agronomy

MDPI

Article

The Impact of Parthenium Weed-Amended Substrates on the Germination and Early Growth of a Range of Pasture and Crop Species

Boyang Shi [1,2,*], Kunjithapatham Dhileepan [2] and Steve Adkins [1]

[1] School of Agriculture and Food Sciences, The University of Queensland, Brisbane 4072, Australia; s.adkins@uq.edu.au

[2] Biosecurity Queensland, Department of Agriculture and Fisheries, Ecosciences Precinct, Brisbane 4102, Australia; kunjithapatham.dhileepan@daf.qld.gov.au

* Correspondence: Boyang.Shi@daf.qld.gov.au

Abstract: Parthenium weed (*Parthenium hysterophorus* L.) is an internationally important invasive weed native to the tropical and sub-tropical Americas, and invasive in more than 30 countries. This weed has serious adverse influences on rangeland and agricultural crop production, on human and animal health, and on the biodiversity of natural communities. Parthenium weed leaf litter can reduce seedling emergence and affect the early growth of a wide range of pasture and crop species. Soil collected from a heavily infested parthenium weed area was shown to reduce seedling emergence of a wide range of test plants (lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), curly windmill grass (*Enteropogon acicularis* L.), and liverseed grass (*Urochloa panicoides* P.Beauv.)) by between 20 to 40%; however, the soil had no effect on the subsequent growth of the surviving test plants. Soil amended with dried parthenium weed leaf litter reduced the emergence of test species by ca. 20 to 40%, but it had no effect on the growth of the surviving test plants. One week after emergence, the growth of all test species was stimulated by 9 to 86% in the leaf litter-amended soil with the increased growth matching the increased amounts leaf litter amended. In parthenium weed-infested landscapes, the substrate (soil) is affected by the plant during the growing season by the release of allelopathic chemicals. However, the plant's litter can affect the community outside of the growing season, first through a residual allelopathic activity, but also by a fertilizing effect as litter breakdown occurs. This study demonstrates the significant ability of parthenium weed to affect plant communities throughout the year and, when considered over several years, this may lead to the creation of a complete monoculture of the weed.

Keywords: parthenium weed; allelopathy; infested soil

Citation: Shi, B.; Dhileepan, K.; Adkins, S. The Impact of Parthenium Weed-Amended Substrates on the Germination and Early Growth of a Range of Pasture and Crop Species. *Agronomy* **2021**, *11*, 1708. https://doi.org/10.3390/agronomy11091708

Academic Editors: Jeffrey Weidenhamer, Sajid Latif and Leslie A. Weston

Received: 11 August 2021
Accepted: 24 August 2021
Published: 27 August 2021

1. Introduction

Parthenium weed (*Parthenium hysterophorus* L.; Asteraceae) is an annual herbaceous plant believed to have originated from the area surrounding the Gulf of Mexico or from central South America [1]. This weed now has a pantropical distribution and is found in more than 80 countries [2]. It is recognized as a major weed in Southern and Eastern Africa, Southern and South East Asia, the Middle East, the Pacific, and Australia [3]. Parthenium weed is known to have substantial negative impacts on agricultural crop production [3], the rearing of grazing animals [3–5], and on human health [5]. In addition, many natural ecosystems have been invaded and negatively impacted by parthenium weed [6]. The reasons for the species' success in its introduced range has been attributed to its high rate of production of viable seeds, a long life in the soil seed bank, a variable short to long lifecycle allowing for the production of seeds under nearly any environmental condition, a tolerance to a range of environmental stresses, the absence of natural enemies, the use of numerous vectors for seed dispersal, considerable genetic diversity within its populations, and the plant's allelopathic activity [7].

Allelopathy is a phenomenon observed in many plant species, which involves the release of chemicals into the local environment through root exudation, plant residue and litter decay, or by leaching from aerial plant parts, which can all affect the growth and development of neighboring plants [8]. The success of aggressive plant species such as parthenium weed with respect to invasion and establishment in new regions is thought to involve their ability to suppress and to replace other species within the existing plant community. The role of aerial and underground parts of parthenium weed plants, and their residues, has been investigated with respect to allelopathy on several occasions [2,9,10]. It is known that allelochemicals can be washed from parthenium weed vegetative parts by rain, naturally released from their roots [9,11], and emitted from decaying plant residues [9,11–15]. Root release is thought to play an important role in placing allelochemicals within the soil rhizosphere, from where they disperse to modify the local soil environment [16]. These root-emitted allelochemicals can then be further altered by soil attributes such as soil moisture content, which can change their mobility and biological availability [16].

Numerous studies have shown that when a direct application of an aqueous parthenium weed leaf extract is made to seeds of a wide range of test plant species, their germination is reduced, and seedling growth is delayed [17]. Further studies have shown that the germination and seedling growth of several plant species can also be reduced when aqueous solutions, made from various parts of the parthenium weed plant, are added by irrigation to the growing medium [18,19]. However, the value of such experiments has been brought into question [2] because these aqueous extracts applied directly to seeds or to soil also contain enzymes, salts, and amino acids that can also affect growth, and substantially higher doses of the potential allelochemicals are often used, much higher than are normally found under natural conditions.

When leaf litter from parthenium weed, sicklepod (*Cassia obtusifolia* L.), and chaff-flower (*Achyranthes aspera* L.) was combined and then incorporated into a soil into which several wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) cultivars were sown [20], seed germination was unaffected, but the growth of the young plants was significantly reduced. In another study, the growth of several Brassicaceae species was significantly reduced when they were grown in a soil amended with parthenium weed residues [21]. There are only a few studies that have looked at the soil collected from a parthenium weed-infested site and its impacts on crop growth. In one study, the rhizosphere soil collected from a parthenium weed-infested site near a water channel, and a sample collected from a nearby infested but dry site, showed a greater inhibition of maize growth when studied in a pot trial, suggesting that better growing conditions could increase the production of allelochemicals [22]. Another study showed that the amounts of soil nitrogen and organic matter were significantly higher in a parthenium weed-infested site as compared to a non-infested site [23].

The main aims of this study were to (1) investigate seed germination and the subsequent growth of a range of pasture species and crops in soil collected from a parthenium weed-infested and non-infested rangeland; (2) investigate seed germination and growth of a range of pasture species and crops in soils amended with varying quantities of parthenium weed leaf litter.

2. Materials and Methods

2.1. Impact of Soil from a Parthenium Weed-Infested Area on Germination and Growth of Test Plant Seedlings (Experiment 1)

Soil collection: Soil samples were collected from a parthenium weed-infested site (brown-grey dermosol, pH 6.0, 23% moisture content, 11% organic matter, 5.44% C, 0.54% N) and a parthenium weed-free site (brown-grey dermosol, pH 6.3, 21% moisture content, 9% organic matter, 4.07% C, 0.37% N). The two sites at Kilcoy (26.94° S, 152.49° E) in South-East Queensland, Australia were separated by 15 m and were both at an altitude of *ca.* 230 m above sea level. Both sites were originally native grassland and had been under continuous grazing for at least 33 years, with parthenium weed present in the area for at least 28 years. The selected parthenium weed-infested site had a history of supporting parthenium weed populations of *ca.* 15 plants m^{-2} for at least the past 5 years, whereas the parthenium weed-free site had been free from parthenium weed for the same period. The climate at both sites was subtropical (ranging from 7 to 37 °C), with 950 mm of rainfall during the 2015–2016 summer season when the soil samples were collected.

Soils samples from 1 m^2 quadrats taken from the parthenium weed-infested (n = 25) and the non-infested site (n = 25) were collected at random. From each quadrat, five individual soil cores (each 8 cm in diameter and 20 cm in depth) were collected using a soil auger. In each quadrat, samples were collected from each of the four corners and one from the center of the quadrat, yielding a total of 125 soil cores each from the infested and non-infested sites. The soil samples were collected from a top (0–10 cm depth) and a bottom section (10–20 cm depth) from each site, and the samples for each depth and each quadrat were then mixed and placed into separate plastic bags, sealed, and transported to the laboratory. The samples were then individually passed through a 1 mm sieve and used immediately in a glasshouse trial.

Test species: Lettuce (*Lactuca sativa* L.; Great Lakes) and maize (*Zea mays* L.; Early Extra Sweet F1) seeds were obtained from Mr Fothergill's Seeds Pty. Ltd. (South Windsor, New South Wales) and were regarded as crop species. Curly windmill grass (*Enteropogon acicularis* L.) and liverseed grass (*Urochloa panicoides* P.Beauv.) were obtained from Progressive Seeds Pty. Ltd. (Mount Crosby, QLD, Australia) and Herbiseeds (Twyford, Berkshire, England), respectively, and were regarded as an Australian native (curly windmill grass) and nonnative (liverseed grass) grass species.

After purchase, all seed lots were stored in an environmentally controlled seed store (at 15 ± 1 °C and 15 ± 3% relative humidity) to maintain their viability and to overcome any dormancy present at the time of purchase. Before experimental use, seed lots were X-rayed (Faxitron, Faxitron Bioptics, LLC, Tucson, AZ, USA) to determine aspects of quality, including the proportion of filled seeds (Figure A1). If necessary, seed lots were cleaned to yield batches with >90% seed fill. A germination test was then performed. In this test, three replicates of 30 seeds from each species were evenly placed onto an agar medium (10 g L^{-1}, 15 mL per dish; Sigma-Aldrich, St. Louis, MO, USA) contained in 9 cm diameter plastic Petri dishes. The Petri dishes were then sealed around their edges with Parafilm and incubated at 25/20 °C (day/night) using a matching 12/12 h photoperiod (light intensity of *ca.* 100 µmol m^{-2} s^{-1}) for 14 days in a germination incubator (Thermoline, QLD, Australia). The germination percentages were recorded daily and only seed lots yielding >90% germination were used in the following studies.

Glasshouse test: Seeds of each of the four test species were surface sterilized in a sodium hypochlorite solution (2% *v/v*) for 5 min, followed by washing several times with sterile water. The seeds were then sown to a depth of 1.5 cm, in lots of 10, into 192 black plastic pots (8 cm diameter) each containing 300 g of one of the four collected soil samples, and then moistened to field capacity with tap water. There were 48 pots per test species, 24 for the non-infested site and 24 for the infested site, with 12 from the 0 to 10 cm layer and 12 from the 10 to 20 cm layer, yielding 12 replications per treatment. All pots were placed in a glasshouse at 27/22 ± 5 °C (day/night) with a matching 14/10 h photoperiod and soil moisture maintained at field capacity. The seedling emergence (taken to represent germination) was recorded after 7 days. Seedlings were then thinned to two seedlings of uniform size per pot and the seedlings were allowed to grow under the same conditions for a further 28 days. Every 7 days, three replicate pots were removed from the trial, the seedling shoot lengths measured, and the total dry biomass of the plants determined. Any other plant species that emerged in the pots were removed as soon as they appeared.

2.2. Impact of Parthenium Weed Leaf Litter on Test Plant Germination and Growth (Experiment 2)

Leaf litter preparation: Seeds of parthenium weed (Clermont biotype) were obtained from the University of Queensland seed collection and were surfaced-sterilized by shaking in a sodium hypochlorite solution (2% *v/v*) for 5 min, followed by washing several times with sterile water. The seeds were then sown (five seeds per pot) into 30 black plastic pots (14 cm diameter, AVONA, Garden City Plastics, QLD, Australia) containing a UC potting compost (washed sand, sphagnum peat moss, and a stock fertilizer mixture at the rate of 8 kg m^{-3}), wetted to field capacity with tap water, and then placed into a glasshouse set at 27/22 ± 2 °C (day/night) with a matching 14/10 h photoperiod. When seedlings were *ca.* 2 cm tall, they were thinned to one seedling per pot. These plants were then allowed to grow under the same conditions until they were 57 to 84 days old. All 30 plants were then harvested, with their leaves (except the newly expanding leaves) placed into paper bags (replicate collections), and then dried at 35 °C for 72 h. These leaves were ground into 1 mm^2 sized particles and were immediately used in the glasshouse trial the following day.

Glasshouse trials: All test species used were the same as described above in Experiment 1. One day after oven-drying, the three replicates of the parthenium weed leaf litter were incorporated into the UC potting compost at a rate of either 1.0, 2.0, or 5.0 g kg^{-1} of potting compost that was used to fill the 14 cm diameter black plastic black pots (AVONA, Garden City Plastics, QLD, Australia). The dry parthenium weed leaf litter was mixed only into the uppermost 3 cm of the 1 kg compost mass that had been added to each pot. The quantity of leaf litter added per mass of soil was determined based on the amount of leaf litter likely to have fallen onto the surface area of 20 parthenium weed plants per m^2 in the field. Once prepared, the compost was moistened to field capacity with tap water and the pots placed into a glasshouse at 27/22 ± 2 °C (day/night) with a matching 14/10 h photoperiod. Three replicate pots of each of the four parthenium weed residue treatments (0, 1, 2, or 5 g) were prepared, so there were 48 pots in total. Ten surface-sterilized seeds of maize, lettuce, curly windmill grass, and liverseed grass (shaken in a 2% sodium hypochlorite solution for 5 min and then washed several times with sterile water) were then sown into the pots to a depth of *ca.* 1.5 cm (into the middle of the leaf litter layer). When germination was complete, and seedlings were *ca.* 2 cm tall (7 days after planting), they were counted and then thinned to one representative seedling per pot. After a further 30 days of growth, the plants were harvested, and their shoot lengths and total plant dry biomass were determined by oven-drying at 60 ± 2 °C for 7 days. C and N concentrations were analyzed by a TruMac Carbon and Nitrogen Determinator (LECO Corporation, St. Joseph, MI, USA).

Experimental design and data analysis: A completely randomized design was used in both experiments with three replicates per treatment. All experiments were conducted in a glasshouse at the EcoSciences Precinct, Dutton Park, Queensland. Prior to presentation, all datasets were transformed using a general linear model, with test species and treatment as the main blocks, using the Minitab statistical package (Version 17, USA). Furthermore, two sample *t*-tests were undertaken on seedling emergence, growth, and biomass datasets to examine differences between treatments (soil from parthenium weed-infested and weed-free areas).

3. Results

3.1. Impact of Soil from a Parthenium Weed-Infested Area on Germination and Growth of Test Plant Seedlings (Experiment 1)

Seedling emergence: The final seedling emergence proportion of all test species (lettuce, maize, curly windmill grass, and liverseed grass) was significantly reduced in soil from the parthenium weed-infested site (*ca.* 65%) as compared to the soil from the parthenium weed-free site (98%; Figure 1). The soils from the 10 to 20 cm depth had the same inhibitory effect as did the soils from the 0 to 10 cm depth for the parthenium weed-infested site (Figure 1). In both soil profile depths, the emergences of liverseed grass and maize were inhibited the most (by 44%) and least (by 18%), respectively (Figure 1).

Seedling growth: Following a further 7 days of growth for the remaining two seedlings per pot, it was observed that three of the four test species were inhibited in their growth, as assessed by shoot length (Figure 2) and biomass accumulation (Figure 3) in the soil collected from the parthenium weed-infested field site, with maize the only species not affected. However, from 14 days onward, there was no significant difference in the seedling growth between the soil from parthenium-infested and non-infested sites. There was also no significant difference between the soil collected from 0 to 10 cm and 10 to 20 cm at either the parthenium weed-infested or the non-infested site for all species (Figures 1 and 2).

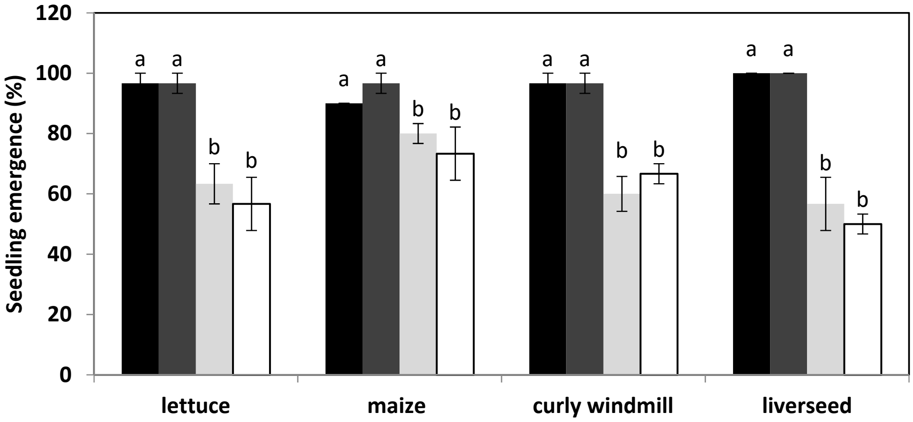

Figure 1. Seedling emergence percentage for lettuce, maize, curly windmill grass, and liverseed grass when seeds were sown into soil collected from a parthenium weed-infested site, either collected from the 0 to 10 cm (light grey) or 10 to 20 cm depth (white), and in soil collected from a non-infested soil collected from the 0 to 10 cm (dark grey) or 10 to 20 cm (black). Error bars represent two standard deviations from the mean, as calculated for 24 replicate pots of 30 sown seeds. Means within species that do not share the same letter are significantly different from one another at $p > 0.05$.

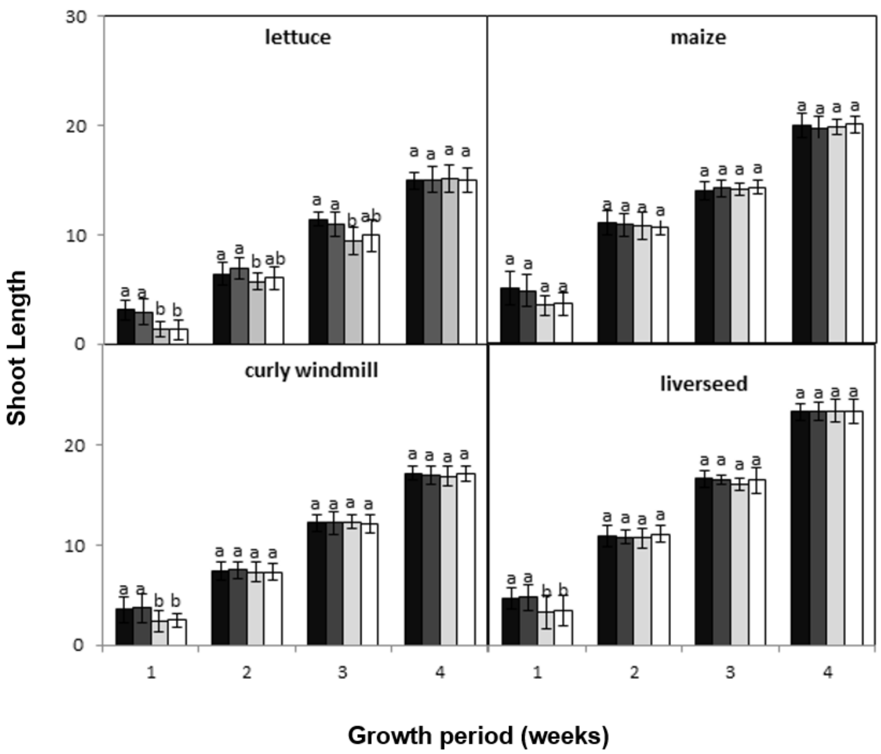

Figure 2. Shoot length attained by lettuce, maize, curly windmill grass, and liverseed grass seedlings when grown in a soil from a parthenium weed-infested site, either collected from the 0 to 10 cm (light grey) or 10 to 20 cm (white) depth; and from a non-infested soil collected from the 0 to 10 cm (dark grey) or 10 to 20 cm (black) depth. Error bars represent two standard deviations from the mean as calculated for three replicates of six seedlings in three pots. Means within species and growth periods that do not share the same letter are significantly different from one another at $p > 0.05$.

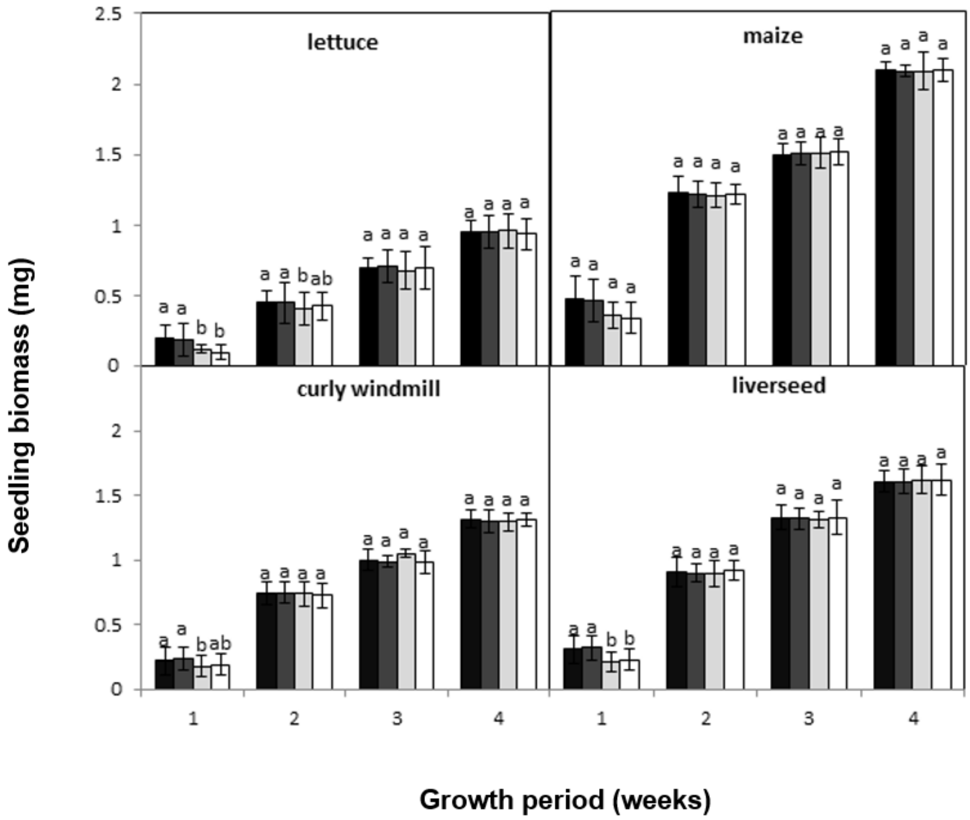

Figure 3. Seedling biomass production from lettuce, maize, curly windmill grass, and liverseed grass seedlings when grown in a soil from a parthenium weed-infested site, either collected from the 0 to 10 cm (light grey) or 10 to 20 cm depth (white) and from a non-infested soil collected from the 0 to 10 cm (dark grey) or 10 to 20 cm (black). Error bars represent two standard deviations from the mean as calculated for three replicates of six seedlings in three pots. Means within species and growth periods that do not share the same letter are significantly different from one another at $p > 0.05$.

3.2. The Impact of Parthenium Weed Leaf Litter on Germination and Growth of Test Plants (Experiment 2)

Seedling emergence: Seedling emergence from lettuce, maize, curly windmill grass, and liverseed grass were all inhibited when they were sown into the parthenium weed leaf litter-amended compost (Figure 4), with seedling emergence percentages decreasing with the amount of leaf litter incorporated. In the 5.0 g of leaf litter-amended compost, inhibition of seedling emergence was significantly greater (*ca*. 60%) than in the 1.0 g of leaf litter-amended compost (78%), but not greater than the 2.0 g of leaf litter-amended compost (72%), and there was no significant difference between the 1.0 and 2.0 g of leaf litter-amended composts. The greatest inhibition in seedling emergence was observed for lettuce and liverseed grass, while maize and curly windmill grass were least affected (Figure 4).

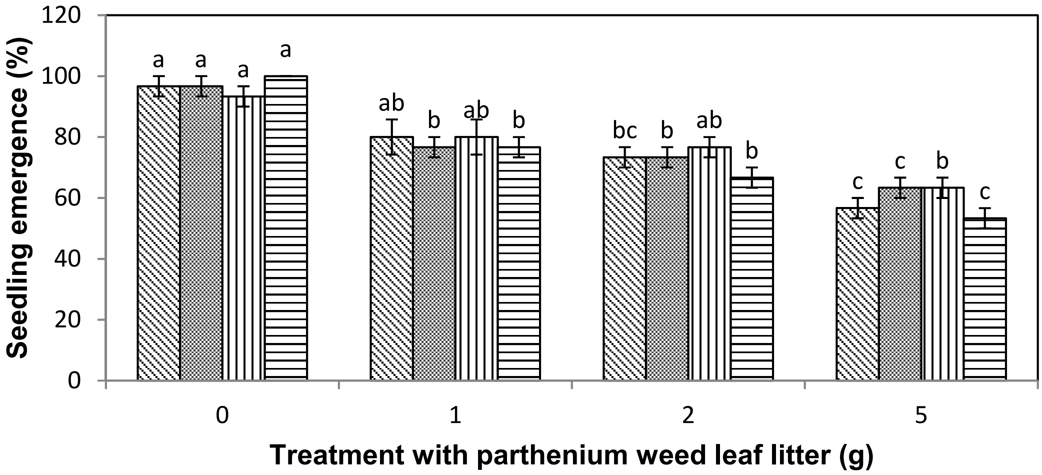

Figure 4. Seedling emergence of lettuce (▧), maize (▩), curly windmill grass (◫), and liverseed grass (⊟) when sown into compost amended with different amounts of parthenium weed leaf litter (either 0, 1, 2, or 5 g) for 30 days. Error bars represent two standard deviations from the mean as calculated for three replicates of three seedlings. Means within each treatment that do not share the same letter are significantly different from one species to another at $p > 0.05$.

Seedling growth: Shoot growth (Figure 5) and biomass production (Figure 6) for lettuce, maize, curly windmill grass, and liverseed grass were stimulated when they were grown in the leaf litter-amened compost, especially in the 5.0 g of leaf litter-amended compost. However, there was no significant difference between the 1.0 and 2.0 g of leaf litter-amended soils, except for liverseed grass. In addition, there was a significant difference in shoot growth between the compost without leaf litter and soil containing 1.0 g of leaf litter, expect for curly windmill grass.

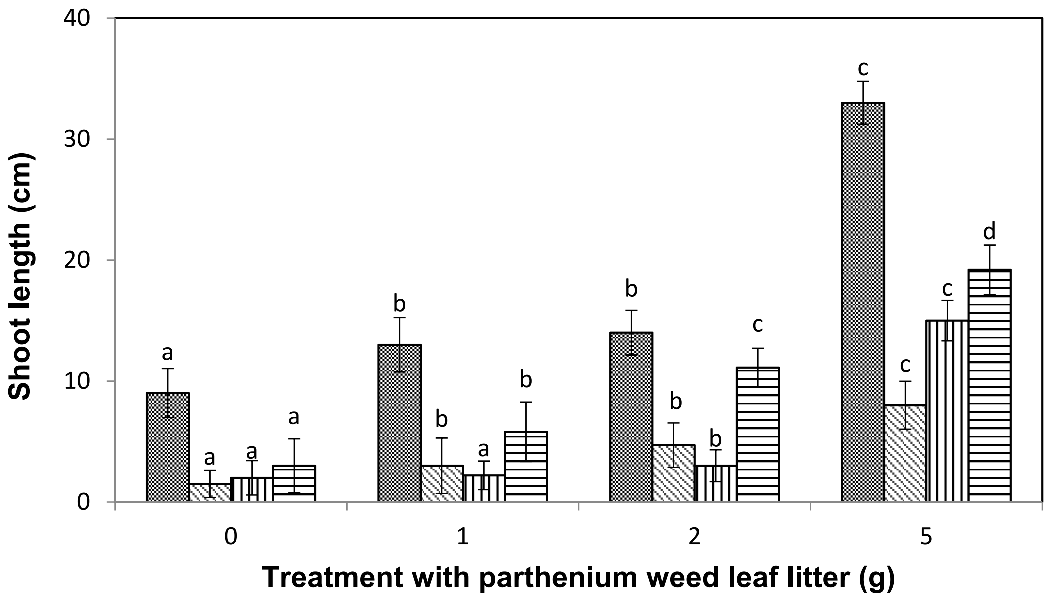

Figure 5. Shoot length of maize (▩), lettuce (▧), curly windmill grass (◫), and liverseed grass (⊟) when sown into compost amended with different amounts of parthenium weed leaf litter (0, 1, 2, and 5 g) for 30 days. Error bars represent two standard deviations from the mean as calculated for three replicates of three seedlings. Means within each treatment that do not share the same letter are significantly different from one species to another at $p > 0.05$.

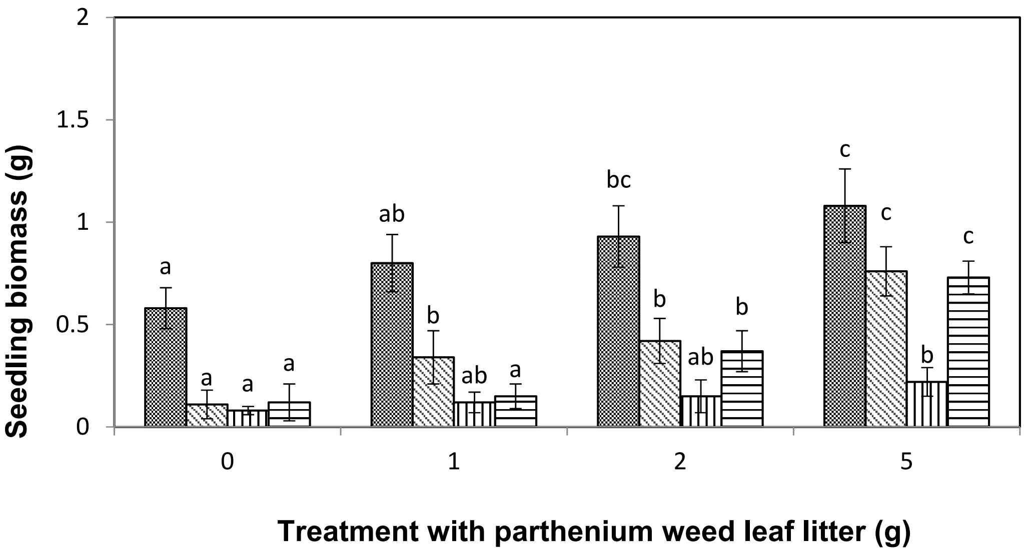

Figure 6. Seedling biomass of maize (▩), lettuce (▧), curly windmill grass (▥), and liverseed grass (▤) when they were sown into compost amended different amounts of parthenium weed leaf litter (0, 1, 2, and 5 g) for 30 days. Error bars represent two standard deviations from the mean as calculated for three replicates of three seedlings. Means within each treatment that do not share the same letter are significantly different from one species to another at $p > 0.05$.

Carbon and nitrogen concentrations: The C and N concentrations of the lettuce, curly windmill grass, and liverseed grass plants increased significantly when grown in the compost containing 5.0 g of parthenium weed leaf litter (Table 1). However, there were no significant differences in C and N concentration in any species when grown in the compost containing 1.0 or 2.0 g of parthenium weed leaf litter.

Table 1. Carbon and nitrogen concentrations of maize, lettuce, curly windmill grass, and liverseed grasses seedlings when they were grown with different amounts of parthenium weed leaf litter (0, 1, 2, and 5 g per pot) for 30 days. Means are shown plus or minus two standard deviations from the mean and calculated for nine replicates of five seedlings and from duplicate experiments using each biotype. Means within treatments that do not share the same letter are significantly different from one another at $p > 0.05$.

	Lettuce		Maize		Curly Windmill		Lambs Quarter	
Treatment (g)	C (%)	N (%)	C (%)	N (%)	C (%)	N (%)	C (%)	N (%)
0	23.80 ± 1.65 a	0.12 ± 0.01 a	27.10 ± 1.72 a	0.14 ± 0.01 a	24.10 ± 1.86 a	0.11 ± 0.03 a	25.20 ± 1.66 a	0.13 ± 0.01 a
1	27.20 ± 1.65 ab	0.15 ± 0.02 ab	25.70 ± 3.80 a	0.13 ± 0.02 a	26.80 ± 2.79 ab	0.13 ± 0.05 ab	27.20 ± 1.58 ab	0.14 ± 0.03 ab
2	29.40 ± 0.91 ab	0.16 ± 0.11 ab	29.8 ± 3.90 a	0.16 ± 0.02 a	29.70 ±1.03 ab	0.16 ± 0.01 ab	29.2 ± 0.86 ab	0.17 ± 0.03 ab
5	32.60 ± 3.11 b	0.22 ± 0.03 b	29.60 + 5.57 a	0.18 ± 0.03 a	31.40 ± 1.96 b	0.18 ± 0.06 b	30.80 + 2.72 b	0.21 ± 0.04 b

4. Discussion

Soil collected from a long-term, heavily infested parthenium weed area was shown to reduce seedling emergence of a wide range of test plants by between 20 and 40% (Figure 1); however, the soil had no further effect on the growth of the surviving test plants. In a second study, a compost amended with dried parthenium weed leaf litter reduced seedling emergence of the same test plant species by between 20 and 40% (Figure 4); however, the compost had no further effect on the growth of surviving test plants. Interestingly, one week after emergence, the growth of all test species was stimulated by 9% (lettuce) to 85% (maize) in the amended soil with the increased growth matching the increased amounts of leaf litter amendment (Figure 5). These results suggest that in parthenium weed-infested landscapes, the substrate can be affected by the weed both during the growing season by the release of allelopathic chemicals, and the dead plant litter can affect the community outside of the growing season, first through an allelopathic activity, but also by a fertilizing effect as litter breakdown occurs.

4.1. Impact of Soil from a Parthenium Weed-Infested Area on Germination and Growth of Test Plant Seedlings (Experiment 1)

Seedling emergence of the three species (lettuce, curly windmill grass, and liverseed grass) was reduced by ca. 40% when their seeds were sown into a soil collected from a parthenium weed-infested site and compared to the emergence achieved in an analogous soil from a non-infested site (Figure 1); however, seedling emergence of one test plant (maize) was only reduced by 20% (Figure 1). This suggests that the parthenium weed population (especially those that may be present in the seed bank) was affected and reduced seedling emergence by the parthenium weed community. A previous study reported a similar inhibitory effect of parthenium weed-infested soil on the germination of maize [22]. The present study showed that soil from the 0 to 10 cm and the 10 to 20 cm depth had equal inhibitory effects on seedling emergence, suggesting that the allelochemicals were evenly distributed in the top 20 cm profile of soil.

Following 1 week of growth, and as assessed by shoot length attainment (Figure 2) and biomass production (Figure 3), the growth of maize was unaffected by the soil taken from the parthenium weed-infested site, while growth of the three other species was reduced by 30 to 45%. However, in the subsequent weeks, the growth of all species in soil from the parthenium weed-infested and non-infested sites was not significantly different. The reduction in the inhibitory effect may be due to the degradation of the allelochemicals in the soil medium [16]. A previous study showed that one of the parthenium weed allelochemicals, parthenin, is rapidly degraded in soil after collection and is considered to have a low persistence in soil [24]. Furthermore, in another study, parthenin and coronopilin could not be detected in soil samples collected from the upper soil layer of a heavily infested parthenium weed site [25]. Stigmasterol, a well-known phytosterol, has been discovered recently from parthenium weed plants and may have impacts on the growth of test plants [26]. An additional possibility is that, unlike seedling germination, seedling growth is less sensitive to the effect of the allelochemicals. It is known that one mode of action of the organic acids produced by parthenium weed roots is the inhibition of cell division [27], which may be more critical in the process in seed germination than in seedling growth. Again, there were no effects of soil collection depth upon growth inhibition. A previous study [10] observed that aqueous leaf extracts from parthenium weed had a much greater effect on seed germination than on seedling growth.

It was interesting to note that the most resistant species to the effect of parthenium weed allelochemicals was maize, the largest-seeded species tested. In a previous study investigating the allelopathic capacity of the tree of heaven (*Ailanthus altissima* (Mill.) Swingle), it was reported that during germination, species with large seeds were more resistant to allelopathic substances than were small seeds [28], and Adkins and Sowerby [10] also reported the large-seeded sunflower (*Helianthus annuus* L.) to be more resistant to parthenium weed extracts during germination than several smaller seeded species were.

The outcome of the allelochemical inhibition of germination in all four test species in soil collected from a parthenium weed-infested site was to reduce the emerging plant population size by up to 40%, but little impact was observed on the further growth of the surviving plants. Thus, under parthenium weed infestations, some degree of inhibition on seedling emergence would be expected.

4.2. Impact of Parthenium Weed Leaf Litter on Test Plant Germination and Growth (Experiment 2)

Seedling emergence from all test plant species was reduced by 20 to 40% when in soil containing parthenium weed leaf litter (Figure 4). This suggests that the leaf litter produced by one to two parthenium weed plants can reduce seedling emergence from other plant species that may be present in the seed bank. A major source of these chemicals is undoubtedly the aerial parts where certain allelochemicals are contained within the multitude of trichrome hairs that cover the surface of the leaves and stems that can be released directly to the soil during rainfall [29–31]. Additionally, continuing inputs of compounds may also play an important role in the growth inhibition under the field conditions where parthenium weed is growing with either crops or pasture species. Maize was least affected, while liverseed grass was most affected (Figure 4). This result is consistent with the previous experiments where the maize germination was least affected in terms of its germination and growth in soil collected from a parthenium weed-infested site [32]. In line with maize but in contrast with the other test species, another study [20] showed that parthenium weed leaf litter could not significantly reduce the germination of wheat or pea seeds.

There is evidence that the inhibition of maize growth in soil collected from a parthenium weed-infested site is greater in soils with higher water availability for plant growth, indicating that parthenium weed produces more allelochemicals under better growth conditions [22]. Additionally, a mixture of parthenium weed with sicklepod and chaff-flower leaf litter incorporated into soil showed significant growth inhibition of wheat and pea [20]. Kobayashi [16] reported that allelochemicals may be altered by soil micro-organisms, which can modify the structure of allelochemicals.

Following 1 week of growth, based on shoot length attainment (Figure 5) and biomass production (Figure 6), the further development of all test species was stimulated by the leaf litter, with the increase in biomass correlating with an increase in the amount of parthenium weed leaf litter used as the amendment (Figure 5). The reduction in the inhibitory effects may be due to the degradation of the allelochemicals from the leaf litter in the soil medium, as previously argued [24]; however, there were higher levels of C and N found in the plants grown with the higher level (5 g) of parthenium weed leaf litter than in samples with the lower levels (0 to 2 g) of parthenium weed leaf litter (Table 1). Most soil chemistry features examined in invaded sites have not been found to be significantly different to those of noninvaded sites [33–35]. However, soil nitrogen (N) dynamics, including N-mineralization and microbial biomass N, have been shown to be significantly different in invaded sites, including changes in the vegetation present in grassland communities [36]. Growth stimulation may be attributable to the fact that parthenium weed residues have been reported to contain high levels of N, P, and K [37], which could provide a fertilization effect to the growth of the test plants once the allelochemicals have broken down. In a previous study, wheat and pea seedlings showed higher initial growths in parthenium weed leaf-amended soils as compared to those growing in unamended soils; however, from 60 days after sowing, plants showed higher growth rates in unamended soil [20]. The compost used in this present study was fertilizer-free, so the effect of extra NPK may have been easily seen in the leaf litter treatments.

5. Conclusions

Soil collected from a parthenium weed-invaded area was shown to reduce seedling emergence of a wide range of plant types, including both crop and pasture plants, introduced and native species, but it had no effect on their subsequent growth. Thus, parthenium weed infestations have the potential to reduce plant populations through a reduction in their germination rate. Compost amended with parthenium weed leaf litter was also shown to reduce seedling emergence of a wide range of plant types, but it had no effect on their subsequent growth. This inhibition of germination by leaf litter has the potential to reduce the population size of other plant species in a parthenium weed-infested site. This study demonstrates the significant ability of parthenium weed to suppress the seedling density of crops and pasture species due to its allelopathic capacity.

Author Contributions: Conceptualization, B.S., K.D. and S.A., data curation, B.S.; methodology, B.S., K.D. and S.A., formal analysis, B.S., resources, K.D. and S.A., supervision, S.A., writing—original draft, B.S.; validation, B.S.; writing—review and editing, K.D. and S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The study was funded by the School of Agriculture and Food Sciences, The University of Queensland, and Biosecurity Queensland, Department of Agriculture and Fisheries. The authors would like to thank Laura Wendling for providing funding for soil sample analysis.

Conflicts of Interest: The authors assert that there are no conflict of interest.

Appendix A

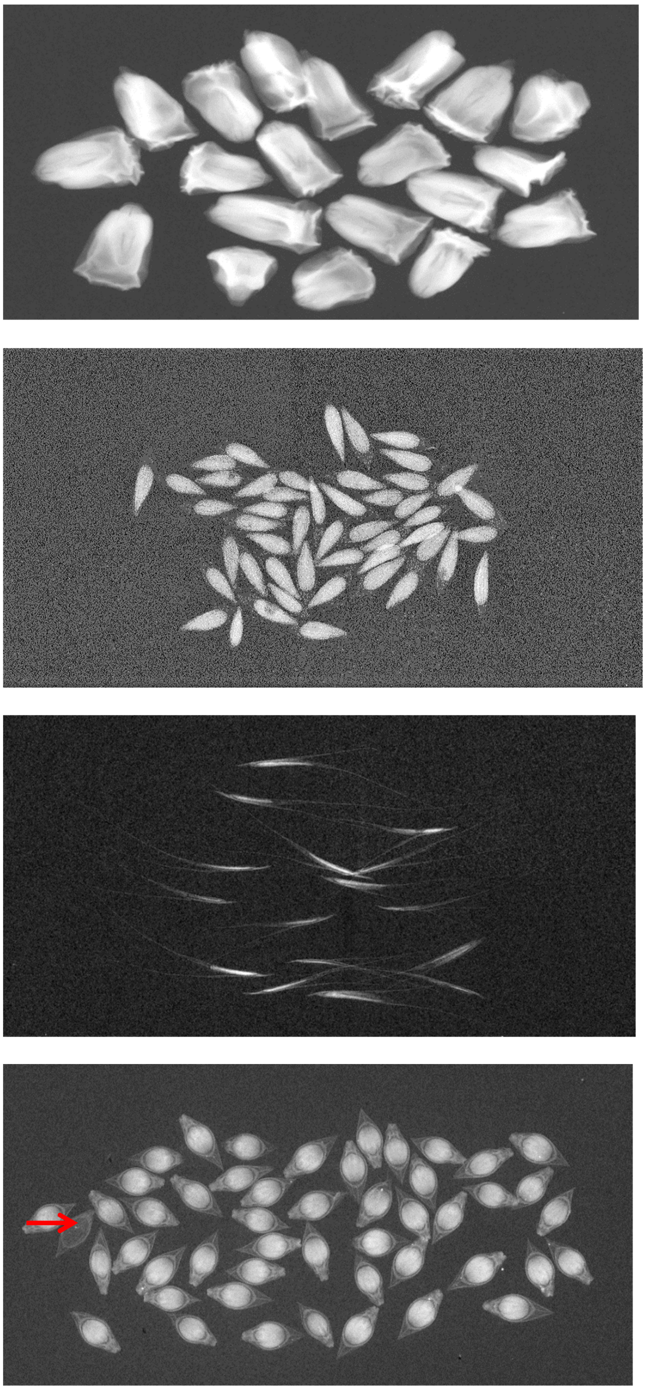

Figure A1. X-ray images of maize (*Zea mays* L.), lettuce (*Lactuca sativa* L), curly windmill grass (*Enteropogon acicularis* L.), and liverseed grass (*Urochloa panicoides* P.Beauv) seed lots used in the bioassay experiments. The images show >95% seed fill for the four seed lots. The red arrow points to a nonviable seed.

References

1. Navie, S.; McFadyen, R.; Panetta, F.; Adkins, S.; Shepherd, R. A comparison of the growth and phenology of two introduced biotypes of *Parthenium hysterophorus*. In Proceedings of the 11th Australian Weeds Conference, Victoria, Australia, 30 September–3 October 1996; pp. 313–316.
2. Shi, B.; Aslam, Z.; Adkins, S. The Invasive Potential of Parthenium weed: A Role for Allelopathy. In *New Developments in Allelopathy Research*; Price, J.E., Ed.; Nova Science Publishers: New York, NY, USA, 2015.
3. Adkins, S.; Shabbir, A. Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). *Pest Manag. Sci.* **2014**, *70*, 1023–1029. [CrossRef]
4. Chippendale, J.; Panetta, F. The cost of parthenium weed to the Queensland cattle industry. *Plant Prot. Q.* **1994**, *9*, 73–76.
5. Navie, S.C.; McFadyen, R.E.; Panetta, F.D.; Adkins, S.W. The effect of CO_2 enrichment on the growth of a C3 weed (*Parthenium hysterophorus* L.) and its competitive interaction with a C4 grass (*Cenchrus ciliaris* L.). *Plant Prot. Q.* **2005**, *20*, 61–66. [CrossRef]
6. Belgeri, A.; Navie, S.C.; Vivian-Smith, G.; Adkins, S.W. Early recovery signs of an Australian grassland following the management of *Parthenium hysterophorus* L. *Flora-Morphol. Distrib. Funct. Ecol. Plants* **2014**, *209*, 587–596. [CrossRef]
7. Bajwa, A.A.; Chauhan, B.S.; Farooq, M.; Shabbir, A.; Adkins, S.W. What do we really know about alien plant invasion? A review of the invasion mechanism of one of the world's worst weeds. *Planta* **2016**, 1–19. [CrossRef] [PubMed]
8. Rice, E.L. *Allelopathy*, 2nd ed; Academic Press: Orlando, FL, USA; London, UK, 1984.
9. Kanchan, S.D.; Jayachandra. Allelopathic effects of *Parthenium hysterophorus* L. I. exudation of inhibitors through roots. *Plant Soil* **1979**, *53*, 27–35. [CrossRef]
10. Adkins, S.; Sowerby, M. Allelopathic potential of the weed, *Parthenium hysterophorus* L., in Australia. *Plant Prot. Q.* **1996**, *11*, 20–23.
11. Kanchan, S.D.; Jayachandra. Allelopathic effects of *Parthenium hysterophorus* L.: Part IV. identification of inhibitors. *Plant Soil* **1980**, *55*, 67–75. [CrossRef]
12. Pandey, D.K.; Palni, L.M.S.; Joshi, S.C. Growth, reproduction, and photosynthesis of ragweed parthenium (*Parthenium hysterophorus*). *Weed Sci.* **2003**, *51*, 191–201. [CrossRef]
13. Kohli, R.K.; Batish, D.R.; Singh, H.P.; Dogra, K.S. Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. *Biol. Invasions* **2006**, *8*, 1501–1510. [CrossRef]
14. Joshi, B.; Pandey, N.; Rao, P. Allelopathic effect of weed species extracts on germination, growth and biochemical aspects in different varieties of wheat (*Triticum aestivum* L.). *Indian J. Agric. Res.* **2009**, *43*, 79–87.
15. Pandey, D.K. Inhibition of salvinia (*Salvinia molesta* Mitchell) by parthenium (*Parthenium hysterophorus* L.). II. Relative effect of flower, leaf, stem, and root residue on salvinia and paddy. *J. Chem. Ecol.* **1994**, *20*, 3123–3131. [CrossRef]
16. Kobayashi, K. Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biol. Manag.* **2004**, *4*, 1–7. [CrossRef]
17. Channappagoudar, B.; Jalageri, B.; Biradar, N. Allelopathic effect of aqueous extracts of weed species on germination and seedling growth of some crops. *Karnataka J. Agric. Sci.* **2005**, *18*, 916–920.
18. Khan, N.; Hashmatullah; Naveed, K.; Hussain, Z.; Khan, S. Assessment of allelopathic effects of parthenium (*Parthenium hysterophorus* L.) plant parts on seed germination and seedling growth of wheat (*Triticum aestivum* L.) cultivars. *Pak. J. Weed Sci. Res.* **2012**, *18*, 39–50.
19. Wakjira, M.; Berecha, G.; Tulu, S. Allelopathic effects of an invasive alien weed *Parthenium hysterophorus* L. compost on lettuce germination and growth. *Afr. J. Agric. Res.* **2009**, *4*, 1325–1330.
20. Gupta, S.; Narayan, R. Effects of applied leaf biomass of *Parthenium hysterophorus*, *Cassia obtusifolia* and *Achyranthes aspera* on seed germination and seedling growth of wheat and pea. *Allelopath. J.* **2010**, *26*, 59–70.
21. Singh, H.P.; Batish, D.R.; Pandher, J.K.; Kohli, R.K. Phytotoxic effects of *Parthenium hysterophorus* residues on three Brassica species. *Weed Biol. Manag.* **2005**, *5*, 105–109. [CrossRef]
22. Safdar, M.E.; Tanveer, A.; Khaliq, A.; Naeem, M.S. Allelopathic action of parthenium and its rhizospheric soil on maize as influenced by growing conditions. *Planta Daninha* **2014**, *32*, 243–253. [CrossRef]
23. Timsina, B.; Shrestha, B.B.; Rokaya, M.B.; Münzbergová, Z. Impact of *Parthenium hysterophorus* L. invasion on plant species composition and soil properties of grassland communities in Nepal. *Flora* **2011**, *206*, 233–240. [CrossRef]
24. Belz, R.G.; van der Laan, M.; Reinhardt, C.F.; Hurle, K. SoiL degradation of parthenin-does it contradict the role of allelopathy in the invasive weed *Parthenium hysterophorus* L.? *J. Chem. Ecol.* **2009**, *35*, 1137–1150. [CrossRef] [PubMed]
25. Shi, B. *Invasive Potential of the Weed Parthenium Hysterophorus: The Role of Allelopathy*; The University of Queensland: Brisbane, Australia, 2016.
26. Ramamurthy, S.K.; Sridhar, C. Isolation of Stigmasterol from *Parthenium hysterophorous* plant. *Pharma Innov. J.* **2018**, *7*, 149–151.
27. Mersie, W.; Singh, M. Effects of phenolic acids and ragweed parthenium (*Parthenium hysterophorus*) extracts on tomato (*Lycopersicon esculentum*) growth and nutrient and chlorophyll content. *Weed Sci.* **1988**, *36*, 278–281. [CrossRef]
28. Heisey, R.M. Allelopathic and herbicidal effects of extracts from tree of heaven (*Ailanthus altissima*). *Am. J. Bot.* **1990**, *77*, 662–670. [CrossRef]
29. Yaduraju, N.; Sushilkumar, P.B.M.; Gogoi, A. *Parthenium hysterophorus*–distribution, problem and management strategies in India. In Proceedings of the Second International Conference on Parthenium Management, University of Agricultural Sciences, Bangalore, India, 5–7 December 2005; pp. 6–10.

30. Bashar, H.; Juraimi, A.S.; Ahmad-Hamdani, M.S.; Uddin, M.K.; Asib, N.; Anwar, M.; Rahaman, F. A Mystic Weed, Parthenium hysterophorus: Threats, Potentials and Management. *Agronomy* **2021**, *11*, 1514. [CrossRef]
31. Bajwa, A.A.; Weston, P.A.; Gurusinghe, S.; Latif, S.; Adkins, S.W.; Weston, L.A. Toxic potential and metabolic profiling of two australian biotypes of the invasive plant parthenium weed (*Parthenium hysterophorus* L.). *Toxins* **2020**, *12*, 447. [CrossRef] [PubMed]
32. Mersie, W.; Singh, M. Allelopathic effect of parthenium (*Parthenium hysterophorus* L.) extract and residue on some agronomic crops and weeds. *J. Chem. Ecol.* **1987**, *13*, 1739–1747. [CrossRef]
33. Osunkoya, O.O.; Akinsanmi, O.A.; Lim, L.S.; Perrett, C.; Callander, J.; Dhileepan, K. *Parthenium hysterophorus* L. (Asteraceae) invasion had limited impact on major soil nutrients and enzyme activity: Is the null effect real or reflects data insensitivity? *Plant Soil* **2017**, *420*, 177–194. [CrossRef]
34. Ojija, F.; Manyanza, N.M. Distribution and Impact of Invasive Parthenium hysterophorus on Soil Around Arusha National Park, Tanzania. *Ecol. Evol. Biol.* **2021**, *6*, 21–27.
35. Etana, A.; Kelbessa, E.; Soromessa, T. Impact of *Parthenium hysterophorus* L. (Asteraceae) on soil chemical properties and its distribution in a reserve area: A case study in Awash National Park (ANP), Ethiopia. *J. Soil Sci. Environ. Manag.* **2015**, *6*, 116–124.
36. Srivastava, P.; Raghubanshi, A. Impact of Parthenium hysterophorus L. invasion on soil nitrogen dynamics of grassland vegetation of Indo-Gangetic plains, India. *Environ. Monit. Assess.* **2021**, *193*, 1–16. [CrossRef] [PubMed]
37. Biradar, D.; Shivakumar, K.; Prakash, S.; Pujar, B. Bionutrient potentiality of *Parthenium hysterophorus* and its utility as green manure in rice ecosystem. *Karnataka J. Agric. Sci.* **2006**, *19*, 256–263.