Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 542 – 549

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Allelopathic effects of the invasive plant Wedelia (*Sphagneticola trilobata* L.) aqueous extract on common beans (*Phaseolus vulgaris* L.)

K.R.S. Perera[1], R.M.C.S. Ratnayake[2], U.P.K. Epa[1*]

[1]Department of Zoology and Environmental Management, University of Kelaniya 11600, Dalugama, Kelaniya, Sri Lanka
[2]Department of Plant and Molecular Biology, University of Kelaniya 11600, Dalugama, Kelaniya, Sri Lanka

Received – April 07, 2023; Revision – June 11, 2023; Accepted – June 20, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).542.549

KEYWORDS

Sphagneticola trilobata

Allelopathy

Phaseolus vulgaris

Germination

Growth

Seed

Yield

Weeds

ABSTRACT

Sphagneticola trilobata (L.) Pruski is an alien invasive weed with aggressive growth habits, environmental stress tolerance, and the ability to synthesize allelochemicals. However, in many parts of the world, this plant is still recommended for use in composting, phytoremediation, and as an ornamental ground cover in gardens. The present study investigated the allelopathic effect of *S. trilobata* on the seed germination, growth and yield of *Phaseolus vulgaris* L. To analyze the allelopathic effects of *S. trilobata* on *P. vulgaris* seed germination, hundred seeds of *P. vulgaris* were exposed to different concentrations of the aqueous extracts of fresh and dry *S. trilobata* (2.5×10^2, 5.0×10^2 and 7.5×10^2 g/L) in Petri dishes for five days. The impact of *S. trilobata* aqueous extract on the growth and yield of *P. vulgaris* was also studied with seedlings planted in a compost soil mixture. Results of the study suggested negative impacts of *S. trilobata* extracts on *P. vulgaris* seed germination, growth and yield. *P. vulgaris* seed germination was significantly lower in the treatments than in the control ($p<0.05$). Further, *P. vulgaris* plants treated with fresh plant extracts at 5.0×10^2 and 7.5×10^2 g/L concentrations had significantly lower shoot height, growth rate, leaf area, fresh shoot weight, dry shoot weight, root length, pod length and yield ($p<0.05$) than controls. From the results of the study, it can be concluded that *S. trilobata* aqueous extracts have a dose-dependent allelopathic effect on *P. vulgaris* seed germination, growth, and yield and among the dry and fresh plant extracts, fresh plant aqueous extracts have a more significant allelopathic impact. As *S. trilobata* contains water-soluble allelochemical, it should not be used in biofertilizer production, phytoremediation, or as live mulch.

* Corresponding author

E-mail: epa@kln.ac.lk (U.P.K. Epa)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

1 Introduction

Sphagnaticola trilobata (L.) Pruski, formerly known as *Wedelia trilobata*, is one of the world's top 100 most environmentally destructive invasive alien species (Lowe et al. 2000). *S. trilobata* belongs to the Asteraceae family and is found in Asia, South America, and the Pacific. *S. trilobata* is a creeping and climbing perennial herb that grows up to 30 cm high and 4 m long. This mat-forming plant frequently creates a dense ground cover (Figure 1) and may short-climb trees or other vegetation. *Sphagnaticola* species have antimicrobial, insecticidal, larvicidal and trypanosomicidal properties and are used for medicinal purposes (Huang et al. 2006; Maldini et al. 2009; Toppo et al. 2013) and as a decorative ground cover in many parts of the world (Wagner et al. 1990; Macanawai 2013). It is also used as a cover crop in rubber and tea plantations to control soil erosion in Asia (Nayanakantha 2007). Furthermore, it is used for ground cover in Middle Eastern countries, which helps stabilize the soil and prevent it from moving due to wind. The use of *S. trilobata* as a ground cover is getting increasingly popular in various countries, including the Kingdom of Saudi Arabia (Swaefy and Basuny 2011).

However, *S. trilobata* has become a noxious weed in many countries, covering vast areas in agricultural lands, along coastal areas, rail and roadsides, garbage dumps, open grounds and other disturbed habitats (Wu et al. 2008; Macanawai 2013; Hossen et al. 2020; Gao et al. 2022). It has also been found on the recently developed volcanic islands, atolls, limestone, continental, and even small uninhabited offshore islands in most Pacific Island countries and territories (Wagner et al. 1990). The aggressive growth pattern of *S. trilobata* is attributed to its tolerance to environmental stresses and ability to synthesize allelochemicals (Zeng et al. 1994; Wu et al. 2008; Wang et al. 2012). Allelochemicals have biological effects on other organisms, many of which are still unknown (Waller 2003; Ferguson et al. 2013). Because of its significant impact on plant development and yield reduction in natural and agricultural settings, allelopathy of invasive species has gained increased attention (Waller 2003; Wang et al. 2012; Shahena et al. 2021; Gao et al. 2022). Aqueous plant extract is one of the most commonly utilized ways to assess plant species' allelopathic effects (Zeng et al. 1994; Nie et al. 2004; Ilori et al. 2010; Ullah et al. 2021).

As *S. trilobata* invades agricultural fields and is used as a ground cover, compost production (Setyowati et al. 2014a,b; Hussain et al. 2020; Setyowati et al. 2021) and phytoremediation (Dissanayake et al. 2002), it is a necessity to study its allelopathic effects on cultivated crop varieties. *Phaseolus vulgaris* L. is a popular herbaceous annual crop grown worldwide for its edible beans as dry seed and unripe fruits. It was hypothesized that the allelopathic biomolecules of *S. trilobata* aqueous extract negatively affect the seed germination, growth and yield of *P. vulgaris*. Therefore, the present study was conducted to investigate the allelopathic effect of *S. trilobata* on the seed germination, growth and yield of *P. vulgaris*.

2 Materials and Methods

2.1 Preparation of aqueous extracts from wet and dry plant parts of *S. trilobata*

Aerial parts of *S. trilobata* were collected from the home gardens of the Gampaha district, Western Province, Sri Lanka, from November to December 2020. A voucher specimen of *S. trilobata* was deposited at the Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka. The flowers, dried yellow leaves, and dead stems were removed, and fresh *S. trilobata* parts were chosen, washed, and cut into 1cm pieces. Seventy-five grams of plant parts were mixed with 100 mL of distilled water and ground well. The resulting solution was filtered and centrifuged, and a stock solution of 7.5×10^2 g/L

Figure 1 *S. trilobata* (a– Densely grown *S. trilobata* in a home garden in Sri Lanka, b- Flower of *S. trilobata*)

of aqueous extract of fresh plant parts was prepared. Fresh pieces of *S. trilobata* were also air-dried for seven days at room temperature (28°C), and a stock solution of 7.5×10^2g/L of aqueous extract was prepared. Another two test solutions were prepared using these stock solutions with concentrations of 2.5×10^2 g/L and 5.0×10^2 g/L.

2.2 Effect of fresh and dry *S. trilobata* aqueous extracts on *P. vulgaris* seed germination

The experiment was conducted using four sets of Petri dishes; three sets were used as treatments and one as a control. Three replications were used for each treatment and control. *P. vulgaris* seeds (Sanjaya variety-bush bean type) were surface sterilized with 5% sodium hypochlorite for 20 minutes before being washed with distilled water several times. A Whatman No.1 filter paper and one hundred *P. vulgaris* seeds were placed on each 70 mm Petri dish. Thirty mL of test solutions (2.5×10^2 g/L, 5.0×10^2 g/L and 7.5×10^2 g/L) and distilled water were added daily into complementary treatments and controls, respectively. The remaining solution added the previous day was removed using an injection cylinder. All Petri dishes were arranged in a completely randomized design. The germination of *P. vulgaris* seeds was recorded upon the emergence of radicle every 24 h for five days.

2.3 Effect of fresh *S. trilobata* aqueous extracts on *P. vulgaris* growth and yield

Four sets of pots filled with the topsoil compost mixture (1:1) were prepared in triplicates to estimate the effect of different *S. trilobata* fresh aqueous extract concentrations (2.5×10^2 g/L, 5.0×10^2 g/L, and 7.5×10^2 g/L) on the growth and yield of *P. vulgaris* plants. Five germinated *P. vulgaris* seeds were planted in each pot (1.98×10^{-2} m²), and pots were arranged in a completely randomized block design. One hundred mL of the test solutions and distilled water were added into three complementary treatments and to the controls on each other day, respectively. As a fertilizer, 100 g of compost was added to each experimental pot once a week. The shoot height of plants was measured, and a digital image (Sony Cyber-shot DSC-H300 20.1 MP, Digital Camera) of the second leaf of each plant was taken weekly. ImageJ software was used to calculate the leaf area in cm^2 (Schneider et al. 2012). Also, pods produced in the plants were harvested, and the lengths of the pods were measured using a thread and meter ruler. The weights of pods were measured using an electric balance (Sartorius CP224S), and the yield of *P. vulgaris* was calculated. All plants were removed after 50 days, and the above and below-ground parts were weighed to calculate each replicate's fresh shoot and root weight. The shoot and root were air-dried in an oven at 70°C for three days before their dry weights were measured. Soil pH (HACH - HQ 40d), organic matter content (Schulte and Hopkins 1996) and total nitrogen (Bremner 1996) in each experimental pot were measured biweekly.

2.4 Statistical analysis

Analysis of variance (ANOVA) was conducted using MINITAB (version 14) software to compare seed germination, growth and yield of *P. vulgaris* exposed to different concentrations of aqueous extracts of *S. trilobata* and the significance of the variation was tested at 0.05 level using Tukey's pairwise comparison. Pearson correlation test was carried out to assess the dose dependency of impacts observed in *P. vulgaris* growth and yield to aqueous extracts of *S. trilobata*.

3 Results and discussion

In this study, the invasive *S. trilobata* had negative allelopathic effects on the seed germination, growth and yield of *P. vulgaris*.

3.1 Effect of fresh and dry *S. trilobata* aqueous extracts on *P. vulgaris* seed germination

Aqueous extracts of fresh and dry *S. trilobata* significantly inhibited the germination of *P. vulgaris* seeds. The number of germinated seeds and the seeds germination ratio in seeds treated with aqueous extracts of *S. trilobata* was substantially lower ($P<0.05$, one-way ANOVA) than in the control (Table 1). These findings suggest the presence of phytotoxic germination inhibitory substances in the aqueous extracts of *S. trilobata*.

Table 1 Effect of *S. trilobata* aqueous extracts on *P. vulgaris* seeds germination and seed germination ratio after five days of exposure

Concentration (g/L)	Fresh plant parts aqueous extract of *S. trilobata*		Dry plant parts aqueous extract of *S. trilobata*	
	Number of germinated seeds	Germination ratio	Number of germinated seeds	Germination ratio
Control	84±0.3^a	100±0.0^a	80±0.7^a	100±0.0^a
2.5×10^2	55±0.3^b	65±0.4^b	53±0.3^b	66±0.4^b
5.0×10^2	31±1.3^c	37±1.6^c	33±0.9^c	41±1.1^c
7.5×10^2	9±0.6^d	10±0.4^d	8±0.3^d	10±0.4^d

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test ($P<0.05$)

The dry and fresh aqueous extract at the concentration of 7.5×10^2 g/L had the highest negative impact on the seed germination of *P. vulgaris*, while the lowest effect was reported at a concentration of 2.5×10^2 g/L. This may be attributed to the higher sensitivity of plants to phytotoxic biomolecules during seed germination and the initial stages of development. Results agree with the findings of Kwembeya et al. (2013), who reported that *Lantana camara* L. negatively affected the germination and growth of Blackjack, *Bidens pilosa* L. when added to the soil under glasshouse conditions. According to Nie et al. (2004), dissolved biomolecules of *S. trilobata* reduced the protective enzyme activities of plant seeds. When exposed to phytotoxic compounds, plants cannot successfully protect the cell membrane from the active oxygen, damaging the membrane and decreasing seed vitality.

In this study, as shown by the results of the Pearson correlation test, the allelopathic effect of *S. trilobata* was dose-dependent, and the number of germinated seeds decreased with increasing concentrations of both types of aqueous extracts. The percentage germination of *P. vulgaris* seeds exposed to aqueous extracts of *S. trilobata* and control gradually increased with time. However, seed germination of *P. vulgaris* in control was always higher than in the treatments. The inhibition of seed germination was higher in seeds treated with an aqueous extract of fresh *S. trilobata* than the dry *S. trilobata.* Drying plant leaves may have reduced the concentrations or effects of allelochemicals in the *S. trilobata*.

The concentration-dependent inhibitory effects of *S. trilobata* extracts on *P. vulgaris* seed germination demonstrated the allelopathic potential of this plant. These results contradict the findings of Krishnan and Rajalakshmi (2021), who exposed cowpea seeds to *S. trilobata* extract (2 – 10 %) and did not find any negative impact on the seed germination. These findings suggest that the phytotoxic effects of *S. trilobata* extracts varied among the tested plants.

3.2 Effect of fresh aqueous extracts of *S. trilobata* on *P. vulgaris* growth and yield

Results of the seed germination study revealed that maximum inhibition in the seed germination was reported in the seed treated with the highest dose (7.5×10^2 g/L) of fresh aqueous extract of *S. trilobata*. Based on its effectiveness, this dose was selected for the pot study under natural conditions, and *P. vulgaris* seedlings were treated with 7.5×10^2 g/L of fresh aqueous extract of *S. trilobata* and found that treated seedlings started to die from the third week and out of 15, only nine plants survived. In contrast, none of the control plants died until the end of the experiment. The shoot heights of the plants treated with the aqueous extracts were significantly lower than that of the control ($p<0.05$). Like seed germination, the fresh extracts at concentrations of 7.5×10^2 g/L and 2.5×10^2 g/L had the strongest and weakest adverse effects on the shoot height of *P. vulgaris*, respectively. A comparison of the *P. vulgaris* plant's height at the end of the experiment is given in Table 2.

The changes in the surface area of the second leaf of *P. vulgaris* treated with aqueous extracts of *S. trilobata* with time are shown in Figure 2. Initially, the size of the second leaf of *P. vulgaris* was comparatively smaller, and later on, it gradually increased until it slowed down in the sixth and seventh weeks. The lowest leaf area was recorded in the plants exposed to 7.5×10^2 g/L of aqueous extract. In contrast, the concentration of 2.5×10^2 g/L had the lowest impact on the leaf area.

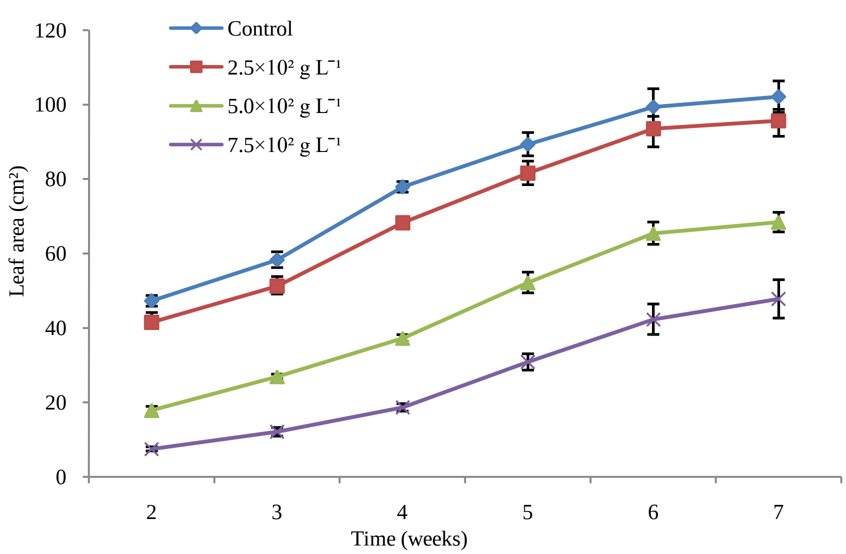

Figure 2 Variation in the surface area (Mean±SEM) of the second leaf of *P. vulgaris* exposed to aqueous extracts of *S. trilobata* with time

Table 2 Effect of fresh aqueous extracts of *S. trilobata* exposure on the *P. vulgaris* plant height

Concentration of *S. trilobata* aqueous extract (g/L)	Height (cm)
Control	73.3±0.9[a]
2.5×10^2	67.7±1.2[b]
5.0×10^2	46.6±1.2[c]
7.5×10^2	37.7±1.3[d]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Table 3 The second leaf surface area of *P. vulgaris* plants after the seventh week

Concentration of aqueous extract of *S. trilobata* (g/L)	Leaf area (cm²)
Control	102.11±4.23[a]
2.5×10^2	95.70±0.33[a]
5.0×10^2	68.40±2.63[b]
7.5×10^2	47.78±5.16[c]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letters in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Table 4 Effect of fresh aqueous extracts of *S. trilobata* on the various growth parameters of *P. vulgaris* within seven weeks of the experimental period

S. trilobata aqueous extract concentration (g/L)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Length of pods (cm)	Yield (g)
Control	8.43±0.27[a]	1.67±0.02[a]	14.7±0.39[a]	0.44±0.02[a]	0.14±0.01[a]	11.5±0.27[a]	20.31±2.17 [a]
2.5×10^2	7.86±0.25[a]	1.57±0.03[a]	13.2±0.44[a]	0.41±0.01[a]	0.13±0.01[a]	10.8±0.16[a]	17.71±1.04[a]
5.0×10^2	5.12±0.35[b]	1.17±0.05[b]	11.3±0.50[b]	0.32±0.01[b]	0.11±0.06[b]	8.0±0.13[b]	8.05±0.72[b]
7.5×10^2	3.44±0.36[c]	0.87±0.11[c]	8.5±0.66[c]	0.25±0.02[c]	0.08±0.01[c]	4.6±0.25[c]	1.79±1.20[c]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Exposure to the higher concentrations of aqueous extracts of *S. trilobata* significantly reduced the leaf area of *P. vulgaris* compared to the control (P<0.05). Although 2.5×10^2 g/L of *S. trilobata* aqueous solution reduced the leaf area compared to the control, this difference is insignificant (Table 3). The allelopathic effect of *S. trilobata* aqueous extract affects plant cell structure, division and elongation, growth regulation systems, metabolism, photosynthesis, mineral ion uptake, amino acid, and nucleic acid synthesis (Cheng and Cheng 2015).

The *P. vulgaris* plants in the control pots began to flower at the beginning of the fourth week. While the plants treated with 2.5×10^2 g/L *S. trilobata* aqueous extract reached the flowering stage at the end of the fourth week and was followed by plants treated with 5.0×10^2 g/L and 7.5×10^2 g/L which were showing flowering at the end of the fifth week. The effect of aqueous extract of fresh *S. trilobata* on growth parameters of *P. vulgaris* plants, such as fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, length of the pod and yield, are presented in Table 4.

All the concentrations of *S. trilobata* aqueous extracts have an inhibitory effect on the *P. vulgaris* shoot growth, which was indicated by lower weights of fresh and dry shoots in the plants treated with *S. trilobata* aqueous extracts compared to the control. Although 2.5×10^2 g/L aqueous extract of *S. trilobata* reduced the fresh and dry shoot weights of *P. vulgaris*, this was not significantly different from the controls. Furthermore, *P. vulgaris* root growth was significantly reduced by the application of the *S. trilobata* aqueous extracts at 5.0×10^2 g/L and 7.5×10^2 g/L concentrations, as evidenced by shorter root lengths and lower fresh and dry root weights compared to controls (P<0.05). The lowest dose of *S. trilobata* aqueous extracts (2.5×10^2 g/L) showed no significant differences from the control plants regarding root length, fresh root weight and dry root weight.

Higher concentrations of *S. trilobata* aqueous extracts (5.0×10^2 and 7.5×10^2 g/L) also reduced the length of the pods and the yield of *P. vulgaris* as compared to the 2.5×10^2 g/L aqueous extract and control plants (P<0.05). No statistically significant difference (P>0.05) was reported in the average yield of *P. vulgaris* seedlings exposed to 2.5×10^2 g/L aqueous extract and control plants. The lower primary productivity due to lower shoot and root development may reduce the yield of plants exposed to higher concentrations of aqueous extracts of *S. trilobata*.

All the measured growth parameters of *P. vulgaris* had a strong significant negative correlation with different concentrations of *S. trilobata* fresh aqueous extracts (P<0.05). The growth parameters of *P. vulgaris* exposed to *S. trilobata* aqueous extracts decreased as the concentrations of aqueous extracts increased (Table 5). Similarly, the aqueous extract of *Wedelia* also significantly inhibited the growth of chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* L.), and green gram (*V. radiata* L.) seedlings with a more pronounced effect at higher concentrations (Shahena et al. 2021).

Table 5 Correlation between the various concentrations of *S. trilobata* fresh aqueous extracts and various growth parameters of *P. vulgaris* within seven weeks experimental period

Growth parameter	Correlations (r) between concentration and growth parameters*	P value
Shoot height	-0.976	0.024
Leaf area	-0.976	0.024
Fresh shoot weight	-0.975	0.025
Dry shoot weight	-0.978	0.022
Root length	-0.990	0.010
Fresh root weight	-0.984	0.016
Dry root weight	-0.976	0.024
Length of the pod	-0.967	0.033
Yield	-0.980	0.020

*According to the Pearson correlation test, all the growth parameters are significantly (P<0.05) negatively correlated to the concentrations of aqueous extract of fresh S. trilobata.

Table 6 Various physicochemical parameters of soil collected from *P. vulgaris* growing pots

S. trilobata aqueous extract concentration (g/L)	pH	Organic matter content (%)	Total nitrogen (%)
Control	7.06±0.04	6.27±0.10	1.86±0.35
2.5×10^{2}	7.08±0.07	6.56±0.14	1.88±0.15
5.0×10^{2}	7.09±0.02	6.84±0.11	1.84±0.24
7.5×10^{2}	7.11±0.01	7.04±0.29	1.93±0.21

Values in the columns are not significantly different as per Tukey's pairwise significant difference test (P<0.05)

Significantly low seed germination, poor growth and yield were recorded in the *P. vulgaris* plant treated with the *S. trilobata* aqueous extracts; this might be attributed to the presence of the allelopathic biomolecules such as coumarin derivatives, diterpene, sesterpenes and sesquiterpene lactones that are present in *S. trilobata* aqueous extracts (Shahena et al. 2021). Hossen et al. (2020) indicated the presence of two phenolic compounds, i.e. vanillic acid and gallic acid, from the *Wedelia chinensis*(Osbeck) Merr aqueous extract, which are the main constituents of the allelopathic effects. Allelochemicals present in the aqueous extracts of *S. trilobata* could disrupt the photosynthetic activities, mineral absorption, cell division, and energy metabolism of the *P. vulgaris* plants (Pasiecznik 1999; Cheng and Cheng 2015). Due to the disturbance in the plant, as mentioned earlier, weak development of the root system and reduction of the leaf area was reported and this could have a significant effect on the overall growth, fruiting time and yield of the *P. vulgaris*. Pu et al. (2022) have observed a low growth rate and yield of tomatoes in the presence of *S. trilobata*. Similar results were observed when rice (*Oryza sativa* L.) was fertilized with green manure manufactured using *W. trilobata* (Nie et al. 2004). Extracts of *W. chinensis* also affected the shoot growth of lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), and rapeseed (*Brassica napus* L.) (Hossen et al. 2020).

3.3 Physicochemical parameters of soil

There were no significant changes in the various physicochemical parameters such as pH, total nitrogen and organic matter content of the soil collected from *P. vulgaris* growing pots (Table 6).

According to the results, soil quality has not changed by adding different concentrations of *S. trilobata* extracts. This suggests that the toxic effects of *S. trilobata* extracts are mainly because of the allelochemicals in the extract.

Conclusion

This study indicates the presence of water-soluble phytotoxic substances in the aqueous extracts of *S. trilobata*, which show allelopathic effects on the seed germination, root and shoot development and final yield of *P. vulgaris.* Fresh plant aqueous extracts had a more significant allelopathic effect than the dry plant extracts of *S. trilobata*. As *S. trilobata* contains water-soluble allelochemicals, it should not be recommended in biofertilizer production, phytoremediation, or live mulch.

Conflicts of Interest

The authors declare no conflict of interest.

References

Bremner, J. M. (1996). Nitrogen-total. Methods of soil analysis: Part 3. *Chemical methods*, *5*, 1085–1121.

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science*, *6*, 1-16. https://doi.org/10.3389/fpls.2015.01020

Dissanayake, U.S., Tennakoon, K.U., & Priyantha, N. (2002). The potential of two invasive plant species, *Lantana camara* L. and *Wedelia trilobata* L., for selective heavy metal uptake. *Ceylon Journal of Science*, *29*(1), 11–17.

Ferguson, J.J., Rathinasabapathi, B., & Chase, C.A. (2013). Allelopathy: How Plants Supress Other Plants, University of Florida for protected area practitioners' future course. *Annals of the Missouri Botanical Garden*, *90*, 67–76.

Gao, L, C.M., Zeng. L., Zhang, Q., Zhu, H., Gu, X., & Peng, C. (2022). Adaptation of the invasive plant (*Sphagneticola trilobata* L. Pruski) to a high cadmium environment by hybridizing with native relatives. *Frontiers in Plant Science*, *28*(13), 905577. https://doi.org/10.3389/fpls.2022.905577.

Hossen, K., Das, K. R., Okada, S., Iwasaki, A., Suenaga, K., & Kato-Noguchi, H. (2020). Allelopathic potential and active substances from *Wedelia chinensis* (Osbeck). *Foods*, *9* (11), 1591.https://doi.org/10.3390/foods9111591

Huang, X., Ou, S., Tang, S., Fu, L., & Wu, J. (2006). Simultaneous determination of trilobolide-6-O-isobutyrates A and B in *Wedelia trilobata* by gas chromatography. *Chinese Journal of Chromatography*, *24*(5), 499–502. https://doi.org/10.1016/s1872-2059(06)60021-7

Hussain, N., Abbasi, T., & Abbasi, S.A. (2020). Evaluating the fertilizer and pesticidal value of vermicompost generated from a toxic and allelopathic weed ipomoea. *Journal of Saudi Society of Agricultural Science*, *19*, 43-50. https://doi.org/10.1016/j.jssas.2018.05.005

Ilori, O.J., Otusanya, O.O., Adelusi, A.A., & Sanni, R.O. (2010). Allelopathic Activities of Some Weeds in the Asteraceae Family. *International Journal of Botany*, *6*, 161-163. https://doi.org/10.3923/ijb.2010.161.163

Krishnan, L.S., & Rajalakshmi, R. (2021). Effect of *Wedelia trilobata* on the germination and seedling growth of Cowpea. *International Journal of Creative Research Thoughts*, *9*, 4298-4303.

Kwembeya, A., Rugare, J. T., & Mabasa, S. (2013). Allelopathic Effects of Lantana (*Lantana camara*) on Blackjack (*Bidens pilosa*) and Pearl Millet (*Pennisetum glaucum*). *Asian Journal of Agriculture and Rural Development, 3*(8), 2013: 543-553.

Lowe S., Browne M., Boudjelas S., & De Poorter, M. (2000). 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. The Invasive Species Specialist Group (ISSG), Species Survival Commission (SSC) of the World Conservation Union (IUCN).

Macanawai, A.R. (2013). Impact of *Sphagneticola trilobata* on Plant Diversity in Soils in South-East Viti Levu, Fiji. *Journal of Life Science*, *7*(6), 635-642.

Maldini, M., Sosa, S., & Montoro, P.(2009). Screening of the topical anti-inflammatory activity of the bark of *Acacia cornigera* Willdenow, *Byrsonima crassifolia* Kunth, *Sweetia panamensis* Yakovlev and the leaves of *Sphagneticola trilobata* Hitchcock. *Journal of Ethnopharmacology*, *122*(3), 430-433 pp.

Nayanakantha, N.M.C. (2007). Alien invasive plants and their potential threat to biodiversity in rubber plantations. *Bulletin of the Rubber Research Institute of Sri Lanka*, *48*, 61-66.

Nie, C.R., Zeng, R.S., Luo, S.M., Li, H.S., Hong, M.Q., & Cheng, L.Q. (2004). Allelopathic potentials of *Wedelia trilobata* L. on rice. *Acta Agronomica Sinica*, *30*, 942-946.

Pasiecznik, N.M. (1999). Prosopis-pest or providence, weed or wonder tree? *European Tropical Forest Research Network Newsletter*, *28*, 12-14.

Pu, L., Cheng, L. Li A., Liang, S., Wei, Q., Wu, S., He, L., Xu, X., Lei, N., & Chen, J. (2022). Effects of clonal integration on allelopathy of invasive plant *Wedelia trilobata* under heterogeneous light conditions. *Plant Ecology*, *15*(3), 663–671. https://doi.org/10.1093/jpe/rtab028

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). "NIH Image to ImageJ: 25 years of image analysis". *Nature Methods*, *9*, 671-675.

Schulte, E. E., & Hopkins, B. G. (1996). Estimation of soil organic matter by weight loss-on-ignition. *Soil organic matter: Analysis and interpretation*, *46*, 21–31. https://doi.org/10.2136/sssaspecpub46.c3

Setyowati, N., Muktamar Z., & Wiyanti E. (2021). Compost of yellow creeping daisy (*Wedelia trilobata*, L.) weed to increase organic tomato yield. *International Journal of Current Research*, *4*, 980-983. https://doi.org/10.47191/ijcsrr/V4-i8-15

Setyowati, N., Muktamar Z., Oktiasa S., & Ganefianti, D.W. (2014b). Growth and yield of chili pepper under different time application of wedelia (*Wedelia trilobata*) and siam weed (*Chromolaena odorata*) organic fertilizers. *International Journal on Advanced Science, Engineering and Information Technology,4*(6), 13-16.

Setyowati, N., Muktamar, Z., Suriyanti, B., & Simarmata M. (2014a). Growth and yield of chili pepper as affected by weed based organic compost and nitrogen fertilizer. *International Journal on Advanced Science, Engineering and Information Technology, 4*(2), 84-87.

Shahena, S., Rajan, M., Chandran, V., & Mathew, L. (2021). Allelopathic effect of *Wedelia trilobata* L., on the germination and growth of *Cicer arietinum*, *Vigna unguiculata*, and *Vigna radiata* seedlings. *Journal of Applied Biology and Biotechnology,9*(2), 93-114.https://doi.org/10.7324/JABB.2021.9209

Swaefy, H. M. F., &Basuny, A. M. (2011). Effect of Biofertilization on *Wedelia trilobata* L. Plant Resistance to Salinity. *Australian Journal of Basic and Applied Sciences,5*(11), 571-578.

Toppo, K.I., Guptha, S., Karkun, D., Agrawal, S., & Kumar, A.(2013). Antimicrobial activity of *Sphagneticola trilobata* (L.) *pruski, against some human pathogenic bacteria and fungi, International Quarterly of Life Sciences*, *8*(2), 695-700 pp.

Ullah, M. S., Sun, J., Rutherford, S., Ullah, I., Javed, Q., Rasool, G., Ajmal, M., & Du, D. (2021). Evaluation of the allelopathic effects of leachate from an invasive species (*Wedelia triobata*) on its own growth and performance and those of a native congener (*W. chinensis*). *Biological Invasions, 23*(10), 3135-3149.

Wagner, W., Herbst, D., & Sohmer, S. (1990). *Manual of the flowering plants of Hawai'i*. (1st ed., p. 1952). University of Hawaii Press and Bishop Museum Press, Honolulu.

Waller, G.R. (2003). Introduction-reality and future of allelopathy. In F.A. Macias, J.C.G. Galindo, J.M.G. Molinillo, & H.G. Culter (Eds.), *Allelopathy, Chemistry and Mode of Action of Allelochemicals* (pp. 1-12), CRC Press, New York..

Wang, R., Rehman, S.U., Liang, X., Song, Y., Su, Y., Baerson, S., & Zeng, R. (2012). Effects of simulated acid rain on the allelopathic potential of invasive weed *Wedelia trilobata*, *Allelopathy Journal, 30*(1), 23-32.

Wu, J. R., Peng, S.L., Zhao, H.B., & Xiao, H.L. (2008). Allelopathic effects of *Wedelia trilobata* residues on lettuce germination and seedling growth. *Allelopathy Journal, 22* (1), 197-204.

Zeng, R.S., Lin, X.L., &Luo, S.M. (1994). Studies on the allelopathic effects of *Wedelia chinensis* aqueous extraction. *Journal of South China Agricultural University,15*, 26-30.