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Allelopathic potential assessment of root exudates and rhizosphere soil of *Turnera subulata*

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The overuse of herbicides for weed control can leads to the evolution of herbicide resistant weeds as well as the impacts upon human health. In this regard, the use of herbicide can be minimized by implementing the allelopathic concept in agriculture. In this study, the evaluation of the allelopathic potential of root exudates and soil rhizosphere under *Turnera subulata* (white alder) by using Plant Box Method and Rhizosphere Soil Method, respectively were carried out under laboratory experiment. Each experiment was conducted in Completely Randomized Design (CRD) with three replicates. Allelopathic activities were tested on selected bioassay species; mustard (*Brassica chinensis*), weedy rice (*Oryza sativa*), wheat grass (*Triticum aestivum*), and common Malaysia rice variety (MR303). Results showed that the root exudates and rhizosphere soil of *T. subulata* exhibited different trend of allelopathic activities on the growth of selected bioassay species. The regression coefficient of determination (r^2) was found being the highest on the effect of root exudates of *T. subulata* on the radicle and hypocotyl length of wheat grass (0.7895 and 0.7946, respectively). The radicle and hypocotyl length of wheat grass were inhibited by 62.6% and 84.6% respectively compared to control. The radicle and hypocotyl length of all bioassay species were significantly decreased ($p < 0.05$) when plants were grown with increasing amount of *T. subulata* rhizosphere soil. At amount of 50 mg rhizosphere soil, the highest inhibitory activities were exhibited by MR303, followed by weedy rice, wheat grass and mustard (where the inhibitory percentages were 28.6, 28.1, 7.5 and 6.5 compared to control respectively). This proves that the root exudates of *T. subulata* possess allelopathic effects and able to negatively affect the growth tested bioassay species. The results provide significant information on the utilization of *T. subulata* for sustainable weed management.

Keywords: Allelopathy, allelochemicals, *Turnera subulata*, rhizosphere soil, root exudates.

INTRODUCTION

Weeds have become the major problem in agricultural sector across the world. Weeds reduce the crop yields and affect the agricultural productivity (Sairah et al. 2014). Currently, the usages of synthetic herbicides to control weeds have been increased among the farmers all around the world. The practise has triggered environmental and human health concerns as well as the increasing number of herbicide-resistant weeds. Drinking water sources could also become contaminated resulting in negative effects on plants, fish birds and others. For these reasons, allelopathy has been introduced as a new approach to control weeds (Imen et al. 2019). The use of allelopathic concept can be carried out by utilizing nature products directly, interaction of leaf debris of allelopathic plants toward unwanted plants or via allelochemicals as biopesticides (Singh et al. 2010).

Allelopathy is a natural phenomenon in which a plant or tree produce and release phytotoxins into their surrounding and influence the biological and agricultural systems (Otusanya et al. 2014). The phenomenon is also refers to as beneficial or harmful effects of one plant on another plants, both crop and weed species (Ferguson et al. 2003). Those phytotoxins known as allelochemicals are chemicals compound, released by plant or tree through a number of ways including root exudates, volatilization, leaching and decomposition or decaying (Rice, 1984). Allelochemicals are also known as plant secondary metabolites and can be synthesized by fungi, viruses, microorganisms and plants (Otusanya et al. 2014). Allelochemicals play an important role in the regulation of plant diversity and in the sustainable agriculture. Each plant produces allelochemicals which can enter other plants through a number of ways but the amount of produced allelochemicals can vary between plants (Khan et al. 2009). Previous studies have confirmed the presence of allelochemicals in leaves, stems, flowers, roots and buds of plant (Aldrich, 1984). These allelochemicals can either stimulate or inhibit the germination and growth of the receiver plants (Nornasuha et al. 2017; Nurul Ain et al. 2017). Allelochemicals are free from the harmful effects as compared to the pesticides in use, yet have potential to be used as bio pesticide (Khan et al. 2011).

Turnera subulata (Family: Passifloraceae) is a perennial herb with simple leaves (de Brito Filho et al. 2014). In this study, *T. subulata* (white alder) which have white petals and stained at the base was used as a donor plant (MyBIS, 2020). In Brazil, its leaf extract is used as an alternative traditional medicine for several types of chronic diseases such as diabetes, hypertension, chronic pain and general inflammation (Natalia et al., 2016). The flower of this plant can attract 28 species of insects, predominantly bees (Clemens and Petrucio, 2006). Thus, this plant have been used as an alternative for insect's biological control in paddy field (Badrulhadza et al. 2018). Tsun and Fai (2012) proved that the total pool of phenolic compounds, including flavonoids, in the *T. subulata* extracts were capable in scavenging free radicals and reducing oxidants. To date, there are limited studies that have been conducted in relation to root exudates and rhizosphere soil of *T. subulata*. Thus, this study was carried out to evaluate the allelopathic potential of root exudates and soil rhizosphere of *T.subulata* by using plant box and rhizosphere soil methods.

MATERIALS AND METHODS

Donor plant

Turnera subulata plants (white alder) were planted in the polybag by using stem cutting at February 2020 in UniSZA Herbal Garden, Tembila, Besut, Terengganu. After 8 weeks, plants with attached root were used for plant box method and the soil surrounding the root (rhizospheric soil) was used in rhizosphere soil method.

Recipient plants

Seeds of four bioassay species were collected and were pre-germinated prior to bioassay testing. Seeds of mustard (*Brassica chinensis*) were purchased from Green World Genetics Sdn. Bhd and wheat grass (*Triticum aestivum*) was purchased from WHT Wellgrow Seeds. Whilst, seeds of weedy rice (*Oryza sativa*) and MR303 rice seeds were obtained from Kawasan Pembangunan Pertanian Bersepadu Terengganu Utara (IADA KETARA), Jerteh, Terengganu.

Seed Germination

Four different bioassay species of seeds were pre-germinated prior to testing. *Brassica chinensis* seeds were soaked in distilled water for three hours, and germinated on filter paper for 48 hours. Seeds of *O. sativa*, *T. aestivum* and MR303 were soaked in distilled water for 24 hours and germinated on filter paper for 48 hours. The floated seeds were considered as non-germinate and discarded.

Plant Box Method

The root exudates from the donor species were evaluated for their allelopathic potential using the plant box method that was introduced by Fujii et al. (2007). The roots of *T. subulata* were placed in a nylon gauze tube. Tubes were laid in a corner of a plant box (magenta box; 60 mm width × 60 mm length × 95 mm height). A control sample was prepared without a donor plant in another box. Agar powder (Nacalai Tesque, Kyoto, Japan) was used as media and were prepared in 0.75% w/v and autoclaved at 121°C for 15 minutes. The agar gel was cooled and sowed with 33 pre-germinated seeds of bioassay species, respectively. The sowing position is shown in Figure 1. All tested seeds were incubated at 25°C for five days, with 12 h/ 12 h photoperiod. All experiments were carried out in three replicates. After five days, the hypocotyl and radicle length of each bioassay species were measured and recorded.

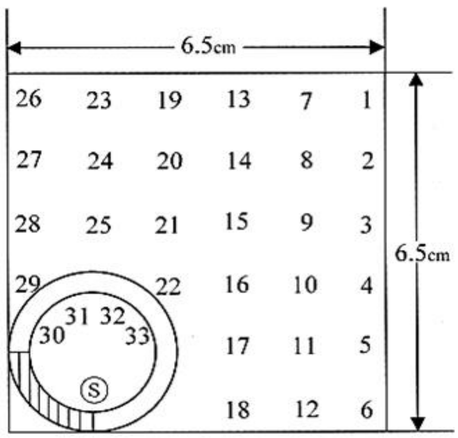

Figure 1: Sowing position of bioassay species and 's' denoted for donor plant (which is *Turnera subulata*) (Fujii et al. 2007)

Rhizosphere Soil Method

The allelopathic potential of rhizosphere soil under *T. subulata* was determined using Rhizosphere Soil Method. Approximately 5 g, 10 g and 50 g of sieved rhizosphere soil of *T. subulata* were placed in the six well multi-dish plates (Nalge Nunc Intl., Roskilde, Denmark) respectively. The agar powder (Nacalai Tesque, Kyoto, Japan) were used as medium in this experiment. The media was prepared in 0.75% w/v and autoclaved at 121°C for 15 minutes. The first layer of agar (5 mL) was added into each well of the six-well multidish and allowed to be solidifying at room temperature. Then, 5 mL (second layer) of agar medium were added to each well of multi-dish. After the agar solidified, five seeds of the pre-germinated bioassay species were sowed on top of the agar (Fujii et al. 2004). There were three replicates in each of the amount of rhizosphere soil. The photoperiod for this experiment was 24-hour dark for five days. After five days, the hypocotyl and radicle length were measured and recorded.

Statistical Analysis

All experiments were conducted in Completely Randomized Design (CRD) and data regarding the percentage growth of seedlings by the donor plants were subjected for the Simple Linear Regression (SLR) for plant box method and one-way analysis of variance (ANOVA) using SPSS software, version 21 for rhizosphere soil method. Separation of treatment means from the control at 0.05 probability level were conducted using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Plant Box Method

In this study, the growth of bioassay species can be seen clearly throughout the transparent magenta box. Results showed that the growth of radicle and hypocotyl lengths in close proximity to the donor plant was inhibited significantly. As the closer they were to the donor, the more the inhibition were measured. Wang et al. (2019) also reported that allelochemicals released by the donor plants affect the photosynthesis and enzyme activities of recipient plants which indirectly interferes the growth of radicle and hypocotyl. Similar results were found by Ishak et al. (2016), which reported the inhibition of radicle length of *Tridax procumbens*, *Ageratum conyzoides* and *Emilia sonchifolia* by 98%, 68% and 77% respectively, when sowed closer to the *Leucaena leucocephala* source.

Turnera subulata root exudates significantly (<0.05) inhibited the radicle and hypocotyl length of all bioassay species when assessed by the plant box method. Figure 2 (a-h) and 3 (a-h) shows the relationship between the radicle/ hypocotyl elongation of bioassay species with the distance from the root of *T. subulata*. The regression coefficient of determination (r^2) on all radicle and hypocotyl length of bioassay species exceeded 0.7, with the highest r^2 values for both radicle (0.7895) and hypocotyl (0.7946) of wheat grass (Figure 2(h) and Figure 3(h)). Meanwhile, the percentage inhibition of radicle and hypocotyl growth was the highest both on mustard which are 89% and 91.5% respectively compared to control. Radicle growth of weedy rice and MR303 were decreased by 84% and 76% respectively. Whereas the hypocotyl growth of these two species were both decreased by 87%.

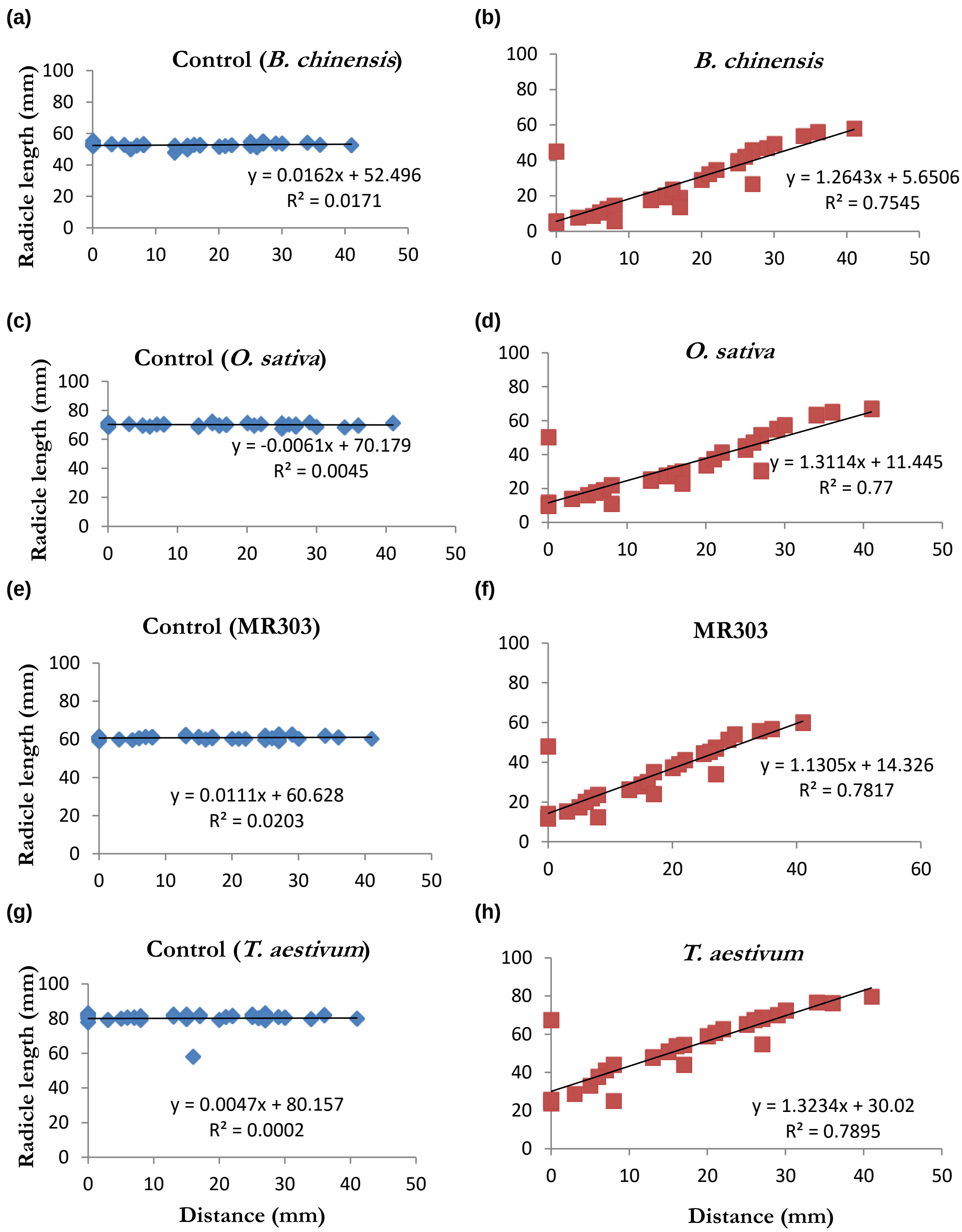

Figure 2 (a-h):The relationship between radicle elongation of selected bioassay species grown in plant box containing agar with *T. subulata* as a donor plant. Intercept (radicle) and slope of

regression line (trend) represents the level of activity

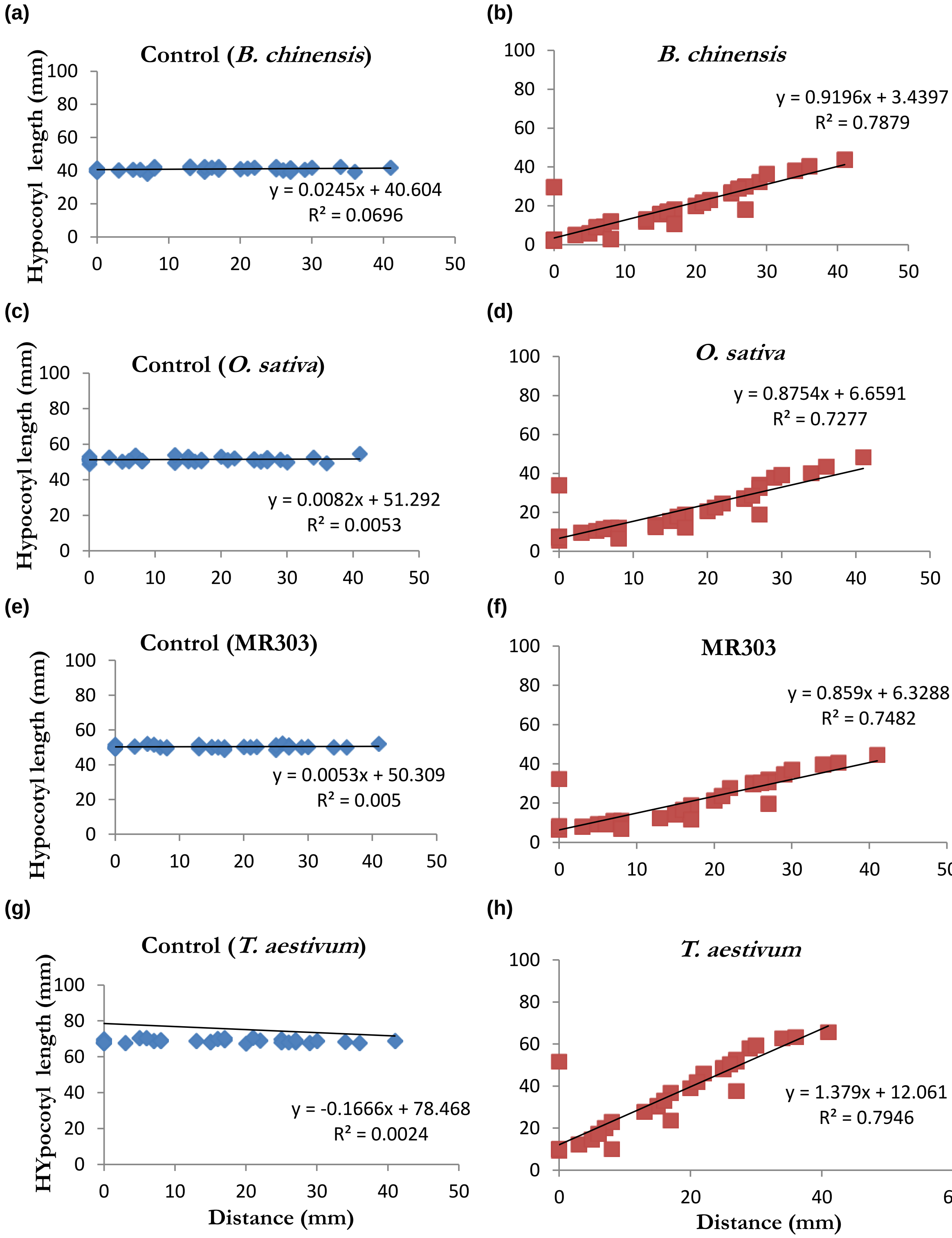

Figure 3 (a-h): The relationship between hypocotyl elongation of selected bioassay species grown in plant box containing agar with *T. subulata* as a donor plant. Intercept (hypocotyl) and slope of regression line (trend) represents the level of activity.

The allelopathic behavior of root exudates of a donor plant can be described in terms of intercept, slope, regression line, and regression coefficient. Regression equation with small intercept and small slope suggest the strong inhibitory allelopathic activity (Sairah et al. 2014). High intercept value indicates weak allelopathic potential (all control) whereas low intercept value indicates strong allelopathic potential (all radicle and hypocotyl growth). For example, the radicle length of wheat grass without the presence of *T. subulata* (which serve as control in this research) show high intercept value (80.16). However, with the presence of *T. subulata* as the source plant in this research, the radicle length of wheat grass show low intercepts value (30.02). Thus, strong allelopathic activity was exhibited by *T. subulata* on the radicle length of wheat grass. According to Shiraishi et al. (2002), a very few plant species with allelopathic effects satisfy these two conditions (intercept and slope value). This result proved that the root distance is an important factor to measure the effectiveness of allelopathic plant against the growth of other plants.

Rhizosphere Soil Method

This method was performed to identify the allelopathic potential of rhizospheric soil under *T. subulata*. This allelopathic activity indicates the release of allelochemicals by the donor plant to the soil. From this study, the allelochemicals released by *T. subulata* affects the growth of radicle and hypocotyl of the bioassay species. Leslie et al. (2012) reported that the root cells can rapidly generate and release large quantities of allelochemicals in response to stress or local rhizosphere conditions. This was supported by Syuntaro et al (2010) that allelopathic activity is affected by the presence of soils. The increasing amount of rhizosphere soil of *T. subulata* suppressed the radicle and hypocotyl length of all tested bioassay species (Table 1). At concentration of 50 mg, the inhibitory activity of rhizosphere soil against the radicle length of MR303 was found to be the highest (28.6%), followed by weedy rice (28.1%), wheat grass (7.5%) and mustard (6.5%) as compared to the control. Meanwhile, the inhibitory activity of rhizospheric soil against hypocotyl length showed similar result. Inhibitory against MR303 was the highest (39%), followed by mustard (31%), weedy rice (26.5%) and wheat grass (8.3%) compared to the control. In comparison, the percentage inhibition of rhizosphere soil was slightly lower than the plant box method (root exudates). This may be due to the allelochemicals of *T. subulata* in the soil have been metabolize by the soil microbes, which reduces its concentration. Dixon et al. (2010) found that the rhizosphere soil condition is influenced by root secretions and contains associated soil microorganisms. It is proved that the soil microorganisms affect the allelochemicals content in the rhizospheric soil. This can be concluded that the inhibitory percentage of radicle and hypocotyl length increased with the increase of the rhizosphere soil amount

Table 1: Allelopathic effects of different amount of rhizosphere soil from *T. subulata* (white alder) on bioassay species by Soil rhizosphere method. (mean ± standard error)

Bioassay species	Treatments	Length			
		Radicle(mm)		Hypocotyl (mm)	
Brassica chinensis (mustard)	Control	46.98±0.44	a	35.98±0.37	a
	5mg	41.62±0.26	b	32.0±1.65	b
	10mg	39.58±0.28	c	29.40±1.30	c
	50mg	40.51±0.34	d	24.84±2.55	d
Oryza sativa (weedy rice)	Control	69.04±0.17	a	51.44±2.79	a
	5mg	61.40±0.24	b	48.31±1.27	b
	10mg	58.10±0.21	c	45.87±2.05	c
	50mg	49.58±0.19	d	37.82±1.59	d
MR303 (rice variety)	Control	60.60±0.21	a	49.07±1.43	a
	5mg	56.18±0.20	b	43.47±1.58	b
	10mg	48.47±0.19	c	37.58±1.17	c
	50mg	43.29±0.33	d	29.91±1.65	d
Triticum aestivum (wheat grass)	Control	80.71±0.23	a	69.13±1.81	a
	5mg	77.73±0.27	b	66.18±1.96	b
	10mg	71.9±0.28	c	60.67±2.28	c
	50mg	74.63±0.27	d	63.41±.87	d

Note: Means within the columns followed by same alphabet were not significantly different (p>0.05) according to DMRT. Values given in the table were mean of all the parameters over that of the control.

CONCLUSION

This study shows that *T. subulata* possess significant allelopathic potential. Specifically different allelopathic activities were observed against *B. chinensis, O. sativa*, *T. aestivum* and MR303. All bioassay species indicated significant inhibition on radicle and hypocotyl growth when the distance was closer towards *T.subulata* root in plant box method. Concomitantly, the rhizosphere soil method revealed that the inhibition of radicle and hypocotyl length was dose-dependent. Finally, the results suggested that the allelopathic potential of *T.subulata* should be explored in order to be integrated into sustainable weed management system. Further studies are needed to identify the compounds present in *T. subulata* root exudates and soil rhizosphere, as well as to conduct the bioassay experiment under greenhouse and field conditions.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

WZAWAH performed the experiments, data collection and wrote the manuscript. NY designed the experiments, performed data analysis and wrote the manuscript. MASS also wrote and finalized the manuscript. KAA read and approved the final version.

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