JOURNAL OF AGROBIOTECHNOLOGY 2022, VOL 13(1S):9-16.
e-ISSN: 2180-1983
http://dx.doi.org/10.37231/jab.2022.13.1S.308
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Evaluation of Allelopathic Potential of *Pluchea indica* on *Brassica chinensis* and *Zea mays*

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Received: 31/07/2022, Accepted: 08/09/2022 Available Online: 16/10/2022

ABSTRACT

Weeds have become one of the leading causes of crop production losses, including the cost of weed management, weed crop competition, and weed intervention with crop management practices. Moreover, herbicide use in agricultural systems is currently being debated due to the potential adverse effects on the environment and human health. The utilization of the allelopathic phenomenon is widely considered an alternative weed management strategy. However, allelopathic study of *Pluchea indica* plant was limited. *P. indica* is a flowering plant under family Asteraceae, locally known as Beluntas. Thus, this study was conducted to determine the allelopathic effects of the aqueous leaf extract of *P. indica* on the seedling growth of tested plant species and to investigate the effect of *P. indica* leaf debris on the growth of tested plant species. In this study, the allelopathic potential of *P. indica* leaves were investigated through petri dish bioassay and soil bioassay method. The two tested plant species were chosen for the bioassay: monocotyledonous; maize *(Zea mays*) and dicotyledonous; mustard (*Brassica chinensis).* These tested plant species were chosen for the experiment due to their known quick seedling growth behaviours and commonly used in allelopathic studies. The result showed that as the concentration of aqueous leaf extract increased, the inhibition percentage of the radicle length of *B. chinensis* and *Z. mays* were increased. However, stimulatory effect was observed on the radicle length of *B. chinensis* and *Z. mays* when 1.3%and 2.5% w/w *P. indica* leaf debris were applied. Thus, aqueous leaf extract and leaf debris of *P. indica* have potential allelopathic effects on the growth of *B. chinensis* and *Z. mays*. Further studies need to be conducted on other bioassay species especially weed species, to elucidate the allelopathic potential of *P. indica* in weed control.

Keywords: Allelopathy, *Pluchea indica*, beluntas, aqueous extract, soil bioassay

INTRODUCTION

Allelopathy is a biological phenomenon that occurs when living species in an environment interact chemically, and it was considered for solving pest and weed problems in future sustainable agriculture (Macías et al., 2019). The biosynthesis of allelochemicals, their structures, exudation into the environment and fate, mode of action, biological activities, and breakdown process are all aided by the allelopathic phenomenon. Allelopathy also

consists of data on secondary metabolite-driven interactions between plants (including intra-species interactions like autotoxicity), plants and microbes, plants and insects, and plants and viruses (Jabran, 2017). Allelopathy is important in weed control, crop protection, and crop restoration. Scientists that involved in allelopathic analysis are focussing on the appropriate manipulation of allelopathy in advancing the crop productivity and environmental protection through eco-friendly management of weeds, pests, crop diseases, nutrient conservation in cropland, and the synthesis of new agrochemicals based on natural products (Mushtaq & Siddiqui, 2018).

Plant organs such as roots, rhizomes, leaves, stems, bark, flowers, fruits, and seeds release allelochemicals into the environment (Albuquerque et al., 2011). Allelopathic interactions, which impact the distribution and abundance of species in plant communities, are thought to be key to the success of many invasive plants (Svensson et al., 2013). Plant growth and development, particularly germination and early seedling growth, are affected by these chemicals as well as cause imbalance in soil fertility and limitation of microbial population in soil (Laizer et al., 2021). Allelochemical production is crucial in determining the vegetation's diversity, dominance, and natural succession in agro ecosystems.Thus, information on allelopathic activity and the allelochemicals released has the potential to be further investigated to develop and improve weed management strategies (Yusoff & Sahid, 2015).

Pluchea indica (L.) Less (Asteraceae) is a perennial shrub plant with a wide range of branches that is indigenous to much of Asia, India, and northern Australia (CABI, 2021). This species is commonly known as Beluntas in Malaysia. *P.indica* leaves are short-stalked, obovate, thick papery, and have a serrated border and tapering base. When young, the leaves are bright green; and when mature, they are pale green and very aromatic when crushed (Chan et al., 2022). *P. indica* extracts were discovered to have potential as an anti-tuberculosis action (Mohamad et al., 2011) and possesses therapeutic characteristics that could be further developed into nutraceutical, diet supplement, or cosmetic goods (Normala et al., 2011). Previous study found that the aerial parts and leaves of *P. indica* contain caffeoylquinic acids, phenolic acids, flavonoids and thiophenes (Chan et al., 2022). Due to these medicinal properties, this plant may have allelopathic potential. Previous studies reported that medicinal plant species posses strong allelopathic potential (Islam et al., 2018). Since there is limited study regarding the allelopathic activity of *P. indica*, this research was carried out to evaluate the allelopathic potential of *P. indica* leaf. This research is conducted in two types of bioassay methods which are laboratory and soil bioassay.

MATERIALS AND METHODS

Sample collection and preparation

Pluchea indica plants were collected from Taman Herba, Universiti Sultan Zainal Abidin Kampus Besut, Malaysia at the coordinate 5°45'11.5" N, 102°37'42.0" E. *P. indica* was chosen as the donor species for this research. The leaves were washed, and oven dried at 60 ºC overnight before being stored at room temperature (28 ±2°C) in the laboratory. The seeds of *Brassica chinensis* (mustard) and *Zea mays* (sweet corn) were purchased from Green World Genetics Sdn. Bhd. Company. Both seeds served as receiver bioassay plant species in this research. *B. chinensis* seeds are the dicot tested bioassay species whilst *Z. mays* seeds are the monocot tested bioassay species.

Laboratory bioassay

Approximately 10 g of dried leaves were ground before being soaked for 72 hours with 200 mL distilled water at 4°C. The extracts were filtered through one layer of filter paper before being centrifuged at 4032 *g* for 15 minutes. The supernatant from each plant species was collected and filtered using a filter pump through one layer of 0.2 µm cellulose membrane filter (Whatman). The extracts were stored at 4°C until use (Yusoff & Ismail, 2015).

The following are the three concentrations of aqueous extracts utilised in the experiment: - 12.5 g/L, 25.0 g/L, and 50.0 g/L. Distilled water was used as control in this experiment. To wet the filter paper in the Petri dishes, 5 mL of each aqueous extract were used. There were three replicates for each concentration, with ten seeds in each petri dish. The petri dishes were incubated at 28 ^{0}C ± 2 (with 12 hours photoperiod) and checked daily. A seed was considered germinated when the radicle length was more than 2 mm. After seven days, the hypocotyl and radicle length of receiver species were measured and recorded (Yusoff & Ismail, 2015).

Soil bioassay

The dried leaves of *Pluchea indica* were ground separately and then mixed in a seedling tray with 200 g soil (composition: 61.7% sand, 28.2% clay, 10.1% silt; 0.43% total Nitrogen and pH 4.9). In the experiment, three concentrations of leaf debris were used: 0.6%, 1.3% and 2.5% w/w. The soil without leaf debris served as the control. Ten pre-germinated *B. chinensis* and *Z. mays* seeds were sowed and watered regularly. The tray was placed in the room growth chamber (temperature: 22 ± 2 °C , light density: 80-100 lm/W, relative humidity: 60 ±10%) with 12 hours photoperiod. After seven days, the seedlings were thinned to five seedlings per hole. After two weeks, the seedling growth (shoot and root length) was measured (Yusoff & Ismail, 2015).

Statistical Analysis

Both experiments were conducted in the Completely Randomized Design (CRD) with three replications. The experimental results were subjected to a one-way ANOVA, and the Duncan Multiple Range Test was used to compare means at the 5% significance level. The statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, USA).

The calculation for the inhibitory effect was as follows: (Aslani et al.,2014).

$$I = 100\ (C - A)/C$$

where "I" stands for the inhibition percentage, "C" for the bioassay species' control mean radicle and hypocotyl length, and "A" for the treatment's mean radicle and hypocotyl length.

RESULTS AND DISCUSSION

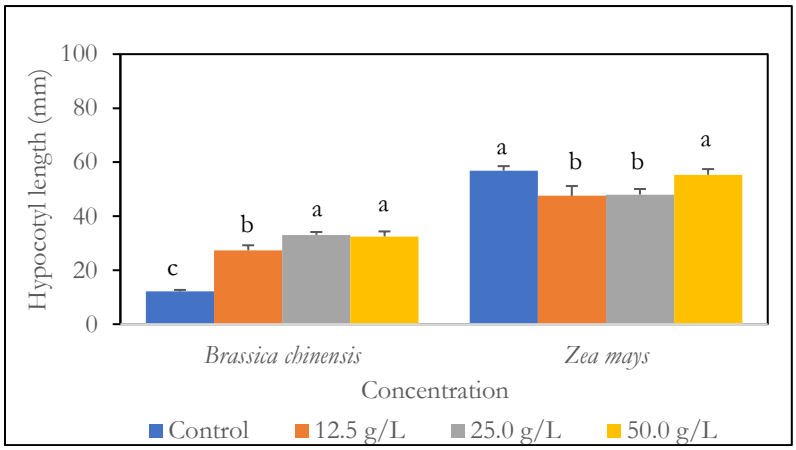
Effect of the aqueous leaf extract of *P. indica* on the receiver species

The results of the laboratory experiments, as shown in Fig. 1, indicated that the hypocotyl length of *Brassica chinensis* and *Zea mays* depended on the concentration of *Pluchea indica* aqueous leaf extract used. Based on Table 1, when compared to the control, the hypocotyl length of *B. chinensis* was significantly stimulated ($\rho<0.05$) by -125.3% , -172.6% , and -167.7 % at concentrations of 12.5 g/L, 25.0 g/L, and 50.0 g/L of *Pluchea indica* aqueous leaf extract, respectively. Following that, the leaf extract of *P. indica* at concentrations of 12.5 g/L and 25.0 g/L significantly inhibited ($\rho<0.05$) the hypocotyl length of *Z. mays* by 16.4% and 15.6%, respectively, when compared to the control (Table 1). However, the aqueous leaf extract of *P. indica* significantly affected the hypocotyl length of *Z. mays* with low effectiveness (3% inhibition rate) at 50.0 g/L concentration.

Fig. 1 indicated that the radicle length of *B. chinensis* and *Z. mays* was inversely proportional to the concentration of *P. indica* aqueous leaf extract used. The radicle length of *B. chinensis* and *Z. mays* progressively decreased when the concentration increased. As the concentration of aqueous leaf extract increased, the radicle length of *B. chinensis* and *Z. mays* were significantly decreased compared to control. More than 50% inhibition of radicle length compared to control was exhibited by 50.0 g/L of *P. indica* aqueous leaf extract on the radicle length of *Z. mays*. Whilst, at concentrations of 12.5, 25.0 and 50.0 g/L of *P. indica*, the radicle length of *B. chinensis* where inhibited by more than 50%.

P. indica leaf extract influences the growth of *B. chinensis* and *Z. mays* in a dose-dependent manner. According to Yuliani and Rahayu (2018), 0.125%, 0.50% and 1.0% concentrations of *P. indica* leaf extract inhibited the seed germination in *Amaranthus spinosus*. *Pluchea indica* inhibited the germination and growth of weed species such as *Mimosa pudica* and *Ruellia tuberosa* in a previous study by Yuliani et al. (2009). *P. indica* was discovered to contain phenol compounds such as coumarin, benzoic acid, salicylic acid, and vanilic acid, which may inhibit the growth of *B. chinensis* and *Z. mays* (Yuliani & Rahayu, 2018). Kumbhar & Patel (2012) reported that phenolic compounds could influence the germination and establishment of the crop through the disruption of enzyme and respiration activity. Moreover, the receiver's radicle length was more sensitive than the hypocotyl length. This was because the radicle was the first organ exposed to the extract and has highly permeable tissue compared to other organs (Islam et al., 2019; Nishida et al., 2005).

(a)



(b)

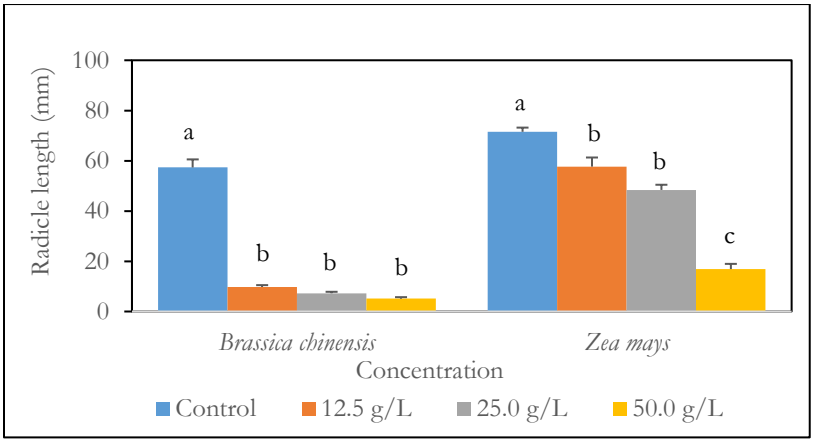


Fig. 1. The effect of *P. indica* leaf aqueous extract on the (a) hypocotyl length and (b) radicle length of *B. chinensis* and *Z. mays*. Means with the same letters in the graph bar for each receiver species are not significantly different at ϱ>0.05.

Table 1. The effect of different concentrations of the *P. indica* leaf aqueous extract on the percentage inhibition of hypocotyl and radicle length of *B. chinensis* and *Z. mays*.

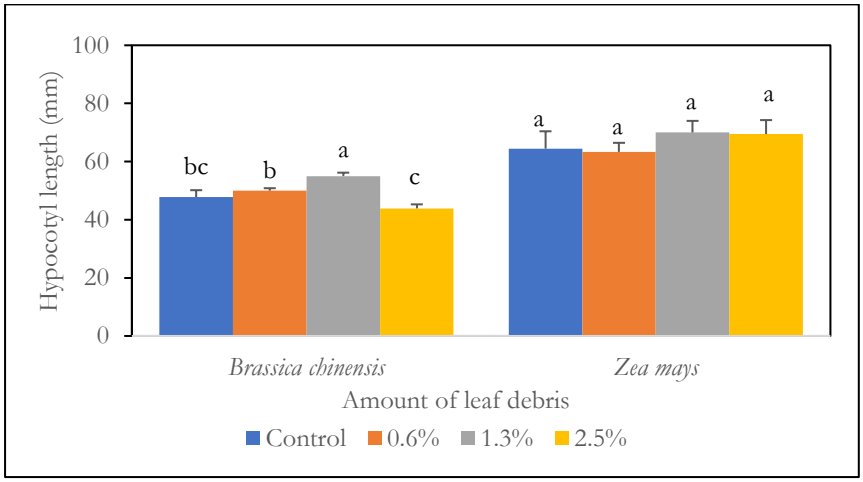
Bioassay species	**Concentration of aqueous leaf extract (g/L)**	**Percentage of inhibition (%)**	
		Hypocotyl length	Radicle length
Brassica chinensis	12.5	-125.3	82.9
	25.0	-172.6	87.3
	50.0	-167.7	91.0
	Control	0.0	0.0
Zea mays	12.5	16.4	19.4
	25.0	15.6	32.3
	50.0	2.7	76.4
	Control	0.0	0.0

Effect of the leaf debris of *P. indica* on the receiver species

Fig. 2 shows that 1.3% of *P. indica* leaf debris significantly stimulated less than 20% the hypocotyl length stimulation (ϱ<0.05) on *B. chinensis*. There was no significant effect on *B. chinensis* hypocotyl length compared to control when applied with 0.6% and 2.5%of *P. indica* leaf debris. Meanwhile, no significant effect was demonstrated on the hypocotyl length of *Z. mays* when treated with *P. indica* leaf debris (Fig.2). In addition, when 1.3% and 2.5%of *P. indica* leaf debris were applied, the radicle length of *B. chinensis* were significantly increased compared to the control with -160.6% and -166.7%, respectively. Similar trend of stimulatory effect was observed on the radicle length *Z. mays* when when were applied with 1.3% and 2.5% *P. indica* leaf debris.

These findings contradicted previous findings that allelopathic plant leaf debris inhibitory effectiveness was related to plant debris concentrations, with larger quantities having a stronger inhibitory impact (Begum et al., 2021). However, Kobayashi (2004) reported that the activity of allelochemicals in soil depends on several factors such as leaching, degradation, soil pH, organic matters and others, and this could be that the bioactive concentration of the allelochemicals in the soil being degraded. Ambika (2002) reported that freshly decomposed leaves of *C. odorata* inhibited crop growth. However, after 60 days, the crop growth was promoted after the *C. odorata* leaves were fully decomposed. Besides, Sodaeizadeh et al. (2009) reported that the maximal phytotoxic effect of *Peganum harmala* soil integration occurred one to three days after the decay began, and phytotoxicity was initially reduced on the seventh day before disappearing on the fifteenth day.

(a)



(b)

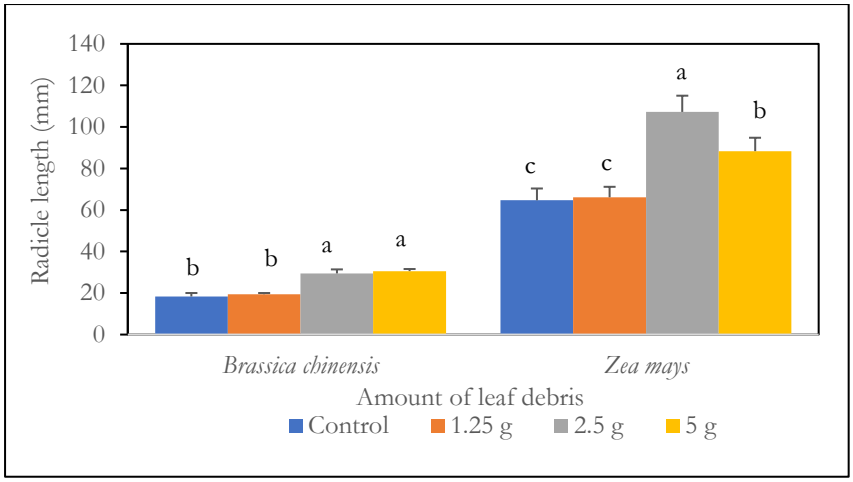


Fig. 2. The effect of *P. indica* leaf leaf debris on the (a) hypocotyl length and (b) radicle length of *B. chinensis* and *Z. mays*. Means with the same letters in the graph bar for each receiver species are not significantly different at ϱ>0.05.

Table 2. The effect of different concentrations of the *P. indica* leaf debris on the percentage inhibition of hypocotyl and radicle length of *B. chinensis* and *Z. mays*.

Bioassay species	**Amount of leaf debris (w/w %)**	**Percentage of inhibition (%)**	
		Hypocotyl length	Radicle length
Brassica chinensis	0.6	-104.7	-106.1
	1.3	-115.1	-160.6
	2.5	-108.1	-166.7
	Control	0.0	0.0
Zea mays	0.6	1.7	-102.2
	1.3	-108.6	-165.8
	2.5	-107.8	-136.6
	Control	0.0	0.0

CONCLUSION

The inhibitory activities of *Pluchea indica* aqueous leaf extracts and leaf debris were concentration and bioassay species dependent. Aqueous leaf extract of *P. indica* at all concentrations showed more than 50% inhibition compared to control on the radicle length of *Brassica chinensis*. The highest concentration of *P. indica* aqueous leaf extract (50 g/L) showed more than 50% inhibition of control towards the radicle length of *B. chinensis* and *Zea mays*. However, *P. indica* aqueous leaf extract exhibited low allelopathic activity on the hypocotyl length of *B. chinensis* and *Z. mays*. Whilst there wasstimulatory allelopathic effect exhibited by leaf debris of *P. indica* on the growth of *B. chinensis* and *Z. mays*. There wasno significant effect of *P. indica* leaf debris on the hypocotyl length of *Z. mays*. This study found that the allelopathic activity of *P. indica* was more profound on the growth of *B. chinensis*.

P.indica leaves have allelopathic potential towards the growth of *B. chinensis* and *Z. mays*. In the future, it was recommended that to test allelopathic activity of *P. indica* on many bioassay species, including weed species for weed control. Further study also needs to be conducted under greenhouse and field conditions. Even though

allelochemicals have been utilised as ecologically benign herbicides for decades, natural herbicides generated from allelochemicals are few and far between.

ACKNOWLEDGMENTS

We would like to thank Universiti Sultan Zainal Abidin for providing the facilities throughout this study.

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How to cite this paper:
Salim, M.R., Yusoff, N., & Wan Abdul Halim, W.Z.A. (2022). Evaluation of allelopathic potential of *Pluchea indica* on *Brassica chinensis* and *Zea mays*. *Journal of Agrobiotechnology*, *13*(1S), 9-16.