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Bioherbicidal interference of plant extracts and residue on seed germination and seedling growth of an invasive alien weed (*Parthenium hysterophorus* L) in Ethiopian agriculture

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Abstract
The study investigated bioherbicidal inhibition potential of two plant species extract and residue **(***Azadirachata indica* leaves and oil extracts, *Brassica carinataa* seed extract and residue on *P. hysterophorus* growth at *in vitro* and field pot experiment. The extracts of 6, 8 and 10% (w/v) obtained from shade dry leaves and seeds. The seeds oil was donated by Leather and Leather product Institute of Maazama oils in Ethiopia. Germination, shoot and root growth of Parthenium treated by plants extract were significantly (p<0.0001) lower than seeds receiving distilled water. Highest germination suppression was found from all concentrations of *A. indica* seed oil followed by 10% aqueous extracts of *B. carinata* seed and *A. indica* leaves, where germination was reduced by (89.3, 67% and 51%), respectively compared to control. Moreover, the field pot experiment results had also shown a significant inhibition on all growth parameters of Parthenium. Among the suppressed growth the height was suppressed by (78, 68.5 cm) seed oil and 10% *A. indica* leaves, respectively. Similarly, the biomass reduced by (49.2, 43.5gm per plant). Therefore, it can be concluded from the present study that *A. indica* and *B. carinata* are promising plant species as a natural herbicide for the management of noxious weed *Parthenium*.

Keywords: *Azadirachata indica*, *Brassica carinataa*, field, growth inhibition, *in vitro*, *Parthenium hysterophorus*

Introduction
Parthenium (*Parthenium hysterophorus* L.) is an herbaceous invasive weed believed to have originated in tropical America and now occurs widely in India, Australia, East and South Africa. It is a procumbent, diffused leafy herb, 0.5 -2 m tall, bearing alternate, pinnatified leaves, belonging to the family Compositae. Parthenium is believed to have been introduced to Ethiopia in 1976/77 with army vehicles from Somalia and has become a serious weed in both arable and grazing lands (Tamado, 2001 [18]; Taye, 2002) [25]. Since its introduction in 1976 into Ethiopia parthenium weed has been reported as relentlessly spreading throughout the agricultural lands, forests, orchards, poorly managed arable crop lands and rangelands, almost throughout the country (Tefera, 2002) [21].

The successful spread of parthenium in so many parts of the world including Ethiopia has mainly been attributed to its allelopathic properties, which enables it to compete effectively with crops and pasture species (Batish *et al*., 2005b) [17]. It was also reported to cause severe crop losses. Sorghum grain yield losses between 40 and 97% were reported in Ethiopia if parthenium is left uncontrolled throughout the season (Tamado, 2002) [19]. As a result of its efficient biological activity and adaptability to varying soils and micro environments, *P. hysterophorus* tends to replace the dominant flora in a wide range of habitats cutting across state boundaries and agro-climatic regions. Wherever it invades, it forms a territory of its own by replacing the indigenous natural flora, including medicinal herbs utilized by people as a source of medicine (Oudhia, 2000) [13].

Usual weed control methods; manual or mechanical kill only the top growth with little effect on tubers. In addition, use of synthetic herbicides although has undoubtedly enhanced the much-needed crop production, yet have led to a number of toxicological and environmental problems causing ill effects on human health (Macias *et al*., 2003) [23]. Therefore, efforts are being made worldwide to search for safer and environmentally benign chemicals that have relatively shorter half-life.

In this direction, biologically active natural products such as allelochemicals are fast, being tested for weed management since they are environment friendly and have different modes of action, besides they also exhibit immense diversity and biological activity (Dayan, 2003) [24].

Allelochemicals emancipated as residues, exudates and leaches by many plants from leaves, stem, roots, fruit and seeds reported to interfere with growth of other plants (Asgharipour *et al*., 2010) [2]. Studies on the allelopathic grasses of *Imperata cylindrica* (L.) Beauv and *Desmostachya bipinnata* (L.) Stapf demonstrated to have the potential to reduce the distribution of *P. hysterophorus;* and aqueous extracts of these grasses had a strong inhibitory effect to germination and growth of the target weed (Javaid *et al*., 2005; Anjum *et al*., 2005). Another research revealed that aqueous leaf extracts of allelopathic trees *viz*., *A. indica* (L.) A. Juss., *Ficus bengalensis* L., *Melia azadarach* L., *Mangifera indica* L. and *Syzygium cumini* (L.) Skeels, have the potential to decline germination and seedling growth of *Parthenium* (Shafique *et al*., 2005) [15].

The essential oils can also be used as viable weed control technology under organic farming systems (Tworkoski, 2002) [22]. A study on the herbicidal effect of volatile oils from leaves of *Eucalyptus citriodora* against the noxious weed *P. hysterophorus* showed that a concentration of 5.0 nl ml^{-1} *Eucalyptus* oil completely inhibited the germination (Singh *et al*., 2005) [17] computer software, at probability. Later on, a study conducted on the inhibitory effects of the husk extracts of 7 rice varieties on growth of barnyard grass (*Ehinochola curssgali* L. Beauv.) was meaningful (Jonghan *et al*., 2005) [5]. Further adverse effects of water extract of different *Brassica* species against germination and growth of cut leaf ground cherry weed (*Physlis anagulata* L.) have also been reported (Marwat *et al*., 2008) [12].

Even if today, allelopathy is recognized as appropriate natural herbicide technology to control weeds using chemicals released from various plant part's, in Ethiopia no efforts has been made to explore the allelopatic potential of some useful plants such as *A. indica* and *Brassica carinataa* on the growth of *P.hysterophorus*. Therefore, the present study was conducted to investigate the suppressive potential of two plant species extract and residue i.e. **(***Azadirachata indica* leaves and oil extracts, *Brassica carinataa* seed extract and residue on seed germination and seedling growth of *P. hysterophorus* under *in vitro* and field pot experiment. And to determine the feasibility of using plant extracts as an effective and low-cost technology for small holder framers to manage the *Parthenium* weeds.

Materials and Methods

Site description

The *in vitro* test was conducted in Plant Pathology Laboratory, Mekelle University. Subsequently, the field pot experiment was conducted at Kemisse district in North Wollo of Amhara Region, Ethiopia to assess the response of *P. hysterophorus* seedlings to treatments of *A. indica* leaves, seed oil and *B. carinata* residue used as growth medium. The experimental site lies at 10^{0}43'32" N latitude and 39^{0}52'52"E longitude. Altitude of the area ranges from 1424 -3200 meter above sea level with mean annual rainfall 600-1100 mm and mean annual minimum and maximum temperatures are 27 °C and 36 °C (Bureau of Agriculture, 2000) [6].

Extract preparation

Leaves of *A. indica* were collected from mid part of the tree from plantation forest field at Kemisse. Then prior to extraction the leaves were washed with water and kept to dry well in light shade to its appropriate moisture level at the laboratory of Leather and Leather product Institute of Maazama oils in Addis Ababa, Ethiopia. The dried leaves were chopped into pieces using grinder. 10g of chopped leaves were weighted using aluminum tray and transferred to juice maker. Subsequently soaked in 100 ml distilled water and run for 10 minutes for extraction using blender mode to obtain 10% w/v aqueous extract. Afterward the extract was transferred into flask and placed on a shaker for 10 hours. The extract was then left for 1 hr to settle before filtration and filtered through muslin cloth followed by Whatman No.1filter paper. The extract was considered as stock solution and a series of solution with different strengths (6 and 8%) were prepared by dilution to obtain a10% w/v aqueous extract, 10 g crushed dry leaves of *A. indica* was soaked in 100 ml) (Shafique *et al*; 2005) [15].

A. indica oils were donated by Leather and Leather product Institute of Maazama oils since this institute prepares the *A. indica* oils for other research purposes in the country. Seeds of *B. carinata* was extracted followed the same procedure as mentioned above for *in vitro* test; while for field experiment seeds of *B. carinata* were sown on pots one season before the main experiment. At maturity the whole part of the plants were uprooted and chopped into small pieces. Then 2 kg of the *B. carinata* chopped residues were incorporated with other soil types for *parthenium* planting media and treated as *Brassica* treatments.

In vitro bioassays

The top of paper method was used for germination and early seedling growth of *Parthenium*. 30 Petri-dishes (9 x 18cm size) were washed with 70% ethanol and cleaned three times with distilled water and incubated until dries. The sterilized Petri-dishes were arranged in completely randomized designed lined with filter papers moistened with 1.2 ml of the respective concentration (6, 8 and 10%) of each extract in three replication patterns, while in case of control only 1.2 ml of distilled water was applied (personal communication with Ethiopian researcher; Shafique *et al*; 2005) [15]. Simultaneously on the top of moistened paper thirty seeds of *parthenium* were placed in each Petri-dish and maintained under laboratory conditions at 23ºc temperature. The plates were regularly checked to kept seeds moist and added the extracts of respective concentration every two days interval to facilitate germination and seedling establishment. After two weeks the numbers of germinated seeds were counted; and by taking 10 randomly selected plants from each Petri-dish shoot and root length were measured.

Field pot experiment

Twenty-four plastic pots (27x25 cm size) were prepared for growth medium purpose. Twenty-one pots were filled with 2 kg of sandy loam, 3 kg of decomposed forest soil and 3 kg of black top soil and the remain three pots were filled with 2 kg of the *B. carinata* chopped residues, 2 kg of sandy loam, 2 kg of decomposed forest soil and 2 kg of black top soil since considered as residual treatment. The whole pots were arranged in a completely randomized designed under natural conditions. The pots were arranged in each replication with

50 cm spacing; the pot soils were watered at 5 days interval for one month before planting, to create good mixed soil composition and moisture for the seedlings. Simultaneously the *parthenium* seeds were seeded on 0.125 m^2 large plastic bucket seed-bed for transplanting purpose. The seed beds were covered day and night for seven days with light steel sheet coil, then after one week the shade was applied only during the night time until one month to protect small animals which distract the seedlings, then the seedlings were completely exposed to full sunlight and allowed to stabilized the environmental conditions for three weeks. After the appearance of three to four true leaves, three *parthenium* seedlings per pot were transplanted to minimize the risk of germination failure.

After proper establishment of the seedlings thinning was made to maintain one seedling per pot. The seedlings were watered every two days interval until the stage of maturity. When the Parthenium plants grow well and produced full size leaves, the plants were sprayed at a rate of 80 ml of the extracts per plot on the first-round spray. Similarly, the second-round spray was carried out three weeks later. Data for plant height, spikelet, biomass and seed yield were taken.

Statistical analysis

The collected row data's normality was checked, and then transformation performed. After data transformation for all growth parameters of *in vitro* and field pot data the analysis of variance, coefficient of variation and comparisons of treatments means were done by using SAS version 9 computer software, at probability significance level of α=0.05 (SAS Institute, 2012) [24].

Results and Discussion

***In vitro* test**

Statistical analysis of the data revealed that all the tested concentrations of *A. indica* leaves, seed oil and *B. carinata* seed extract had significant (p<0.0001) inhibition effect on germination, shoot and root growth of *P. hysterophorus* (Table 1). The most effective treatment in suppressing germination of *P. hysterophorus* were all the three different concentrations of *A. indica* seed oil followed by 10% aqueous extracts of *B. carinata* seed *and A. indica* leaves, where germination was reduced by (89.3, 67% and 51%), respectively compared to control. Recent study on inhibition potential of *A. indica* had shown that at higher concentration the germination of *P. hysterophorus* highly suppressed (Knox *et al*., 2010) [10]. The effect may probably be due to disrupting potential of allelochemicals on the plant's ability to germination. In addition, the oil vapors increased water loss leading to wilting and dead of seeds.

Table 1: Effect of plant extracts and seed oils on germination, shoots and root growth

Bioherbicides	Concentration	Germination (%)	Shoot length (cm)	Root length (cm)
Control (water treated)	0%	9.0a	1.55a	0.46a
Azadirachata indica leaves	6%	5.1b	0.0b	0.0b
	8%	4.2cd	0.0b	0.0b
	10%	3.9de	0.0b	0.0b
Azadirachata indica oil	6%	0.7f	0.0b	0.0b
	8%	0.7f	0.0b	0.0b
	10%	0.7f	0.0b	0.0b
Brassica carinataa seed	6%	4.7bc	0.0b	0.0b
	8%	4.0cde	0.0b	0.0b
	10%	3.3e	0.0b	0.0b
LSD		0.74	0.08	0.02
CV		11.8	29.4	22.2
R^2		0.98	0.99	0.99
P -value		***	***	***

Means within columns not sharing the same letter show significance difference and *** indicates significant difference at p < 0.0001.

Moreover, the lowest (0, 0cm) shoot and root lengths, respectively were recorded from seeds treated with leaves and seed oil extracts compare to seeds receiving distilled water (1.55, 0.46 cm) growth were showed, respectively. At all concentrations of *A. indica* seed oil the germination, shoot and root lengths of *P. hysterophorus* were completely retarded. Study conducted on the herbicidal effect of volatile oils from leaves of *Eucalyptus citriodora* against the noxious weed *P. hysterophorus* and found that a concentration of 5.0 nl ml^{-1} *Eucalyptus* oil completely inhibited the germination (Singh *et al*., 2005) [17]. The reductions in shoot and root length may be attributed the reduced rate of cell division and cell elongation due to the presence of allelochemicals on leaves and oil extracts.

Generally, the *in vitro* findings revealed that all tested bioherbicide inhibit germination, shoot and root growth, the effect was most pronounced by *A. indica* oil at all concentration followed by extracts of higher concentration *B. carinata* seed and *A. indica* leaves compared to distilled water treated seeds.

Field pot experiment

Analysis of the field pot experiment data revealed that *A. indica* leaves, seed oil and *B. carinata* residue had significantly (p<0.0001) reduced the height, biomass, seed yield, number of spikelets, harvesting index and number of branches per plant (Table2).

The height growth remarkably reduced by (78, 68.5 and 57.7 cm) due to spray of seed oil *A. indica* 10% leave extract and *B. carinata* residue respectively compares to control (78cm height). A similar inhibition treatment effects was observed in biomass of *parthenium* where decline by (49.2, 43.5 and 36.2gm per plant) respectively compare to control 49.2gm per plant (Table2). Such difference may be related to specific allelopathic compounds being produced in *A. indica*. These results support the earlier findings that aqueous extracts of *A. indica* leaves of low concentrations were least toxic exhibiting not strong impact on biomass. However, seedling biomass of *P. hysterophorus* was significantly reduced at 10% extract of *A. indica* leaves extract (Oudhia, 2000) [13].

Table 2: Effect of plant extracts, seed oil and residue on growth parameters of *parthenium*

Bioherbicides	Concentration	Height (cm)	Biomass (gm)	Seed yield (gm)	Spikelet (No)	Harvest index	Branch (No)
Control (water treated)	0%	78.0a	49.2a	2.5a	983.7a	5.0a	1.0a
Azadirachata indica leaves	6%	45.2b	22.7b	0.9b	368.7b	4.1a	0.7b
	8%	38.2b	24.0b	0.7b	265.7b	2.7b	0.8b
	10%	9.5cd	5.7d	0.1c	55.5c	0.6c	0.2c
Azadirachata indica oil	6%	0.0d	0.0d	0.0c	0.0c	0.0c	0.0c
	8%	0.0d	0.0d	0.0c	0.0c	0.0c	0.0c
	10%	0.0d	0.0d	0.0c	0.0c	0.0c	0.0c
Brassica carinataa residue	2kg	20.3c	13.0c	0.3c	99.5c	2.1b	0.5b
LSD		12.5	7.0	0.27	110	1.03	0.27
CV		29.7	28.2	28	28.4	32.3	36.7
R^2		0.96	0.97	0.97	0.97	0.94	0.92
P-value		***	***	***	***	***	***

Means within columns not sharing the same letter show significance difference and *** indicates significant difference at $p< 0.0001$.

The overall seed yield means treated by leaves extract, seed oil and residue were significantly lower than the water treated, the highest suppression were *A. indica* seed oil followed by 10% leaves extract concentration and residue by (2.5, 2.4 and 2.3 gm per plant) respectively from the control 2.5 gm (Table2). The concomitant decrease in seed yield of the plant may be explained by the fact that as less shoot and root fresh yield was produced by the plant, less assimilates were partitioned to the shoot portion and resulted in less photosynthesis, thereby decrease seed yield. This may also be associated to the lower number of branches per plant, number of capsules per plant, and number of seeds weight per capsule, which thus contribute to reduced seed yield.

The other obstructed growth parameters were number of spikelet's per plant and harvesting index. Similarly, like other growth parameters complete inhibition were observed in all levels of oil. Moreover *A. indica* 10% leave extract and *Brassica* residue reduced spikelet and harvesting index by (928.2, 884.2 and 4.4, 2.9) compared to control (5.0, 983), respectively (Table 2). This could be due to the development of short stature, lower number of leaves as well as lower number of branches resulted from spray of extracts might have an effect on flower development and finally on the number of spikelet's per plant. In addition, assimilation and utilization of growth factors after extract application might have disfavor the movement of nutrients in the plant and stressed growth of productive tillers these results decrease in the harvesting index.

The effects of bioherbicide after sprayed were observed deliberately until all the plants reached at harvested to see either it recovers after some months or completely died. Finally, the plants treated by *A. indica* oil did not regenerate after death but some other *parthenium* plant treated by *A. indica* leave extract at different concentration and *B. carinata* residue exist until harvest however, they were not seed productive and not show good growth performance (Table 2). This might be resulted due to more and vigorous allelopathic potential of *A. indica* plant extract and *B. carinata* residue that disfavor regeneration of *parthenium* weed. That is the importance of this study of bioherbicide for their selection against *parthenium* for future.

The field pot study results revealed a similar inhibition effect to *in vitro* experiment. Both the *in vitro* and pot result realizes the poor *parthenium* growth even complete growth hindrance to majority of seedlings. So allelochemicals release from living, dead plant materials and accumulates in the soil beneath of this tree and residue had adversely affected the germination and growth of *parthenium*. The present study also revealed that the possibility of using the allelochemicals directly or as a structural lead for the discovery and development of environmentally friendly bioherbicide to control one of noxious weeds of *P. hysterophorus* in Ethiopia.

Conclusion

The study demonstrates that the overall growth parameter means of *parthenium* plant treated by *A. indica* leaves, seed oil, *B. carinata* extract and residue were significantly lower than the control. The effect was most pronounced by *A. indica* oil at all concentration level resulted 100% seed dormancy (0.0, 0.0 cm of shoot and root growth) were recorded in *in vitro* test and 100% mortality at actively growth stage of the *parthenium* after the first spray in the field pot experiment, followed by 10% concentration level of *A. indica* leaves extract and *B. carinata* residue were highly suppressed most growth parameters compared to control. In addition, the plant extracts indicated the highest suppressions on different growth parameters and seed production of *parthenium* plant. Therefore, it can be concluded from the present study that *A. indica* and *B. carinata* residue are the promising plant species possessing weed suppressing ability and is worth exploiting as a natural herbicide for the management of noxious weed *P. hysterophorus*.

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Conflict of Interest

Not available

Financial Support

Not available

Reference

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