ALLELOPATHIC POTENTIAL OF *Silybum marianum* L. AGAINST THE SEED GERMINATION OF EDIBLE LEGUMES

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ABSTRACT

A laboratory based trial using water extract was conducted to investigate the allelopathic potential of Silybum marianum L. on kidneybean (Phaseolus vulgaris L.), mungbean (Vigna radiata L.), chickpea (Cicer arietinum L.) and soybean (Glycine max L.) during April 2011, in the Weed Science Research Laboratory, Department of Weed Science, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan. Fresh plants of S. marianum were collected, dried and ground. Then the powder was soaked in tap water @ 55g/liter and 110g/liter. Ten seeds each of kidneybeans, mungbean, chickpea and soybean were placed in Petri dishes separately and different concentrations of extracts were applied according to the requirements. A control treatment (0 g/liter) was also included for comparison. The experiment was laid out in completely randomized design (CRD) with three replications and treatments. The results showed that with increasing the leaves extract concentration of S. marianum, a significant decrease was noted in the germination percentage, mean germination time (MGT), germination index (GI) and seed vigor index (SVI) of the test species. The tolerance order of the mungbean and soybean against the extract concentration of S. marianum was higher than the other two species, while the chickpea was more susceptible to the toxicity of S. marianum. Therefore, it was noted that the infestation of S. marianum can pollute the soil by accumulating toxic chemicals and affect the agro-biodiversity. Our findings suggest that preventing spread of S. marianum is the better weed management method in legume crops in order to avoid any post germination problem.

Key words: Allelopathy, inhibition, legumes, *Silybum marianum* L., seed vigor index.

INTRODUCTION

Allelopathy is derived from the Greek words “allelo” and “pathy” meaning reciprocal sufferings of two organisms especially plants. It is defined as the direct or indirect stimulatory or inhibitory effects of one plant on another through release of chemical compounds (allelochemicals) that escape into the environment (Rice, 1984) by

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leaching from above ground parts, stem flower, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Ferguson and Rathinasabapathi, 2003). Scientists have a consensus on the fact that secondary metabolites may function as allelochemicals. Some examples include terpenoids, phenolics, alkaloids, fatty acids, steroids and polyacetylenes (Kohli, 1998). These natural plant products may provide clues to new and safe herbicide chemistry or growth hormones development.

Most of the weed species have inhibitory effects on crops, yet some weed species also exhibited stimulatory effects on the seed germination, growth and yield of crops. The weeds influence the crop plants by releasing phytotoxins from their seeds, decomposing residues, leachates, exudates and volatiles (Narwal, 2004). Weeds compete for light, nutrients, moisture and space with the crop and thus cause severe losses in crop yields. Besides, weeds can also affect a crop's growth by releasing allelochemicals into the growing environment (Rice 1984; Kadioglue *et al.,* 2005). All plant parts of the weed including leaf, stem, root, and fruit have allelopathic potential (Alam and Islam, 2002; Tinnin and Muller, 2006). Allelopathic efficacy of weeds on germination and seedling growth of crops vary from weed to weed (Hamayun *et al*., 2005). The allelopathic effects of various parts of same weed also differ for their effects on germination and initial growth of plants (Aziz *et al.,* 2008). However, various parts of weeds show different behavior in exerting their allelopathic effects on crops (Veenapani, 2004). Weeds also exert allelopathic effects on crop seed germination and growth by releasing water-soluble compounds into the soil (Batish *et al*., 2007).

Silybum marianum L. (Milk thistle) is a common weed of our crops; it emerges from October to December (Khan *et al.* 2009) and invades winter crops and vegetables, such as wheat, lentil, chickpea, potato, mungbean and pea etc. *Silybum marianum* poses serious threat to these crops because it has got comparatively a heavier biomass than the crop plants, as a result it not only captures maximum resources from the crop plants but also add allelochemicals in to the surrounding soil through root exudates and continuous growth and fall of leaves. Allelopathic effects of different weeds on wheat and chickpea and other beans crops have been reported in the literature (Singh *et al*., 2005) but no such research has yet been conducted on the allelopathic effects of *S. marianum* specifically on kidneybean, mungbean, soybean and chickpea crops.

Keeping in view the recognized importance of weeds allelopathy, an experiment was conducted under laboratory conditions with the objectives to screen out *S. marianum* L. water extracts for their allelopathic status and to assess their effect on seed status like seed germination, germination index (GI), mean germination time (MGT) and seed vigour index (SVI) of some edible legumes.

MATERIALS AND METHODS

Collection of plant material

Silybum marianum L. plants were collected randomly from the research fields of New Developmental Research Farm of the Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan. Whole plant samples were collected by cutting the plants at the base (above the ground) with a sickle. Then plants were washed with tap water to wash down the dust and other particles and then dried in shade. After drying the whole plants (roots, stems, leaves, and fruits) were first chopped and then ground with the help of grinder. The final ground sample was kept in paper bag for further use in the experiment.

Preparation of water extracts of *Silybum marianum* L.

The dried powder of the *Silybum marianum* L. plants was weighed, and mixed in tap water @ 110 and 55 g L^{-1} to make two concentrations at room temperature for 24 hrs. The final water extracts of the different parts of *Silybum marianum* L. were obtained by filtering process through 10- and 60-mesh sieves. Both of the water extracts concentrations (110 and 55 gL^{-1}) were individually bottled and tagged for further process in the experimentation.

Experimental setup

The study was carried out in the Weed Science Research Laboratory, Department of Weed Science, Khyber Pakhtunkhwa Agricultural University, during April 2011. Ten seeds each of kidneybeans, mungbeans, chickpea and soybean were put separately in petri dishes (each having 9cm dia) containing three layers of Whatman No.1 filter paper. The petri dish were replicated three times. Three mL of the extract as per treatment were added to each petri dish and tape water was used as a control treatment for comparison. During the period of 10 days the petri dishes were observed daily and an equal amount of extract was added to each petri dish as needed to prevent seeds or seedlings from drying out. The germination data was recorded on daily basis for a week. After 7 days the seedlings were uprooted and washed with water. The length of the shoots was measured in centimeters.

Data parameters and formulae

Germinated seeds were counted daily. The seeds were considered as germinated when the radical size was 2 mm. The number of germinated seeds was recorded every 24 h, and these seeds were discarded after data collection. Seven days after sowing the percentage data were calculated using the formula,

$$\text{Germination \%} = \frac{\text{Germinated seed}}{\text{Total seed}} \times 100$$

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981),

$$MGT = \frac{\sum Dn}{\sum n}$$

where n is the number of seeds that emerged on day D, and D is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983),

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \text{- - - - - - -} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Seedling vigor index (SVI) was calculated according to the following formula of Abdul-baki and Anderson (1973).

$$SVI = \text{Germination} \times \text{Radical length (cm)}$$

Statistical analysis

The collected data were analyzed through Analysis of Varaiance (ANOVA) techniques and the least significant difference (LSD) at ($P \leq 0.05$) was computed by procedure mentioned by (Steel *et al*., 1997).

RESULTS AND DISCUSSIONS

Germination (%)

The analysis of variance of the data showed that percent germination of all the tested species was significantly affected by the extract of *Silybum marianum* and their different concentrations. Means separation test at ($P \leq 0.05$) showed that maximum (68.35 %) germination was noted for mungbean, which was statistically at par with the effect on kidneybean (68.33), and minimum germination (40.00%) was observed for chickpea. The data for the concentration mean showed that the percent germination of the all tested species decreased with the increasing concentrations of *Silybum marianum* (Table-1). Highest concentration mean (90.00) was recorded for 0 gL^{-1} (control) while the lowest concentration mean (25.00) was deciphered for 110 gL^{-1} i.e. significantly different the control treatments (0 gL^{-1}). As for the interaction of extract concentration and species tested, the highest value (95.00) was recorded for 0 gL^{-1} x Soybean and minimum (5.00) was noted for 110 g L^{-1} x Chickpea. The presence of allelochemicals has been explored many times and many scientists are of the view that allelopathins are present in various parts of plants and can greatly affect the receiver plants in many ways. However, allelochemicals are synthesized in plants as secondary metabolites and located in certain specialized organs of donor plants (Kobayashi, 2004). These results are supported by the findings of Kadioglue *et al*. (2005). They reported inhibition in the germination rate and final germination of lentil, chickpea, and wheat with different plant part extracts of different broad and narrow leaf weeds.

Table-1. Effect of *Silybum marianum* L. extract concentrations x species tested on germination (%).

Species tested	**Extract concentrations**			**Specie Mean**
	0 g L^{-1}	**55 g L^{-1}**	**110 g L^{-1}**	
Kidneybean (*Phaseolus vulgaris* L.)	90.00 a	70.00 ab	45.00 bc	68.33 a
Mungbean (*Vigna radiata* L.)	90.00 a	70.00 ab	47.00 bc	68.35 a
Chickpea (*Cicer arietinum* L.)	85.00 ab	30.00 cd	5.00 d	40.00 b
Soybean (*Glycine max* L.)	95.00 a	85.00 ab	7.00 d	61.66 a
Concentration Means	90.00 a	63.75 ab	25.00 b	

LSD $_{0.05}$ value for species = 16.79, LSD $_{0.05}$ value for concentrations = 39.03, LSD $_{0.05}$ value for Interaction = 29.08

Mean germination time (MGT)

The value of Mean germination time (MGT) expresses the rapidity of the germination i.e. the lower the value of the mean germination time the earlier will be the germination. The results reported here clearly indicated that *Silybum marianum* was very toxic at all levels investigated, with significant increase in MGT. Analysis of variance of the data revealed that *Silybum marianum* extract had a significant effect on means of the tested species (Table-2). Means of the species showed that maximum MGT (4.29) was recorded for kidneybean which was statistically at par with chickpea (4.00), while lowest MGT (2.82) was observed for mungbean. The result showed that the different extract concentration also had significant effect on the MGT of the species, whenever the extract concentration increases a remarkable decrease was found in the MGT values. The highest MGT mean value (4.79) was recorded under the concentration of 110 g L^{-1} followed by 55 g L^{-1} and the maximum value (2.73) for the concentration mean was noted in the 0 g L^{-1} (control). The interaction exhibits that maximum (5.90) value of MGT attained by the treatment 110 gL^{-1}x soyabean, while the minimum value (2.27) was investigated for the combination 0 gL^{-1} x soybean.The results are supported by the findings of Babar *et al*., (2009) who stated that chickpea seeds soaked in root extract of *Asphodelus tenuifolius* Cav. took more time for germination. These results are also in agreement with those of Stavrianakou *et al*. (2004). They reported inhibition of germination, germination index and increase in germination time of chickpea and lentil with the extract of different weeds.

Table-2. Effect of *Silybum marianum* L. extract concentrations x species tested on mean germination time (MGT).

Species tested	Extract concentrations			Specie Mean
	0 g L^{-1}	55 g L^{-1}	110 g L^{-1}	
Kidneybean (*Phaseolus vulgaris* L.)	3.61 bc	3.50 bc	5.78 a	4.29 a
Mungbean (*Vigna radiata* L.)	2.55 bc	2.42 bc	3.50 bc	2.82 b
Chickpea (*Cicer arietinum* L.)	2.50 bc	5.50 a	4.00 ab	4.00 a
Soybean (*Glycine max* L.)	2.27 c	2.60 bc	5.90 a	3.59 ab
Concentration Means	2.73 c	3.50 b	4.79 a	

LSD $_{0.05}$ value for species = 1.103, LSD $_{0.05}$ value for concentrations = 0.129, LSD $_{0.05}$ value for Interaction = 1.91

Germination Index (GI)

Germination index (GI) is directly correlated with germination percentage. Thus greater the value of GI, the greater will be germination percentage. The statistical analysis of data revealed significant differences in GI due to concentrations of *Silybum marianum*, species tested and their interaction. The data on GI (Table-3) showed that the highest species mean (3.0) was observed in mungbean treatment followed by soyabean (2.64), Like the species mean the concentration means also varied and posed a significant affect on the value of GI. The maximum GI value (3.37) was found under the control treatments (0 gL^{-1}), while minimum GI value (0.75) was investigated under the concentration of 110g L^{-1} of *Silybum marianum* extract, showing a high distinction in results and great influence of alleopathic compound that were present in the extract of *Silybum marianum*. The data for interaction revaled that the highest value (3.43) of GI was noted under the combination of 0 gL^{-1} concentration x soybean, while minimum (0.07) was computed for the combination of 110 gL^{-1} concentration x soyabean. Similarly, Tanveer *et al.* (2008) also reported minimum GI and germination percentage of rice when applied with leaf leachates of *Xanthium strumarium.*

Table-3. Effect of *Silybum marianum* L. extract concentrations x species tested on germination index (GI) of the species.

Species tested	Extract concentrations			Specie Mean
	0 g L^{-1}	**55 g L^{-1}**	**110 g L^{-1}**	
Kidneybean (*Phaseolus vulgaris* L.)	2.76 bc	2.19 cd	0.92 def	1.96 b
Mungbean (*Vigna radiate* L.)	3.98 ab	3.10 abc	1.91 cde	3.00 a
Chickpea (*Cicer arietinum* L.)	2.33 cd	0.58 ef	0.12 f	1.01 c
Soybean (*Glycine max* L.)	3.43 a	3.41 abc	0.07 f	2.64 ab
Concentration Means	3.37 a	2.32 a	0.75 b	

LSD $_{0.05}$ value for species = 0.83, LSD $_{0.05}$ value for concentrations = 1.32, LSD $_{0.05}$ value for Interaction = 1.44

Seed vigor index (SVI)

Like the previously discussed parameters, the SVI was also significantly affected by the influence of *Silybum marianum* water extract, and whenever the concentration increased the values of SVI for all the tested species decreased in the investigation. Analysis of variance of the data revealed that *Silybum marianum* extract had significant effect on means of the tested species (Table-4). Means of the species showed that maximum SVI value (439.1) was recorded for mungbean, while lowest SVI (261.8) was calculated for chickpea. Furthermore the different extract concentration also had significant effect on the SVI of the species. The highest SVI mean value (698.7) was recorded under the concentration of 110 g L^{-1} followed by 55 g L^{-1} and the minimum value (80.8) computed for the 110 gL^{-1}concentration. The interaction data presented that maximum (760.0) value of SVI attained by the treatment 0 gL^{-1}x soyabean, while the minimum value (12.50) was investigated for both the combination 0 gL^{-1} x soybean and 0 gL^{-1} x chickpea. The present findings got support from the earlier studies of Mubeen *et al*. (2011) who found significantly minimum seedling vigor index (SVI) for rice seeds which were soaked in leaf extract of *Trianthema portulacastrum.* Effects of leaf extracts of various weeds on germination, radicle and plumule extension of field crops have also been reported earlier by (Singh *et al.* (2005b).

Table-4. Effect of *Silybum marianum* L. extract concentrations x species tested on Seedling vigor Index (SVI).

Species tested	**Extract concentrations**			**Specie Mean**
	0 g L^{-1}	**55 g L^{-1}**	**110 g L^{-1}**	
Kidneybean (*Phaseolus vulgaris* L.)	720.0 a	322.0 cd	186.0 cde	409.13 a
Mungbean (*Vigna radiate* L.)	680.0 a	525.0 ab	112.5 de	439.1 a
Chickpea (*Cicer arietinum* L.)	635.0 ab	138.0 de	12.50 e	261.83 b
Soybean (*Glycine max* L.)	760.0 a	391.0 bc	12.50 e	387.83 a
Concentration Means	698.7 a	344.0 b	80.8 c	

$LSD_{0.05}$ value for species = 113.7, $LSD_{0.05}$ value for concentrations = 196.5, $LSD_{0.05}$ value for Interaction = 196.9

CONCLUSION

The conclusions made in light of the results obtained were that *Silybum marianum* L*.* contains toxic and water soluble compounds. These compounds could be released by rain or irrigation and dissolved in water under field conditions and pollute the upper fertile zone of the soil, which resulted in the germination failure or seedling growth retardation in the legume and other crops. Therefore, pro-active preventative management of *Silybum marianum* L. is direly required in legume crops.

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