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Phytotoxic Effect of Aqueous Extract of *Lantana Camara* L. Leaves on Germination and Early Growth of Rice (*Oryza sativa* L. cv. Cempo Merah)

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Abstract

Background: The use of aqueous extracts from certain plants in controlling weeds is gaining attention in eco-friendly agriculture due to their allelopathic activity. One of the plants that has been investigated as a potential bioherbicide for controlling various weeds is *Lantana camara* L. Its extract has been tested on several non-targeted plants and has been shown to inhibit their germination. This study aimed to examine the effect of bioherbicide (aqueous extract of *Lantana camara* L. leaves) on rice (*Oryza sativa* L. cv. Cempo Merah) as a non-targeted plant. **Methods:** This experimental study used a completely randomized design with three replications. Concentration series of the aqueous extracts from *L. camara* L. leaves (0%, 25%, 50%, 75%, and 100%) were tested on rice seeds that were germinated on petri dishes for 14 days. **Results:** The results showed that the aqueous extract of *L. camara* L. leaves had a phytotoxic effect on germination and early growth of rice (*Oryza sativa* L. cv. Cempo Merah). **Conclusions:** The highest concentration exhibited the greatest phytotoxicity. Allelopathy chemicals in *L. camara* L. leaves were responsible for these effects.

Keywords: Bioherbicide; germination; growth; *L. camara* L.; rice cv. Cempo Merah

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Introduction

Rice (*Oryza sativa* L. cv. Cempo Merah) is one of the fundamental staple foods consumed by over half of the world's population, including Indonesia. It is an excellent source of carbohydrates, proteins, vitamins, and minerals (Chaudhari et al., 2018). Indonesia has several pigmented rice varieties, including black rice, brown rice, and red rice, which originate from different cultivars. However, most people prefer and are accustomed to consuming white rice. On the other hand, the intense pigmentation of rice represents the phenolic and flavonoid contents, both of which provide health benefits for the prevention of chronic diseases, such as type II diabetes and obesity (Pradipta et al., 2020). Red rice has been reported to contain higher levels of iron, magnesium, calcium, and zinc than white rice (Raghuvanshi et al., 2017). It was also reported to have excellent antioxidant properties due to its high total phenolic and total flavonoid contents, as well as DPPH scavenging activity. This could make it a part of the diets of individuals with diabetes and other non-communicable diseases, given its lower glycemic index compared to white rice (Raghuvanshi et al., 2017). In maintaining health and increasing immunity, red rice is widely popular and consumed by people. Due to the high demand for red rice, efforts are necessary to increase rice production and prevent crop failure.

Apart from abiotic stress (drought, flooding, salinity, and heavy metals), another challenge faced in rice farming was the presence of weeds (Khasanah & Rachmawati, 2020b; Palei et al., 2024; Pathak et al., 2021; Widayat & Purba, 2015). Therefore, weeds must be controlled effectively to prevent them from interfering with rice growth and reducing crop yields. Several types of weeds have been identified as growing in rice fields, including *Cyperus difformis*, *Cyperus iria,* and *Echinochloa crus-galli* (Umiyati et al., 2023). Yurlisa (2021) reported that at least 51 species of weeds (from 42 genera and 21 families) were identified in rice fields, based on a literature review of studies conducted over 8 years from 2012 to 2020 in eight different locations in Indonesian rice fields. Among these weeds, *Cynodon dactylon* (L.) Pers., *Fimbristylis miliacae*, and *Monochloa vaginalis* were the dominant weed species (Yurlisa, 2021).

The use of bioherbicides derived from certain plants to control weeds is gaining more attention in eco-friendly agriculture, rather than synthetic herbicides (Hasan et al., 2021). Some plants produce allelochemicals that serve as bioherbicides through allelopathy activity (Gindri, Coelho, Uarrota, et al., 2020). *Lantana camara* L. is a perennial aromatic shrub of the Verbenaceae family, considered one of the main invasive plants. This invasive weed grows rapidly and uncontrollably; therefore, one of the efforts to control it is to optimize its use (Patel, 2011). Most people use *L. camara* as an ornamental plant because of its beautiful, colorful flowers. Furthermore, each part of this plant contains numerous bioactive compounds responsible for various therapeutic applications (Lonare et al., 2012). Another critical potential use of this plant is as a bioherbicide to control the growth of multiple weeds (Mishra, 2015).

The allelopathic effect of bioherbicides is not only accepted by weeds but also by the main cultivated crop. Information regarding the impact of bioherbicides on non-targeted plants is still limited. As a bioherbicide, the extract of *L. camara* L. has been tested on several non-targeted plants, such as *Raphanus sativus*, *Capsicum annum*, and *Lycopersicon esculentum* (Senarathne et al., 2011). *S*ome selected crops, including *Oryza sativa* L., *Triticum aestivum* L., and *Vigna sinensis* (L.) (Hossain & Alam, 2010), were shown to inhibit their germination. Ahmed et al. (2007) also reported on some food crops, such as Indian mustard (*Brassica juncea* (L.) Czern., garden cucumber (*Cucumis sativus* L.), black gram (*Phaseolus mungo* L.), radish (*Raphanus sativus* L.), asparagus bean (*Vigna unguiculata* (L.) Walp.), and chickpea (*Cicer arietinum* L.), that received an allelopathic effect from this plant.

The use of herbicides to inhibit weed growth around rice was thought to disrupt rice growth. This study utilized non-targeted plant rice (*Oryza sativa* L. cv. Cempo merah), one of the red pigmented rice cultivars that has been studied for its good nutritional value, including amino acids, both essential and non-essential (Agustin et al., 2021). Studies on abiotic stress in this plant have been reported to show its resistance to abiotic stress (Khasanah & Rachmawati, 2020a, 2020b; Rachmawati et al., 2018). To evaluate the resistance of the plant to the bioherbicide effect, this study aimed to investigate the phytotoxic effect of a bioherbicide from the aqueous extract of *L. camara* L. leaves on rice (*Oryza sativa* L. cv. Cempo merah) by evaluating germination and early growth parameters.

Methods

This experimental study was conducted in February 2022 in the Ngaliyan district of Semarang. This study used a completely randomized design. Each treatment group consisted of three replications, with 30 seeds per replication, totaling 90 seeds. The independent variable was the application of aqueous extract from *L. camara* L. leaves with various concentrations. Meanwhile, the dependent variable was several parameters of germination and growth parameters of rice 'Cempo Merah'. Several conditions, which were set as control variables, included the frequency and volume of watering after treatment (8 mL/day), air temperature (28-30°C), humidity (85%), and water pH (7).

Preparation of aqueous extract of Lantana camara L. leaves.

Healthy leaves of *Lantana camara* L. were collected in the morning, taken from the yard of UIN Walisongo Semarang, then washed under running tap water to remove dust and other contaminants on the leaf surface. Then, the cleaned leaves were dried at room temperature (28-30°C) for 2 days in the shade, followed by an additional 2 days of drying in an oven at 40°C Next, the leaves are crushed into powder using an electric blender. The powder (5 grams) was macerated in 200 mL of distilled water (w/v) in an Erlenmeyer flask and then kept at room temperature (28-30°C) for 24 hours. The solution mixture was filtered and then centrifuged at 3600 rpm for 10 minutes. The resulting supernatant was assumed to be a crude extract of *Lantana camara* leaves with a concentration of 100%. Furthermore, the extract was diluted with distilled water to obtain extracts with various concentrations, namely 25%, 50%, and 75%. The extract concentration of 0% consisted of distilled water only and served as the control.

Rice germination

Rice seeds were surface-sterilized using 0.5% KMnO4 for 2 minutes, then washed three times with tap water (Wang et al., 2011). A total of 30 sterile rice seeds were germinated in a petri dish (diameter 90 mm) on one layer of Whatman No. Filter paper. 1 and 2 layers of tissue paper at room temperature for 14 days. Each Petri dish was treated by dousing 4 mL of extract with a predetermined concentration. All petri dishes were closed and placed in the shade for 2 days. To maintain the moisture of the filter paper, 8 mL of distilled water was added every day.

Parameter measurement

The parameters measured in this study consisted of germination percentage (GP), germination index (GI) or germination speed index, mean germination time (MGT), and vigor index (VI), calculated using the formula (Table 1). The indicator of germination was the emergence and growth of the radicle, approximately 2 mm in length. The number of germinated seeds and shoot length were observed daily for 14 days. At the last observation (day 14), five germinating seeds were randomly selected from each petri dish for later measurement, including root and shoot lengths, as well as wet and dry weights of the seedlings.

Table 1. The formula of GP, GI, MGT, and VI

Parameters	Formula	Reference
Germination percentage (GP)	$GP\ (\%) = \frac{N_i}{N} x\ 100\%$	(El-Kenany & Salma, 2013) (Han et al., 2023)
Germination index (GI)	$GI = \sum \frac{G_i}{D_i}$	(Gupta, 1993) (Han et al., 2023)
Mean Germination Time (MGT)	$MGT\ (day) = \frac{\sum G_i\ x\ D_i}{\sum G_i}$	(Parera et al., 2023) (Aref et al., 2011) (Han et al., 2023)
Vigor Index (VI)	$VI = FGP\ x\ (SL + RL)$	(Gupta, 1993) (Uprety et al., 2021)

Where **Ni** represented the accumulate number of germinated seeds on Di; **N** represented the total number of examined seeds; **Gi** represented the number of seeds germinated on Di; **Di** represented the number of days from the beginning of the experiment; **FGP** represented the final germination percentage; **SL** represented the shoot length; and RL represented the root length).

Data analysis

All data obtained were analyzed using SPSS version 16 to get the mean and standard deviation. The data obtained from each parameter were tested using the One-Way ANOVA test at a p-value of 0.05, after meeting the parametric assumption tests. Duncan's test was carried out if the data from the One-Way ANOVA test showed a p-value < 0.05.

Result

The germination percentage was considered a measure of seed population viability, calculated as the number of seeds germinated per total seeds sown, expressed as a percentage. The results showed that the aqueous extract of *L. camara* L. leaves inhibited rice seed germination in the early stage (Figure 1). On day 2, a significant difference in germination percentage was observed, with the lowest germination percentage shown by the treatment of aqueous extract of *L. camara* L. leaves at 100% concentration ($p < 0.05$) (Table 2). On day 5, the control achieved maximum germination, while the other treatments reached maximum germination between day 8 and day 10 of germination (Figure 1). In this study, the final germination percentage on day 14 of germination showed insignificant differences, as indicated by the fact that nearly all treatments had germination percentages exceeding 90% (Table 2).

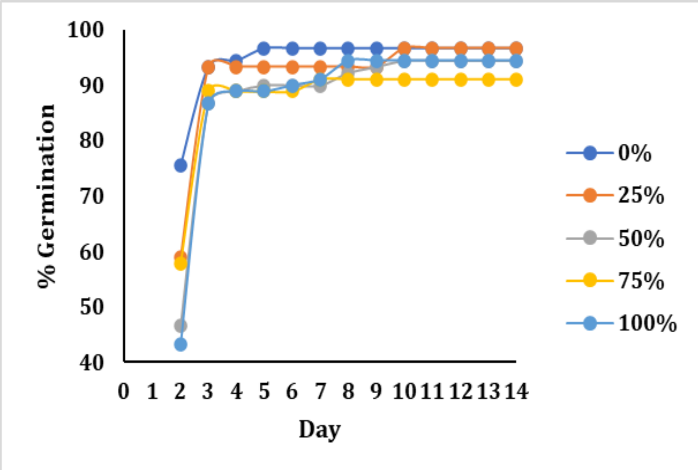

Figure 1. Germination percentage of rice seeds after being given aqueous extract of *L. camara* L. leaves for 14 days

Table 2. GP on day-2, FGP, GI, MGT, and VI of rice seeds after being given aqueous extract of *L. camara* L. leaves

Treatments	GP (%) (day-2)*	FGP (%) (day-14)	GI*	MGT (day)	VI*
0%	75.56 ± 1.28 c	96.67 ± 0.00	13.32 ± 0.33 b	2.28 ± 0.09	2562.63 ± 33.62 e
25%	58.89 ± 1.47 b	96.67 ± 0.00	12.38 ± 0.35ab	2.62 ± 0.09	1481.15 ± 10.29 b
50%	46.67 ± 1.76 a	94.44 ± 1.92	11.39 ± 0.81 a	2.83 ± 0.42	1599.06 ± 26.34 c
75%	57.78 ± 1.46 b	91.11 ± 5.09	11.87 ± 1.20 a	2.47 ± 0.24	1943.97 ± 18.30 d
100%	43.33 ± 1.53 a	94.45 ± 6.94	11.23 ± 0.75 a	2.81 ± 0.43	686.91 ± 37.56 a

Note: n=3; *) showed significant difference ($p<0.05$); different letters showed substantial differences based on Duncan's test ($p<0.05$)

There was a significant difference in the germination index and vigor index, with the lowest score observed in the treatment of 100% ($p < 0.05$) (Table 2). Based on Duncan's test, there was a significant difference in germination index between the extract treatment of *L. camara* leaves and the control. It is worth noting that rice seeds treated with the extract at 75% showed a higher vigor index than the other extract treatments, approaching that of the control. The higher the mean germination time, the longer the time needed for the seeds to germinate. Although the results showed no significant difference in the mean germination time, there was a tendency for 30 seeds treated with 100% extract to take longer to germinate compared to seeds without treatment (control) (Table 2).

The other results showed that the aqueous extract of *L. camara* leaves inhibited the growth of rice seedlings, as indicated by their shoot length (Figure 2). Although the germination percentage of seeds treated with *Lantana* extract from day 3 to day 8 increased to nearly the same level as the control, the shoot lengths of seeds treated with the extract were shorter than those of the control, which was visible from day 5 to day 14 (Figure 2). During the 14-day germination period, the shortest shoot length was observed in the treatment with 100% concentration. Rice seeds treated with the extract at a 75% concentration exhibited a significantly higher shoot length than the other treatments, approaching the control value.

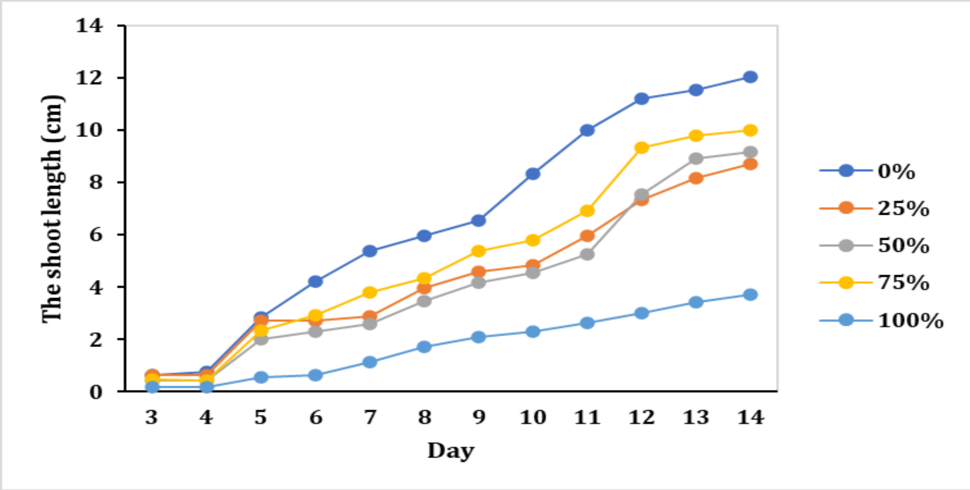

Figure 2. The shoot length of rice seedlings after being given aqueous extract of *L. camara* L. leaves for 14 days

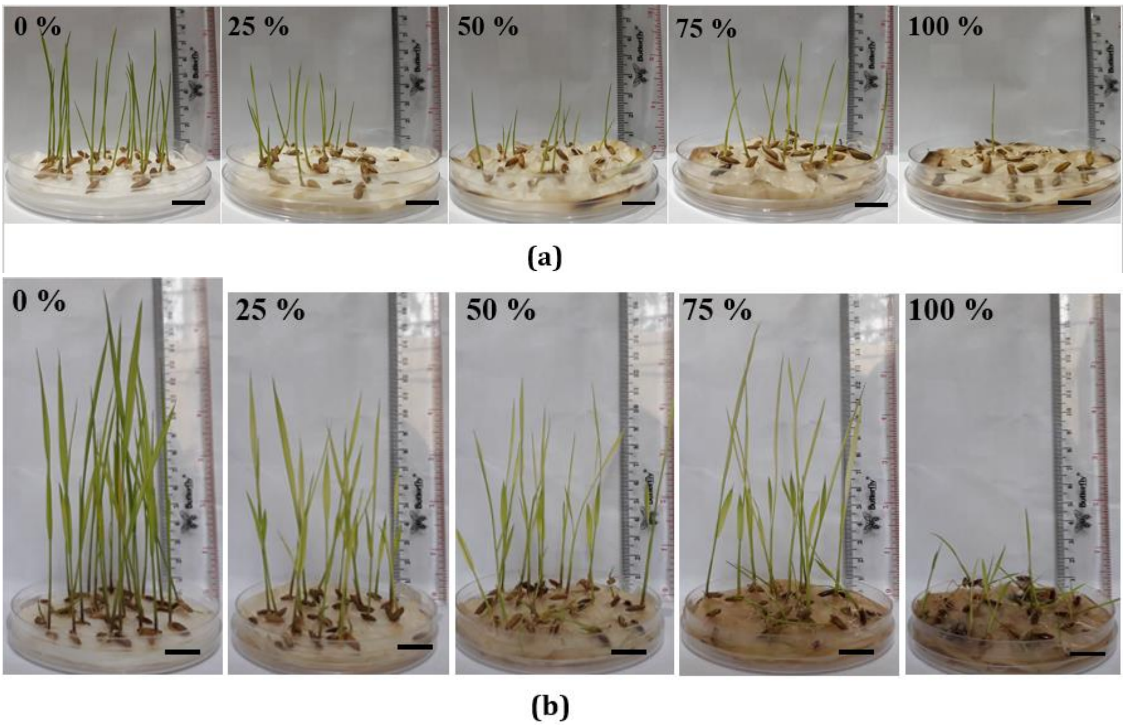

Figure 3. The morphological appearance of rice seedlings after being given aqueous extract of *L. camara* L. leaves: (a) on day-7; (b) on day-14, scale bars: 10 cm

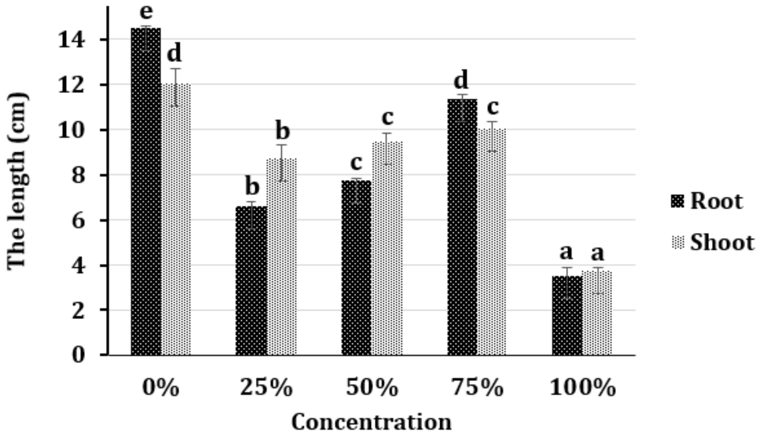

Figure 4. The root and shoot lengths of rice seedlings after being given aqueous extract of *L. camara* L. leaves on day 14. Note: n=3; different letters showed significant differences based on Duncan's test (p< 0.05).

Morphological appearance of rice seedlings exposed to aqueous extract from *L. camara* L. leaves on day-7 and day-14 could be observed clearly in Figure 3. The pale, yellowish-green leaves and abnormal growth were visible on the seedlings treated with 25%, 50%, and 100% concentrations. The seedlings treated with 100% showed the worst development of all treatments. The shoot and root lengths of rice seedlings after treatment with the extract showed a significant difference ($p < 0.05$) (Figure 4). The fresh and dry weights of the shoots and roots of rice seedlings, after being treated with the extract, also showed a significant difference ($p < 0.05$) (Figure 5). These parameters decreased by 25 to 100% after exposure to the aqueous extract of *L. camara* leaves compared with the control. The worst impact was seen in the treatment of 100%.

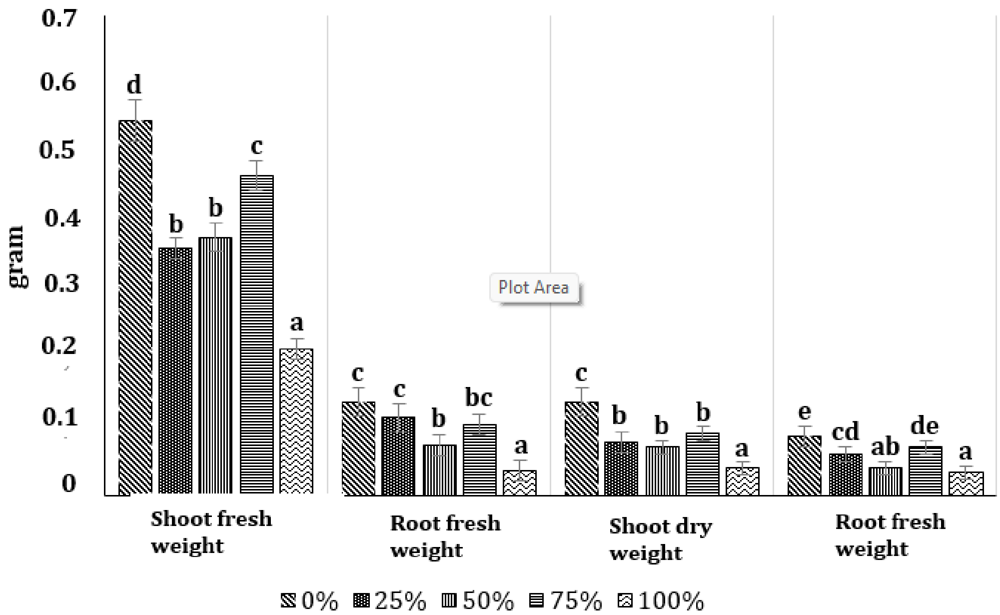

Figure 5. The fresh and dry weights of the shoots and roots of rice seedlings after being given aqueous extract of *L. camara* L. leaves on day 14. Note: n=3; different letters showed significant differences based on Duncan's test (p<0.05).

Discussion

Seed germination is the initiation process of the first developmental phase in the higher plant life cycle, and it is a crucial stage in determining seedling formation (Wolny et al., 2018). Seeds would begin to germinate under favorable environmental conditions (suitable light, temperature, water, pH, and humidity) and internal factors (suitable seed maturity and regulation of plant hormones, such as abscisic acid and gibberellin, breaking seed dormancy and promoting seed germination (Koger et al., 2004; Skubacz & Daszkowska-Golec, 2017; Weitbrecht et al., 2011). Therefore, this study used germination and early growth as parameters to evaluate the effect of the aqueous extract of *L. camara* L. leaves on a non-targeted plant, rice (*Oryza sativa* L. cv. Cempo Merah).

The results of this study showed that the aqueous extract of *L. camara* L. leaves had a phytotoxic effect on the germination and early growth of rice (*Oryza sativa* L. cv. Cempo Merah). The greatest phytotoxicity was shown by the highest concentration, 100%. The results were supported by previous studies examining the phytotoxic effect of *L. camara* L. extract on several crops, including non-targeted plants. Tadele (2014) reported that a 75% aqueous extract of *L. camara* L. leaves significantly reduced the seed germination percentage of Eragrostis tef by 13.5% compared to the control after 14 days ($p < 0.01$). Ngonadi et al. (2019) also reported that a 40% extract of *L. camara* L. leaves significantly inhibited maize seed germination by 16.7% compared to the control after 8 days ($p < 0.05$). Hossain & Alam (2010) also reported that on day 7 of germination, seed germination percentages of *Oryza sativa* L., *Triticum aestivum* L., and *Cucurbita pepo* L. were treated with aqueous extract of *L. camara* L. leaves with 50%, 75% and 100 % concentrations—seed germination percentages of *Vigna sinensis* (L.) Hassk. *Amaranthus tricolor* L. was also reduced after treatment with the extract at 75% and 100% (Hossain & Alam, 2010). The germination percentage of maize seeds was found to be lower (26.67%) compared to the control after being treated with an aqueous extract of *L. camara* L. leaves at 50% and 75% concentrations for two weeks (Mistica et al., 2023).

This aqueous extract also affected the germination index and vigor index, with the lowest score observed in the treatment of 100% ($p < 0.05$). A previous study revealed that water extracts of *L. camara* L. leaves, at concentrations of 1.25%, 2.5%, 3.75%, and 5%, caused a decrease in the germination speed index of *Biden pilosa* seeds (Gindri, Coelho, Uarrota, et al., 2020)—the seed germination index of four vegetable species, such as *Triticum turgidum* ssp. Durum, *Hordeum vulgare*, *Cicer arietinum*, and *Lens culinaris* decreased after being treated with an aqueous extract of *L. camara* L. leaves at lower concentrations of 10%, 25%, and 50%. Even at higher concentrations of 75% and 100%, no germination occurred (Talhi et al., 2020). This leaf extract also affected the germination process of oats (*Avena sativa* L.) by decreasing the germination speed index and seed viability, leading to oxidative stress through increased reactive oxygen species production (Gindri, Coelho, & Uarrota, 2020). Germination index and tolerance index of *black gram* (*Vigna mungo* L.) were found to be decreased with an increase in the concentrations (10%, 20%, 40%, 80%, and 100%) of this aqueous leaf extract (Nawab & Yogamoorthi, 2016). Seeds with a greater germination index were considered to have better vigor (Gupta, 1993).

The vigor index indicated the seed's ability to germinate quickly and evenly, even under unfavorable environmental conditions (Dadlani & Yadava, 2023). Seeds with a greater vigor index were also considered to be more vigorous. A decrease in seed vigor index was one of the impacts of the allelopathic effect from the plant (Khasanah & Rahmawati, 2023). Unlike other study in which treatment of allelopathy extract from sedge grass significantly increased the mean germination time of soybean seeds (Darmanti et al., 2015), on this study was insignificant difference ($p>0.05$) for mean germination time of rice after treated with aqueous extract of *L. camara* L. leaves (Table 2). Nevertheless, the mean germination time of the treatments with *L. camara* L. extracts tended to be higher or longer than that of the control. An increased mean germination time is correlated with the final germination percentage, and it could be used as a good indicator of seed vigor in canola (Amirmoradi & Feizi, 2017).

This aqueous extract also affected the shoot and root lengths of rice seedlings, with the lowest score obtained at a 100% concentration ($p < 0.05$). The shoot and root lengths of several crops such as *Triticum aestivum*, *Lens culinaris*, *Brassica campestris,* and *Oryza* *sativa* L. were decreased after being treated with aqueous extract of *L. camara* leaves with 2%, 4%, 6%, 8%, and 10% (Acharya et al., 2022). The length of the seedling could be attributed to the seed vigor index and the rate of seedling emergence (Gupta, 1993). Both fresh and dry weights of the shoots and roots of rice seedlings decreased after exposure to an aqueous extract of *L. camara* leaves at concentrations of 25% to 100%, compared with the control. The 100% concentration treatment showed the lowest score. Exposure to *L. camara* L. extract has also been investigated for its potential to reduce the wet and dry weights of *Abelmoschus esculentus* and *Vigna unguiculata* seedlings (Bhattacharya et al., 2020).

Allelopathy chemicals or phytochemicals in *L. camara* L. leaves were responsible for the emergence of these effects. Bhuvaneswari & Giri (2018) had been reported the presence of the phytochemicals in methanol and aqueous extracts of *L. camara* L. leaves, such as tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, carbohydrate, anthraquinone, polyphenol, and glycoside, presented in high intensity of the color based on qualitative phytochemical analysis. The leaves of *L. camara* L. were investigated to have a high total phenols (324 ± 22.68 mg/gm) compared to other chemical compounds (alkaloids, tannins, saponins, and flavonoids) (Bhuvaneswari & Giri, 2018). Lopes et al. (2022) reported that the high total phenolic content in *Ricinus communis* leaves extract was thought to play a role in its allelopathic activity and showed the most potent inhibitory effect on the germination and growth of *Bidens bipinnata*. Phenolic compounds exhibited herbicidal activity by inhibiting germination through the disruption of the photosynthetic process and cell division, inducing the production of reactive oxygen species (ROS), and inhibiting the generation of detoxifying enzymes and growth hormones (Tucuch-Pérez et al., 2023). Phytochemicals such as flavonoids, alkaloids, terpenoids, tannins, saponins, and glycosides in methanol and n-hexane extracts of aerial parts of *Chenopodium glaucum* L. were highly phytotoxic to radishes (Ullah Khan et al., 2023). This decrease was caused by the delay in the initiation of germination, cell division, and elongation (Ullah Khan et al., 2023). Triterpenoids such as Lantadene A and B were the possible compounds conferring the herbicidal properties of *L. camara* (Gindri, Coelho, Uarrota, et al., 2020; Sharma et al., 2007). According to Mishra (2015), *L. camara* contains some chemical constituents, such as β-pinene, 1,8-cineole, cinnamic acid, dipentene, ferulic acid, palmitic acid, and ρ-coumaric acid, which have a role in inhibiting seed germination and plant growth.

Unlike weeds, rice was a non-targeted plant in which the inhibitory effect from the allelochemicals of bioherbicide should be avoided. This study showed that after treatment with the aqueous extract of *L. camara* leaves at concentrations of 25%, 50%, and 75%, the seeds of rice cv. Cempo Merah still germinated and grew, although the growth was not as maximal as in the control. This proved that the local plants, such as rice cv. Cempo Merah may have potential tolerance to allelopathy exposure. Plants that grew and developed together with species producing allelopathic compounds have generally developed a certain level of tolerance through the evolutionary process (Hickman et al., 2021) as the results of research by Weerakoon et al. (2018), who investigated nine varieties of Sri Lankan Rice (*Oryza sativa* L.), indicated moderate resistance to herbicides. (2024) also reported that there were no symptoms of phytotoxicity in rice (*Oryza sativa* L. cv Rojolele Srinuk) after treatment with the herbicide ethyl pyrazosulfuron at 10%.

Conclusions

This study concluded that the aqueous extract of *L. camara* L. leaves significantly inhibited early seedling growth and germination of rice cv. 'Cempo Merah' in a concentration-dependent manner. The greatest phytotoxicity was shown by the highest concentration, 100%. To determine the resistance of rice to bioherbicide exposure from aqueous extracts of *L. camara* L. leaves, further research was needed to evaluate antioxidant mechanisms and levels of oxidative compounds in seedlings.

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Declaration statement

The authors reported no potential conflict of interest.

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