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Research Article

Allelopathic potential of apus bamboo leaf extract towards *Asystasia gangetica* and *Cyperus rotundus*

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ABSTRACT

Weeds pose a significant challenge in agricultural activity by competing with cultivated crops for essential resources. The conventional use of chemical herbicides has demonstrated adverse impacts on the environment and human health, prompting a shift toward exploring bioherbicides. This study aimed to assess the inhibitory effects of Apus bamboo (Gigantochloa apus Kurz) leaf extract on the pre-germination phase of Brassica juncea, Asystasia gangetica, and Cyperus rotundus through bioassay and pot experiment. The research design employed a completely randomized design (CRD) with a single factor representing the concentrations of Apus bamboo leaf extract (% w/v). The concentrations tested were 0%, 5%, 10%, 15%, and 20%. Each concentration was replicated three times, resulting in 15 experimental units. Bioassay results revealed the capacity of Apus bamboo leaf extract to inhibit and disrupt B. juncea seed germination. The pot experiment results demonstrated the inhibitory effects of the extract, significantly affecting A. gangetica germination and population, A. gangetica total fresh and dry weight, and C. rotundus total dry weight. The extract exhibited strong inhibition, starting at a concentration of 15% for A. gangetica and 10% for C. rotundus. The findings suggested that Apus bamboo leaf extract holds significant potential as a bioherbicide.

Keywords: allelochemical; bioherbicide; inhibitory effect; weed control

INTRODUCTION

Weeds, commonly considered wild plants, frequently emerge near agricultural lands, posing a significant challenge to agricultural activity. Uncontrolled weed growth competes with cultivated plants for essential resources such as nutrients, water, and sunlight. The negative impact of weed growth results in reduced harvest yields and detriment to cultivated plants, necessitating effective control measures (Palijama et al., 2018). Controlling weeds through chemical herbicide application often had adverse consequences for the environment and human health (Yuliyanah & Meikawati, 2015). Therefore, an environmentally friendly approach is crucial for addressing weed-related issues, with the development of bioherbicides emerging as a promising solution. Recent studies have shown that integrating bioherbicides into weed management strategies can significantly enhance crop yields while minimizing ecological disruption (Srikrishnah & Begam, 2019; Campos et al., 2023). This shift towards biological control methods reflects a growing recognition of the need for sustainable agricultural practices that protect both human health and the environment.

Bioherbicides are biological formulations that employ living organisms, such as bacteria, fungi, or specific natural substances, to inhibit weed growth. This approach is environmentally friendly as it avoids using synthetic chemicals that can contaminate soil and water. Bioherbicides also have the potential to support sustainable agriculture by reducing dependency on chemical herbicides (Cordeau et al., 2016; Darmanti, 2018). The process of weed control using bioherbicides involves isolating and cultivating organisms with allelopathic capabilities against specific weeds (Kostina-Bednarz et al., 2023). Allelopathy is the influence of chemical compounds that can inhibit plant growth (Qu et al., 2021). Bioherbicides can selectively inhibit weed growth without affecting cultivated plants (Sari & Suhendra, 2021). Furthermore, the efficacy of bioherbicides can be enhanced by understanding the specific interactions between allelochemicals and target weed species (Scavo & Mauromicale, 2021; Lopes et al., 2022).

Bamboo contains allelopathic compounds that can affect organisms both directly and indirectly. The allelopathic effect of bamboo can either inhibit or stimulate growth through chemical compounds released into the environment (Kristiana, 2019). The selective nature of allelopathic compounds on organisms depends on compound concentration, type, and the response of plant species (Darmanti, 2018). The abundant phytochemical content in bamboo leaf waste around agricultural areas makes it a potential candidate for use as a bioherbicide, offering an environmentally friendly solution to inhibit weed growth and support sustainable agriculture. This allelopathy also affects the germination patterns of cultivated plants, a crucial criterion for bioherbicides not to impact the growth and development of the main plant (Cai & Gu, 2016).

Bamboo biomass shavings waste is known to contain phenolic compounds (1.56%), fatty acids (29%), methyl ester (27.03%), linoleate (12.13%), and phytol (3.62%), presenting potential as a natural bioherbicide. Bamboo leaves also contain anthraquinone, stimulating the growth of new cells (Zhang et al., 2010). Phenolic compounds constitute the main group of allelopathy, consisting of phenols, flavonoids, hydroxycinnamic acids, hydroxycinnamic benzoates, phenylpropanoids, coumarins, and tannins (Perveen et al., 2019). Phenolic compounds inhibit weed growth, strengthen plant cells, and prevent abnormal plant cell growth (Macías et al., 2019; Rasouli et al., 2016). Bamboo leaf waste emerges as a promising raw material for bioherbicide production due to its abundant phytochemical content. This research expected an effective concentration of apus bamboo leaf extract, serving as a sustainable alternative for weed control, particularly targeting Chinese violet *(Asystasia gangetica)* and purple nutsedge *(Cyperus rotundus)* in agricultural fields. This approach not only provides an effective solution to weed-related problems but also supports environmental preservation and agricultural ecosystem balance. This study aimed to assess the inhibitory effects of Apus bamboo (*Gigantochloa apus* Kurz) leaf extract on the pre-germination phase of *Brassica juncea*, *Asystasia gangetica*, and *Cyperus rotundus* through bioassay and pot experiment.

MATERIALS AND METHODS

The research was conducted from September to November 2023 at the Ecotoxicology Waste and Bioagents Laboratory, Cikabayan Greenhouse, Cikabayan and Leuwikopo experimental site, IPB University. The materials used in this research included apus bamboo leaves *(Gigantochloa apus* Kurz*)*, Chinese violet *(Asystasia gangetica)* as broadleaf weed, purple nutsedge *(Cyperus rotundus)* as sedge weed, mustard green seeds *(Brassica juncea)*, and methanol (CH_3OH) as solvent. The mustard green seed was used for the bioassay experiment, while Chinese violet and purple nutsedge were used for the pot experiment.

Experimental design

The research was conducted using a Completely Randomized Design (CRD) with one factor, which was the concentration of apus bamboo leaf extract, comprising five levels, namely K0 (concentration at 0% or control), K1 (concentration at 5%), K2 (concentration at 10%), K3 (concentration at 15%), and K4 (concentration at 20%). Each treatment was replicated three times, resulting in 15 experimental units for each bioassay and pot experiment.

Experimental procedure

The Apus bamboo *(Gigantochloa apus* Kurz*)* leaf was collected from the Cikabayan Garden, air-dried for about one week until obtaining the dry weight (Nursal & Juwita, 2006), and then chopped the dried leaves into powder. The extraction was performed using the maceration method by soaking the material in 90% methanol at a ratio of 1:4 (w/v) and incubating it for 24 hours. The extract was separated using a filter to isolate the solid from the filtrate. The maceration process was repeated twice for another 24 hours and filtered again. The filtrate from these two maceration steps was evaporated using a rotary evaporator until all methanol evaporated, resulting in a concentrated extract (macerate). The macerate was then stored in a sterile container and kept in a silica gel desiccator (Olaleye, 2007).

The bioassay experiment was conducted as an initial procedure to test the bioactivity (allelopathy) of the substances extracted from bamboo leaves. This experiment was carried out by growing test seeds in a petri dish lined with Whatman paper. Mustard green (*Brassica juncea*) seeds were used for the experiment due to its sensitivity to the chemical treatments. The extract concentrations refer to weight per volume (w/v) obtained by dissolving the macerate (g) in distilled water (mL). The concentration treatments of the extract were 0% (control), 5%, 10%, 15%, and 20%, with three replications. Each treatment contained 20 seeds in each Petri dish. The treatment application was dripping 3 mL of extract onto the seeds until the seeds and the paper were moist but not flooded. The observation and measurement on bioassay were seed germination percentage, plumule length, and radicle length for each treatment. The seeds and paper were kept moist during observation, and distilled water was added to the paper if necessary. Observations were conducted once a week for two weeks.

For the pot experiment, seeds of Chinese violet (*Asystasia gangetica*) and purple nutsedge (*Cyperus rotundus*) tuber were prepared and collected from the Cikabayan and Leuwikopo experimental sites. Pot and soil were used as the planting medium. Treatment solutions based on concentration from the bioassay experiment were also prepared right before planting. The planting of seed/tuber was performed at a depth of one centimeter and then covered with soil. Then, the treatment was applied by spraying the solutions into the soil surface. Watering was conducted by placing pots in a concave tarp filled with water, allowing the soil to absorb moisture through capillarity until it became wet. Continuous observation was performed weekly until the weeds reached the maximum vegetative phase, approximately four weeks.

The allelochemical compound content of Apus bamboo leaf extract was analyzed by conducting a literature study and reviewing scientific articles, books, and research reports to identify known compounds. The variables of bioassay observation were seed germination percentage and seedling size (plumule and radicle length measured with ImageJ). The variables of pot experiment observation were germination percentage, population, and fresh/dry weight of biomass. The obtained data was analyzed using the Kruskal-Wallis test at a 5% significance level with RStudio software. Post-hoc testing was performed for significantly different results using the Dunn's test at a 5% significance level.

RESULTS AND DISCUSSION

Potential allelochemical constituents

Bamboo leaves contain various bioactive compounds that contribute to their potential allelochemical properties, influencing the growth of neighboring plants. Key constituents include flavonoids such as isoorientin, vitexin, flavone C-glycosides, and phenolic acids like caffeic, ferulic, and cinnamic acid, which exhibit significant phytotoxic effects. Understanding these allelochemicals is crucial for elucidating their ecological roles and exploring applications in sustainable agriculture. The table below summarizes the primary allelochemical constituents found in bamboo leaf extracts, highlighting their significance in ecological interactions and agricultural applications.

Table 1. Potential allelochemical constituents of bamboo leaf extract.

Compound	Type / Group	Reference
Flavone C-glycosides	Flavonoids	(Wang et al., 2012; Zhao et al., 2022)
Isoorientin	Flavonoids	(Wang et al., 2012)
Isovitexin	Flavonoids	(Wang et al., 2012; Gong et al., 2015)
Orientin	Flavonoids	(Wang et al., 2012; Gong et al., 2015)
Vitexin	Flavonoids	(Wang et al., 2012; Gong et al., 2015)
Luteolin	Flavonoids	(Tanaka et al., 2014; Gong et al., 2015)
4-Hydroxycinnamic acid	Phenolic	(Toan et al., 2018; Ma et al., 2020)
Caffeic acid	Phenolic	(Gong et al., 2015; Chen et al., 2022)
Chlorogenic acid	Phenolic	(Zhang et al., 2010; Gong et al., 2015)
Gallic acid	Phenolic	(Hu et al., 2011; Uddin et al., 2011)
Cinnamic acid	Phenolic	(Toan et al., 2018)
Ferulic acid	Phenolic	(Gong et al., 2015; Toan et al., 2018)
Coumaric acid	Phenolic	(Gong et al., 2015; Puig et al., 2018)
Saponins	Saponins	(Cechin et al., 2022; Hidayah & Hafshah, 2023)
Tannins	Tannins	(Tongco et al., 2016; Novakoski et al., 2020)
Alkaloids	Alkaloids	(Ummah et al., 2017; Hidayah & Hafshah, 2023)

Bioassay experiment

The bioassay experiment of Apus bamboo leaf extract was conducted to evaluate the presence of allelochemicals and test their inhibitory activity on the seed germination of tested plant seeds. This research used the mustard green seed as a bioassay indicator. The results of the bioassay experiment are presented in Table 2.

Table 2. Seed germination and seedling size of mustard green at 7 days after sowing (DAS).

Parameter of observation	K0	K1	K2	K3	K4
Seed germination (%)	71.67a	11.67b	0.00b	0.00b	0.00b
Reduction (%)	-	83.72	100.00	100.00	100.00
Plumule length (mm)	37.97a	5.64b	0.00b	0.00b	0.00b
Reduction (%)	-	85.15	100.00	100.00	100.00
Radicle length (mm)	48.47a	4.47b	1.67b	0.00b	0.00b
Reduction (%)	-	90.78	96.55	100.00	100.00

Note: K0 (control), K1 (extract 5%), K2 (extract 10%), K3 (extract 15%), K4 (extract 20%). Data was collected at seven days. According to Dunn's test, values followed by the same letter(s) in each row are not significantly different at p ≤ 5%.

Based on the bioassay observations, the application of apus bamboo leaf extract treatments (K1, K2, K3, K4) sequentially reduced seed germination by 83.72%, 100%, 100%, and 100%, respectively. A similar trend was observed in seedling size, with plumule length reduced by 85.15%, 100%, 100%, and 100%. Radicle length was also reduced by 90.78%, 96.55%, 100%, and 100%. The zero values, indicating a 100% reduction in several parameters, occurred because the seeds did not germinate. The results of the Kruskal-Wallis test (α=0.05) showed that the Apus bamboo leaf extract treatment significantly affected seed germination, plumule length, and radicle length of mustard green seedlings. Those indicated that the extract contained bioactive compounds that act as allelochemicals and affect germination. Apus bamboo leaves contain phenolic compounds, flavonoids, and tannins that can inhibit seed germination (Rawat et al., 2017; Li et al., 2022). Phenolic and flavonoids are allelochemical compounds that effectively inhibit germination enzymes (Kristanto, 2006; Ghimire et al., 2020).

Similar research involving the application of bamboo leaf waste solution on soybeans with various treatment concentrations did not show a significant effect on seed germination, seedling length, and germination time (Cahyanti, 2019). Inhibition of germination caused by phenolic compounds has different mechanisms depending on the type of seeds (Chenyin et al., 2023). It has been found that phenolic compounds such as tannic acid, coumaric acid, and vanillic acid can inhibit mitochondrial activity and affect the hypocotyl growth of green beans. Vanillic acid can inhibit Ca^{2+} transport in mitochondria but does not affect respiration (oxidative phosphorylation) (Demos et al., 1975; Abenavoli et al., 2010). Phenolic compounds can also inhibit seed metabolism, including inhibiting endosperm breakdown and damaging germination enzyme catalysts (Einhellig, 2003).

In addition to inhibition from the content of bamboo leaf extract compounds, direct inhibition can also be influenced by the difficulty of seeds in the imbibition process. The higher the concentration given, the lower the extracellular water potential and the higher the intracellular water potential. This causes the entry of water into the seed to be inhibited due to the difference in water potential between the cells inside and outside the seed (Saux et al., 2020; Bhatt et al., 2022; Lestari et al., 2023). The results of this bioassay served as the basis for conducting pot experiments on the target weeds.

Pot experiment

The pot experiment was conducted to test and evaluate the inhibitory performance of the extract on weeds in soil, where the soil in the pots represented the actual conditions of its application as a bioherbicide. Based on the observation, applying bamboo leaf extract at several concentrations can reduce seed germination in both weeds. Based on the result (Table 3), bamboo leaf extract application treatments (K1, K2, K3, K4) experienced successive reductions in Chinese violet seed germination by 9.10%, 50.01%, 54.55%, and 72.73%, respectively. This reduction in seed germination also occurred when bamboo leaf extract was applied to purple nutsedge tubers. The treatments (K1, K2, K3, K4) reduced the seed germination of purple nutsedge by 29.63%, 33.33%, 44.44%, and 44.44% respectively. Bamboo leaf extract can inhibit the germination process of weed seeds because it contains main compounds consisting of flavonoids, alkaloids, saponins, and tannins. Its flavonoid content is 5.57% of the fresh leaf weight (Gong et al., 2015; Romansyah et al., 2019). Flavonoids and tannins have the potential to be compounds for bioherbicides, and the isolated flavonoids reduce seedling growth and root development (Shah & Smith, 2020). As found in mahogany, its leaf extract treatment can control *Ageratum conyzoides* due to its high flavonoid content (Kusumaningsih, 2022). Flavonoids can also denature proteins in cells and destroy cell membranes (Parwata et al., 2022; Arsy et al., 2023).

Table 3. Seed germination at 15 DAS and plant population of weeds at 30 DAS.

F	Seed germination (%)		Population (plants per pot)	
	Chinese violet	*Purple nutsedge*	*Chinese violet*	*Purple nutsedge*
K0	48.89a	90.00	8.67a	18.33
K1	44.44ab	63.33	8.67a	14.33
K2	24.44bc	60.00	3.67b	10.33
K3	22.22bc	50.00	4.00b	13.33
K4	13.33c	50.00	5.67ab	10.67
P-value	0.022	0.391	0.012	0.086
CV (%)	52.75	41.11	38.42	29.15

Note: K0 (control), K1 (extract 5%), K2 (extract 10%), K3 (extract 15%), K4 (extract 20%), CV (coefficient of variation). According to Dunn's test, values followed by the same letter (s) are not significantly different at p ≤ 5%.

The delayed germination also made the weed population periodically different. The number of plants per pot on final observation of Chinese violet showed a reduction starting from 10% concentration (K1, K2, K3, K4), which were 0%, 57.67%, 46.14%, and 34.60%, respectively. Meanwhile, the number of plants on purple nutsedge (K1, K2, K3, K4) experienced a reduction of 21.82%, 43.64%, 27.28%, and 41.79%, respectively. Although the results showed a decrease, the treatment did not significantly affect the germination and population of purple nutsedge based on the Kruskal-Wallis test. Those effects occurred because the tubers of purple nutsedge have a robust dormancy mechanism, allowing them to remain viable even under adverse environmental conditions. This dormancy is often attributed to the accumulation of phenolic compounds and other secondary metabolites that not only contribute to the tubers' survival but also may inhibit the effective action of bioherbicides (Jangaard et al., 1971; Ali et al., 2023).

Figure 1 shows that the control always has the highest population both in Chinese violet and purple nutsedge from 5-30 days after sowing (DAS). Based on Figure 1, the inhibition showed linear values as the concentration of bamboo leaf extract until 30 DAS. This means that the higher the concentration, the higher the inhibition, and the smaller the population because of delayed germination. The delay in germination was caused by the phytotoxicity of bamboo leaf extract, which interferes with physiological processes necessary for seed germination, such as enzyme activity and nutrient uptake, and disrupts normal growth patterns in these weed species (Guo et al., 2013; Macías et al., 2019).

(a) (b)

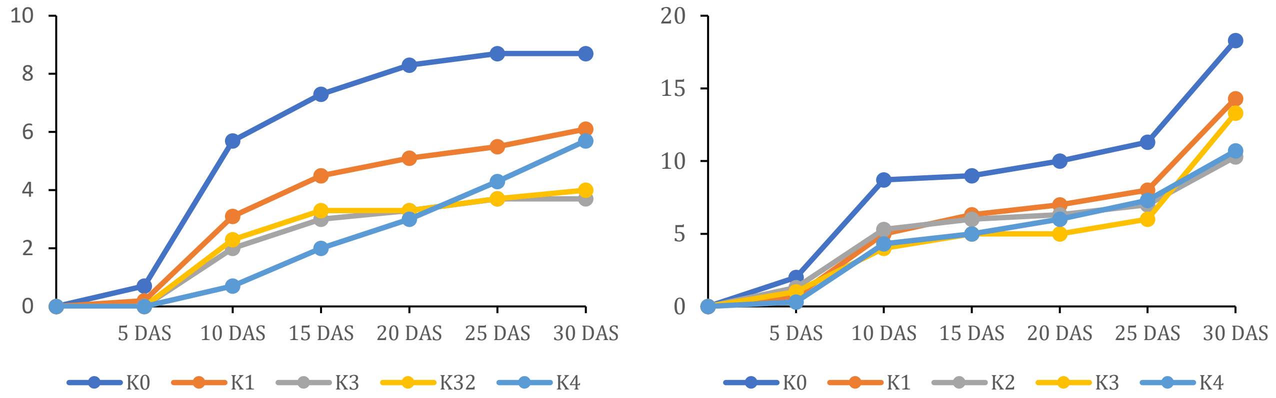
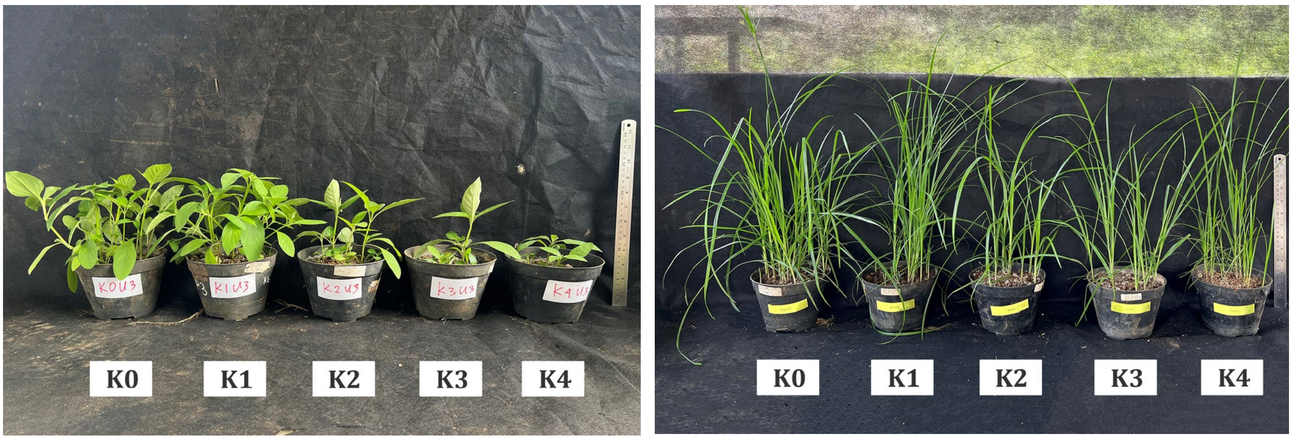


Figure 1. Periodic population (number of plants per pot) of (a) Chinese violet and (b) purple nutsedge.



(a) (b)

Figure 2. Population (number of plants per pot) of (a) Chinese violet and (b) purple nutsedge at 30 DAS.

This finding aligns with the study by Jose et al. (2016), who reported that bamboo leaf extract contains phenolic acids, which effectively inhibit the growth of *Mimosa bimucronata*. At least there are 14 phenolic acid compounds found in bamboo leaf extract: benzoic acid, benzene acetic acid, salicylic acid, p-hydroxybenzoic acid, Vanillic acid, p-Coumaric acid, protocatechuic acid, syringic acid, gallic acid, m-Coumaric acid, vanillylmandelic acid, 3-Methylmandelic acid, 3.4-Methylenedioxymandelic acid, and trans-Ferrulic acid. The population started to rise after 20 DAS because the seeds and tuber were free from the solvent of bamboo leaf extract. This phenomenon is explained by

Hasan et al. (2021), who state that bioherbicides based on organism extract are easily degradable in the environment. In some cases, this organic product is not feasible in the field. Ahn et al. (2021) mentioned that the previous study indicated that phenolic allelochemicals could be degraded in the soil after 10 days. This is mainly due to microorganism activities, such as bacteria and fungi, that can consume organic chemicals.

Biomass fresh and dry weight

Biomass reflects the growth and accumulated photosynthates in plants during the growth process. The high morphological growth of weeds means that the materials that make up the weeds will form high biomass. High biomass indicates that the photosynthesis reaction process that produces photosynthate or material that forms weeds runs smoothly and can be distributed to all weed parts (Sari et al., 2017). The dry weight reflects the pattern of plants accumulating products from the photosynthesis process and integrates with other environmental factors. Dry weight is obtained after the oven-drying for 48 hours at a temperature of 60 °C. The oven-drying removes all the water in the weeds, indicating only the weight of the remaining organs.

Based on Table 4, significant results were obtained in the biomass fresh (stem, leaf, total) and dry (leaf, total) weight of the Chinese violet. The application of bamboo leaf extract effectively inhibited the growth of Chinese violet, as indicated by the reduction in total fresh biomass weight by 10.92%, 48.39%, 52.39%, and 65.37% for treatments K1, K2, K3, and K4, respectively. Its strong inhibition was also indicated by the reduction in dry biomass weight by 16.03%, 44.31%, 52.76%, and 68.51%, respectively. The application of bamboo leaf extract, starting at a concentration of 15%, effectively inhibited the growth of Chinese violet. It's presumed that the higher the concentration, the more potent inhibition, as shown at the lower biomass values.

Table 4. Fresh and dry weight of Chinese violet biomass.

F	Fresh weight (g)				Dry weight (g)			
	Root	Stem	Leaf	Total	Root	Stem	Leaf	Total
K0	5.89	5.20a	13.17a	24.26a	0.85	0.64	1.93a	3.43a
K1	5.74	4.69ab	11.18ab	21.61ab	0.78	0.55	1.56ab	2.88ab
K2	3.37	2.27abc	6.88abc	12.52abc	0.43	0.50	0.98bc	1.91ab
K3	3.84	1.87bc	5.84bc	11.55bc	0.53	0.22	0.87bc	1.62b
K4	2.79	1.11c	4.50c	08.40c	0.37	0.10	0.62c	1.08b
P-value	0.169	0.047	0.040	0.042	0.116	0.057	0.037	0.049
CV (%)	43.97	67.53	50.60	49.98	47.72	69.85	51.55	49.12

Note: K0 (control), K1 (extract 5%), K2 (extract 10%), K3 (extract 15%), K4 (extract 20%), CV (coefficient of variation). According to Dunn's test, values followed by the same letter (s) are not significantly different at P ≤ 5%.

Table 5. Fresh and dry weight of purple nutsedge biomass.

F	Fresh weight (g)				Dry weight (g)			
	Root	Tuber	Leaf	Total	Root	Tuber	Leaf	Total
K0	6.88a	4.4	18.84	30.12	1.10	0.98a	2.99	5.07a
K1	2.78ab	3.27	11.57	17.63	0.54	0.80a	1.84	3.18ab
K2	1.44b	2.05	10.43	13.92	0.48	0.16b	1.48	2.12b
K3	3.01ab	2.95	10.45	16.42	0.61	0.61ab	1.60	2.82b
K4	2.55ab	2.25	10.34	13.86	0.45	0.47ab	1.55	2.47b
P-value	0.042	0.071	0.177	0.055	0.139	0.018	0.138	0.045
CV (%)	61.27	37.37	38.25	38.72	47.59	52.39	39.60	39.19

Note: K0 (control), K1 (extract 5%), K2 (extract 10%), K3 (extract 15%), K4 (extract 20%), CV (coefficient of variation). According to Dunn's test, values followed by the same letter (s) are not significantly different at P ≤ 5%.

The results from Table 5 showed a similar trend but indicated no significant difference in the fresh biomass weight of purple nutsedge weeds, according to the Kruskal-Wallis test. Significant results were observed in purple nutsedge biomass's tuber and total dry weight. The application of bamboo leaf extract effectively inhibited the growth of Chinese violet, as indicated by the reduction in total fresh biomass weight by 37.27%, 58.18%, 44.37%, and 51.28% for treatments K1, K2, K3, and K4, respectively. The effectiveness of bamboo leaf extract application begins at 10% concentration, impacting and inhibiting purple nutsedge growth.

Research indicates that allelochemicals can preferentially inhibit root growth over shoot growth in weeds, which is critical since root development is essential for nutrient and water uptake (Yang & Kong, 2017). Secondary metabolite compounds can also disrupt cellular processes in target plants, leading to reduced seedling vigor and, ultimately, lower biomass accumulation (Zhang et al., 2015). Based on the percentage of growth reduction, the potential for increased inhibition of Chinese violet and purple nutsedge needs further study, as the values are linearly related to the extract concentration. Further research on higher concentrations may result in either greater or reduced inhibition, potentially identifying the optimum concentration. Additionally, experiments on non-weed plants may be necessary to determine a safe concentration that won’t harm them.

CONCLUSIONS

Based on the results and discussion, Apus bamboo leaf extract contains various compounds, including phenolics, flavonoids, saponins, tannins, and alkaloids, which potentially exhibit allelopathic activity, as indicated by the inhibitory effects observed in the bioassays, where the tested seeds of *Brassica juncea* experienced reduced germination and seedling size. Apus bamboo leaf extract significantly affects and inhibits the germination of *Asystasia gangetica* and reduces its population. The extract reduces the growth of *Asystasia gangetica,* starting at a concentration of 15%, while for *Cyperus rotundus*, the inhibition begins at 10%. The values show a trend of stronger inhibition with higher concentrations. Further studies might be needed to evaluate bamboo leaf extract during the post-emergence phase of weeds.

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