

Allelopathic effect of parthenium weed (*Parthenium hysterophorus L.*) leaf litter on the seedling emergence and growth of wheat (*Triticum aestivum L.*)

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Abstract. Parthenium weed (*Parthenium hysterophorus L.*) is a pantropical weed from Central America that has invaded more than 40 countries in the world. The invasiveness and competitive advantage of this weed are attributed to its ability to produce large number of seeds, tolerance to environmental stresses, environmental and climate change adaptability, and allelopathic properties. Phenolics and sesquiterpene lactones are two major classes of allelopathic compound produced by parthenium weed and these chemicals are known to have significant negative effect on germination, seedling emergence and growth of various plants species. The allelopathic properties of parthenium weed had been investigated in various past studies, however, most of the studies conducted the experiment under laboratory conditions with the allelopathic effect being tested by applying the aqueous extract of the weed directly on the test plants. This study aimed to examine the allelopathic effect of the leaf litter of parthenium weed under more natural conditions. Fresh leaves were oven dried and crushed to crude powder, hereafter referred to as leaf litter. The leaf litter were incorporated into the upper layer of the media of Greenfingers compost bark in Anova pots. In to this were sown the seeds of the test plans then set to germinate. Another treatment without leaf litter was set up as a series of control. The treatments were arranged in simple randomized design in the glasshouse. After one week, any seedling produced were counted and thinned to five seedlings per pot. The plants produced from these seedlings were harvested after further 40 days of growth and the growth parameter including root length, shoot length, and dry weight were measured. ANOVA analysis of the result showed that the measurable growth reduction increased as the amount of parthenium weed leaf litter increased but variability between results existed. The uncontrollable factors and seasonal conditions may cause this variability, in addition to the seed quality. The chemicals contained within the parthenium weed leaf litter has been shown to affect crop growth presumably through allelopathic activity under natural field conditions.

1. Introduction

Parthenium weed is considered as a cropping, grazing, and environmental weed. It has spread to more than 40 countries (Bajwa *et al.* 2018). In Queensland Australia, 8.2 million ha of parthenium weed infestation can be found across the central highlands, with scattered infestations found in the northern and eastern parts of the State (Department of Environment and Energy 2018). Sporadic infestations can also be found in New South Wales, Northern Territory and Western Australia (Australian Weed Committee 2012). In India, parthenium weed has become a major problem in all States, from where it has further spread to neighbouring countries by vehicles or as a contaminant of transported seeds

(Adkins & Shabbir 2014). This weed also ruthlessly affects the grazing and cropping lands of Eastern African countries, with the worst infestation occurring in Ethiopia (Adkins & Shabbir 2014).

The successful establishment of parthenium weed in fields is supported by several factors, such as its numerous seed production, genetic diversity, tolerance to stresses, and its allelopathic potential (Bajwa *et al.* 2016). Parthenium weed competes with major field crops in Australia, on the India subcontinent, and in Africa (Bajwa *et al.* 2016). While controlling this weed requires an integrated physical, chemical, and biological approach, the infestation sites also need to be quarantined to prevent spread to other locations (Parsons & Cuthbertson 2001). In Australia, parthenium weed control focuses on the prevention of spread supported through government policy and cultural control methods. The detection and eradication programs had been successfully implemented in New South Wales by having strong legislative, extension, and management process, as well as establishing service such as machinery wash-down facilities to ensure cleanliness of the machinery that is moving within and between States (Parsons & Cuthbertson 2001; Blackmore & Johnson 2010). The strong enforcement of the policy that prevents spread, such as the Biological Control Act 1985 or the Environment Protection and Biodiversity Conservation Act 1999, also enforce the control measures. However, in many developing countries, these types of control measures for parthenium weed fail because of weak regulation enforcement and/or the weed had become too widely spread before the control measures were put in place. In India, the control measures include physical (hand weeding, burning, cutting), chemical (herbicide, bioherbicide), and biological control (Mexican beetle, mycoherbicide), but the success rate is limited (Batish *et al.* 2012), whereas parthenium weed control requires long-term community participation and currently such a control approach is not available in India (Batish *et al.* 2012). In Ethiopia, the research on parthenium weed and weeds in general is limited. The use of herbicides is also restricted to large-scale farms because the small farmer cannot afford the high price of chemicals (Tamado 2001).

The physical methods of control often become the only options for parthenium weed control in lesser developed countries. This method generates high biomass waste that can affect the soil. The interest of this present research project is on the allelopathic effect of parthenium weed plant residues in the soil and its subsequent effect on crops that are planted therein. The residues from the weed are also rich in organic compounds that can alter the chemical and nutrient status of the soil (Batish *et al.* 2002). Residues have high amounts of nitrogen, phosphorus, potassium, and other nutrients essential for plant growth (Kishor *et al.* 2010). These nutrients increase the possibility of parthenium weed being used as a soil amendment and as a green fertilizer, combined with the quality of weed suppressing compound created by the allelopathy (Saravanane *et al.* 2007) as long as the allelopathy doesn't affect the crop. Parthenium weed residues that fall onto soil surface create a layer of organic matter that can remain there or become incorporated into the soil as breakdown occurs. Either on the soil surface or in the soil the litter releases the allelopathic compounds to the soil environment. In cropping areas, the residues that are present may then affect the succeeding crop, and as a result, drastically reduce crop production.

The past research on parthenium weed allelopathy is dominated by studies that simply administer crude plant extracts made from the weed and place them directly onto the germinating seeds in Petri dishes, which has very little similarity to what actually happens in nature (Willis 2007). Hence, this present research study intends to use an experimental design that resembles more accurately natural conditions. The aim of this study is to investigate the effect of parthenium weed leaf litter on the seedling emergence and growth of selected crops for their allelopathic and organic compounds.

2. Material and Methods

2.1 Germination Test

The seeds used in this study was wheat (acquired from UQ Gatton Plant Science Laboratory seed store). Prior to use, all seed lots were handpicked (pressed with a finger to examine the seed fill), then further examined with a Faxitron X-Ray machine (Faxitron Bioptics, LLC, Arizona, USA) to determine the quality and seed fill percentage. Petri dishes (90 mm diameter, Sarstedt Australia Pty Ltd) were layered with two Whatman filter papers (90 mm, Labserv) and moistened with 10 mL

distilled water. Ten seeds were placed on the filter paper, with five replicate dishes. The Petri dishes were then put in a germination incubator (Thermoline Scientific, Australia) set at 15/20°C with a 12/12-hour photoperiod. After 7 days, germinated seeds were counted.

2.2 Glasshouse Trials

This study was conducted in a glasshouse from March to September 2018 in UQ Gatton Plant Nursery Unit. The experiment was conducted twice. Test plant for the first experiment were planted on the 9th April 2018 and harvested on 10 May (30 days after sowing). For the second experiment, the seeds were sown on 13th July 2018 and harvested after the same number of days as in the first experiment.

The experiment was designed with a completely randomized design. There were four treatments in this experiment: media mixed with 3 g of parthenium weed leaf litter (3P treatment), media mixed with 5 g of parthenium leaf litter (5P treatment), media mixed with 5 g of peat moss (PT treatment), and control treatment where the media had no additional compound mixed. The PT treatment was added with the consideration that parthenium weed could probably add organic compound to the media. In order to see whether the seedling emergence and growth were affected by the allelopathic compounds or by the organic compound of parthenium weed, PT treatment was added as a non-allelopathic organic compound. There were six replicates (represented by pot) for each treatment, and there were five plants in each pot.

2.2.1 Leaf litter collection:

Parthenium weed leaves were harvested from the Queensland Department of Agriculture and Fisheries' parthenium weed trial site in Helidon Spa (27°33'33.9''S - 152°05'22.6''E, elevation 172 m) from March to June 2018. For the first experiment, fresh parthenium weed leaves were harvested once on 7 March 2018, while for the second experiment, the leaves were harvested in small amount once every week in June 2018. The fresh leaves were separated from the stem, placed in a paper bag, and then dried in a laboratory oven (Thermoline Scientific, Australia) at a temperature of 40°C for 72 hours. After being dried, the leaves were then crushed by hand until they became a crude powder. The peat moss used in this experiment was Tref Peat Moss (Fine, Garden City Plastics, QLD, Australia). The leaf litter was then weighed into lots of 3 g and 5 g and the peat moss was weighed into lots of 5 g, then stored in separate sterile bottles and kept at room temperature until used.

2.2.2 Media preparation:

This experiment used one kg of Green Fingers SLCS composted bark (fines, 1 to 5 mm) media placed into each of 72 black ANOVA pots (14 cm diameter, Garden City Plastics, QLD, Australia). The leaf litter and peat moss were emptied from the plastic jar onto the surface of the media, then mixed thoroughly in a 3 cm depth with a wooden stick. Ten seeds of wheat were sown in 1 cm depth, and at least 1 cm apart in the media. The pots were watered to field capacity and maintained at this level throughout the study. After 7 days, the germinated seedlings were counted and expressed as the seedling emergence percentage, then thinned to five plants per pot. The seedlings were then left to grow for a further 30 days. The seedlings were then harvested, cleaned from the media and the shoot and root length measured. The plants were then dried in an oven (Thermoline Scientific, Australia) for 72 hours at a temperature of 40°C, then dry weights were determined.

2.3 Data analysis

Seedling emergence percentage was determined by the number of seedlings emerging 1 week after sowing. Shoot length was measured from the shoot base to the tip of the plant and the root length was measured from the base to the longest root tip. Altogether, including that for dry weight, all datasets were analyzed for Analysis of Variance using the R Studio statistical program with alpha 0.05. Data that showed significant difference was then further analyzed with a T-Test to determine significant differences between groups.

3. Results

The seedling emergence percentage of wheat varied across treatment. In the first experiment, all treatments on wheat had seedling emergence percentage of 90% or above and no significant differences observed, while in the second experiment, the seedling emergence percentage in the 3 g parthenium weed treatment was lower than the 5 g parthenium weed treatment (65% and 87% respectively). The soil amelioration treatments applied in both experiments did not show a significant difference in seed germination percent achieved.

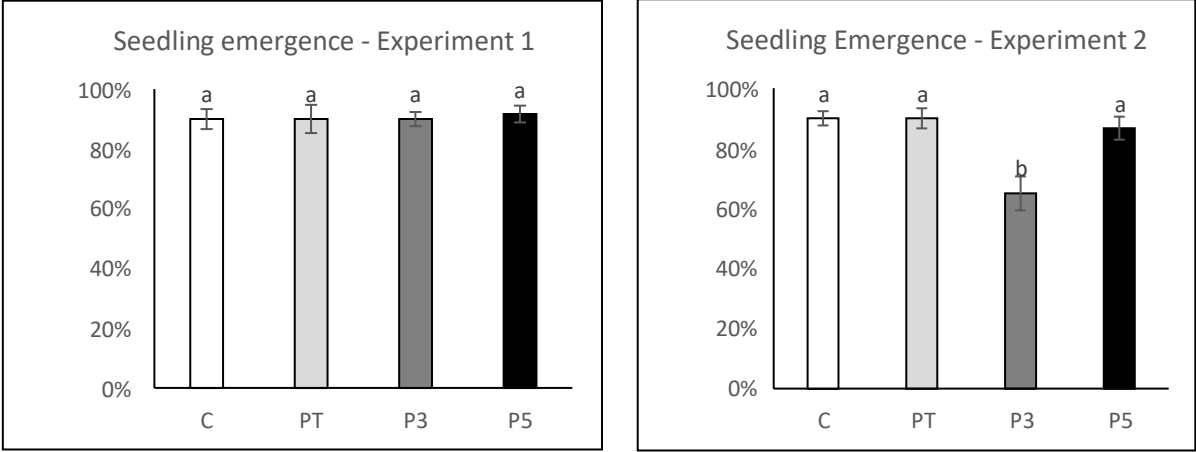


Figure 4.1 The seedling emergence percentage of test plant achieved after 7 days of growth under four treatments (C: Control treatment, PT: peat moss treatment, P3: 3 g of parthenium weed leaf litter treatment, P5: 5 g of parthenium weed leaf litter treatment) and from two experiments run at different times. The bar tags represent two SE of the mean of 6 pots and the letters indicate statistical similarity within a species.

In the first experiment, the root length reduction caused by 3 g of parthenium weed leaf litter was 5.69% while 5 g of leaf litter caused 7.5% of reduction. This reduction is limited compared to the second experiment, where the 3 g caused 41.8% reduction and 5 g caused 46.1%.

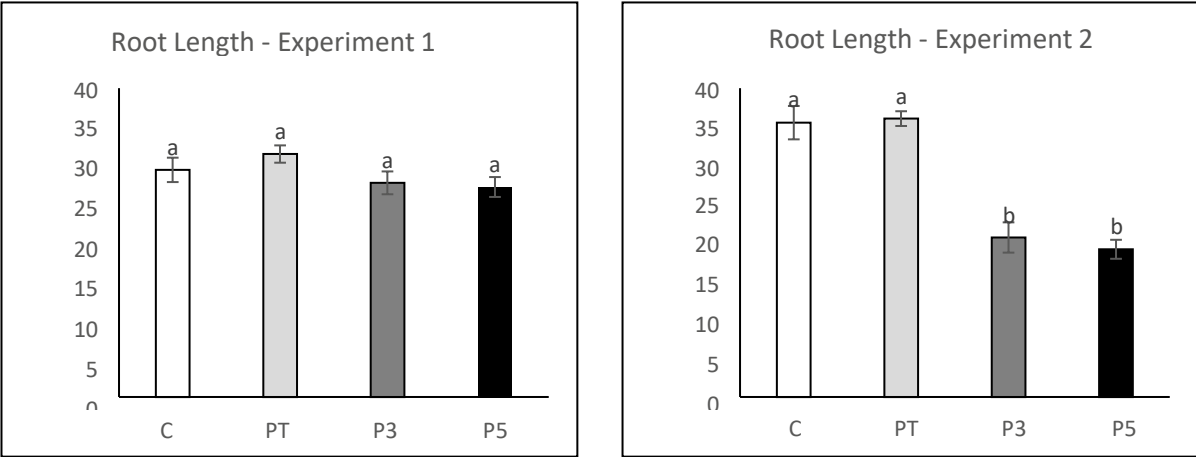


Figure 4.2: The root length (cm) of test plan after 30 days of growth under four treatments (C: Control treatment, PT: peat moss treatment, P3: 3 g of parthenium weed leaf litter treatment, P5: 5 g of parthenium weed leaf litter treatment) and from two experiments run at different times. The bar tags represent two SE of the mean of 6 pots and the letters indicate statistical similarity within a species. For the shoot observation, the analysis showed reduction for both of 3 g and 5 g treatments in experiment 1 ($p=0.002$) and experiment 2 ($p=0.42$).

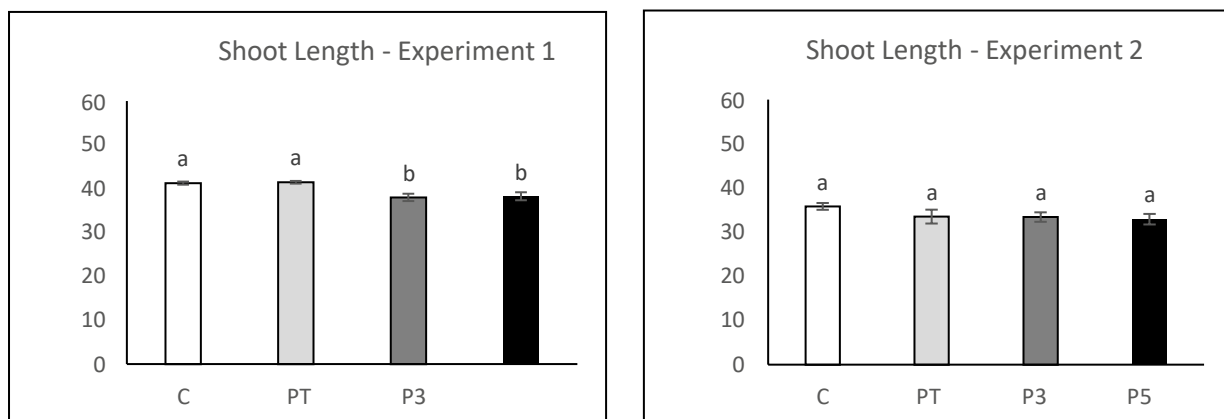


Figure 4.3: The shoot length (cm) of test plant achieved after 30 days of growth under four treatments (C: Control treatment, PT: peat moss treatment, P3: 3 g of parthenium weed leaf litter treatment, P5: 5 g of parthenium weed leaf litter treatment) and from two experiments run at different times. The bar tags represent two SE of the mean of 6 pots and the letters indicate statistical similarity within a species.

In the first experiment, dry weight in 3 g and 5 g treatments experienced 11% and 13% reduction respectively compared to the control treatment ($p=0.003$). The reduction was bigger in experiment 2 where 3 g treatment reduced the dry weight by 13% and 5 g by 28%, but no significant difference between treatments.

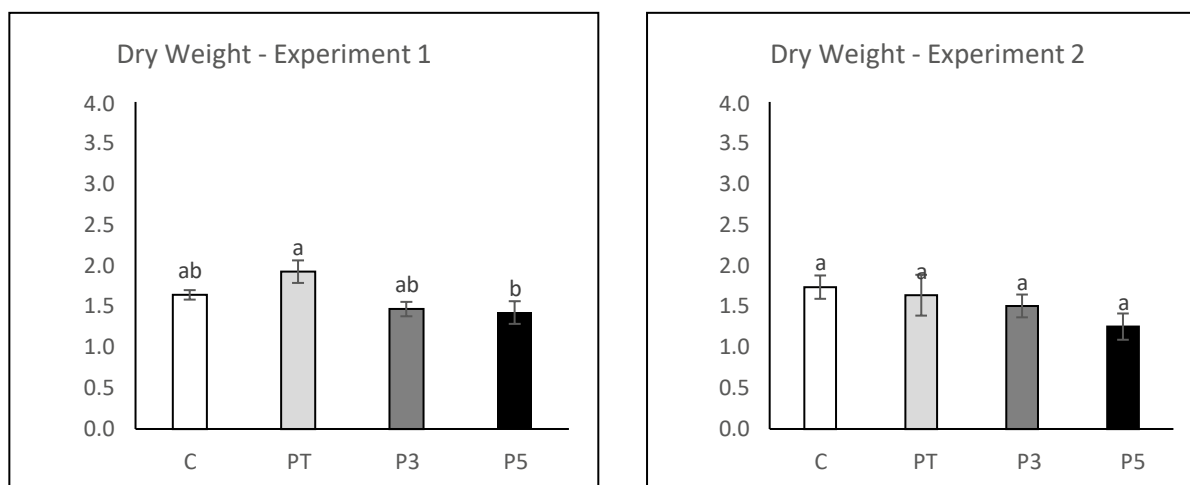


Figure 4.4: The dry weight (gr) of test plant achieved after 30 days of growth under four treatments (C: Control treatment, PT: peat moss treatment, P3: 3 g of parthenium weed leaf litter treatment, P5: 5 g of parthenium weed leaf litter treatment) and from two experiments run at different times. The bar tags represent two SE of the mean of 6 pots and the letters indicate statistical similarity within a species.

3.1 Seedling Emergence

There could be several explanations for the varied results of seedling emergence. Firstly, the emergence percentage reduction is caused by the external factor, specifically leachate from the parthenium weed leaf litter; or secondly, the internal factors such as seed quality and susceptibility. Phenolics are the main allelopathic compounds present in the parthenium weed litter are known to be antagonistic to the action of gibberellin (Williams & Hoagland 1982). Gibberellin is the plant growth regulator (PGR) that regulates the mobilization of sugar reserves from the endosperm to the embryonic axis during early stage of germination in Poaceae species where it is used to drive cell division

(Hopkins & Hüner 2009). Sesquiterpene lactones as the other most important allelopathic compound in parthenium weed litter can impair respiration and affect the activity of certain protease and peroxidase enzymes (Batish *et al.* 2002a) which in turn will interfere with the cellular activity within the seed and reduce germination.

The quality (i.e. seed fill and viability percent, dormancy factors) of the seed is also another crucial factor that determines successful germination of a species. The seed coat permeability, seed metabolism, differential uptake of chemicals and seed size may also determine a species sensitivity to allelopathic compound (William & Hoagland 1982). This may be one of the reasons of lower emergence in P3 treatment in Experiment 2.

3.2 Root Length

The result shows leaf litter of parthenium weed causes root growth retardation effect. There are several possible explanations of the effect of the leaf litter to the root growth and root environment. According to a number of studies, the reason would be; first, direct allelopathic effect of the leachate to the root growth. Two major allelopathic compound in parthenium weed are phenolic and sesquiterpene. Phenolic is water soluble that directly reduce the cell division and elongation in the root by inhibiting the activity of gibberellin and indoleacetic acid that induce growth (Mersie & Singh 1987). The main sesquiterpene in parthenium weed is parthenin. When the leaf litter is incorporated into the growth media, parthenin is released and affects cellular activity of the root tip of the plants (Raouf & Siddiqui 2013). Parthenin can cause abnormality in the mitotic cell division that cause chromosomal distortion, and hence, inhibitory of growth (Raouf & Siddiqui 2013). Additionally, parthenin (or sesquiterpene lactone in general) can react and modify the original properties of amino acid and protein which in turn will cause damage in cellular membrane and dehydrogenase activity in root (Batish *et al.* 2002a). Second, the leachate can also disturb the nutrient availability for the plants by affecting the microbial activity in the rhizosphere which have big role in nitrogen fixation and nitrification (Batish *et al.* 2002b). The leachates from parthenium weed reduce the growth rate of nitrogen fixating bacteria like azotobacter by lowering auxin production (Kanchan & Jayachandra 1981). The nitrification process by *Nitrosomonas sp.* is also susceptible to parthenium weed leachate (Kanchan & Jayachandra 1981). Increasing phenolics in the soil can decrease the soil pH, which affect the microbial activity (Batish *et al.* 2002b; Singh *et al.* 2003).

The root length was found to be more sensitive to the allelopathy than the shoot length in various experiments and test plants. The similar result was recorded on species such as wild oats and cobbler's peg (Batish *et al.* 2002a), chickpea and radish (Batish *et al.* 2002b), and teff (*Eragrostis tef* Zucc., native grain to Ethiopia) (Tefera 2002). The higher sensitivity is possibly because the root has direct contact to the parthenium weed litter that release phenolics to the media and the cell in the root absorb the compounds from the environment earlier than the other part of the plant.

3.3 Shoot Length and Dry Weight

Shoot length was reduced as an increasing amount of parthenium weed leaf litter is incorporated into the soil. The leachates with allelopathic compounds from the leaf litter affecting the plants in several ways, including the disturbance in cellular activity (which then affect transpiration and photosynthesis) and reducing the availability of the nutrient to the plant.

Parthenin can cause damage in cellular membrane and result the loss of chlorophyll in the leaves (Batish *et al.* 2002a). The seedling growing in parthenin has decreased chlorophyll content and it also affects photosynthetic activity (Batish *et al.* 2002a). Though it is not clear whether the decreasing chlorophyll content is due to the direct inhibition of allelochemical to the synthetic pathway or the degradation of the already formed chlorophyll (Batish *et al.* 2001). Moreover, the test plant (mung bean) that was treated with parthenin also experienced impaired cellular respiration, reduced protein content, and problem with enzymes activity, especially peroxidase and protease (Batish *et al.* 2001). Like many other sesquiterpenes, parthenin can form a bonding with and transfer toxic alkyl group to amino acid and protein, hence damaging the metabolism and inhibit growth (Singh *et al.* 2002).

Dry weight reduction varied among the treatments and experiment. Biomass accumulation is the function of the photosynthetic efficiency and the resources uptake, both the nutrient from the soil or

the amount of light (Squire 1990). The presence of allelopathic compounds affect both of these processes. In the soil system, parthenium weed leachates can alter the chemical status, decreasing pH, as well as alter the accumulation and availability of the nutrient (Batish *et al.* 2002). As mentioned, allelopathic compound can also interfere with photosynthetic activity by reducing the chlorophyll content in the leaves, which will reduce the plant's ability to convert nutrient into biomass. In this experiment, the wheat seedling underwent 5 g parthenium weed treatment also showed the chlorosis symptoms and a general weight loss.

3.4 Organic Compound and Seasonality

By comparing the peat moss with parthenium weed treatments, it can be determined whether adding parthenium weed leaves would provide the organic compound and promote the growth of the test plants. This idea originated from a number of studies which conclude that parthenium weed has a high amount of organic compound that may become the source of nutrient. Batish *et al.* (2002) demonstrated that parthenium weed leaves on low concentration in soil (3 g parthenium weed in 100 g soil) increase the amount of organic carbon, potassium, and organic matter, but is negatively correlated with pH, manganese, iron, phosphorus, and nitrogen. However, the leachates compounds from parthenium weed can also undergo several types of transformation, such as toxification, detoxification, absorbed to clay particles, or provide carbon for microbe (Batish *et al.* 2002; Singh *et al.* 2005). In this experiment, on concentration of 5 g in 1 kg soil, dried parthenium weed leaf litter decreased the root length, shoot length, and dry weight of the test plant. It can also be established that on this concentration, parthenium weed ability to provide nutrients for the plants is overshadowed by its allelopathic effect. The allelopathic effect from the leaf litter is evidently stronger than the nutrient it provides.

The activity of allelopathic compounds is complex. It is influenced by environmental stress, soil microbe, plant community and many other biotic and abiotic factors (Filep *et al.* 2016) which can increase or decrease its toxicity and availability (Batish *et al.* 2002; Singh *et al.* 2005). Moreover, allelochemical production is also found to be fluctuated following seasonal dynamics (Filep *et al.* 2016), which may explain difference in results between experiment 1 and 2.

Soil is a complex system with physical, chemical, and biological activity occurring at the same time, and the wide range of uncontrolled external factor such as temperature, humidity, and radiation may result in findings of this experiment varied widely from the known literature, which were mostly conducted by growing test plants in an incubator or growth chamber. The uncontrolled environmental and seasonal conditions may affect the seedling emergence and thus potentiality influence the results of following experiments.

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