*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 3 Number 3 (2014) pp. 389-401**
**http://www.ijcmas.com**

**Original Research Article**

# Allelopathic potential of *Silybum marianum* and its utilization ability as a bio herbicide

**M.A.Elhaak[1*], Mohsen K.H.Ebrahim[2], Fatma Elshintinawy[3] and H.Mehana[1]**

[1]Botany Dept., Fac. Science, Tanta University, Egypt
[2]Biology Department, Faculty of Science, Taibah University, Almadinah Almunawwarah, Saudi Arabia, Permanent: Botany Dept., Fac. Science, Tanta University, Egypt
[3]Department of Biology Faculty of Science Taif University KSA Permanent: Botany Dept., Fac. Science, Tanta University, Egypt
***Corresponding author***

**A B S T R A C T**

The wheat (*Triticum astivum*) cultivars; Saka93, Saka61 and Gmiza9 grains are from Sakha research center. While seeds of its six common weeds (*Vicia sativa*, *Avena fatua*, *Phalaris minor*, *Trifolium resupinatum*, *Euphorbia heliscopia*, *Malva parviflora*) inaddition to *Silybum marianum* plant samples were collected from the wheat fields at Al-Garbia Governorate, Egypt during the flowering and fruiting stages. Distilled water and 0-80% ethanol and acetone concentrations as cold or hot at 70-90◦ C were used to extract the phenolic compounds of *S. marianum* plants parts. Results indicated that extracted amount of phenolic compounds from the seeds, flowers, leaves, and stem of *Silybum marianum* by cold and hot acetone > ethanol > water with a highest from plant flowers and a lowest from plant stem. Cold acetone extracted greater phenolic compounds than the hot one giving it the priority for using while hot ethanol or water extracted the highest phenolic compounds compared with their cold ones. Linear and significant relationship was between extracted phenolic compounds from the different plant organs and acetone or ehanol concentrations. Gimiza 9, Sakha 61 and Sakha 93 wheat cultivars exhibited significantly different germination percentages due to the different extracts of *Silybum marianum* organs. The germination percentages of the three wheat cultivars were slightly or not affected by the different plant extracts. Meanwhile, ethanol extracts completely inhibited the germination of phalaris seeds while leaf extract did the same and completely inhibited the germination of Vicia and Malva. Acetone extract especially for leaf, flowers and seeds of *Silybum marianum* plant in both 20and 30% concentrations completely inhibited the germination of the studied weeds seed. Other plant organs (root and stem) acetone extracts had reduced the germination of the weeds seed especially those of Phalaris. However, *Silybum marianum* plants have an allelopathic effect for wheat crops and weeds in the field and severely on the accompanied weeds. Study suggests the use of extracts from *Silybum marianum* plant, especially cold acetone, as a save natural biological herbicide.

**Keywords**

*Silybum marianum*; phenolic compounds; wheat; allelopathy.

## Introduction

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). It is a major weed in sugar beet wheat and canola (*Brassica napus* L.) causing large yield reductions (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 bicolour* L.) (Inam and Hussain, 1988).

Milk thistle known as *Silybum marianum* (L.) Gaertn., a member of the family Asteraceae. In older literature, as well as some modem European works, it is cited as Carduus marianus L.. Over the years, several other plants have been referred to as milk thistles, but authorities now reserve that common name for this species. Also, it must not be confused with the blessed or holy thistle, which is Cnicus benedictus L., an entirely different plant, although the similarity of the religiously inspired common names is confusing. Members of this genus grow as annual or biennial plants. The erect stem is tall, branched and furrowed but not spiny. The large, alternate leaves are waxy-lobed, toothed and thorny, as in other genera of thistle. The lower leaves are cauline (attached to the stem without petiole). The upper leaves have a clasping base. They have large, disc-shaped pink-to-purple, rarely white, solitary flower heads at the end of the stem. The flowers consist of tubular florets. The phyllaries under the flowers occur in many rows, with the outer row with spine-tipped lobes and apical spines. The fruit is a black achene with a white pappus.

S. marianum is by far the more widely known species. Milk thistle is believed to give some remedy for liver diseases (e.g. viral hepatitis) and the extract, silymarin, is used in medicine. Mild gastrointestinal distress is the most common adverse event reported for milk thistle. The incidence is the same as for placebo. A laxative effect for milk thistle has also been reported infrequently. Milk thistle, also known as the Marian, St. Mary's, or Our Lady's thistle, is a tall herb with prickly leaves and a milky sap. Milk thistle is native to the Mediterranean region of Europe but naturalized in California and the eastern United States.

## Habitat and Genus of milk thistle members

Native to the Mediterranean, grows wild throughout Europe and is widely naturalized in California and Australia. Milk thistle thrives in open areas. Also cultivated as an ornamental plant, milk thistle prefers a sunny position and self-seeds readily. The flower heads are picked in full bloom in early summer. Seeds are collected in late summer. Milk thistle fruit consists of ripe seed of S. marianum (L.) freed from the pappus, and its preparations in effective dosage. The preparation contains silibinin, silydianin, and silychristin. Milk Thistle seeds were consumed by European wet nurses to insure a healthy milk supply. The heads of this Thistle formerly were eaten, boiled,

treated like those of the Artichoke. Milk Thistle seeds help stimulate protein synthesis in the liver. They even can help reverse the damage done from eating poisonous mushrooms or from carbon tetrachloride, which destroy liver cells and usually cause death. When Milk Thistle seeds are used within 48 hours, the survival rate is almost 100%. When fed to animals that had partial hepatectomies, their livers grew back more quickly. Milk Thistle is a good supplement to use to protect the liver when needing to take pharmaceutical drugs.

The erect stem is tall, branched and furrowed but not spiny. The large, alternate leaves are waxy-lobed, toothed and thorny, as in other genera of thistle. The lower leaves are cauline (= attached to the stem without petiole). The upper leaves have a clasping base. They have large, disc-shaped pink-to-purple, rarely white, solitary flower heads at the end of the stem. The flowers consist of tubular florets. The phyllaries under the flowers occur in many rows, with the outer row with spine-tipped lobes and apical spines. The fruit is a black achene with a white pappus.

Only two species are currently classified in this genus: *Silybum eburneum* Coss. & Dur., known as the Silver Milk Thistle, Elephant Thistle, or Ivory Thistle *Silybum eburneum* Coss. & Dur. var. hispanicum *Silybum marianum* (L.) Gaertner, the Blessed Milk Thistle, which has a large number of other common names, such as Variegated Thistle.

A number of other plants have been classified in this genus in the past but have since been relocated elsewhere in the light of additional research. *S. marianum* is by far the more widely known species. It is believed to give some remedy for liver diseases(e.g. viral hepatitis) and an extract, silymarin, is used in medicine. The adverse effect of the medicinal use of milk thistle is loose stools.

The present study conducted to evaluate and determine the germination of the commonly cultivated wheat cultivars and the associated weed under different extracts concentration of the different plant organs of milk thistle. The study also aimed to determine the photo toxicity and stability of milk thistle under heat treatment.

## Materials and Methods

The present study was carried out at Botany Department, Faculty of science, Tanta university, Egypt. Grains of three types of wheat (*Triticum astivum*) cultivars; Saka93, Saka61 and Gmiza9 which under taken for the present study are from Sakha rseach center. Also, seeds of the six common weeds associated to wheat (*Vicia sativa, Avena fatua, Phalaris minor, Trifolium resupinatum, Euphorbia heliscopia, Malva parviflora*) were collected from the wheat fields at Al-Garbia Governorate, Egypt. While samples of *Silybum marianum* were also collected from naturally growing plant at those wheat fields at Al-Garbia Governorate, Egypt during the flowering and fruiting stages of the plant. We separated samples into the main plant parts (roots, stems, leaves, flowers and seeds).

### *S. marianum* samples extraction

Three extracts were prepared by using three different solvents (Distilled water, Ethanol 80%, Acetone 80% ) each extract was carried out under cold and hot at 70-9o◦C and solvents was added to the dried powder of *S. marianum* plants parts,

shaken will, and leaved 2hours for hot extract and 24 hours the cold ones. Then all extracts were filtrated with Whatman filter papers no. 2. The ethanol and acetone solvents were evaporated by using rotatory evaporator and the produced dried extracted materials dissolved in the suitable amounts of distilled water and completed to a definite volume and this solution was considered as the 100 percentage. Hot and cold-water extracts were completed to a similar volume by adding distilled water and this water solution was also considered as 100 percentage. The 100% extracts concentrations were adjusted to be 5g per 100ml water, then two dilutions of this extracts (20% and 30%) are prepared by dilution with distilled water.

**Germination experiment:**

The extracts were applied on Wheat cultivars grains and the weeds seed as irrigation by the different extracts and different concentrations for several times during germination period, (two weeks) whenever needed. The grains and seeds germinated in Petri dishes and observed for percentage of germintion for two weeks. When we could not observe any additional germination experiment stopped. All experiments occur at room temperature. Control experiment has distilled water instead of plant extracts. The germination percentages were recorded daily until the end of the experiment, after two weeks.

**Chemical analysis**

Total phenolic content was estimated in each part of *Silybum marianum* plant. Total phenolic were estimated quantitavely using the method described by Jindal and Singh (1975). A known weight (0.5g) of the dried tissues of each plant part (root, stem, flower and leaves) was extracted by 95% ethanol three times. The clear supernatants were combined and completed to a known volume . Then 1ml from this extract was mixed with 1 ml foline reagent and 1 ml $NA_2CO_3$ (20%), then completed to a known volume with distilled water .Thereafter, the absorbance was measured at 650 nm after 30 minutes. A standard curve was prepared by using different concentrations of pyrogallol as the previous procedure and used for the determination of the total phenolic compounds content (mg/g dry mass) in the plants.

**Statistical analysis**

The result were statistically analyzed using two ways analysis of variance (ANOV) to determine the F test, LSD at 0.05 level and the degree of significance for the obtained variations by different extract solvent, solvent concentration and their interactions. Also, correlation and regression coefficients were applied for investigating the significance of the relationships between the studied variables. All of statistical methods were according to the method described by Bishop(1983), while the analysis was carried out by SPSS statistical package .

## Results and Discussion

**Effect of solvent characteristics on phenolic compounds extraction**

Solvents of different basic capacities as acetone, ethanol and water were used to extract the phenolic compounds of the different plant organs of *Silybum marianum* plant dry powders. The solvents applied as cold or hot and on different concentrations .

**Effect of solvent type:**

*Silybum marianum* plant organs dry samples were extracted by each acetone, ethanol and water. The represented data in Figure (1) indicates that acetone extracted a greater amount of the phenolic compounds from the seeds, flowers, leaves, and stem of *Silybum marianum* as a cold extract. Ethanol followed acetone in th amount of extracted phenolic compounds, while water came after them and extracted the least amount of phenolic compounds from the different organs of *Silybum marianum*.

The highest extracted amounts of phenolic compounds by cold acetone were from the plant flowers followed by seeds while the lowest ones extracted from the plant stem.

It is also important to note that the amount of extracted phenolic compounds by the cold acetone was greater than that amount extracted by the hot acetone fom thedifferent organs of *Silybum marianum.* On the opposite the hot ethanol and water extracted a remarkable high amount of phenolic compounds in comparison with the cold ethanol or water solvent. However, hot water extract more than double amount of stem phenolic compounds in comparison with the cold water. Each of cold and hot ethanol and water extracted higher amounts of the phenolic compounds from *Silybum marianum* plant flowers *Silybum marianum* and the least from the plant stem.

**Effect of solvent concentration**

An increasing concentration of both acetone and ethanol solvents from 0 to 80% was used to extract the phenolic compounds from the different organs of *Silybum marianum* plant. The mean of the extracted phenolic compounds from the different plant organs (Fig. 2) showed a progressive increase in the extracted amount of phenolic compounds with the increase in the concentration of both acetone and ethanol solvent. The relationship between concentration of both solvent and the amount of extracted phenolic compounds was linear and significant as indicated by the $R^2$ values of the regression equation. Acetone extracted greater amount of phenolic compounds from the plant in comparison with ethanol, especially at high concentrations as also indicated from the slopes of the regression equation of the relatinship.

The represented data in Figure (3) indicated that hot acetone and ethanol 80% concentration extracted the highest amount of phenolic compounds from the different *Silybum marianum* plant organs. Also, this concentration (80%) of the cold acetone and ethanol extracted the highest amounts of phenolic compounds from the different plant organs except from the stem where 60% concentration of cold acetone extracted that highest amount followed by 80% concentration. All of the used solvent concentrations extracted high amounts of phenolic compounds from *Silybum marianum* flowers in comparison with the extracted amounts from the other plant organs.

**Germintion of wheat**

The germination of the three commonly cultivated wheat cultivars in the Nile Delta in Egypt followed for studying the effect of the extracted phenolic compounds from the different organs of *Silybum marianum* by the different solvents. The extracted phenolic compounds from the plant organs

by the most extractable solvent, cold acetone and hot ethanol, applied in two concentrations (20 and 30%) and were used on the grain of the wheat cultvars.

The three wheat cultivars (Gimiza 9, Sakha 61 and Sakha 93) showed significantly different germination percentages after treatment grains with both cold acetone and hot ethanol solventsfor phenolic compounds of the root, stem, leaves, flowers and seed of *Silybum marianum* plant extracts (Table 1). The germination percentage of the wheat cultivars showed 100% germination percentage under the control (water). Also, increasing concentration of ethanol extract of the different organs of *Silybum marianum* led to more inhibition in the three wheat cultivars grains percentage of germination. The least germination percentages were exhibited 20and 30% of *Silybum marianum* leaf extracts. Germination percentages (70,70), (40,20) and (40,40) were for Gimiza9, Sakha 61 and Sakha 93respectively showing effectiveness of the used extract concentrations on Sakha 61in comparison with the other two cultivars which showed no differences.

On using acetone extracted phenolic compounds from the different organs of *Silybum marianum*, the percentage of the grains of the three studied wheat cultivars decreased significantly (Table 1). The least germination percentage was 30% and was for Gimiza 9 grains and by extract of *Silybum marianum* leaf. The increase in extract concentration from 20 to 30% did not led to a recognized inhibition in the germination percentage. In both Sakha 61 and 93the least ger4mination percentages were 40 and 70% by 20% leaf extract and 60 and 70% by 30% stem extract respectively.

**Germination of weeds**

The seeds of common weeds in the wheat fields *Vicia sativa, Malva parviflora,* and *Phalaris moir* germination percentages were greatly affected by the treatment with ethanol, acetone and water extracts of the different *Silybum marianum* plant organs (Table 1).

Water extract of the different organs of *Silybum marianum* led to 100% of the Malva seeds germination percentage, while water extract of *Silybum marianum* flowers and seeds completely inhibited the germination of phalaris seeds. Leaf water extract inhibited seed germination of Vicia and phalaris to 40%. Water extract of the other plant organs of *Silybum marianum* did not show any remarkable effect on the germination of the three weeds.

Ethanol extracts of the different organs of *Silybum marianum* completely inhibited the germination of phalaris seeds while leaf extract did the same and completely inhibited the germination of Vicia and Malva. The increase in ethanol extract from 20 to 30% of the different plant organs led to more inhibition in the germination of the three weed seeds.

Acetone extract especially for leaf, flowers and seeds of *Silybum marianum* plant in both 20and 30% concentrations completely inhibited the germination percentage of the three studied weeds seed. Other plant organs (root and stem) acetone extracts had reduced the germination of the three weeds seed especially those of Phalaris.

The weeds influence the crop plants by releasing phyto toxins from their seeds, decomposing residues, leachates, exudates and volatiles (Narwal, 2004). Weeds can

**Fig.1** The variation of extracted phenolic compounds content by hot or cold acetone, ethanol and water

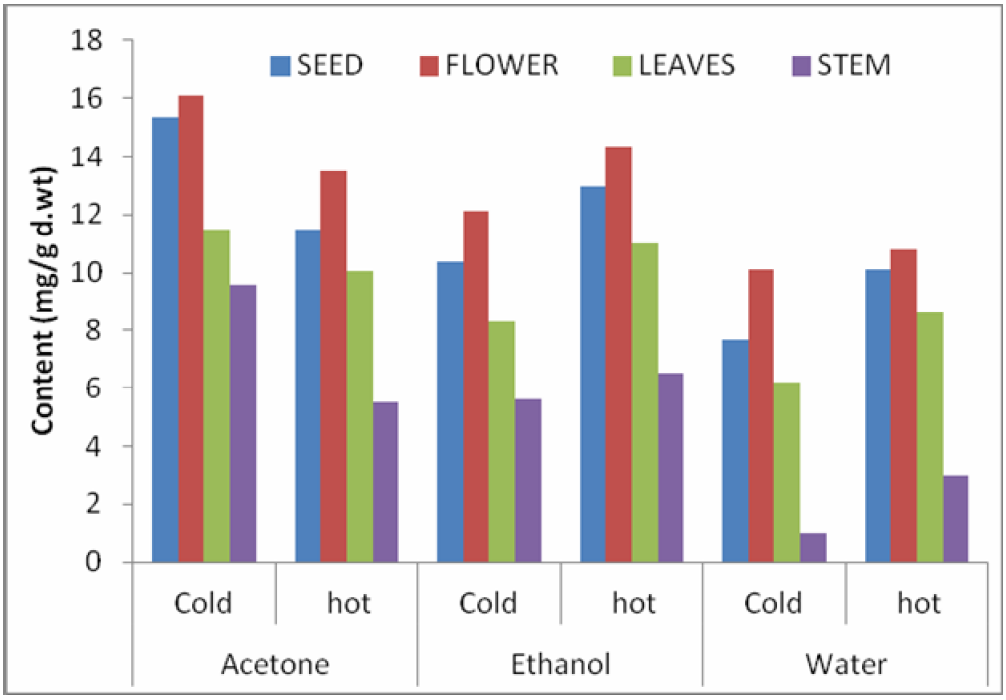


**Fig.2** The variation of extracted phenolic compounds content by the different concentrations of acetone and ethanol

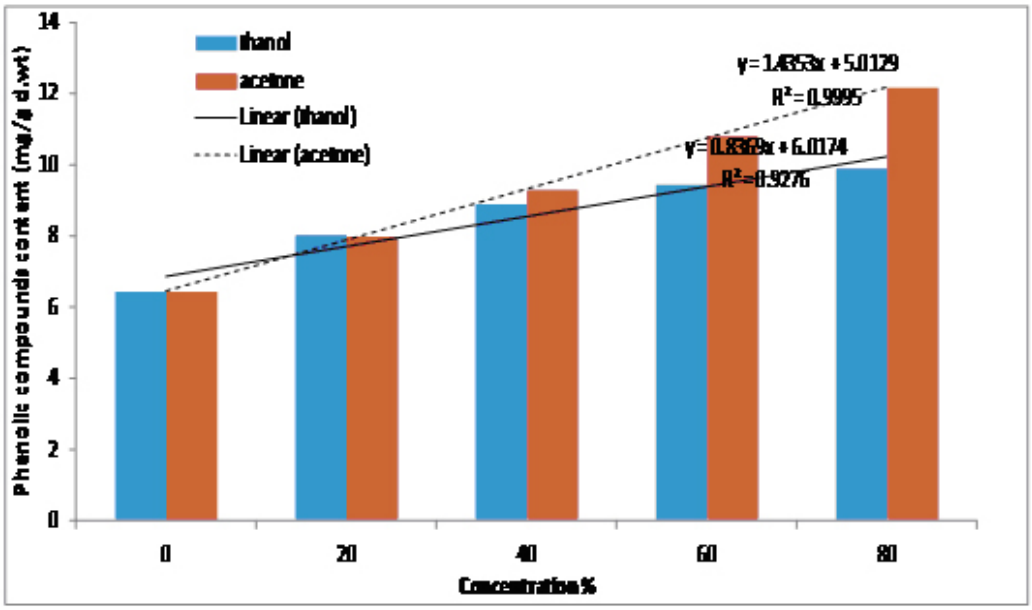

**Fig.3** The variations of extracted phenolic compounds content by hot or cold acetone, ethanol and water from the different organs of *Silybum marianum*.

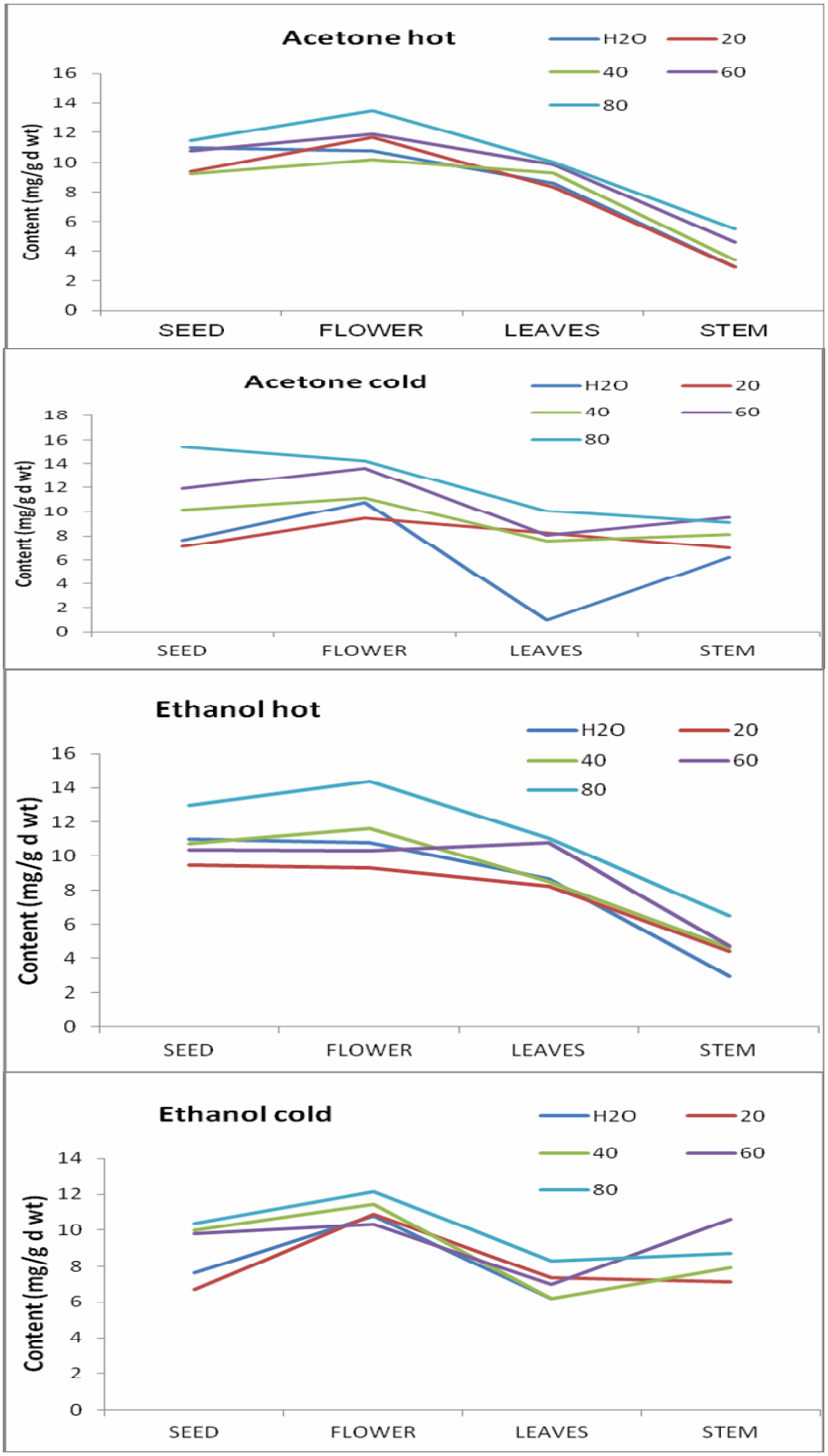

**Table.1** The variations in the germination percentages of some wheat cultivars and weed species under the effect of water, acetone and ethanol extracts of *Silybum marianum* different plant organs

| Species | | Parts | water | acetone | | ethanol | |
|---|---|---|---|---|---|---|---|
| | | | | 20% | 30% | 20% | 30% |
| GMIZA 9 | wheat cultivars | CONTROL | 100 | 100 | 100 | 100 | 90 |
| | | ROOT | 100 | 100 | 100 | 100 | 70 |
| | | STEM | 80 | 100 | 90 | 100 | 100 |
| | | LEAF | 60 | 90 | 60 | 100 | 100 |
| | | FLOWER | 70 | 100 | 80 | 100 | 100 |
| | | SEED | 90 | 100 | 80 | 100 | 90 |
| SAKA 61 | | CONTROL | 100 | 100 | 100 | 80 | 100 |
| | | ROOT | 100 | 100 | 90 | 100 | 90 |
| | | STEM | 100 | 90 | 80 | 100 | 100 |
| | | LEAF | 100 | 40 | 40 | 100 | 90 |
| | | FLOWER | 80 | 90 | 80 | 100 | 90 |
| | | SEED | 90 | 90 | 70 | 100 | 80 |
| SAKA 93 | | CONTROL | 100 | 100 | 100 | 90 | 100 |
| | | ROOT | 100 | 100 | 90 | 100 | 100 |
| | | STEM | 100 | 90 | 80 | 100 | 100 |
| | | LEAF | 100 | 70 | 60 | 100 | 90 |
| | | FLOWER | 90 | 100 | 90 | 100 | 90 |
| | | SEED | 90 | 80 | 90 | 100 | 80 |
| VICIA SATIVA | | CONTROL | 80 | 100 | 100 | 90 | 100 |
| | | ROOT | 80 | 0 | 0 | 60 | 50 |
| | | STEM | 100 | 0 | 0 | 100 | 90 |
| | | LEAF | 40 | 0 | 0 | 40 | 0 |
| | | FLOWER | 80 | 0 | 0 | 90 | 0 |
| | | SEED | 100 | 0 | 0 | 70 | 0 |
| MALVA SP | Weed species | CONTROL | 100 | 100 | 100 | 100 | 100 |
| | | ROOT | 100 | 0 | 0 | 100 | 100 |
| | | STEM | 100 | 0 | 0 | 40 | 0 |
| | | LEAF | 100 | 0 | 0 | 10 | 0 |
| | | FLOWER | 100 | 0 | 0 | 10 | 0 |
| | | SEED | 100 | 0 | 0 | 10 | 0 |
| PHALARIS MINOR | | CONTROL | 100 | 100 | 100 | 80 | 80 |
| | | ROOT | 40 | 0 | 0 | 60 | 60 |
| | | STEM | 70 | 0 | 0 | 70 | 20 |
| | | LEAF | 10 | 0 | 0 | 50 | 40 |
| | | FLOWER | 0 | 0 | 0 | 10 | 0 |
| | | SEED | 0 | 0 | 0 | 0 | 0 |

also affect a crop's growth by releasing allelochemicals into the growing environment (Rice 1984; Kadioglue *et al.*, 2005). Phenolic compounds are the most important secondary product that affects other plants growth in their environment (e.g. Kohli, 1998). Acetone, ethanol and water as basic solvents used to extract the phenolic compounds from the different organs of *Silybum marianum* plant dry powders in cold or hot concentrations indicated that acetone extracted a greater amount of the phenolic compounds from the seeds, flowers, leaves, and stem of the plant as a cold extract. Ethanol followed acetone and extracted a remarkable amount of the plant phenolic compounds while water came after them as it extracted the least amount of phenolic compounds from the different organs of *Silybum marianum*. This will indicate to the inhibition capacity of the extraction of the three solvent to the germination of plants and gave acetone extraction the highest capacity. In addition, stability and level of phytotoxicity or allelopathic capacity of milk thistle vary with methods for extract preparation and solvent or media as suggested by Shamima and Asaduzzaman (2012) . The highest extracted amounts of phenolic compounds by cold acetone were from the plant flowers followed by seeds while the lowest one was from the plant stem. Exhibition of different allelochemicals of the different plant organs was also found by many authors (e. g. Aziz et al. 2008). Veenapani (2004) reported that various parts of weeds show different behavior in exerting their allelopathic effects on crops. It is also important to note that the amount of extracted phenolic compounds by the cold acetone was greater than that amount extracted by the hot acetone from the different organs of *Silybum marianum.* On the opposite the hot ethanol and water extracted a remarkable high amount of phenolic compounds in comparison with the cold ethanol or water solvent. However, hot water extract more than double amount of stem phenolic compounds in comparison with the cold water. Each of cold and hot ethanol and water extracted higher amounts of the phenolic compounds from *Silybum marianum* plant flowers *Silybum marianum* and the least from the plant stem. All plant parts of the weed including leaf, stem, root, and fruit have different amounts of allelochemical (Alam and Islam, 2002; Tinnin and Muller, 2006).

The concentration of both acetone and ethanol solvents was critical in the extraction of phenolic compounds. However, mean of the extracted phenolic compounds from the different plant organs showed a progressive increase in the extracted amount of phenolic compounds with the increase in the concentration of both acetone and ethanol solvents with a linear and significant relationship. Acetone extracted greater amount of phenolic compounds from the plant in comparison with ethanol, especially at high concentrations.

The hot acetone and ethanol at 80% concentration extracted the highest amount of phenolic compounds from the different *Silybum marianum* plant organs while other concentrations of the used solvents extracted high amounts of phenolic compounds from *Silybum marianum* flowers.

*Silybum marianum* grow naturally in the fields of the three commonly cultivated wheat cultivars in the Nile Delta in Egypt. The extracted phenolic compounds from *Silybum marianum* plant organs by the

most extractable solvent, cold and hot acetone, ethanol and water applied in two concentrations (20 and 30%) affected slightly the germination percentages of grain of the three wheat cultvars. The three wheat cultivars (Gimiza 9, Sakha 61 and Sakha 93) exhibited significantly different germination percentages due to the phenolic compounds of the root, stem, leaves, flowers and seed of *Silybum marianum* plant extracts. The water extracts of *Silybum marianum* significantly affected percent of germination for all of the tested cultivated and weed species as indicated by *Rahamdad Khan et al.* (2011). The germination percentage of the three wheat cultivars was 100% under the control (water) and concentrations of ethanol extracts of the different organs of *Silybum marianum* inhibited differently the percentage of germination of the three wheat cultivars grains. The 20 and 30% of *Silybum marianum* leaf extracts caused the least germination percentages for the grains of the three cultivars. Germination percentages were reduced (70,70), (40,20) and (40,40) for Gimiza9, Sakha 61 and Sakha 93respectively showing effectiveness of the used extract concentrations on Sakha 61in comparison with other two cultivars which showed no differences. The inhibition in germination after acetone extracted phenolic compounds from the different organs of *Silybum marianum***,** was remarkable and significant. The least germination percentage was 30% and was for Gimiza 9 grains and by extract of *Silybum marianum* leaf. The increase in extract concentration from 20 to 30% did not led to a recognized inhibition in the germination percentage.

The germination of common weeds in the wheat fields *Vicia sativa, Malva parviflora*, and *Phalaris moi r* was greatly inhibited by the treatment with ethanol, acetone and water extracts of the different *Silybum marianum* plant organs. Water extract of the different organs of *Silybum marianum* did not affect the germination percentages and led to 100% in Malva, while water extract of *Silybum marianum* flowers and seeds completely inhibited the germination of phalaris seeds. Leaf water extract inhibited seed germination of Vicia and phalaris to 40% which is also found by Shamima and Asaduzzaman (2012) for canola and ryegrass.

Ethanol extracts of the different organs of *Silybum marianum* completely inhibited the germination of phalaris seeds while leaf extract did the same and completely inhibited the germination of Vicia and Malva. The increase in ethanol extract from 20 to 30% of the different plant organs led to more inhibition in the germination of the three weed seeds. Acetone extract especially for leaf, flowers and seeds of *Silybum marianum* plant in both 20and 30% concentrations completely inhibited the germination percentage of the three studied weeds seed.

Other plant organs (root and stem) acetone extracts had reduced the germination of the three weeds seed especially those of Phalaris. The previous data may indicate to use the extracted phenolic compounds from *Silybum marianum* plant as a save natural biological herbicide as also suggested by many authors as Kohli (1998) who reported that these natural plant products may provide clues to new and safe herbicide chemistry or growth hormones development. The data showed also, that the effective parts of *Silybum marianum* are considered a waste as plant leaves and flowers.

## References


Alam, S.M. and E. Islam. 2002. Effects of aqueous extract of leaf stem and root of nettle leaf goosefoot and NaCl on germination and seedling growth of rice. Pak. J. Seed Technol. 1: 47-52.

Alam, S.M. and E. Islam. 2002. Effects of aqueous extract of leaf stem and root of nettle leaf goosefoot and NaCl on germination and seedling growth of rice. Pak. J. Seed Technol. 1: 47-52.

Austin, M.P., Fresco, L.F.M., Nicholls A.O., Groves, R.H. and Kaye, P.E. 1988. Competition and relative yield estimation and interpretation at different densities and under various nutrient concentrations using *Silybum marianum* and *cirsium vulgare*. *J. Ecol.,* 76: 157–171.

Aziz, A., A. Tanveer, M. Ali, M. Yasin, B.H. Babar and M.A. Nadeem. 2008. Allelopathic effect of cleavers (*Galium aparine*) on germination and early growth of wheat (*Triticum aestivum*). Allelopathy J. 22: 25-34.

Chambreau, D. and MacLaren, P.A. 2007. Got milk thistle? An adaptive management approach to eradicating milk thistle on dairies in King county, Washington state. pp. 107-109. In: *Meeting the Challenge: Invasive Plants in Pacific Northwest Ecosystems* (ed. by Harrington T.B. and Reichard S.H.). General technical report. No. PNW-GTR- 694, June. Pacific Northwest Research Station, Forest Service, United States Department of Agriculture, Portland.

Dodd, J. 1989. Phenology and seed production of variegated thistle, *Silybum marianum* (L.) in Australia in relation to mechanical and biological control. *Weed. Res.,* 29: 255–263.

Groves, R.H. and Kaye, P. E. 1989. Germination and phenology of seven introduced histle species in Southern Australia. *Aust. J. Bot.,* 37: 351–359.

Inam, B. and Hussain, F. 1988. Allelopathic effects of *Silybum marianum* Gaertn. *Sarhad J. Agric.,* 4: 481–494.

Kadioglue, I., Y. Yanar and U. Asav. 2005. Allelopathic effects of weed leachates against seed germination of some plants. J. Environ. Biol. 26: 169-173.

Khan, M.A. and Marwat, K.B. 2006. Impact of crop and weed densities on competition between wheat and *Silybum marianum* Gaertn. *Pak. J. Bot.,* 38: 1205–1215.

Kohli, R.K. 1998. Allelopathy and its implications in agroecosystem *In* A.S. Basra (ed.). Crop Science and Recent Advances. Haworth Press. Inc. pp. 205-209.

Qasem, J.R. and Foy, C.L. 2008. Weed allelopathy, Its ecological impacts and future prospects. *J. Crop Prod.,* 4(2): 43-119.

Rahamdad Khan Muhammad Azim Khan1, Waheedullah1, Muhammad Waqas1, Abdul Majid Khan, Zahid Hussain, Adres Khan and M.A. Raza, 2011. Allelopathic potential of *Silybum marianum* l. against the seed germination of edible legumes Pak. J. Weed Sci. Res. 17(3): 293-302

Rice, E.L. 1984. Allelopathy. 2nd Ed. Acad. Press. Inc. Orlando.Florida, USA.

Shamima S. and Asaduzzaman MD. 2012. Allelopathic studies on milk thistle (*Silybum marianum*). Int. J. Agril. Res. Innov. & Tech. 2 (1): 62-67, June, 2012 Available online at http://www.ijarit.webs.com

Shimi, P., Poorazar, R., Jamali, M. and Bagherani-Torshiz, N. 2006. Evaluating clopyralid as a broad leaf

herbicide in canola fields of Iran. *Pak. J.Weed Sci. Res.,* 12: 307–311.

Tinnin, R.O. and C.H. Muller. 2006. The allelopathic influence of Avena fatua. The allelopathic mechanism. Bulletin Torrey Bot. Club.99: 287-292.

Veenapani, D. 2004. Inhibition in seed germination of *Oryza sativa* (paddy) by two weed species. Flora and Fauna, 10: 11-12.

Young, J.A., Evans ,R.A. and Hawkes, R.B. 1978. Milk thistle (*Silybum marianum*) seed germination. *Weed Sci.,* 26: 395–398.

Suneetha Parameswarappa, Chandrakant Karigar and Manjunath Nagenahalli. 2008. “Degradation of ethylbenzene by free and immobilized”, Biodegrad.19:137–144.

US EPA.,1996. “Priority pollutants”, Code of Federal regulations, Title 40, Chapter 1, Part 423, Appendix A. Environmental Protection Agency, Washington, DC.