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RESEARCH ARTICLE

Effect of aqueous extracts of the invasive weed, creeping daisy (*Sphagneticola trilobata)* on the mortality of earthworm, *Perionyx excavatus*

K.R.S. Perera and U.P.K. Epa*

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ABSTRACT

Sphagneticola trilobata (L.) Pruski is considered as one of the world's worst alien invasive species. The extent of mortality of earthworm *Perionyx excavatus* Perrier exposed to 2.5×10^2, 5.0×10^2 and 7.5×10^2 g/L of aqueous extracts of fresh and dry *S. trilobata* plants was studied. The experiment was carried out in plastic containers containing 3:1 mixture of compost and topsoil. The mortality of all the *P. excavates,* exposed to fresh plant extracts of 5.0×10^2, 7.5×10^2 g/L and dry plant extract of 7.5×10^2 g/L, occurred within four weeks. The percentage mortality of earthworms due to *S. trilobata* extracts was significantly higher than in the control (one-way ANOVA, p=0.05). Coiling, abnormal swelling, mucous secretion, blooding, and fragmentation were noticed in earthworms that were dead due to *S. trilobata* plant extracts. The percentage mortality of earthworms showed a strong positive, linear relationship (r^2=0.98; p=0.05) with the different concentrations of aqueous extracts of *S. trilobata*. *S. trilobata* should not be used in agricultural activities such as use of it as live mulch to improve soil conditions or producing compost fertilizer as the aqueous extracts of *S. trilobata* were highly toxic to *P. excavatus.*

Keywords: Earthworms, Invasive weed, Mortality, *Perionyx excavatus*, Soil, *Sphagneticola trilobata*, Weed toxicity

INTRODUCTION

Sphagneticola trilobata (L.) Pruski (creeping daisy, Singapore daisy, trailing zinnia), earlier named *Wedelia trilobata* is a creeping perennial herb of Asteraceae native to tropical Central and South America (Yan-qiong *et al.* 2005). It is considered as one of the world's worst 100 alien invasive species (GISD 2022). Invasive weeds once established in new environments, pose a significant threat to agriculture, forestry and the aquatic environment (Rao and Chauhan 2015). *S. trilobata* has become naturalized in many wet tropical areas of the world, including Australia, Bangladesh, China, Hawaii, India, Japan, Fiji, South Africa, South Florida, Sri Lanka and the West Indies (LiYing *et al.* 2009) forms mats covering the soil, allowing less chance for survival and growth of other plants (LiYing *et al.* 2009; Macanawai 2013). *S. trilobata* is commonly found in agricultural lands, coastal areas, roadsides, rail sides, open lots, garbage dumps and other disturbed sites. It grows well on almost all soil types, including bare limestone and nutrient poor sandy beaches and swampy waterlogged soils (GISD 2022). The major bioactive components of the plant were germacrene D, α-phellandrene, α-pinene, E-caryophyllene, bicyclogermacrene, limonene, α-humulene, sabinene, β-pinene, camphene, 10-nor-calamine-10-one and γ-morphine (De Silva *et al.* 2012; Verma *et al.* 2014). According to previous studies, bioactive molecules of *S. trilobata* have antimicrobial, insecticidal, larvicidal and antiparasitic activities (Huang 2006; Maldini *et al.* 2009; Toppo *et al.* 2013). However, Setyowati *et al.* (2014a, b) suggested to use *S. trilobata* to produce compost as a substitute for N, P, K fertilizer applications in agriculture. Dissanayake *et al.* (2002) have recommended *S. trilobata* as a phyto-remediator that can be used profitably to treat waste effluents and environments contaminated with heavy metal ions. At present *S. trilobata* is widely introduced as an ornamental or ground cover in gardens in many parts of the world.

However, the high concentration of phytochemicals in *S. trilobata* may have detrimental effect on the soil fauna where it is grown. Among soil organisms, earthworms are mainly used as objects in assessing soil quality because they play an important role in the fertility and structure of soils in general. *Perionyx excavates* Perrier is an epigeic earthworm species that mainly feed at or near the soil surface, on plant litter, dead roots, and other plant debris or animal dung. They produce casts at the soil surface, affecting its roughness and macro-pore distribution. *P. excavates* is one of the most widely used earthworms in compost and vermiwash production in tropical Asia (Reinecke *et al.* 1992; Ananthavalli *et al.* 2019). Because earthworms contribute significantly

Department of Zoology and Environmental Management, University of Kelaniya, Dalugama, Kelaniya, Sri Lanka

* Corresponding author email: epa@kln.ac.lk

to soil quality, it is vital to understand the potential deleterious effects of invasive weeds on their activities and survival. It was hypothesized that an aqueous extract of *S. trilobata* reduces the survival of *P. excavatus* living in the soil-compost mixture. Therefore, the present study evaluated the effect of aqueous extracts of fresh and dry plants of *S. trilobata* on the mortality of *P. excavatus.*

MATERIALS AND METHODS

Preparation of aqueous extracts of wet and dry *S. trilobata*

S. trilobata were collected from home gardens in the Gampaha district, Western province, Sri Lanka, from November to December 2019. A voucher specimen has been deposited at the Department of Zoology and Environmental Management, University of Kelaniya. The flowers, dried leaves and dead stem parts were discarded and fresh *S. trilobata* plants were selected, washed and cut into pieces of approximately 1cm. Seventy-five grams of fresh 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 of aqueous extract of fresh plant parts was prepared. Pieces of fresh *S. trilobata* were also shade-dried for seven days at room temperature (28 °C) and a stock solution of 7.5×10^2 gL^{-1} of aqueous extract was prepared. Solutions with a concentration of 2.5×10^2 gL^{-1} and 5.0×10^2 gL^{-1} were prepared using stock solutions of fresh and dried plant extracts.

Rearing of earthworms

P. excavates used in the experiment were reared in a mixture of 3:1 (wet weight) compost and top soil in plastic barrels. The moisture level of the substrate was maintained around 65-70% throughout the study period by a periodic sprinkling of water. Barrels were placed in a humid and dark room at a mean temperature of 26 °C at the laboratory of the Department of Zoology and Environmental Management, University of Kelaniya. Experimental animals from stock culture were separated from the substrate material by hand sorting, after which worms were washed to remove the adhering material from their bodies. Individuals with well-developed clitella and a wet weight of 430 – 480 mg were selected for the experiment.

Effect of aqueous extract of *S. trilobata* on the mortality of *P. excavatus*

The study was conducted in 24 similar-sized plastic containers ($81\text{x}10^{-3}$ $m^2 \times$ 15cm) filled with a mixture of compost and topsoil in a ratio of 3:1. The containers were randomly arranged and there were three replicates for each treatment and the control. Twenty *P. excavatus* collected from earthworm culture were acclimatized to the test soil under test conditions for 24 h before use. Containers were placed on plastic plates to detect the outside movement of earthworms, if any. Then, 50 mL of each aqueous extract of fresh and dry plants (2.5×10^2, 5.0×10^2, 7.5×10^2 gL^{-1}) of *S. trilobata* were sprinkled separately to the soil in treatment containers once in two days for four weeks. A similar volume of distilled water was sprinkled into the control containers.

Containers were observed daily for surfacing and outside movement of *P. excavatus*. Soil mixtures were disturbed after two and four weeks and the number of *P. excavatus* in each container was recorded. Fresh carcasses of earthworms were observed for any physical damages, color variations and hemorrhages.

Physico-chemical parameters of the soil mixture

Total nitrogen (Kjeldahl method, Bremner 1960), pH (pH meter; IQ 150 pH meter - Spectrum Technologies, Inc) and organic matter content (Allison 1965) of the soil mixture in each container were determined at the end of the experiment.

Statistical analysis

Mortality of *P. excavates* in the treatment and control containers and soil physico-chemical properties were compared using one-way ANOVA followed by Tukey's pairwise comparison test at 95% confidence level. The percentage mortality of *P. excavates* was Arc Sine transformed before the analysis. The relationship between percentages of *P. excavatus* mortality and concentrations of aqueous extract of *S. trilobata* were studied by simple linear regression analysis. All the statistical tests were conducted using MINITAB (version 14) software.

RESULTS AND DISCUSSION

Each *S. trilobata* extract evaluated using the soil test showed a different degree of toxicity to *P. excavates.* One to three earthworms moved to the soil surface after 12 min of addition of the first dose of fresh plant extract of 5.0×10^2 and 7.5×10^2 gL^{-1} and died within the first 20 min of the experiment. Plant extracts sprinkled on the soil surface may have seeped into the soil, exposing earthworms to the toxic compounds of *S. trilobata*. When earthworms are directly exposed to plant extracts, the skin may act as a route to the uptake of toxicants (Jager *et al.* 2003; Vijver *et al.* 2003). None of the earthworms in the control containers moved to the soil surface and died within this period. The death of earthworms may be triggered by the damage caused to the mucopolysaccharide layer of the skin (Mulla *et al.*

2010). The percentage mortality of earthworms in the containers exposed to fresh and dry plant extracts of *S. trilobata* is given in **Table 1**.

None of the earthworms survived in the containers treated with 5.0 × 10^2 and 7.5 × 10^2 gL^{-1} of fresh plant extracts after four weeks showing higher toxicity of fresh plant extracts than dry plant extracts. The toxicity of bioactive compounds of *S. trilobata* that cause anti-annelid effect might have been reduced during the drying process (Hung and Duy, 2012; Mertz *et al.* 2012).

Table 1. Mean (±SD) percentage mortality of *P. excavates* exposed to aqueous extracts of fresh and dry plants of *S. trilobata* in the soil mixture

Type of extract	Concentration (g/L)	After two weeks (%)	After four weeks (%)
Fresh plants	Control	$3±3^a$	$7±3^a$
	$2.5×10^2$	$33±2^b$	$80±3^b$
	$5.0×10^2$	$67±6^c$	100^c
	$7.5×10^2$	$87±2^c$	100^c
Dry plants	Control	$2±3^a$	$3±3^a$
	$2.5×10^2$	$33±2^b$	$80±3^b$
	$5.0×10^2$	$60±4^c$	$90±3^{bc}$
	$7.5×10^2$	$80±4^d$	100^c

Different superscript letters in a column denote significant differences (p=0.05) indicated by One-way ANOVA followed by Tukey's pairwise significant difference test.

The concentrations of each crude extract affect the mortality of earthworms in a dose dependent manner. The percentage mortality of earthworms showed a strong positive, linear relationship with the concentration of aqueous extract of fresh (Linear regression analysis; R^2= 98; P<0.05) and dry (Linear regression analysis; R^2=0.98; P=0.05) plants of *S. trilobata* (**Figure 1**).

There were no significant differences in pH, nitrogen and organic matter content of the soil in the treatment and control containers of both experiments (**Table 2**).

As the pH, nitrogen and organic matter content of the soil mixtures were not significantly different, the mortalities observed in treatment containers could be attributed to the toxic effects of aqueous extracts of *S. trilobata* on earthworms. The anatomical symptoms like coiling, abnormal swelling, mucous secretion, blooding, damaged skin and fragmentation were observed in dead earthworms. Earthworms exposed to agrochemicals (Morowati 2000; Muthukaruppan *et al.* 2005; Reddy and Rao 2008) and chemicals such as lead acetate and Imidacloprid (Yvan *et al.* 2005) have also shown similar anatomical symptoms. However, the aqueous extracts of *Apodytes dimidiate* and *Persicaria hydropiper,* when absorbed through the skin, did not produce any acute

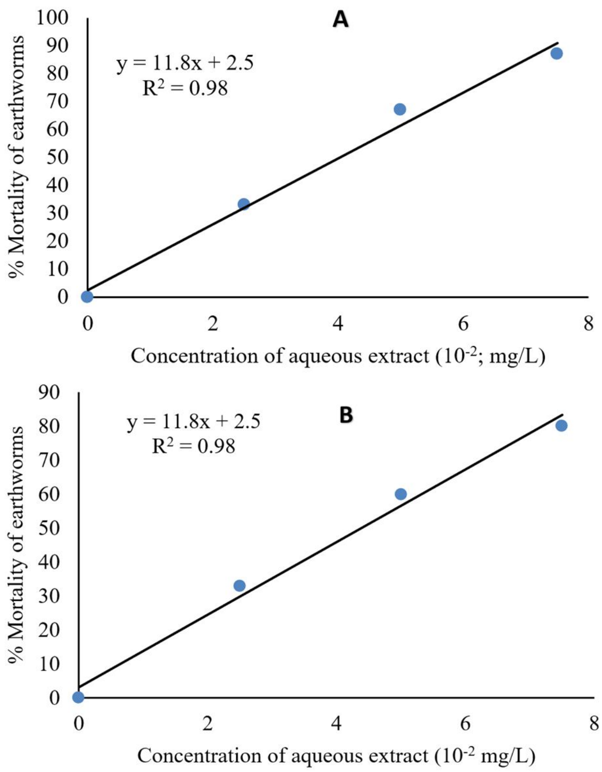

Figure 1. Relationship between concentration of fresh (A) and dry (B) plant extracts of *S. trilobata* and percentage mortality of *P. excavates* after two weeks

Table 2. Mean ± SEM of physico-chemical parameters of soil mixtures exposed to aqueous extracts of *S. trilobata* after four weeks

Type	Concentration (g/L)	pH	Organic matter content (%)	Soil nitrogen (mg/kg)
Fresh plants	Control	7.09±0.06	8.54±0.14	12.9±0.05
	$2.5×10^2$	7.08±0.06	8.75±0.68	11.4±0.18
	$5.0×10^2$	6.99±0.10	8.66±0.19	14.8±0.06
	$7.5×10^2$	7.01±0.08	9.32±0.36	12.9±0.04
Dry plants	Control	7.13±0.04	8.27±0.1	13.2±0.15
	$2.5×10^2$	7.15±0.04	8.52±0.14	11.2±0.05
	$5.0×10^2$	7.16±0.03	8.84±0.11	13.1±0.04
	$7.5×10^2$	7.21±0.02	8.04±0.29	14.5±0.05

Parameters in the columns of both experiments are not significantly different according to one-way ANOVA followed by Tukey's pairwise significant difference test (p>0.05).

toxic effects in *E. fetida* (Brackenbury and Appleton 1997; Govindharaj *et al.* 2022). Therefore, the toxic constituents of *S. trilobata* on earthworms need to be seriously considered in recommending this plant for various applications.

The densely grown mats of *S. trilobata* covering extensive land areas in many countries may antagonistically affect the survival of earthworms that live under these plants. The antagonistic effect of *S. trilobata* on nematode *Meloidogyne incognita* has previously been shown by Silva *et al.* (2008). Therefore, *S. trilobata* should not be used in horticulture and as live mulch to condition the soil and produce biofertilizers. Further research is warranted to investigate the impacts of *S. trilobata* and other invasive weeds on the survival of earthworms and other fauna under natural environmental conditions.

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