HERBICIDAL EFFECT OF Azadirachta indica A. JUSS. ON Phaseolus vulgaris L. AND ITS ASSOCIATED WEED Trianthema portulacastrum

ARTICLE · JULY 2015

HERBICIDAL EFFECT OF *Azadirachta indica* A. JUSS. ON *Phaseolus vulgaris* L. AND ITS ASSOCIATED WEED *Trianthema portulacastrum*

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ABSTRACT
This study evaluated the herbicidal effect of leaf mulch and aqueous extract of Azadirachta indica A. Juss. on the crop Phaseolus vulgaris L. and its associated weed Trianthema portulacastrum L. Fresh leaves of A. indica in its vegetative phase were collected from trees in the University of Ghana Botanical garden, air dried and powered for use as mulch and for the preparation of aqueous extracts. Two concentrations, 0.5% and 1.0 % w/v of Azadirachta leaf mulch and aqueous extracts were prepared and used to determine the percent germination, total plant production and chlorophyll content for crop and its associated weed. Percent germination study was carried out under laboratory conditions using petri dishes and total plant production and chlorophyll content were estimated using pot study in the botanical garden. As a control, distilled water was used for the germination study and air dried garden soil for the pot study. Results indicate a decline of 31% and 67% respectively in germination rate of T. portulacastrum (weed) for both treatments compared to 98% for the control. It was also observed that the 0.5% concentration of leaf mulch played stimulatory effect on P. vulgaris (crop) biomass. 1.0% of leaf mulch reduced the weed biomass by 43%. Finally a decline in the overall chlorophyll content of weed for both treatments was observed. However, the chlorophyll content in crop increased with increasing concentration of A. indica leaf mulch. Potentially, this implies a positive herbicidal effect of A. indica.

Keywords: Allelopathy, *Azadirachta indica* A. Juss., dry leaf mulch, *Phaseolus vulgaris* L., *Trianthema portulacastrum* L., Percent germination, Chlorophyll content.

INTRODUCTION

The word ‘weed’ means any wild plant that grows at an unwanted place for example in fields and interferes with the growth of cultivated plants (Rizvi *et.al.*, 1981). Weeds possess advanced characteristics like production of large number of seeds, rapid seedling growth, fast maturation, dual modes of reproduction and environmental plasticity that enables them to grow, flourish, invade and dominate an important part of natural and agricultural ecosystems (Kholi *et al.,* 2004 and Zimdahl 1999). In agro-ecosystems, crops experience competition with surrounding naturally growing weeds for resources. This results in reduction of crop yield and their quality deterioration (Kholi *et al.*, 2004).

Accounted loss depends on weed species present, timing and duration of competitive interactions, and resource availability (Anaya, 2006). In light of these characteristics of weeds and their risks, it becomes vital to regulate them. Several techniques (e.g. mechanical and chemicals - herbicides) are used for weed control worldwide. Weed control through herbicide use has resulted in many concerns. Research studies have proved that constant use of herbicides have posed problem for health and environment and has affected organisms at genetic level (Sodaeizadeh and Hosseini, 2012). Therefore control of weeds in the field needs revision and introduction of novel control. One form of control is the use of knowledge of allelopathy. Rice (1974) defined allelopathy as a biological phenomenon where one plant inhibits the growth of other plant directly or indirectly through the production of chemical compound known as allelochemicals. Plant parts like foliage, flowers, roots, bark, seeds, mulch, have these allelopathic properties (Ashrafi *et al.*, 2007; Putnam and Tang, 1986; May and Ash, 1990; Mahall and Callaway, 1991; Inderjit, 1996). Generally all the allelopathic plants store their protective chemicals within their leaves, especially during fall. As leaves drop to the ground and decompose, these toxins can affect nearby plants. Some plants also release toxins through their roots, which are then absorbed by other plants and trees. These compounds may enter into the environment and are known to inhibit germination and growth of plant, which is essential feature of all herbicide to control weeds (Sodaeizadeh and Hosseini, 2012).

Most of the allelochemicals like azadirachtin, artemisinin, codeine, vincristine, artopine, scopolamine, and many more are active compounds in various plants in the form of secondary metabolites which can be medicinally important. Plant products therefore can be a possible alternative for synthetic herbicides and these may be used as natural herbicide (Rizvi and Rizvi, 1992). Many efforts have been done to illustrate allelopathic potential of plant at laboratory level; little has been done at field level (lnderjit and Weiner, 2001; Macfas *et al.,* 2007). Most research on allelopathy were focused on the effect of interaction among weed species (Lydon *et al.,* 1997 and Narwal, 1994), weeds and crops (Rice, 1984) and crop species (Hegde and Miller, 1990). The need to reduce harmful environmental effects from the overuse of herbicide has encouraged the development of weed management systems, which are dependent on ecological manipulations rather than agrochemicals (Liebman and Ohno, 1997).

Neem (*Azadirachta indica*), is an evergreen tree native to Southeast Asia. All parts of the plant have been used medicinally for centuries. It is widely used in toothpastes, soaps and lotion today, as well as being a biological insecticide. Wide arrays of biologically active constituents are produced in *Azadirachta indica* like azadirachtin, nimbidin, nimbidolgedunin, etc. (Sankaram, 1987). Because neem contains a number of useful chemicals, with multiple uses and adaptability to diverse habitats and climatic conditions, interest in the tree has increased (Ashrafi *et al.*, 2009). *Azadirachta indica* plant is perhaps one of the most studied and widely used medicinal plants (Sankaram, 1987). Biological and pharmacological activities attributed to solvent extracts and products like oil from the different parts of *Azadirachta* are as diverse as Antiplasmodial, Larvicidal, Fungicidal, Insecticidal, and Anthelminthic (Sankaram, 1987). Furthermore, the bark of *Azadirachta* tree is known to possess tannins and phenolic compounds and exhibits anti-oxidant principles (Nwachukwu, 2009). Although much research has been done on neem in different aspects little information is available concerning the allelopathic potential of neem on crop plants as a natural herbicide (Sankaram, 1997). Few studies have indicated that *Azadirachta indica* leaves possess natural herbicidal properties (Pavankumar *et al.,* 2013 and Bano *et al.,* 2012). This study was therefore carried out to investigate the herbicidal effect of allelopathic substances in the leaf mulch on a crop and its associated weed population. Specifically, the objective of this work was to examine the natural herbicide effect of *Azadirachta indica* leaf mulch on *Phaseolus vulgaris*-a crop plant and its associated weed *Trianthema portulacastrum* commonly known as horse purslane. Khaliq *et al.* (2011) described horse purslane as one of the problematic terrestrial weed widely distributed in Africa, South East and West Asia and Tropical America. This weed infests field crops such as pulses, cotton, sugarcane, direct seeded rice and maize and, could reduce crop yields by 32-60% in most of the agricultural fields (Sethi and Mohnot, 1988; Baylan and Malik, 1989 and Gricher, 2008).

This weed species was found well associated with *Phaseolus vulgaris* in the University of Ghana agricultural fields. Hence, present study seeks to understand natural herbicidal effect of *Azadirachta indica* leaf mulch on weed and the crop growth.

MATERIALS AND METHODS

Fresh leaves of *Azadirachta indica* were collected in its vegetative phase from the trees in the University of Ghana Botanical Garden. The leaves were air dried and powered for the use as mulch and preparation of aqueous extracts.

Germination study

Germination study was done following Richard's function method (Fujii, 2003). Seed of *Phaseolus* and *Trianthema* were cleaned manually to ensure physical purity. Surface sterilization was done with water: bleach solution (10:1) for 15 min and finally rinsed with distilled water for four times. *Phaseolus* and *Trianthema* seeds were kept in 9 cm Petri plates, containing moist Whatman -1 paper, Distil water for control and 5ml of aqueous extract of *Azadirachta indica* leaf powder was added in two concentrations viz. 0.5% and 1% (w/v) for 10 seeds in each Petri plate. Five replicates were maintained for each treatment.

Petri dishes were kept at room temperature, and daily observations were recorded. A seed is considered germinated when the radicle (2mm) is protruded (Hou and Romo, 1998).

Pot Study

Earthen pots with 12 kg soil capacity were filled with 10 kg of air dried soil which was collected from University of Ghana Botanical Garden. Surface sterilized seeds of *Phaseolus* and *Trianthema* in the proportion 10:1 (gms) were sown together in the earthen pots. Irrigation was done daily, equally in all the pots. Three sets of treatments were made:

a) Control: *Phaseolus* + *Trianthema*
b) T1: *Phaseolus* + *Trianthema* + 50 grams of *Azadirachta* leaf mulch (i.e.0.5%)
c) T2: *Phaseolus* +*Trianthema* + 100 grams of *Azadirachta* leaf mulch (i.e.1%)

Thinning was done for *Phaseolus* and *Trianthema* after the first leaf emergence. 15 plants of each species per pot were left to grow. Analysis of biomass and chlorophyll was done on the 20th day after sowing into earthen pots. 10 plants of approximate equal growth and well developed leaves in the terminal position were selected from each pot for estimation of chlorophyll.

Chlorophyll estimation

Chlorophyll was estimated using as described by Arnon method (1949). 100 mg of leaf tissue were suspended in 10 ml of 80% acetone. The mixture was centrifuged (3000 rpm) for 15 minutes. The supernatant was collected and absorbance was recorded at 663 and 645nm in spectrophotometer for total chlorophyll, chlorophyll-a and chlorophyll-b.

Total biomass

Total biomass was estimated by fresh and dry weight analysis. Ten plants of *Phaseolus* of similar height were selected for biomass accumulation on the 20th day after sowing. After recording the fresh weight, plants were dried in an in an oven at 100° F. overnight for examining dry weight. Fresh weight and dry weight were taken for root, stem and leaf (Table 2). For biomass accumulation of *Trianthema*, five plants were selected for analysis.

Statistical analysis

Data were analysed following an analysis of variance (ANOVA) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Seed Germination

Incorporation of *Azadirachta indica* significantly ($P \leq 0.05$) suppressed the germination of weed plant–*Trianthema* when compared with control (Table 1). A significant germination inhibition of 67%in treatment-2 (1.0% *A. indica*) and 31% inhibition in treatment-1 (0.5% *A. indica*) as compare to control were observed. Inhibitory effect on seed germination was observed with increasing concentration of *A. indica*. However, in the case of *Phaseolus*, very little inhibition in germination (15%) even at higher concentration of leaf extract was seen. Germination experiments are widely done to check and evaluate allelopathic potential of plants in the laboratory and this have exhibited promising results (Fujii *et al*., 2004). The present study done on the percent germination of crop and weed species using *A. indica* as a plant with natural herbicidal properties showed positive results.

Table 1: Germination percentage of *Phaseolus* and *Trianthema* seeds at two different concentrations of *Azadirachta* leaf aqueous ex-

Plant name	Control	T1-0.5%	T2-1.0%
Phaseolus vulgaris (crop)	100%	94% (-6)	85% (-15)
Trianthema portulacastrum (weed)	98%	68% (-31)	32% (-67)

Figures given in parenthesis show percentage change over control. Means differed significantly at $P \leq 0.05$.

Pot study

The study was conducted to know effect of *Azadirachta* leaf mulch on the crop (*Phaseolus*) and weed (*Trianthema*). Leaf litter leachates play an important role in farming as mulching is a common traditional farming system in many developing countries of Asia and Africa (Fujii *etal*., 2004). Present experiment was done to mimic field situation.

It was observed that lower concentration leaf mulch (0.5%) played stimulatory effect on crop possibly because of reduced competition due to high nutrient reallocation to crop. Higher concentration (1.0 %) of the *A. indica* leaf mulch significantly reduced the biomass of weed species up to 43% (Table 2). Fresh weight of root and stem were noted to increase in treatment-1 the crop. An overall increase of 27 % growth in crop was noted. In treatment-2, weeds germinated after nine days compared to control. Germination of *Trianthema* seed was delayed in treatment-1 by four days but later, the number of seeds and plant growth increased similar to the control. Sethi and Mohnot (1988) have reported that enormous seeding capacity of this weed allows the mature seed to easily germinate after a short dormancy due to allelochemicals producing multiple generations in any given time. The delay in germination in treatment-1 and treatment-2 could possibly be due to allelopathic crop residues on decomposition produced by a variety of phytotoxins, particularly phenolics in the soil causing side effects (Nelson, 1996). Purvis *et al*. (1985), Thorne *et al*. (1990) and Weston (1996) in similar studies have also noted the potential of allelochemicals to pose a chemical as well as a physical effect on the growth and development of crops and weeds.

Significant ($P \leq 0.05$) difference in the total chlorophyll between control and T2 were noted. Chlorophyll amount increased with increase in concentration of *Azadirachta* leaf mulch (fig.1).

In weed leaf Total chlorophyll of T1 was reduced by 38% when compare to control (fig.2). There was no significant ($P \leq 0.05$) difference between total chlorophyll of Control and T2 even though there was 3% reduction. Similarly chlorophyll a and chlorophyll b were seen reduced to 45% in T-1 when compared to control.

This research demonstrate that dry leaf mulch of *Azadirachta* shows inhibitory effect on *Trianthema* by reducing seed germination and retarding plant growth at higher concentration. It indi-

Table 2: Effect of leaf mulch on fresh weights and dry weight (grams) of *Phaseolus* and *Trianthema*

Plant Part	Control		T-1(0.5%)		T-2 (1%)	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
***Phaseolus* (crop)**						
Root	0.73	0.42	1.04 (42)	0.58 (38)	0.80 (9)	0.45(7)
Stem	7.69	1.19	8.93 (16)	1.45 (21)	7.98 (3)	1.23 (3)
Leaf	14.71	3.31	18.24 (23)	4.21(27)	14.98 (2)	3.37 (2)
***Trianthema* (weed)**						
Root	0.24	0.07	0.23 (-4)	0.065 (-7)	0.14 (-42)	0.04 (-43)
Stem	3.42	0.91	3.22(-6)	0.84 (-6)	1.43 (-58)	0.42 (-54)
Leaf	1.42	0.43	1.29 (-9)	0.40 (-7)	1.01 (-29)	0.29 (-32)

Figures given in parenthesis show percentage change over control. Means differed significantly at P≤0.05.

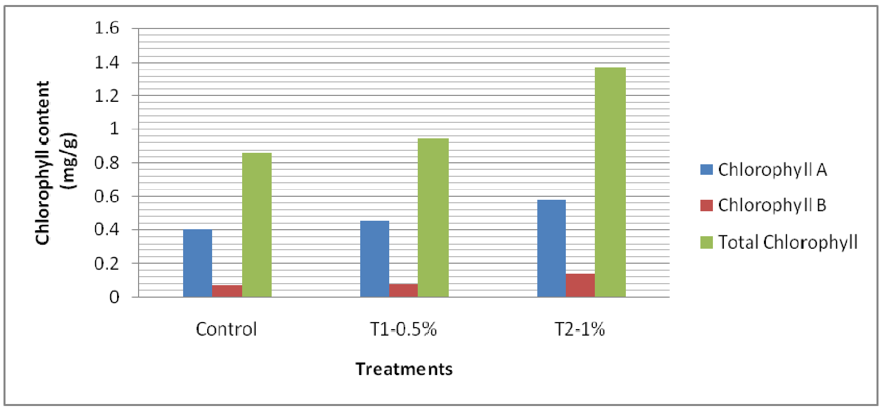

Figure 1: Chlorophyll content in *Phaseolus*

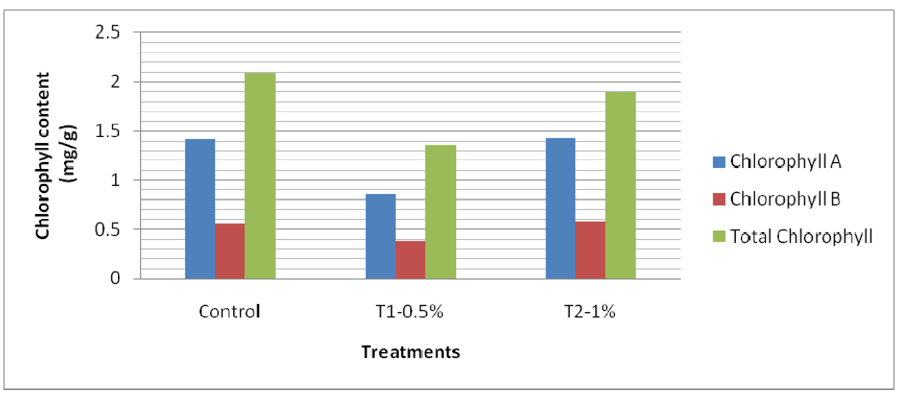

Figure 2: Chlorophyll content in *Trianthema*

cates several biologically active secondary compounds could be responsible for allelopathic effect on *Trianthema*. No significant reduction in crop performance was observed when it was treated with leaf mulch; on the contrary lower concentration (0.5%) had stimulatory effect. These results are supported by Kakati and Baruah (2013), and Bano *et al.* (2013) who reported that neem tree is less inhibitory to crops like green mung (*Phaseolus radiata*) and chickpea (*Cicer arietinum*) and inhibitory to weed biomass and weed population in wheat field, respectively. Similar results are also documented in the work done by Salam and Noguchi (2010) signifying the natural herbicidal effect of *Azadirachta* on few weed species. Allelochemicals are known for their inhibitory as well as stimulatory effect on neighbouring organisms (Rice, 1984). Khan *et al*. (2009) have observed such effects of few medicinal plants. In the present study, *Azadirachta* leaf mulch shows stimulatory effect on *Phaseolus*, as per increase in chlorophyll content and biomass with concentration. At the same time it reduced the growth of weed significantly, this shows that *Azadirachta indica* leaf mulch could be used as natural herbicide. Further work is needed to identify the growth inhibitory substances from the leaf mulch of donor plants before they are considered as having herbicidal properties for commercial use.

RECOMMENDATIONS

- It is recommended that higher aqueous extracts (1.5, 2.0, 2.5 and 3.0 %) of *A. indica* be imposed to *Phaseolus vulgaris* in order to give conclusive inhibitory effect
- Further work is also needed to identify the specific inhibitory allelochemicals present in leaf of *A. indica* that impose growth retardation in *T. portulacastrum*.

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