investigates the effects of aqueous papaya seed solutions (PSS) on seed germination and seedling growth in mung bean (*Vigna radiata*) and wheat (*Triticum aestivum*), with the objective of assessing their potential as natural herbicidal agents for sustainable weed management. PSS were prepared by finely grinding papaya seeds along with the pulp of ripe papaya fruits to obtain a homogeneous mixture. This mixture was then diluted with distilled water to prepare aqueous solutions at concentrations of 25%, 50%, and 75% (w/v), with distilled water alone (0%) as the control. The results indicated that PSS at concentrations of 25% significantly reduced both germination percentage (GP) and germination rate index (GRI) by more than 50% compared to the control. Growth parameters for both plant species were also significantly inhibited at this concentration. In contrast, mean germination time (MGT) and coefficient of velocity of germination (CVG) remained statistically unchanged even at 75% PSS concentration. Mitotic index were also inhibited in a dose-dependent manner, with the most pronounced effects at 50% PSS. These findings suggest that aqueous papaya seed solutions exhibit allelopathic effects and have the potential to serve as sustainable bioherbicides. Their application offers dual benefits: reducing agro-industrial waste and minimizing reliance on chemical herbicides in agricultural practices.

## Introduction

The current trend in agriculture is to find a biological solution to minimize the perceived hazardous impacts of herbicides and insecticides in agricultural production. Many plants produce secondary metabolites that are harmful to other species and help to reduce competition in their natural habitat (Kostina-Bednarz et al., 2023). Chenyin et al. (2023) reported that seeds are the foundation of agroforestry, the beginning of plant growth and development, and also the key link in their life cycle. However, several environmental factors, including temperature changes, oxygen levels, the availability of water, and the absence of physical barriers, significantly influence seed germination (Othman et al., 2023). In the interest of eventually exploiting these chemicals for herbicide use, the present search for natural germination inhibitors was undertaken. Plant-based herbicides offer the potential to reduce reliance on synthetic herbicides, thereby minimizing environmental contamination and alleviating associated human health concerns. These natural compounds have been shown to affect seed germination percentages, as well as the fresh and dry weights of seedlings across various plant species (Cerdeira et al., 2012). Allelopathy is a natural technique which is environmentally friendly. This is one of potential approaches to weed management that may enhance crop productivity (Anwar & Qureshi, 2021). Allelopathic active chemicals may offer innovative chemistry for synthesizing herbicides, insecticides, nematicides and fungicides (Othman et al., 2023). Alkaloids, benzoic and cinnamic acids, glucosinolates, flavonoids, phenolics and terpenes are the most widely available allelochemicals (Anwar & Qureshi, 2021). Glucosinolate chemistry has the potential use for the management of insect pests and plant pathogens (Wolf et al., 1984; Eampracha et al., 2015; Anwar & Qureshi, 2021).

Papaya (*Carica papaya* L.) is a healthy nutritious and flavorful tropical fruit belonging to Caricaceae of Brassicales and is widely cultivated in tropical and subtropical regions (Ma et al., 2021). It is grown both commercially and in the home garden. All parts of papaya including leaf, root, bark, peel, pulp and seed have biological activities such as antiviral, anticancer, antibacterial, antifungal, anti-inflammatory, and antioxidant. Papaya fruit contains a diverse range of amino acids and trace elements (Vij & Prashar, 2015). The leaf extract of eucalyptus has an inhibitory effect on

seed germination and seedling growth of weeds (Ben Ghnaya et al., 2016). Similarly, the bioherbicidal potential of *C. papaya* leaves revealed significant growth inhibition of *Avena fatua* seedlings, while showing no significant effect on the germination of wheat seeds (Anwar et al., 2020). During processing, approximately 300 g/kg of the weight of papaya fruits is discarded as waste, with papaya seeds comprising a significant portion of this byproduct. To improve the overall utilization of papaya, it is worthwhile to explore the potential applications of these otherwise discarded seeds (Lee et al., 2011). As mentioned, there is currently a trend towards searching for new natural plant products to discover and develop new bioherbicides friendly to the environment, papaya seeds represent a promising natural resource for such investigations.

The papaya seed contains various chemical constituents. The seeds and the pulp of *C. papaya* contain benzyl glucosinolate which can be hydrolyzed by myrosinase to produce benzyl isothiocyanate (BITC) (Kumar & Davi, 2017; Ma et al., 2021). Extracts from the sarcotesta, the fleshy seed coat of papaya, and the seeds themselves have been found to contain germination and growth inhibitors that prevent the germination of lettuce seeds (Gherardi & Valio, 1976; Chow & Lin, 1991). The seed is a sensitive plant organ to such bioactive chemical compounds that affects its hypocotyl and epicotyl emergence. The allelopathic effects on seed germination are influenced by several factors, including the types and concentrations of allelochemicals, the species of recipient plants, and prevailing environmental conditions (Ramadan et al., 2018). Mung bean (*Vigna radiata* (L.) R. Wilczek) and wheat (*Triticum aestivum* L.) are agriculturally significant crops with distinct roles: mung bean contributes to nutritional diversity, soil enrichment, and culinary applications, whereas wheat plays a central role in global food security, economic sustainability, and serves as a dietary staple. This study aimed to compare the allelopathic potential of ripe papaya (*Carica papaya* L.) seed and pulp extracts on the germination of mung bean and wheat seeds. The objective was to evaluate their potential as natural, low-cost alternatives to synthetic herbicides, offering an accessible solution for farmers in the region.

## Materials and methods

### 1. Preparation of papaya seed solution

Fresh seeds of ripe papaya (*Carica papaya* L., cultivar:

Holland, the most popular variety in Thailand) were collected from the Thai fruit carts in Pathum Thani, Thailand. The papaya samples were thoroughly washed three times by clean water followed by rinsing once with sterile distilled water (Fig. 1A). Papaya seeds with adhering pulp were ground using an electric grinder (HR2115/02, Philips, Thailand) at medium speed for 1 min to ensure uniform particle size for solution preparation and then transferred to a clean conical flask and stored at 4°C for investigation (Fig. 1B). The sample served as a stock solution of 100% concentration. By subsequent dilution with distilled water of 25, 50 and 75% concentrations were prepared and distilled water was used as control (0%) during the experiment.



**Fig. 1** Preparation of papaya seed solution: (A) Papaya seeds with pulp obtained from ripe papaya fruit; (B) Ground papaya seeds with pulp used for solution preparation

## 2. Seed materials

Mung beans (*Vigna radiata* (L.) R. Wilczek: Ratip) and wheat (*Triticum aestivum* L.: Chia Tai) used in this study were obtained from the local market and soaked in distilled water for 3 h prior to testing. Mung bean is a representative of dicot plant belonging to the legume family (Fabaceae), while wheat is a representative of monocot plant belonging to the grass family (Poaceae). These 2 species serve as model plants in preliminary experiments aimed at identifying substances with potential herbicidal activity for future applications in weed management. Healthy and uniform seeds, selected based on shape and size, were stored at 4°C until use. Both seed types were soaked in distilled water for 3 h prior to testing. The experiment was conducted in the laboratory of Biotechnology Program, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani.

## 3. Data collection and observation

To measure germination percentage, mung bean and wheat seeds were soaked in papaya seed solutions (PSS) at concentrations of 0% (control), 25%, 50%, 75%, and

100%. One sheet of germination paper was placed in each of 5 plastic boxes (13.5 × 13.5 × 9.5 cm), and 20 healthy seeds of uniform size were placed in each box. Each set of seeds was treated with 10 mL of the respective extract, with 5 replicates per treatment. Germination was conducted at room temperature under a 16/8-h light/dark cycle. Distilled water was applied daily to maintain adequate moisture levels. The highest concentration of papaya seed solution (100% PSS) was excluded from the germination assay, as its viscosity prevented uniform distribution across the germination surface. Seed germination was recorded daily for seven days post-sowing to calculate the final germination percentage.

### 3.1 Germination parameters

The germination percentage (GP) refers to the ratio of viable seeds that successfully grow into plants under ideal growing conditions. Germination was considered complete when the seeds exhibited a radical emergence size of  $\geq 2$  mm (International Seed Testing Association; ISTA, 2015). The growth and germination parameters were then recorded. The mean germination time (MGT) refers to the total number of seeds that germinated each day and was included in the computation of seed germination speed. The germination rate index (GRI) illustrates a seed's capacity to control its degree of activity and performance throughout germination and emergence. Co-efficient of velocity of germination (CVG) characterizes the rate at which seeds emerge seedlings during an experimentation. The value of this (index) will increase with rise in the number of emerged seedlings. For the different concentration treatments applied to evaluate the seed germination parameters were analyzed according to Ullah et al. (2023) as follows:

$$\text{Germination percentage (GP)} = \frac{\text{final number of seeds germinated}}{\text{total number of initial seeds}} \times 100 \quad \text{Eq.1}$$

$$\text{Mean germination time (MGT)} = \frac{\sum(N_i T_i)}{\sum N_i} \quad \text{Eq.2}$$

$$\text{Germination rate index (GRI)} = \frac{\sum(N_i)}{i} \quad \text{Eq.3}$$

$$\text{Co-efficient velocity germination (CVG)} = \frac{\sum(N_i) \times \sum(N_i T_i)}{100} \quad \text{Eq.4}$$

$N_i$  = number of seeds germinated on day  $i$   
 $T_i$  = number of days from sowing on day  $i$

### 3.2 Growth parameters

Root and shoot length were determined by uprooting 5 randomly selected seedlings in cm. The

measurements were recorded at 5 days after sowing (DAS) (Ghimire et al., 2023). The length of the shoot was recorded at the end of the trial as suggested by Ullah et al. (2023) seed vigor index (SVI) was calculated by Eq.5 as shown below.

Seed vigor index (SVI) = seedling length (cm) x Germination percentage Eq.5

### 3.3 Determination of mitotic index

The outer dead scales of *Allium cepa* bulb were removed without damaging the root primordia to promote the growth of new roots. The bulbs were grown for 3 days in distilled water, the roots grew up to a length of 2–4 cm and were ready to treat with papaya seed solutions (PSS) treatment at concentrations of 0% (control), 25%, 50% and 75%. The root tips were treated for 2 days and were collected immersed in freshly prepared Carnoy's solution (3:1 v/v, ethanol: glacial acetic acid). Then, about 2-3 mm of the root tips were placed in 1 N HCl for 5 min, macerated with a dissecting needle on a glass slide and allowed to stain in 2% aceto-orcein for 5 min at room temperature. The root tips were squashed down with strong vertical pressure, using the thumb after covering it with a coverslip and paper towel. The pressure was applied to squash the root tip into a single cell layer (Ahmad et al., 2022). Finally, in each radicle tip the mitotic and total cells were counted in 5 fields (400-500 cells) using light microscope to determine the mitotic index (Eq.6).

$$\text{Mitotic index} = \frac{\text{number of dividing cells}}{\text{number of total cells}} \times 100 \quad \text{Eq. 6}$$

### 4. Data analysis

All data were analyzed statistically by analysis of variance using SPSS software (Version 25) and Microsoft Excel 2019. The data was assembled to obtain an average value and analyzed statistically. The factorial experiments were carried out using a randomized complete design with three replications for germination parameters and using a randomized complete design with five replications for mitotic index and growth parameters. Mean comparisons were conducted using Duncan's multiple range test, following a one-way ANOVA, with significance determined at  $p < 0.05$ .

## Results and discussion

Germination parameters are essential for evaluating seed-to-seedling conversion, assessing seed lot quality, and guiding seed pretreatment and nursery management to achieve high germination rates (El-Kassaby et al., 2008). The results for the germination parameters of the mung beans and wheat seeds were evaluated at different concentrations of papaya seed solutions (PSS) for their germination (Fig. 2 and 3). Both mung beans and wheat exhibited similar germination parameters when treated with different concentrations of aqueous papaya seed. The ANOVA results showed significant differences among the germination percentage (GP) and germination rate index (GRI), however, the mean germination time (MGT) and co-efficient velocity germination (CVG) of both plant seeds at 0-25% PSS showed no significant differences. Among the different concentrations of PSS, utilizing a concentration of only 25% led to a reduction of more than 50% in both GP

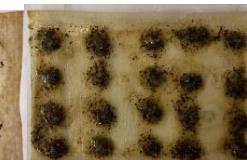
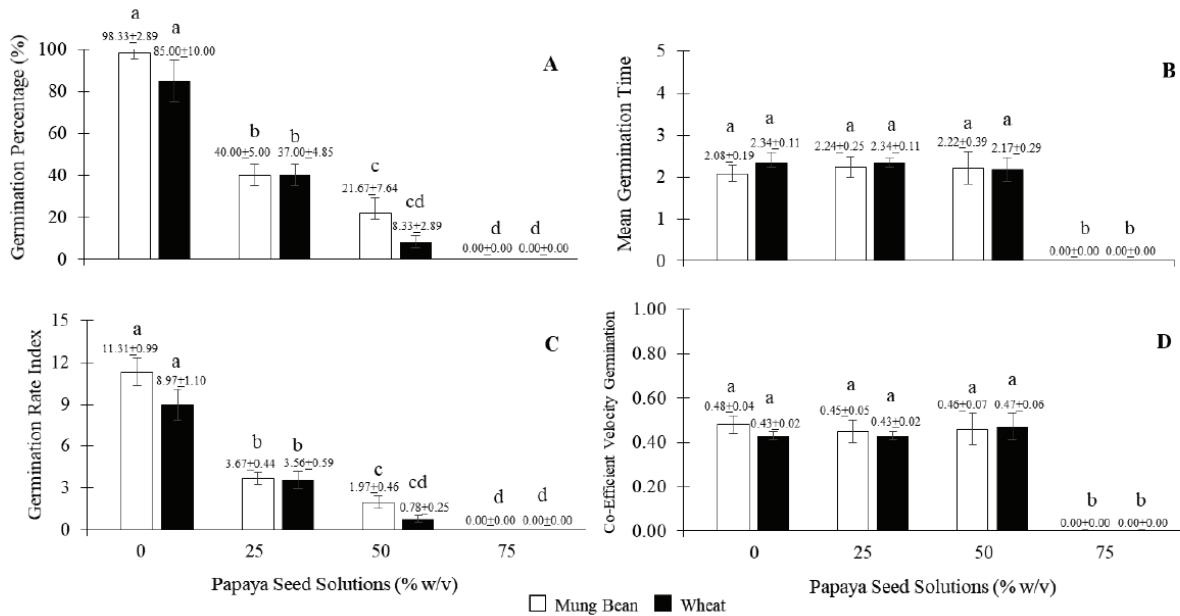
Plant	Papaya seed concentration (% w/v)			
	0	25	50	75
Mung bean				
Wheat				

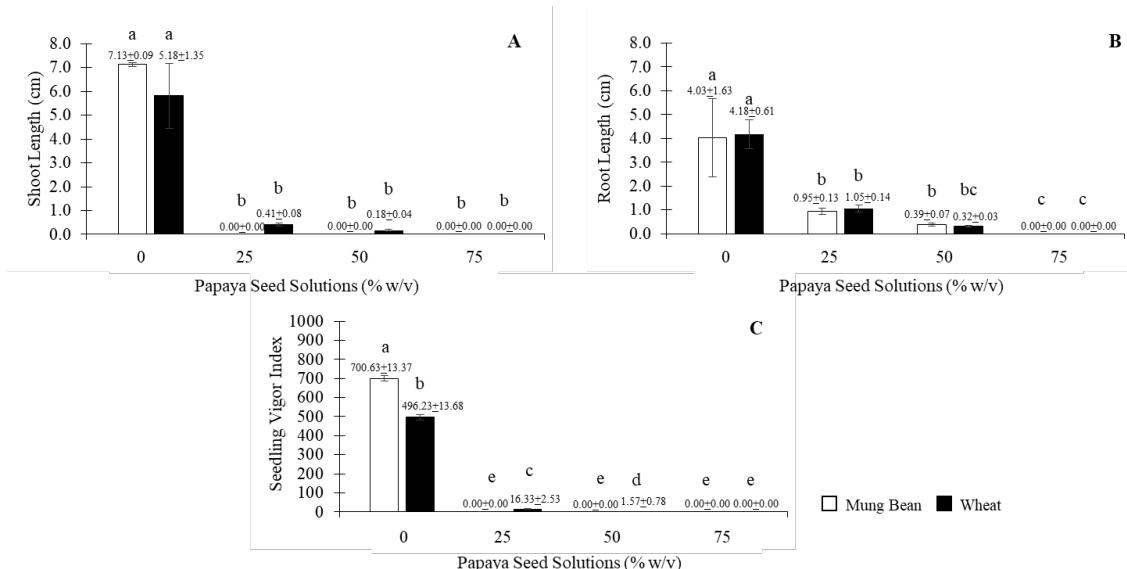
Fig. 2 Effect of different concentrations of papaya seed solutions on mung bean and wheat germination

and GRI levels when compared to the control treatment. The study found that MGT and the CVG did not differ significantly across PSS concentrations up to 50% for both mung bean and wheat. This lack of significant variation suggests that, although higher PSS concentrations markedly reduced overall germination percentage and

seedling growth, the seeds that did germinate did so within a timeframe and at a rate comparable to the control. These results indicate that papaya seed extracts primarily influence the proportion of seeds capable of germinating, rather than affecting the germination speed of viable seeds.



**Fig. 3** The effect on different concentrations of papaya seed solutions (% w/v) on germination percentage (GP), mean germination time (MGT), germination rate index (GRI) and co-efficient velocity germination (CVG). Values were the means ( $\pm$ SE) of 5 independent replications with 20 seeds for each replication. Means followed by different letters in the same graph are significantly different at  $p < 0.05$ , according to the Duncan's multiple range test.



**Fig. 4** The effect on different concentrations of papaya seed solutions (% w/v) on shoot length, root length and seedling vigor index. Values were the means ( $\pm$ SE) of 5 independent replications with 20 seeds for each replication. Means followed by different letters in the same graph are significantly different at  $p < 0.05$ , according to the Duncan's multiple range test.

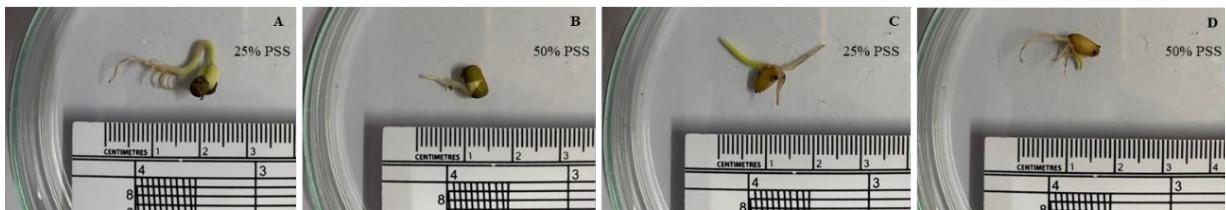


Fig. 5 Morphology of shoots and roots of mung bean (A and B) and wheat (C and D) treated with 25% and 50% papaya seed solutions

The current study's experimental findings showed that different papaya seed concentrations inhibited the germination of mung bean and wheat and also inhibited the growth of both (Fig. 4). The data indicate that increasing concentrations of papaya seed solution (PSS) significantly inhibited growth parameters in both mung bean and wheat. At 25% PSS (Fig. 5A and 5C), shoot length, root length, and seedling vigor index were markedly reduced compared to the control. Complete growth inhibition was observed in mung bean at concentrations of 50% (Fig. 5B and 5D). The wheat exhibited near-complete inhibition at 75% PSS.

The papaya is in the order Brassicales and the family Caricaceae (Aryal & Ming, 2014). Papaya seeds were found to have a considerable amount of benzyl isothiocyanate (BITC), which is formed from benzyl glucosinolate catalyzing myrosinase present in separate compartments in the seeds (Lee et al., 2011). These biochemical compounds from *C. papaya* along with related compounds such as phenethyl isothiocyanate and allyl isothiocyanate, are known to act as germination inhibitors. Additionally, glucosinolates were broken down in the soil and give rise to isothiocyanates, highly toxic to various organisms, including common crop pests and pathogens (Ghidoli et al., 2023). Wolf et al. (1984) reported that BITC, extracted from mature papaya seeds, was capable of inhibiting the germination of velvetleaf (*Abutilon theophrasti*), Corn (*Zea mays*) and soybeans (*Glycine max*). These inhibitory effects may result from reductions in cell size and cell division (Anwar & Qureshi, 2021). The present study also observed similar results, exposure of *Allium cepa* root tip cells to PSS induced a strong dose-dependent cytotoxic effect, significantly suppressing mitotic activity, as illustrated in Table 1. At 25% PSS, the mitotic index decreased by 63.8% compared to the control. Higher concentrations intensified the inhibition, with no significant difference between 50% and 75% PSS, which reduced the mitotic index by 84.6% and 88.6%, respectively. This marked inhibition of cell division likely explains the considerable

reductions in root and shoot growth observed in mung bean and wheat seedlings exposed to PSS. Since cell division in the root meristem is essential for root elongation and overall seedling development, the suppression of mitotic activity by allelochemicals in papaya seed extracts impairs normal growth processes. Consequently, the decreased mitotic index correlates with the inhibited root and shoot elongation, reduced seedling vigor, and overall stunted growth in both crop species, confirming the allelopathic potential of papaya seed extracts as natural growth suppressants.

As mentioned, papaya seeds and pulp contain bioactive compounds such as BITC, phenolic acids, and flavonoids, which have documented phytotoxic and allelopathic effects that inhibit seed germination, seedling growth in various species. Additionally, this biochemical, a sulfur-containing compound abundant in papaya seeds, disrupts cell division and elongation, leading to growth inhibition (Wolf et al., 1984; Salomão & Mundim, 2020). This observation aligns with the findings of Kostina-Bednarz et al. (2023), who suggested that the inhibition of root and shoot elongation is a key indicator of allelopathic activity in the environment. Similarly, Ramos García et al. (2012) identified allyl isothiocyanate (AITC) as one of the key compounds isolated from papaya. Exposure to AITC prompts cells to enter the cell cycle and initiate DNA replication. However, the rate of DNA synthesis is reduced, leading to an increase in S-phase cell populations and delayed seedling growth. Furthermore, cell cycle arrest has been previously linked to plant defense responses (Åsberg et al., 2015).

Table 1 Mitotic index of *Allium cepa* subjected to different concentrations of papaya seed solutions (% w/v)

Papaya seed concentration (% w/v)	Mitotic index
0.00	32.50±3.52 <sup>a</sup>
25.0	11.76±1.53 <sup>b</sup>
50.0	5.00±0.28 <sup>c</sup>
75.0	3.70±0.14 <sup>c</sup>

**Remark:** Values are means (±SE) of 5 independent replications with 20 seeds for each replication, and different letters within the same row differ significantly at  $p \leq 0.05$ .

## Conclusion

This study evaluated the impact of different papaya seed concentrations on germination and growth parameters in mung bean and wheat. At low concentration (25%) of papaya seed solution significantly inhibited key germination and growth parameters in mung bean and wheat, including germination percentage, germination rate index, shoot and root length, seedling vigor index, and mitotic index, by more than 50% compared to control treatments. In addition, the results demonstrate that papaya seed extracts progressively disrupt cell division, with near-complete mitotic arrest at concentration of 25%. These results confirm the potent allelopathic effects of papaya seed extracts, highlighting their potential as natural bioherbicides. These findings underscore the importance of further research and application of allelopathy to advance sustainable farming practices.

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