Title: Potential Suppression Effects of Aqueous Extracts of Selected Plants on Growth of Carrot Weed (*Parthenium hysterophorus* L.) in Arusha, Tanzania

Abstract

Parthenium hysterophorus (Asteraceae:Heliantheae) (parthenium weed), is among of the worst aggressive terrestrial weeds, which has negative impacts on crop and animal production, biodiversity conservation, human and animal health worldwide. Management of this weed become a very difficult because of its mode of spreading since many methods such as chemical .physical, biological and integrated methods has being used without positive impacts. Thus, this study aimed at using suppressive plant which is has allelopathic effects to the weed and environmentally friendly.

Therefore, suppression effect of extracts from four plants namely thorny amaranth (*Amaranthus spinous* L.), neem (*Azadiratchta indica* L.) wheat millet (*Sorghum bicolor*) and marigold flower (*Tagetes erictus* L.) were studied against carrot weed (*Parthenium hysterophorus* L.) in the laboratory and pot experiments which were conducted in Arusha, Tanzania from May to November 2018. Leaf extracts at 25, 50 and 100% concentrations were sprayed on four and eight week ' s seedlings of *P. hysterophorus.* Distilled water was used as control. Root length, shoot length, fresh and dry biomass for seedlings as well as seed germination rate of *P. hysterophorus* were recorded. The highest germination percentages were 95.5% and 93.33% in control treatment for T*. erictus* flower extracts and the leaf extracts respectively. Results showed that, all plant extracts tested indicated high inhibition on growth and germination rate of *P. hysterophorous* with increasing concentrations, from 25 to 100%. However, 100% concentration

was more effective in all plant extracts used. Four weeks seedlings were more susceptible to
foliar sprays compared with eight weeks seedlings. This study concludes that aqueous leaf and
flower extracts of S*. bicolor*, *A. spinous*, *T. erictus* and *A. indica* have the potential to be used as
a source of bio-herbicide against *P. hysterophorus* in Tanzania and other places in the world
affected with this weed.
Keywords: Parthenium, allellopathic, inhibition effects, allelochemicals, Suppression.

1. Introduction

Parthenium hysterophorous L., commonly known as carrot weed is an herbaceous annual weed
native from America but was introduced to Africa, Asia and Australia and establish itself. This
weed can reach a height of 2m, and produce thousands of seeds which remain viable for about
six years in the soil seed bank .(Retief *et al.*, 2013). Generally the weed has the habit to invade
disturbed areas and its incidence is high in the flooded areas (McFadyen 1992). Also this weed
invades farm and grazing lands, and affects biodiversity, animal and human health, agricultural
productivity and food security. This invasive plant species has been reported to originate from
the hybrid of *P. confertum* and *P. bipinnatifidium* (Msafiri *et al.*, 2013). Since its invasion in the
country, *P. hysterophorus* has become a serious threat to biodiversity through degradation of
natural ecosystems (Msafiri *et al.*, 2013). The weed is currently spreading to many habitats
including agricultural lands, protected areas as well as pasture land (Tamado and Milberg, 2000).

P. hysterophorous L. has strong allelopathic effects hence able to establish itself rapidly in new
environments and suppress the growth of other native species. Due to its allelopathic properties,
the plant has ability to colonize soils and inhibits growth of most plant/crop species and cause

injuries to humans and animals (Evans, 1997; Levine*et al.*, 2000; Zavaleta, 2000; Belnap and
Philips, 2001; Maharjan, 2007). High reproductive potential, fast growth rate, adaptation to
new environment and interference by allelopathy are the major contributing factors for rapid
spread and successful establishment of this weed in any ecosystem (Kohli & Rani, 1994).
Several management approaches such as mechanical, chemical, competitive replacement and
biological control have been used to control this weed where only herbicides application is the
common practice (Kumar; 2009). Nevertheless, chemical herbicides such as polyphosphate,
atrazines, 4-D, and Metribuzin are no longer reliable due to the cost and increasing weed
resistance (Vila-Aiub et al., 2008). Therefore, the use of bi-opesticides seems to be a vital
strategy of weed control in recent years. Many plants are known to have herbicidal effects on
other plant species (Vasilakoglou *et al*., 2005; Dhima *et al*., 2006; Javaid *et al*., 2008). Plants
such as *Amaranthas spinous*, *Azadiratchta indica*, and *Tagetes erictus* are reported to have
allelochemicals which, when released to the environment, has effects on the seed germination,
growth and development of other plants.
The present research work aimed to investigate the effect of aqueous extract of leaves and
flowers of *Azadiratchta indica, Amaranthus spinous*, *Sorghum bicolor* and *Tagetes erectus (*leaf
and flower*)* on germination, growth and development of *P. hysterophorus*. Such information is
critically important for the development of biological weed control methods.

2. Method

2.1 Experimental Site

The study was conducted at the Nelson Mandela African Institution of Science and Technology
(NM- AIST) and at Tanzania Pesticides Research Institution (TPRI) in Arusha, Tanzania from

May to November 2018. Experiments were conducted to determine the herbicidal effects of leaf and flowers aqueous extracts of *A. indica, T. erictus S.bicolor and A. spinous* on seed germination, fresh biomass, dry biomass, shoot and root elongation of *Parthenium hysterophorus*. Extracts preparation was conducted at NM-AIST while screen house experiments and part of laboratory work was conducted at Tanzania Pesticide Research Institution. (TPRI).

The experiments were conducted in two different seasons, the first in May-August 2018 and the second in September-November 2018 following complete randomized block design (CRBD).

2.2 *Preparation of extracts*

Leaves of *A. indica, A. spinous*, *T. erictus* and flowers from *T. erictus* plants were collected from the fields in Kikwe village, Arusha (3_o426345N 36 $_o$827934E) and air-dried at room temperature (25_oC) for 20 days. The dried leaves and flowers were grinded separately to powder using laboratory blender. Distilled water was used as extraction solvent whereby 100 g of powdered flowers/leaves were prepared and soaked in 1000 mL of distilled water. The mixture was kept in a conical flask with its top closed and stored in dark room for 72 hours at room temperature and, thereafter, filtered using muslin cloth to obtain a stock solution of 0.1 g mL^{-1} concentration. The stock solution was diluted in three different concentrations of 25, 50 and 100% and named as T2, T3, and T4, respectively. Distilled water was used as a control (T1).

2.2.1 Laboratory bioassays

Extracts were evaluated on *Parthenium* seeds germination using concentrations of 25%, 50% and 100%. Twenty seeds of *Parthenium* were placed in a 7 cm diameter Petri dish plate lined with Whatman No. 1 filter papers moistened with 3 mL of separate concentration of each extract. The control treatments received the same quantities of distilled water. Each treatment was replicated

three times. Plates were incubated in the growth chamber under room temperature(20▪) in
12hours light periods daily. Germinated seeds were counted manually for 14 days every 2 days
intervals and the experiment last for 21 days.

2.2.2 Foliar spray bioassays:

Seeds of *P. hysterophorus* were sown in pots of 10 cm diameter and 30 cm deep each containing
400 g of soil. Initially 20 seeds were sown in each pot which were thinned to 5 uniform seedlings
at the time of harvest. The freshly prepared extracts of *A. spinous, A. indica, S. bicolor and T.
erictus* (leaf and flower) were sprayed on the surface of 4 and 8 weeks old *P. hysterophorus*
plants. Two consecutive sprays were carried out with 5 days intervals each. Control plants were
similarly sprayed with distilled water. Plants were harvested 20 days after spraying.

Figure 1:Pot experiment in screen house

2.3 Determination of shoot length, root length, fresh biomass and dry biomass

Measurements of parameters were taken at the tenth week of growth of *P. hysterophorous*
species in the pot experiment. Roots and shoots for *Parthenium* in each replicate were measured
using a ruler. Fresh biomass was measured using weighing balance thereafter the plants were
placed in a labeled envelop and oven dried at 70 ° C for 3 days. The dry biomass of plants was
recorded and the data obtained was analyzed using statistica Software.

2.4 Germination inhibition/stimulation

Percentage inhibition/stimulation effect on seed germination was calculated using the formula
proposed by Singh & Chaudhary (2011). Inhibition (-) or stimulation (+) = [(Germinated seeds in
extracts - Germinated seed in control)/Geminated seeds in control] x 100.

2.5 Statistical analysis

Significance of the differences in germination percentage, root and shoot length, fresh and dry
biomass of seedlings under different treatments were tested and compared using one way
Analysis of Variance (**ANOVA**). The analyses were done using a statistical package Statistica

version 8. Computed at p=0.05 according to Fischer ' s least significant different test.

3. Results

The results show that plant extracts from different plant species used in this study significantly ($p \leq 0.001$) inhibited germination and seedling growth of *P. hysterophorus*. Root and shoot length, fresh and dry biomass as well as percentage germination of *P. hysterophorous* in control were significantly higher than in the plant extracts treatments.

Results presented in figure 1 indicate that seed germination of *P. hysterophorus* was significantly inhibited by the aqueous extracts of *A. spinous*, *A.indica*, *T.erictus* (leaf and flower) and *S.bicolor*. The inhibitory effect on seed germination trends of *P. hysterophorus* was concentration dependent as increased plant aqueous extracts concentration led to increased inhibitory effect on the germination of *P. hysterophorus*. The highest germination percentages were 95.5% and 93.33% in control treatment for T*. erictus* flower extracts and the leaf extracts respectively.

Inhibition percentage increased significantly ($p \leq 0.001$) from -30.00%, -38.00%, -40.00%, -44.25% to -80.00% at 25.00% concentration and from -66.67, -70%, -80.00%, -83.33% to -95.00% at 100% concentration for *A. indica*, *T. erictus* leafs, *T. erictus* flower, *A. spinous*, and *S. bicol*or extracts respectively (figure 1). Among the plant extracts used, maximum inhibition was observed with *S. bicolor* where 5.00% seeds germinated followed by *A. spinous* (16.67%).

Comparatively, *T. erictus* (flower), *T. erictus* and *A. indica* leaves also reduced significantly the germination percentage by 20%, 30% and 33.33%, respectively, compared with the control treatment (figure 1).

Results presented in figure 4 & 5 revealed that aqueous leaf extract of *A. spinous* significantly ($p \leq 0.001$) reduced dry and fresh biomass of the *P. hysterophorus.* The highest values of fresh and

dry biomass were 19.17, 9.50, 87.33 and 24.67 g for measurements taken at four and eight weeks
of growth, respectively. In the control treatment, the lowest values of fresh and dry biomass of
P. hysterophorus were 0.83, 0.01, 20.33 and 0.43 g for four and eight weeks respectively
recorded at the100% concentration. These results suggest that fresh biomass of *P. hysterophorus*
decreases significantly ($p \leq 0.001$) as the concentration level were increased (figure 4 & 5).
Furthermore, the results revealed that the control (0% concentration) was observed to have high
root and shoot length (9.67 , 10.73 , 8.36 and 8.82 cm, respectively) when compared with high
concentration 100% (figure2 & 3).Moreover, high inhibition rate was observed on *A. indica*
extracts on root and shoot length, fresh biomass and dry biomass with values of 2.64, 3.48, 2.94,
2.95 cm, 1.33, 28.00 and 0.25 and 2.50 g, respectively and eight weeks respectively, as
compared with control which had the values 9.68, 11.40, 19.67 9.50 and 7.93, 8.32, 84.6, 24 g
and 93.33% for root, shoot, fresh biomass and dry biomass, respectively (figure 2,.3,4 & 5).
Results presented in figure 2 & 3 show significant ($p \leq 0.001$) bio herbicidal effects of *T. erictus*
leaf aqueous extract on roots and shoot length of *P. hysterophorus.* The effect on root and shoot
length reduction was observed for both four and eight weeks for *Parthenium* treated with plant
extracts, where the highest values were recorded in the control, which were 9.67 and 6.68 cm in
roots and 11.00 and 8.71cm in shoots, respectively. The lowest root and shoot length were 2.03
and 2.94 cm and 2.17 and 2.38 cm, respectively (figure 2 & 3). Additionally, results also
showed significant reduction in both fresh and dry biomass (figure 4 & 5). Furthermore, the
effects of aqueous flower extracts of *T. erictus* on the root, shoot length, fresh and dry biomass

on *P. hysterophorous was* also studied and the results are as presented on (figure 2 & 3). Both
fresh and dry biomass of *P. hysterophorous* were reduced compared with the control treatment.
The highest fresh biomass values observed in control treatments were 19.67 and 74.67 g for the
four and eight weeks respectively, while the lowest values were 2.17 and 18.67 g observed for
four and eight weeks, respectively, recorded at 100% concentration. The similar inhibition effects
was observed in dry biomass where the highest biomass values were 9.50 and 27.67 g in
control treatment while the lowest values were 0.27 and 0.37 g recorded in treatment with
100% concentration of aqueous flower extracts of *T. erictus* (figure 4 & 5). The lowest root
length (1.63 and 3.50 cm) and shoot length (1.80 and 3.40 cm) of *P. hysterophorus* were in
found in pots treated with 100% (figure 2 & 3).
Results on figure 2, 3, 4 & 5 also showed that *Sorghum bicolor* extracts exhibited strong
inhibition on root length, shoot length, fresh biomass and dry biomass of *Parthenium*. The
highest root and shoot length values were observed in the control treatments that were 7.93 and
11.00 cm respectively while the lowest values were observed at the concentration of 100%. The
same observations were recorded on the fresh and dry biomass where the highest values were
observed in the control treatments and the lowest in 100% concentration.

Figure 1: Inhibitory effects of aqueous extracts of A.indica, T.erictus, A.spinous and S.bicolor on seed germination

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Figure 2. The effects of** aqueous **extracts on root length growth of P. hysterophorous sprayed at 4th week and 8th week of growth and harvested 20 days after spraying

. ***Figure 3: The effects of** aqueous **extracts on Shoot length growth of P. hysterophorous sprayed at 4th week and 8th week of growth and harvested 20 days after spraying***

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4. Discussion

All plant extracts applied showed significance suppression of *P. hysterophorous* seedling growth in pot trials. Four weeks seedling were found to be more susceptible compared with eight weeks seedlings. Results showed that the aqueous leaf extracts of *A. indica, T. erictus, S. bicolor, A. spinous* and *T. erictus* as well as flower extracts of *T. erictus* showed significant effects on seeds germination, reduction in shoot and root length as well as reduced dry and fresh biomass production. Findings obtained in our study are similar with those of Ngodya *et al.* (2016) who reported that germination inhibition, root and shoot length reduction and fresh and dry biomass were decreasing with increasing concentration of *Desmodium* species extracts. Furthermore, Gholami (2011) report that inhibitory effects in roots and shoots were contributed by reduction in cell division. This suggests that bioactive (bio-herbicidal) obtained from aqueous extracts of the investigated plants has a negative effects cell division of *P. hysterophorus.*

Plant extracts have been reported to have inhibitory effects on the growth and development of other plants. For example Siddiqui *et al.* (2009) reported that the aqueous leaf extract of mesquite (*Prosiposis julifora*) at different concentrations cause pronounced inhibitory effects on seed germination and root length on wheat (*Triticum aestivum*). Similarly, *Elisante et al*. (2013) reported the inhibitory effects of *Datura stramonium* extracts on *Cenchrus ciliaris* and *Neonotania wightii* with their increasing concentration. Generally, germination is the results of continuation of metabolic activities and growth of seed tissues which start with absorption of water through diffusion and osmosis hence cause activation of enzymes and increase metabolic activities.

In our experiments, seeds of P*. hysterophorus* supplied with aqueous extracts of *A. indica, T.erictus,* *A. spinous*, and *S. bicolor* affected their germination compared with those supplied with water.
This might be to the reasons that plant extracts had metabolic compounds with inhibition effects.
These findings are similar to the recently study conducted by Ramachandran (2018) who reported
that the germination of *P. hysterophorus* was inhibited due to imbalance of enzymes due to
application of aqueous extracts of *Datural metel*, *Mangifera indica*, *A. indica, T. erictus* and *S.* *bicolor* and *Heliantus annuus* which all showed inhibitory effects on the germination. On the
other hand this study also is in line with the study by Javaid *et al.* (2005) showed a reduction in
Germination of *P. hysterophorus* by aqueous extracts of allelopathic grass *Desmostachya* *bipinnata..* Similarly germination inhibition of *P.hysterophorus* due to aqueous extracts of three
allelopathic grasses namely *Dicanthium annulatum, Cenchrus* *pennisiformis* and *Sorghum helepense* have been reported by Javaid and Anjum (2005).
In this study, we found strong reduction of *P. hysterophorus* biomass by 33.33% using extracts from *A.* *indica*. This reduction is due to the bioactive compounds found in *A. indica* which has bio-herbicides
that suppress the growth of *P. hysterophorus*. Behl *et al*. (2004) reported the presence of bioactive
compound such as Nimbin, Azadirone, Azadirachtins and Salanin in *A. indica*. These bioactive
compounds have strong bio-herbicidal properties and might have caused the suppressive effects in the
Parthenium growth parameters. These findings suggests that aqueous extracts of *A. indica* could be
effective in controlling and managing *P. hysterophorous*.
Extracts from *A. spinous* showed significant effects on the growth of *P. hysterophorus* parameters. For
example, at 50 percent concentration of *A. spinous*, the root length was reduced by nearly 2-folds,

while the dry biomass was reduced nearly by 20-folds (figure4 &5). This could be contributed by the bio-herbicidal present in *A. spinous*. Our results are in agreements with a similar study by Thapar and Singh (2005) who reported that the leaf extracts of *A. spinous* reduced the growth of *P. hysterophorous*. In their study, the suppressive effects of growth were associated with the presences of organic compounds such as amino acids. Another study (Thapar 2005) has reported that the bio-herbicidal present in the leaves of *A. spinous* stimulated lignin biosynthesis which increased the rigidity of the cell wall and limited the cell growth. The effects of aqueous leaf and flower extracts of *T. erictus* on seeds germination and growth of *P. hysterophorus* was investigated. Our findings showed that both extracts inhibited the growth of *P. hysterophorus* nearly by 3-folds at the 100% concentration. These findings are in agreements with the study conducted by Shafique (2011) whose results revealed that the increase of concentration of aqueous extracts of *T. erictus* reduced the root length, shoot length, fresh and dry biomass of *P. hysterophorus* and this was attributed to the concentration of the extract and presence of herbicidal properties found in *T. erictus*. Furthermore, Guzman (1988) revealed that intensity of inhibitory effects of different parts of plants may be due to presence of different phytotoxic compounds such as phenolics, sesquiterpenes and lactones from plant parts. In this study more inhibition on roots, shoots, and germination, fresh and dry biomass was observed when the flower extracts were applied as compared with the leaf extracts. This suggests that flowers of *T. erictus* released stronger bioactive compounds which inhibited the growth parameters compared with leaves.

Sorghum bicolor plant extracts also significantly inhibited the germination and the growth of *P. hysterophorus* root, shoot, fresh and dry biomass and germination as a function of concentration

increase. Our findings suggests that, *S. bicolor* has a great potential to control and manage *P. hysterophorus*. The present results are in agreement with findings of Randhawa *et al.* (2002) who reported that sorghum water extracts at high concentration significantly reduced the germination, root, and shoot length of *Trianthema portulacastrum*.

CONCLUSION

It can be conclude that aqueous leaf and flower extracts of *S. bicolor A. spinous*, *T. erictus* (leafs and flower) and *A. indica* have bio-herbicidal effects on root and shoot length, fresh and dry weight and germination of *P. hysterophorus* in the laboratory and pot experiments. The results show that all the applied plant extracts have potential in the management of *P. hysterophorus* when applied at higher concentrations. Further studies should be conducted under field conditions to ascertain the effectiveness of *S. bicolor A. spinous*, *T. erictus* and *A. indica* in controlling *P. hysterophorus*. Further research on biological management by using other botanicals are needed to come up with proper solution for this noxious weed. The promising plants are recommended for large scale testing in areas where the weed is increasingly becoming a problem.

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