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ALLELOPATHIC STUDIES ON MILK THISTLE (*Silybum marianum*)

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Abstract

Declining crop yield due to weeds and their resistance to herbicides are major constraint for successful crop productions. Milk thistle (*Silybum marianum*) is common weed species in Australian cropping rotation. Allelopathic potentiality of milk thistle on different crops has been documented sporadically, but there is no literature on about ryegrass and canola. Therefore, a laboratory based allelopathic extracts bioassay was conducted. The hot water extracts was prepared from milk thistle plant parts added into water with ration of 1: 10 (plant sample: distilled water) where mixture was heated 10 minutes. After heat treatment samples was immediately sieved and centrifuged and the resulted solution was treated as 100% concentration. Separately, to get the fresh water extract plant sample was added into water (1:10) and kept 24 hours in room temperature. After 24 hours, the sample was sieved and centrifuged and collected samples result was treated 100% concentrations. To obtain 50% concentration, both hot and fresh samples were diluted with distilled water. Therefore the experiment was conducted with five different treatment concentrations (0, 50% hot water extracts, 50% fresh water extracts, 100% hot water extracts and 100% fresh water extracts). The experiment was comprised with RCBD design with three replications under control conditions. During experimental period the allelopathic effects of donor species on germination and seedling growth of ryegrass and canola was observed. Results shows, germination and seedling growth of both receiver species are inhibited by milk thistle extracts. Extracts from fresh water at 100% was more toxic to receiver species followed by 50% concentration of fresh and 100% from hot water extracts. This concentration reduced the root, shoot growth of ryegrass and canola 84.971%, 84.269% and 89.898%, 87.394%, respectively. The result also revealed that allelopathic pattern of hot water extracts was same however; it is less toxic to both receiver species.

Keywords: Allelopathy, milk thistle, ryegrass and canola

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Introduction

Weeds are disliked and are not seen as of such use except for some recognition that they are part of nature (Qasem and Foy, 2008). Although, their ultimate values in natural and agro-ecosystems have not been completely discovered, but many of their negative and positive impacts on both systems are very familiar. To minimize the negative impacts, agricultural crops have increasingly relied on chemical herbicides. However, problems associated with intensive herbicide use include soil and groundwater contamination, development of herbicides resistance weeds and the escalating cost of developing of new herbicides (Worsham, 1989).

Recent assessments of the allelopathic effect of crops and/or weed on weeds have the goal of using naturally produced allelochemicals to reduce reliance on herbicides (Einhelling and Leather, 1988; Putnam and Duke, 1978). In allelopathy, plants provide themselves with a competitive advantage by releasing the phytotoxins into nearly environment (Pratley, 1996). The concept of allelopathy interaction between weeds is interesting. There is evidence that certain weeds species have the potential to be used in solving problems of other weed species and represents an excellent source of natural chemicals that may be involved in developing natural herbicides (Qasem and Foy, 2008).

Milk thistle (*Silybum marianum* Gaertn.) is a winter annual or a biennial noxious weed belonging to *Asteraceae* (Young *et al.*, 1978; Austin *et al.*, 1988; Groves and Kaye, 1989). Its current distribution includes most temperate areas of the world including Australia (Chambreau and MacLaren, 2007). In Australia, it is classified as a declared plant (noxious weed) and particularly prevalent in Victoria and New South Wales, where dense stands can develop on soils of high fertility (Dodd, 1989). Research suggests that ripe fruit of milk thistle contains flavonoids that are used to prepare antihepatotoxic drugs, and have different medicinal value. Apart from the medicinal value, milk thistle may be used for the phytoremediation of polluted). It is a major weed in sugar beet wheat and canola (*Brassica napus* L.) causing large yield reductions (Omtvedt, 1984; Khan and Marwat, 2006; Shimi *et al.*, 2006). However, researches have documented the allelopathic effect of milk thistle on mustard (*Brassica juncea* L.), cucumber (*Cucumis sativus* L.), wheat, and sorghum (*Sorghum bicolor* L.) (Inam and Hussain, 1988) but no such reports exist of milk thistle caused negative effect on annual ryegrass (*Lolium rigidum* L.). The present study was conducted to evaluate and determine the germination and initial growth performance of ryegrass and its associated crop canola under different extracts concentration of milk thistle. The study also aimed to determine the phototoxicity and stability of milk thistle under heat treatment. The significant results will help to create a sustainable weed control option especially against herbicides resistance ryegrass.

Materials and Methods

The allelopathic effects of milk thistle was investigated by implying laboratory study during 10 December 2011 to 15 Decemebr, 2011 at NSW DPI, Wagga Wagga, Australia.

Extracts preparation

Above ground mature milk thistle plants were collected from Wagga Wagga campus of Charles Sturt University, Collected samples were chopped out into 2 cm pieces. 10 g chopped plant samples were added to 100 mL distilled water in a 500 mL volumetric flask and boiled 10 minutes. To get the fresh water extracts, another 10 g chopped samples was added to 100 ml distilled water in a 500 ml) volumetric flask and wrapped with aluminum foil and kept for 24 hours at room temperature (25°C). The heated and soaked extracts were filtered through two layers of cheesecloth used centrifuged for 10 minutes at 5000 rpm using an Eppendorf 5810 bench top centrifuge. It was separately treated with 100% concentration of hot water and fresh water extract, respectively. The 100 % concentration of both extracts (hot and fresh water) was diluted

with distilled water to obtain 50% and 0% (control).

Allelopathy assessment

To know the allelopathic effect of milk thistle annual ryegrass and canola were used as the receiver species. Twenty seeds (non- surface sterilized) of ryegrass and canola were sown into 9 cm petri dishes lined with one layer with one layer of Whatman No. 1 filter paper. Five millilitre of each extract from different concentrated were delivered to each petri dish and distilled water (5 ml) was used as control. Each petri dish with its cover was sealed with a piece of parafilm to reduce evaporation. All dishes were maintained in a control growth room at 21/19°C with day/light for 12 h/12 h. First two days a black polythene was used to cover the all petri dishes, and after two days then cover was removed. Germinated seeds > 1 mm radical were recorded and root and shoot lengths were measured after 5 days of incubation.

Experimental design and statistical analysis

A randomized block design with three replications were used for the experiment. The experiment was repeated twice to confirm the consistent results. All experimental data were subjected to analysis using Genstat 5 (version 13) and treatments means were tested separately with least significant difference (LSD) at 5% level of probability. Percent of germination was calculated as (germinated seeds/total given seeds) X 100.

Results and Discussion

Germination percentages

In our bioassay, the milk thistle extracts significantly inhibited the germination of both species.

Annual ryegrass

Types of extracts concentration noticeably retarded the germination of ryegrass at 3 and 5 days after sowing. Germination of viable ryegrass seeds significantly reduced by fresh water extracts at 100% and 50% concentration followed by 100% extract from hot water extract. The maximum germination percentage 88.33 and 98.33 were obtained by control treatment at 3 and 5 days after sowing, respectively. Fresh water extracts were more toxic compare to hot water extracts for germination of ryegrass. The control gave the highest germination percentage, which was 88.33 and 98.33 at 3 and 5 days, respectively followed by concentration at 50 % from hot water and there was no significance than control. It might be due to the heat treatment diluted the phyto-toxicity of milk thistle and which is less toxic to ryegrass germination process.

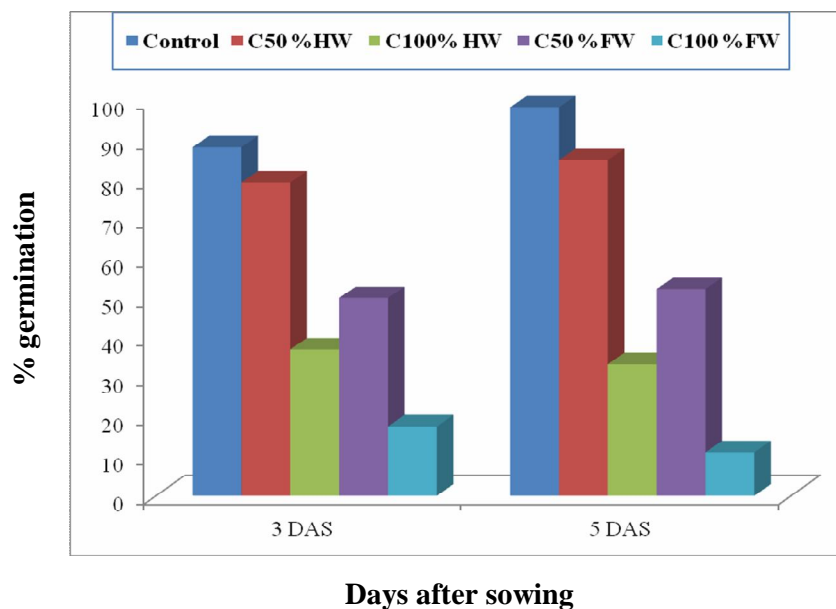


Fig. 1. Effect of milk thistle extracts on germination of annual ryegrass at 3 and 5 days after sowing. LSD at 5% level for 3 and 5 DAS is 18 and 15

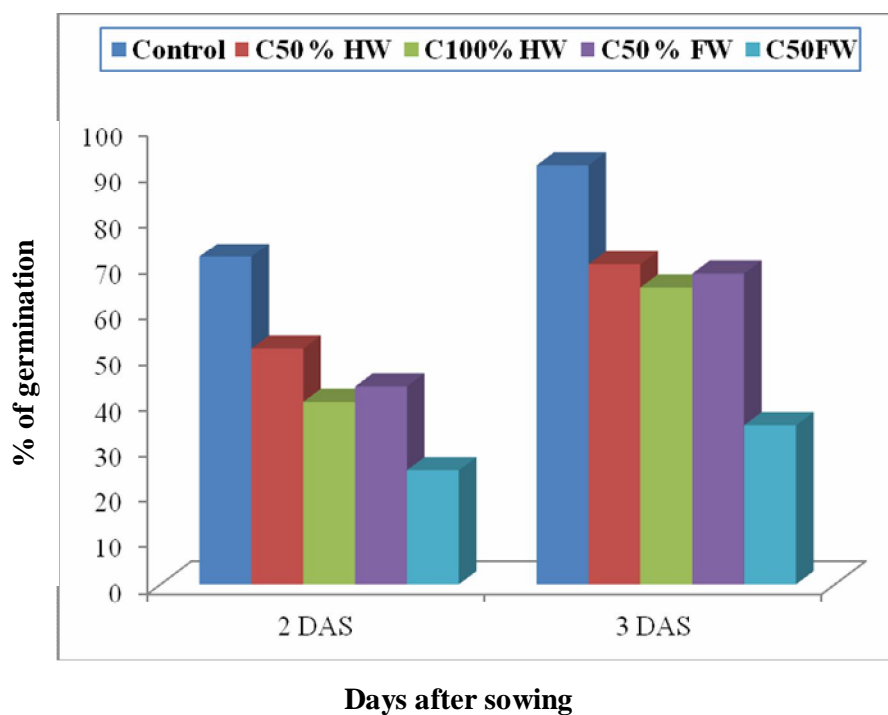


Fig. 2. Effect of milk thistle extracts on germination of canola at 3 and 5 days after sowing. LSD at 5% level for 3 and 5 DAS is 13 and 12

Canola

In canola, the rate of germination was noticeably reduced by all milk thistle extracts at 2 as well 4 days after sowing. Statistically significant lowest percentage of germination 25 and 35 was occurred by extract from fresh water treatment at 100% at 2 and 4 days, respectively. Although at 3 days after sowing there was no statistically noticeable difference among the germination rate caused by concentration at 100%, 50% from hot and 50% from fresh water extracts, but germination rate was significantly reduced by concentration at 100% from fresh water extract. In the contrary, control gave the maximum germination percentages of canola at each observation.

Root and shoot growth

Annual ryegrass

The radical and plumule growth of ryegrass was demonstrably inhibited in both hot and fresh

water extracts especially in fresh water extracts. Significantly, lowest root (12; 45 cm) and shoot length (14; 48 cm) were measured by extracts from fresh water at 100 % and 50 %, respectively (Fig. 3 and 4). These results suggest, due to 24 hours submerged of milk thistle, it released more phytotoxic substances into water that inhibited the seedling growth of ryegrass. The hot water extracts was also inhibited the root growth of ryegrass, although it was less toxic compare to fresh water extracts but root and shoot length was reduced by heat treated extracts at 100 % and 50%. Our finding also reporting that the radical of ryegrass was more sensitive to milk thistle than plumule growth. Similar research also reported separately by Wu *et al.* (1999) and Asaduzzaman *et al.* (2012). They reported that root growth of ryegrass is suppressed more than its shoot growth due to wheat and canola allelopathy.

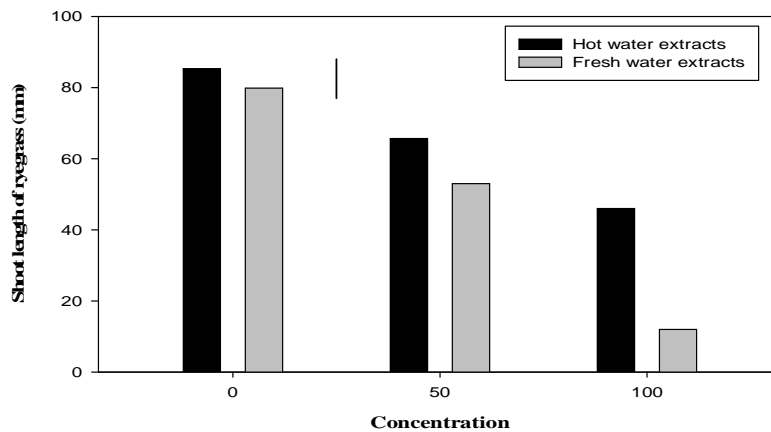


Fig. 3. Allelopathic extract effect of milk thistle on root growth of ryegrass. LSD at 5% level is 11

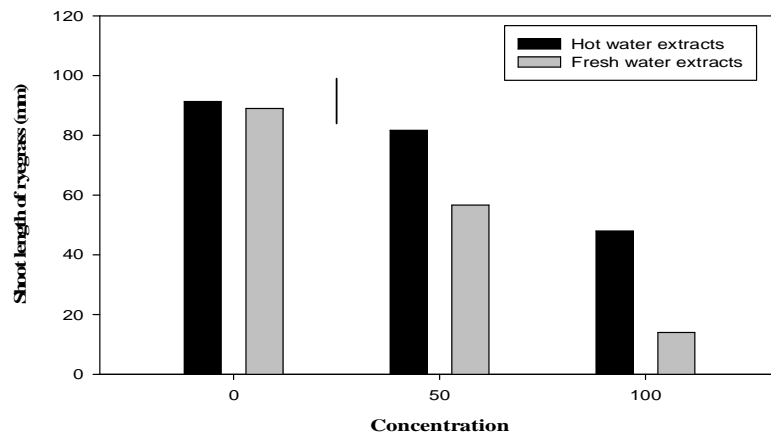


Fig. 4. Allelopathic extract effect of milk thistle on shoot growth of ryegrass. LSD at 5% level is 15

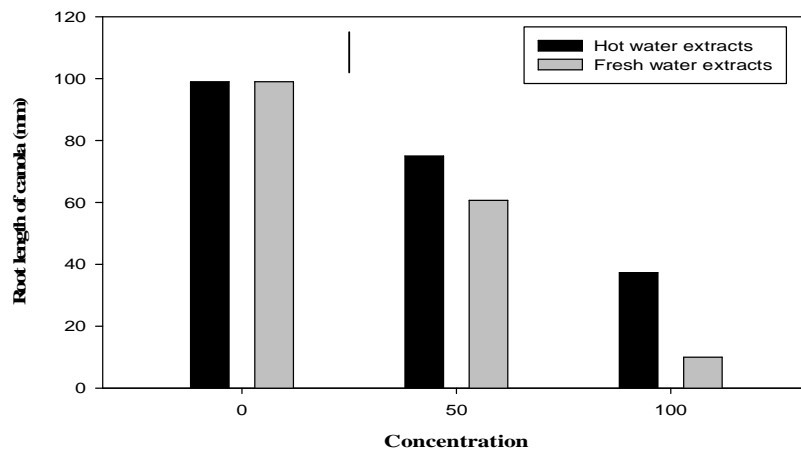


Fig. 5. Allelopathic extract effect of milk thistle on root growth of canola. LSD at 5% level is 13

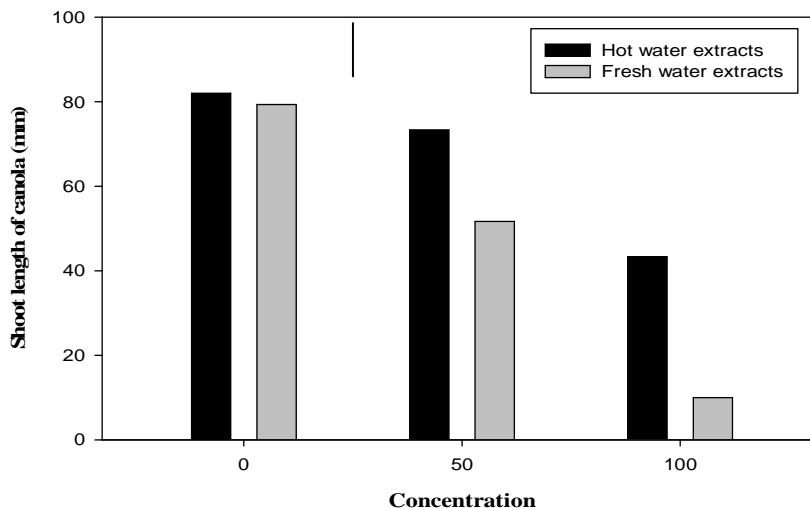


Fig. 6. Allelopathic extract effect of milk thistle on shoot growth of canola. LSD at 5% level is 12.6

Canola

Aqueous extracts from both fresh and hot water samples significantly reduced the root and shoot growth of canola compare to control (Fig. 5 and 6). Similarly, ryegrass, the root and shoot growth of canola was also inhibited by fresh water extracts. Concentration at 100% from fresh water restricted the 89.898% and 87.394% root and shoot length of canola, respectively. Suppression of canola seedling growth may be cause by slow germination that was affected by hydrolysis products of milk thistle into fresh water. Another explanation could be associated with a, increasing extract concentration that increasing the phytotoxicity of donor species, which led to, restricted the cell division and elongation of meristematic tissues of canola. This results are in close to agreement with the findings of Inam and Hussain (1988) who documented the allelopathic

effect of milk thistle and reported that aqueous extracts from the leaves, stems, inflorescences, and roots decreased the germination and early growth of mustard (*Brassica juncea* L.), cucumber (*Cucumis sativus* L.), wheat, and sorghum (*Sorghum bicolor* L.). Separately the maximum root and shoot length were recorded at control followed by extracts from hot water treatment at concentrations 50% and 100%. These results might be due to that, heat treatment diluted or destroy the phytotoxicity of milky substances of donor; therefore, it was less toxic to germination and seedling growth of canola.

Conclusion

In this studies milk thistle demonstrated allelopathic effects on ryegrass and canola including reduced seed germination and reduce

seedling growth. Over all the allelopathic potential of milk thistle increased with increased concentration. In addition, stability and level of phytotoxicity of milk thistle vary on methods for extract preparation and solvent or media. The fresh water extract is more toxic than the heated extract but it is clear that in both condition there is some inhibitory substances presence in milk thistle tissues causing this allelopathicity. These could be used as a potential natural herbicide resource but they must first be identified and their mode of action studies. Future plans are also need to conduct field experiment.

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