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Evaluating Allelopathic Potential of *Piper lolot* Under Different Screening Conditions

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Abstract

Piper lolot is a medical plant species and is widely distributed in many Southeast Asia countries. *P. lolot* is a vigorous-growing plant and curbed the growth of nearby plants in nature. This study aimed to determine the allelopathic effects of this plant (leaf and stem dried powders) under laboratory bioassays and greenhouse conditions against the growth of *Echinochloa crus-galli* and some indicator plants. In laboratory bioassays, at the applied dose (50g/L) of the leaf powders, the root length of *E. crus-galli* and *Vigna radiata* was significantly reduced by over 80%. In greenhouse experiments, the stem and leaf powders significantly inhibited the dry weight of *E. crus-galli* (75%) and (47.6%), respectively. However, at lower concentrations, the powder from leaves and stems slightly promoted the growth of root length in *E. crus-galli*. The inhibitory rate was varied with the indicator plant species and was proportional to the doses of *P.lotot* applied. Further studies on allelochemical identification and isolation from this plant species should be comprehensively studied.

Keywords: Allelopathic potential, extract, *Piper lolot*, bioassays, greenhouse, inhibition

Introduction

Allelopathy, a biochemical interaction involving plants and microorganisms, elicits direct or indirect effects including both inhibitory or stimulatory on the neighboring plant growth in nature. The production and release of chemical compounds (toxins) into the environment from plant species and living organisms drive this phenomenon [1-2]. The allelopathy topic has received much attention from worldwide scientists in recent years since allelopathy was recognized as a possible alternative to synthetic herbicides for weed control [3]. Diverse plant species within ecosystems, spanning leguminous, medicinal, and invasive plants demonstrate substantial allelopathic potential as part of plant defense mechanisms. These mechanisms involve the release of numerous secondary metabolites (allelochemicals), including mineral constituents, responsible for suppressing weeds and enhancing crop yields when directly applied to paddy fields [4]. Crop plants suppress weeds by releasing toxins into the environment through root exudation or from the decomposition of plant residues, as demonstrated by Putnam [5] in over 90 plant species. The inhibitory substances are divided into six specific groups: alkaloids, benzoxazinones, flavonoids, derivatives of cinnamic acid, cyanogenic compounds, ethylene, and seed germination stimulants [5]. Many plants in nature have strong weed-suppressing capabilities; some possess allelopathic potential that inhibited the growth of paddy weeds and significantly increased rice yield. Examples include passion flower (*Passiflora edulis*), alfalfa (*Medicago sativa L.*), fragrant thoroughwort (*Eupatorium cannabium*), buckwheat (*Fagopyrum esculentum*), chinaberry (*Melia azedarach*), kava (*Piper methysticum*), galactia (*Galactia pendula*), neem (*Azadirachta indica*), billygoat weed (*Ageratum conyzoides*), and white lead-tree (*Leucaena leucocephala*) [2-3],[6].

P. lolot, a robust perennial herb in the Piperaceae family, exhibits vigorous growth, occasionally encroaching and impeding the growth of neighbouring plants in nature. With long, upright stems measuring 30–40 cm, the plant emits a fragrant aroma. The dark green stem is characterized by nodal swelling, a round cross-section, and longitudinal grooves, along with short, smooth hair. The simple, alternate leaves are wide-oval, pointed, and asymmetrical, with dimensions of 10–12 cm by 8–11 cm. The upper leaf surface is smooth and shiny, while the lower surface is light green and hairy, featuring entire margins and pinnate veins with 5 basal veins. The central vein divides into 2 lateral veins, curving towards the leaf tip. The petiole, 2–5 cm long, is cylindrical, concave on the upper surface, and wide at the base [6] (Figure 1.). *P. lolot*, indigenous to Southeast Asia, is a distinctive plant that thrives in the wild and is intentionally cultivated for its leaves, valued both as a culinary spice and for medicinal applications. To the best of our knowledge, the compounds found in *P. lolot* include alkaloids, flavonoids, and essential oils, with beta-caryophyllene being the main component [7],[13]. However, few reports have been reported on the allelopathic potential of this plant species. Therefore, the objective of this study was to assess the allelopathic potential of *P. lolot* under laboratory bioassays and greenhouse conditions.

Figure 1. Morphological characteristics of *Piper lolot*

Materials and Methods

The aboveground parts of *P. lolot* were collected in the ecological area in Nho Quan, Ninh Binh (20°18'39"N 105°42'14"E). After being collected, leaf and stem samples were separated, washed, and dried in natural sunlight. After 2-3 days, depending on the temperature, the dry samples were ground into powder about 4mm, then stored in plastic bags and labelled for each bag**.** Seeds, including *E. crus-galli*, Khang Dan 18 rice (*O. sativa*), and green beans (*V. radiata*), were provided by the Agricultural Genetics Institute and the Vietnam National University of Agriculture and used as indicator plants. Before conducting experiments, the germination (%) of all seeds of indicator plants was randomly checked and was shown to be over 90% [7].

Bioassays

The bioassay experiments were performed at the laboratory of the Genetic Engineering Department, AGI, Hanoi, Vietnam. The powders (leaves and stems) of *P. lolot* were separately diluted in 5% agar and distilled water at different concentrations (50 g/L, 25 g/L, 12.5 g/L, and 6.2 g/L) following the previous method [8]. Each diluted solution was poured into the plastic cup. Put it in the UV cabinet for 20 min. Then, kept in cool at 45–50°C and then put 250ml/plastic cup. On the agar surface of the cup, 20 seeds of each indicator plant were evenly grown. Only distilled water was used for the control treatment. The cups were placed in the growth chamber at 25°C, 4000 Lux, light time: 9:00-17:00, and the humidity was adjusted by 75%. After 7 days of sowing, the germination rate, root and stem length, and fresh and dry weight of all tested plants were recorded. All treatments were done with at least three replicates and compared with the controlled treatment [8].

Greenhouse experiments

Three kilograms (3kg) of commercially sterilized soil (pH 4.5–5.8, EC 1.0 ± 0.2, N 1100 ± 100 mg/kg−1, P_2O_4 400 ± 100 mg kg^{-1}) which did not contain any micro-organisms or weed seeds, were used. The soil was air-dried and put in plastic containers (7000 mL, 25 cm in diameter) and then saturated with 1000 mL of tap water. Twenty healthy seeds of *E. crus-galli* were sown at a depth of 1.5 cm. After 2 days, the ground powders of leaf and stem of *P. lolot* at doses of (50 g/m^2, 100 g/m^2, 150 g/m^2, and 200g/m^2) were applied [9]. Each treatment was performed at least thrice. The treatments using only tap water were used as the control. After 30 days, the number of *E. crus-galli* plants, shoot length, and fresh and dry weight were collected and determined.

Soil from paddy fields belonging to the Experimental Station of Agricultural Genetics Institute, Hanoi, Vietnam), where no herbicide had been applied in the previous crop was gathered at 10 cm depth and transferred to a greenhouse (25-30ºC±2). The soil was air-dried, crushed and put in plastic containers (30 cm width x 40 cm length x 30 cm height) and saturated with tap water. After 2 days, the powers of leaves at different concentrations (50 g/m^2, 100 g/m^2, 150 g/m^2, and 200g/m^2) were applied to the containers by scattering on the container surface. Treatments with tap water only were the control. After 30 days, weed biomass including fresh weight and dry weight was determined [8].

Statistical Analyses

The laboratory bioassays were performed with 4 treatments with three replicates. The greenhouse trials were done in a completely randomized design with three replications. The statistical analyses were performed using Excel version 2016, Minitab 1.8, and IRRISTAT version 2010. The inhibition (%) was calculated following the formula:

$$Inhibition\ (\%) = [(1\text{-}sample/control) \times 100]$$

Results and Discussion

Allelopathic potential of the leaves and stems of P. lolot on the growth of E. crus-galli and indicator plants in laboratory bioassays

In this study, we attempted to evaluate the inhibitory effect of *P. lolot* against the growth of some indicator plants including *E. crus-galli, V. radiata* and *O. sativa.* Indeed, among the cultivated species used in this study, *V. radiata* was reported to be highly sensitive to allelochemicals even at low concentrations and to either stimulators or inhibitors used in allelopathic studies [7-9]. while the major paddy weed (*E. crus-galli*) directly competed with rice in the field and is suitable as the tested plant for determining the inhibitory magnitude of the candidate plant species.

Table 1. Allelopathic effect of leaf powders and stem powders of *P. lolot* on the growth of *E. crus-galli* under laboratory bioassays

Dose (g/L)		Germination (%)	Shoot Length (cm)	Root Length (cm)	Dry Weight (g)	Fresh Weight (g)
			E. crus-galli			
Stem	**50**	40.0^{cd} ± 5.0 (-52.9)	2.8^{d} ± 0.8 (-68.1)	0.3^{c} ± 0.1 (-86.3)	0.012^{bc}± 0.001 (-53.8)	0.1^{d}±0.06 (0.0)
	25	38.3^{cd} ± 11.5 (-54.9)	4.6^{cd} ±0.8 (-47.7)	0.5^{bc}± 0.1 (-77.2)	0.015^{d}± 0.002 (-57.6)	0.1^{d} ±0.05 (0.0)
	12.5	53.3^{bcd} ± 7.6 (-37.2)	4.9^{cd} ± 1.6 (-44.3)	0.5^{bc}±0.2 (-77.2)	0.011^{d}±0,001 (-57.6)	0.07^{cd}±0.02 (-30)
	6.25	71.6^{ab} ± 7.6 (-15.7)	8.6^{ab} ± 0.6 (-2.2)	0.6^{bc} ± 0.5 (-72.7)	0.013^{d}±0.001 (-48.7)	0.09^{d}±0.03 (-10)
Leaf	**50**	36.6^{d} ± 7.6 (-56.9)	4.0^{d} ± 0.8 (-54.5)	0.4^{bc}± 0.2 (-81.8)	0.022^{c}±0.002 (-15.3)	0.09^{bcd}±0.06 (-10)
	25	58.3^{bc} ± 7.6 (-31.4)	4.2^{cd} ± 1.1 (-52.2)	0.6^{bc}± 0.1 (-72.7)	0.024^{a}±0.003 (-7.7)	0.11^{ab}±0.1 (+10)
	12.5	67.6^{b} ± 2.5 (-20.4)	6.1^{bc} ± 0.5 (-30.6)	1.0^{bc}±0.2 (-54.5)	0.026^{bc}±0.002 (0)	0.13^{abc}±0.01 (+20)
	6.25	73.3^{ab} ±2.9 (-13.7)	8.6^{ab} ± 0.5 (-2.2)	1.4^{ab}±0.4 (-36.3)	0.025^{bc}±0,001 (-5.1)	0.128^{a}±0.1 (+18.5)
Control		90.0^{a} ± 8.66	8.8^{a} ± 0.4	2.2^{a} ± 0.5	0.026^{bc}±0.003	0.1±0.09^{cd}
			V. radiata			
Stem	**50**	41.6^{e} ± 5.7 (-55.4)	6.6^{b} ± 0.8 (-29.8)	1.1^{c} ± 0.8 (-84.5)	0.2^{d} ± 0.02(-50)	1.4^{d}±0.05 (-69.5)
	25	61.6^{cde} ± 2.9 (-34)	1.4^{de} ± 0.02 (-85.1)	1.5^{c} ± 0.2(-78.8)	0.2^{d} ± 0.02 (-50)	2.5^{c}±0.2 (-45.6)
	12.5	81.6^{abc} ±2.9(-12.5)	3.3^{cd} ± 0.5 (-64.9)	3.5^{b} ± 0.2 (-50.7)	0.3^{bcd} ± 0.05(-25)	3.8^{b}±0.2 (-17.4)
	6.25	86.6^{ab} ±7.6 (-7.1)	5.2^{bc} ± 0.4 (-44.7)	7.1^{a} ± 0.8 (0)	0.3^{ab} ± 0.03 (-25)	5.0^{a}±0.3 (+8.7)
Leaf	**50**	55.0^{de} ±5.0 (-40)	0.9^{e} ± 0.05 (-90.4)	0.3^{c} ± 0.03 (-95.7)	0.3^{ab} ± 0.02 (-25)	1.2^{d}±0.05 (-73.9)
	25	60.0^{de} ±5.0 (-35.7)	1.7^{de} ± 0.5 (-81.9)	0.6^{c} ± 0.1 (-91.5)	0.3^{abc} ± 0.04 (-25)	1.7^{d}±0.1 (-63.0)
	12.5	73.3^{bcd} ±15.2(-21.4)	5.4^{b} ± 1.5 (-42.5)	1.1^{c} ± 0.3 (-84.5)	0.2^{cd} ±0.04 (-50)	2.7^{c} ±0.5(-41.3)
	6.25	98.3^{a} ±2.9 (+5.3)	8.6^{a} ± 0.6 (-8.5)	2.8^{b} ± 0.5(-60.6)	0.4^{a}±0.05 (0)	5.3^{a}±0.3 (+15.2)
Control		93.3^{ab} ± 7.6	9.4^{a} ± 0.5	7.1^{a}	0.4^{a}±0.04	4.6^{a}±0.2
			O. sativa			
Stem	**50**	60.0^{bc}±8.6 (-33.3)	7.1^{ab}±0.6(-10.1)	5.6^{a}±0.5 (+60)	0.193^{a}±0,003 (+1.7)	0.8^{b}±0.09(-33.3)
	25	55.0^{bcd} ±10.0 (-38.9)	4.8^{d}±0.5(-39.2)	2.7^{e}±0.2 (-22.9)	0.18^{ab}±0.01 (-5.3)	0.9^{ab}±0.2 (-25.0)
	12.5	71.6^{ab}±7.6 (-20.4)	7.0^{ab}±0.8(-11.4)	2.3^{bcd}±0.2(-34.2)	0.16^{abc}±0.03 (-15.8)	0.95^{bc}±0.04(-20.9)
	6.25	73.3^{ab}±7.6 (-18.6)	7.6^{ab}±0.1(-3.8)	1.8^{cde}±0.1(-48.5)	0.187^{a}±0.02 (-1.7)	1.1^{a}±0.09 (-8.3)
Leaf	**50**	33.3^{d} ±10.4(-42.8)	5.1^{cd}±0.4 (-35.4)	0.6^{e} ±0.2(-82.8)	0.103^{c}±0.02 (-45.6)	0.2^{d} ±0.06(-83.3)
	25	48.3^{cd} ±7.6(-17.1)	6.3^{bc}±0.3 (-20.2)	1.1^{de}±0.2 (-68.5)	0.143^{abc}±0.03 (-24.6)	0.3^{cd}±0.1 (-75.0)
	12.5	43.3^{cd} ±2.8(-25.7)	7.3^{ab} ±0.4(-7.6)	1.9^{cde}±0.4(-45.7)	0.137^{abc}±0.01 (-28.0)	0.3^{cd} ±0.01(-75.0)
	6.25	43.3^{cd}±7.6 (-25.7)	7.3^{ab}±0.2 (-7.6)	3.0^{bc} ±0.7(-14.3)	0.123^{bc} ±0.02(-35.1)	0.4^{cd} ±0.1(-66.7)
Control		90.0^{a}± 5.0	7.9^{a}±0.3	3.5^{b}±0.7	0.19^{a}±0.01	1.2^{a}±0.2

AI: Average inhibition; SE: Standard errors; "+" implies stimulation (%) and "-" presents inhibition (%) to compare with the control.
The means in the same column with different letters are significantly different by $p < 0.05$ compared with the control.

As presented in Table 1, *P. lolot* had strong allelopathic effects on the growth of some *E. crus-galli* and the tested plant species. However, the magnitude of inhibition fluctuated based on the dose of application. At the highest dose (50g/L) of both stem and leaf powders of *P.lolot*, the growth factors and biomass of *E. crus-galli* and *V. radiata* were significantly reduced, in which the root length of *V. radiata* was the most inhibited (95.4%), *E. crus-galli* (86.3%). Nevertheless, the root length of *O. sativa* was significantly promoted (60%). Notably, the total average inhibition (AI%) of *P. lolot* stems were slightly higher than that of its leaves, which indicated that the stems may contain more allelopathic property or allelochemicals. At the highest dose of application (50L/g), the AI exerted the growth of indicator plants from 56.82% to 57.98%, respectively (Figure 2), and the inhibitory rate was gradually reduced based on the applied doses. On the other hand, the growth inhibition was directly proportional to the doses of *P. lolot* applied and fluctuated with the tested plant species. Our finding was in line with some previous reports on exploiting the biomass of allelopathic plants to manage paddy weeds [2], [4], [9].

Allelopathic potential of the leaves and stems of P. lolot on the growth of E. crus-galli and natural weeds under greenhouse conditions

Table 2 shows the allelopathic effects of the leaf and stem powders of *P. lolot* on the growth of *E. crus-galli*. At 200g/m^2 dose, the stem powders of *P. lolot* suppressed the dry weight of *E. crus-galli* (75.0%), root length (37.9%) and the least inhibition was fresh weight (11.1%) (Table 2). At a dose of application (150g/m^2), root length was reduced by 23.9%, however, the other growth factors of *E. crus-galli* were promoted at lower doses (100 and 50 g/m^2). The leaves of *P. lolot* exerted significantly suppressed the growth of *E. crus-galli* at a dose of application (200g/m^2) from 30-47%. Both root length and dry weight of *E. crus-galli* were inhibited by 20% and 42.8%, respectively. However, the lower doses significantly enhanced the growth of *E. crus-galli* (Table 2).

As presented in Figure 3, at the applied dose (200g/m^2), the biomass fresh weight of natural weeds was significantly minimized by 60%, dry weight (27.2%), while fresh weight and dry weight were slightly inhibited at the dose of application at 150 g/m^2. However, at the lower doses, the biomass of natural weeds was enhanced from 10 to 20% (Figure 3). It implies that *P. lolot* may contain potential nutrients and be used as a promising green manure with multiple benefits comprising soil coverage and nitrogen fixation [2],[8-9].

Recently, numerous studies have evaluated the allelopathic potential of several hundred higher plant species and identified some strong allelopathic plants and promising use for controlling paddy weeds and enhancing rice yield [8-9]. In this study, we found that *P. lolot* powders had a larger inhibitory effect on the root growth than on the shoot growth of the indicator plants. The results were consistent with previous research of Salam and Noguchi [10], who found that extracts from allelopathic plants had a stronger inhibitory effect on root growth as opposed to hypocotyl growth due to the fact that roots are the first organs to absorb allelochemicals from the surrounding environment [10]. Furthermore, Nishida et al [11] found that allelochemicals are more permeable to root tissue than to shoot tissue

[11]. However, it should be noted that the different extract methods may lead to different results due to inherent variances in the metabolic mechanisms involved [12].

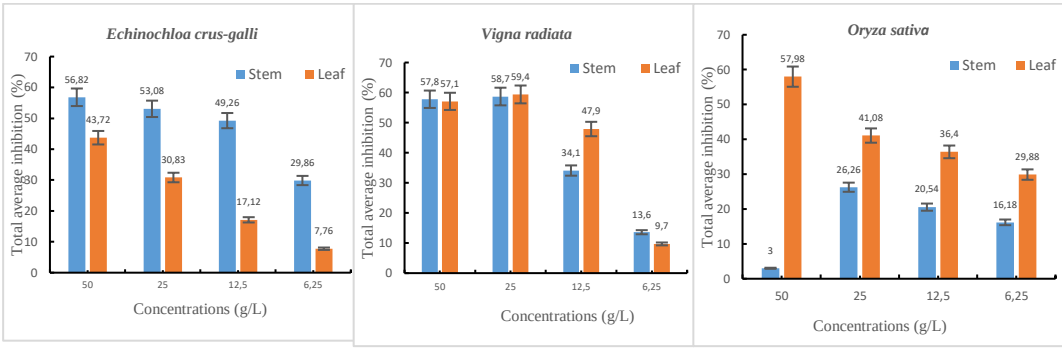

Figure 2. Total average inhibition (%) of leaf powders and stem powders of *P. lolot* on the growth of some indicator plants in bioassays

Table 2. Allelopathic effect of leaf powders and stem powders of *P. lolot* on the growth of *E.crus-galli* under greenhouse conditions

Dose (g/m^2)		Root Length (cm)	Dry Weight (g)	Fresh Weight (g)	AI (%)
Stem	**200**	$26.5^{d} \pm 1.2$ (-37.9)	$0.12^{c} \pm 0.02$ (-75.0)	$0.8^{d} \pm 0.05$(-11.1)	-41.3
	150	$32.5^{c} \pm 1.5$ (-23.9)	$0.25^{bc} \pm 0.05$(+19.0)	$1.7^{bc} \pm 0.1$ (+88.9)	+28
	100	$45.6^{b} \pm 0.2$ (+6.8)	$0.23^{bc} \pm 0.05$ (+19.4)	$1.7^{bc} \pm 0.1$ (+88.9)	+38.4
	50	$47.7^{ab} \pm 0.7$ (+11.7)	$0.33^{ab} \pm 0.05$ (+58.7)	$2.1^{ab} \pm 0.05$ (+133.3)	+67.9
Leaf	**200**	$29.9^{cd} \pm 1.3$ (-30.0)	$0.11^{c} \pm 0.01$ (-47.6)	$0.5^{d} \pm 0.07$ (-44.4)	-40.7
	150	$34.2^{c} \pm 2.7$(-19.9)	$0.12^{c} \pm 0.02$(-42.8)	$1.1^{cd} \pm 0.6$ (+22.2)	-13.5
	100	$46.3^{b} \pm 2.0$ (+8.4)	$0.3^{ab} \pm 0.1$ (+42.8)	$1.8^{ab} \pm 0.07$ (+100)	+50.4
	50	$52.2^{a} \pm 2.4$ (+22.2)	$0.433^{a} \pm 0.05$ (+106.3)	$2.4^{a} \pm 0.05$ (+166.7)	+98.4
Control		$42.7^{b} \pm 3.0$	$0.21^{bc} \pm 0.01$	$0.9^{d} \pm 0.02$	-

AI: Average inhibition; SE: Standard errors; "+" implies stimulation (%) and "-" presents inhibition (%) to compare with the control. The means in the same column with different letters are significantly different by $p < 0.05$ compared with the control

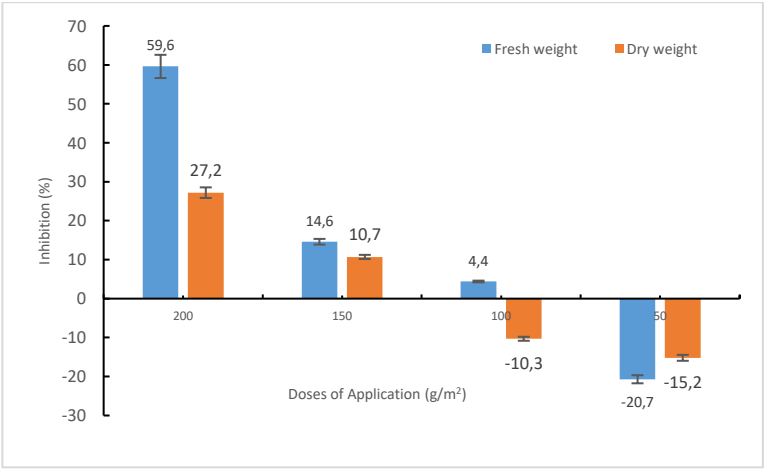

Figure 3. Allelopathic effects of P.lolot leaves powders against the growth of natural paddy weeds in greenhouse conditions

Phytotoxins with similar species possibly be released in large amounts if the appropriate methods are applied. Consequently, the growth inhibition in the indicator plant species is most likely due to allelopathic rather than competitive interference if the competitive factors are minimized. As the result attained, our study suggests that *P. lolot* may be used as a source of natural herbicide to minimize the overuse and dependency on harmful synthetic herbicides and improve soil health and crop yields.

Conclusions

In summary, it is evident that *P. lolot* exhibited allelopathic influence on the growth and development of *E. crus-galli* and indicator plants in both laboratory bioassay and greenhouse conditions. The stem of *P. lotot* had slightly higher allelopathic properties than its leaf under both bioassays and greenhouse conditions. Our findings may aid useful information for further isolating and identifying allelochemicals from this plant species.

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