INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–417/2016/18–2–464–470
DOI: 10.17957/IJAB/15.0139
http://www.fspublishers.org

Full Length Article

Seed Enhancement of *Silybum marianum* and Optimization of Silymarin Extraction

Nazish Jahan[1*], Khalil-ur-Rahman[2], Shahzad M.A. Basra[3], Siddra Sajid[1] and Irfan Afzal[3]
[1]*Department of Chemistry, University of Agriculture Faisalabad, Pakistan*
[2]*Department of Biochemistry, University of Agriculture Faisalabad, Pakistan*
[3]*Department of Agronomy, University of Agriculture Faisalabad, Pakistan*
[*]For correspondence: nazishjahanuaf@yahoo.com

Abstract

The present study elucidates the impact of different priming strategies on seed emergence and growth of milk thistle [*Silybum marianum* (L.) Gaertn.] and also evaluation of the efficacy of various extraction methods for optimal extraction of silymarin from milk thistle seeds. Seeds were primed with distilled water, Humic acid (2%), potassium chloride (2%) and moringa leaf extract (3%) for 24 h. Untreated seeds were used as control. Primed and non-primed seeds were sown in plastic pots. Seed priming treatments significantly improved the performance of milk thistle seeds as indicated by higher values of final emergence and lower values of mean emergence time and time taken to 50% emergence as compared to non-primed seeds. Silymarin was extracted from seeds of milk thistle by applying various techniques like Reflux, Soxhlet, maceration and microwave assisted extraction by using various solvents. Extraction with Soxhlet and microwave assisted techniques were proved better as compared to the others. The results indicated that in both spectrophotometric and HPLC quantification, silymarin contents were found maximum in extracts prepared with methanol by microwave assisted extraction technique followed by Soxhlet.

Keywords: Silymarin; *Silybum marianum;* Seed priming; Extraction methods

Introduction

Silybum marianum (L.) Gaertn., commonly called as milk thistle, is a known hepatoprotective plant and grows natively in the Mediterranean area (Alvarez *et al.*, 2003; Parmoon *et al.*, 2015). Its leaves are large, typically growing 10 cm in width and 30 to 40 cm in length. It is an annual or biennial plant which is naturally occurred in various parts of world and is widely spread in the dry and warm climates of Europe, America and Australia (Radjabian *et al*., 2008). Being an important hepatoprotective medicinal plant, needs to be cultivated at large, to produce the raw material for herbal pharmaceutical industries.

As germination and seedling establishment are the critical stages in the life cycle of plants, thus vigorous seed germination and stand establishment are highly desirable for better yield of crops (Windauer *et al.*, 2007). The optimum temperature required for germination of milk thistle seeds is between 28–29ºC (Heidari *et al.*, 2014), however, germination mostly affected due to broad range of temperature variation in the field environment. Keeping in view the importance of milk thistle for production of flavonoids of the silymarin group, the cultivation and production of this medicinal plant is essential for the pharmaceutical industries. But the hurdle to high yield and production of this plant is the lack of synchronized crop establishment (Foley and Fennimore, 1998: Heidary *et al.*, 2014). Seed priming is being utilized commercially to enhance the speed and uniformity of crop establishment (Afzal *et al.*, 2015). During priming, seeds are hydrated partially for certain period of time followed by redrying that can accelerate their germination when they are subsequently planted. The addition of natural growth promoters like moringa leaf extract during priming can further enhance the performance of seeds in the field (Afzal *et al.*, 2012a; 2015). Recently, Nasiri *et al.* (2014) also found an improvement in the germination potential of milk thistle seeds under saline conditions due to application of salicylic acid as priming agent.

Medicinal plants with less or no side effects than chemical drugs are becoming popular day by day. Various parts of the milk thistle, particularly seeds possessing antioxidant, antifungal and immunomodulator potential are in use of alternative medical practitioners for treatment of variety of disorders. This plant is a natural blend of many pharmaceutically important compounds which may contribute a lot as a natural health healer for treatment of liver disorders. Seeds of this plant possess flavonolignan (silymarin) in high concentration, which is used as strong antihepatotoxic therapeutic agent against almost every kind of human liver disease (Schuumann *et al.*, 2003; Pradhan

To cite this paper: Jahan, N., K.U. Rahman, S.M.A. Basra and S. Sajid, 2016. Seed enhancement of *Silybum marianum* and optimization of silymarin extraction. *Int. J. Agric. Biol.*, 18: 464–470

and Girish, 2006; Qato *et al*., 2008). In addition to its antioxidant properties (Varga *et al*., 2006), it has been reported to have high anti-tumor promoting activity (Roy *et al*., 2012). Many studies have also reported that silymarin is an effective antiviral treatment for hepatitis C virus (Wagoner *et al*., 2010; Ramasamy and Agarwal, 2008) and having anti-inflammatory, antifibrotic (Fuchs *et al*., 1997) and antiproliferative effects (Tyagi *et al*., 2002).

The extraction step is very important to increase the quality of products. During extraction desired molecules diffuse into solvent phase from the bulk herb. Therefore, extraction method needs to be characterized by optimizing the extraction rate with appropriate solvents (Barreto and Clausen, 2002). Various methodologies like reflux extraction, Soxhlet extraction, pretreatments with Soxhlet, maceration (with and without shaking) and microwave assisted extraction can be applied for optimal production of silymarin (Dibert *et al*., 1989; Alvarez *et al*., 2003; Wallace *et al*., 2005; Mani *et al*., 2007).

Keeping in view the importance of milk thistle in the world including Pakistan, the Present study was carried out to explore the potential of seed priming on emergence potential, yield and yield related traits of milk thistle under our own climatic conditions. Furthermore, the efficacy of various extraction methods was also evaluated for optimal extraction of silymarin from milk thistle seeds.

Materials and Methods

Two independent experiments were conducted to explore the potential of priming on emergence and growth of milk thistle and optimize the extraction of silymarin from milk thistle seeds.

Experiment 1

Effect of seed priming on emergence, stand establishment and growth of milk thistle seed: Pot experiment was conducted under net house conditions in the Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan. Earthen pots were filled with 15 kg of a mixture of soil and compost (3:2 ratios). Primed and unprimed seeds of milk thistle were sown in the pots. The experiment was laid out in completely randomized design with five replications. Pots were irrigated uniformly when needed. Daily emergence of seeds was counted.

Seed Priming Protocol: Seeds of milk thistle were soaked in aerated solutions of 2% humic acid, 2% potassium chloride, 3% moringa leaf extract in a beaker for 24 h. Fresh moringa leaf extract was prepared by following the extraction method of Basra *et al.* (2011). For hydropriming, seeds were soaked in water for 24 h. The solutions were continuously aerated with aquarium pump. After soaking, the seeds were thoroughly washed with distilled water and dried under forced air. Untreated seeds were taken as control.

Measurements: Number of seedlings emerged were counted daily according to the seedling evaluation Handbook of Association of Official Seed Analysts (1990). Time to 50% emergence (E_{50}) was calculated following the formulae of Coolbear *et al*. (1984). Mean emergence time (MET) was calculated following Ellis and Robert (1981).

Agronomic traits: Four plants were selected at random from each pot. Number of leaves was counted and then averaged to get number of leaves. Numbers of days from emergence to peak flowering were taken as time to maximum flowering was completed. In each pot the time of maturity was recorded from different sites and then average was calculated which is almost 150 days (5 months)

Experiment II

Optimization of extraction method of Silymarin from milk thistle seeds: Second experiment on optimization of extraction method of silymarine from milk thistle seeds was conducted in the Department of Chemistry and Biochemistry, University of Agriculture Faisalabad. For this purpose, five extraction techniques i.e. reflux, Soxhlet, seed pretreatment, maceration and microwave-assisted extraction were employed for the extraction of silymarin from milk thistle seeds. Extraction was carried out with methanol and ethanol solvents.

i. Extraction with reflux: Dry powder (30 g) of seeds was refluxed with 100 mL of *n*-hexane for defatting and this defatted powder was extracted with ethanol and methanol solvents. After extraction, the extract were filtered, concentrated in a rotary evaporator and stored in refrigerator for further analysis (Aslam *et al*., 2012).

ii. Extraction with soxhlet: A Soxhlet apparatus, equipped with a 500 mL boiling flask, was used for extraction. Powdered seeds (30 g) was placed in a cellulose extraction thimble, 300 mL of *n*-hexane was used as defatting solvent. Then this defatted powder was treated with 300 mL of ethanol and methanol solvents (Cagdas *et al*., 2011).

iii. Extraction with maceration: Milk thistle seeds (10 g) were defatted with 30 mL *n*-hexane by maceration with and without shaking method. Then this defatted powder extracted with 30 mL solvent. Different time periods like 24, 36 and 48 h and solvents like methanol and ethanol were applied to get the optimum extraction. Extracts were filtered and concentrated using rotary evaporator.

iv. Soxhlet with pretreated seeds: Seeds were treated with 1.2% sodium hydroxide, 1.5% sulphuric acid, 2% sodium bicarbonate as alternatives of n-hexane defatting. Seeds soaked in solvents were placed in orbital shaker at 60°C for 24, 36 and 48 h. After the pretreatment, samples were filtered and centrifuged for 10 min. Then seed residue was air dried for 24 h at room temperatures. This pretreatment was done for defatting, after this seeds were extracted with Soxhlet technique (Subramaniam *et al*., 2008).

v. Microwave assisted-extraction: Powdered seeds (5 g) soaked in 95 mL solvent were placed in microwave oven made for extraction purpose. Extraction was carried out with

two irradiance time (1 and 2 min) and with two different solvents (methanol and ethanol). Firstly seeds were defatted and then extract was prepared (Aslam *et al*., 2012).

Quantification of Extracts

A simple, rapid and sensitive spectrophotometric method was used for estimation of silymarin. To make the standard curve, the solutions of silymarin (10 to100 µg/mL) were prepared in methanol. Solutions of silymarin extracted from seeds of milk thistle (10 µg/mL) in methanol were prepared and their absorbance was noted at 287 nm by spectrophotometer and concentration of silymarin was calculated with the help of standard graph.

The estimation of silymarin was also performed on HPLC (Shimadzu, Japan) consisting of pump LC-10AT, UV-VIS detector 2SPD-10AV. The analysis of silymarin samples was carried out by using C18 column. A mixture of phosphoric acid-methanol-water was used as mobile phase. The elution was made in an isocratic mode at a flow-rate 1 mL/min and the UV-detector was used with wavelength at 288 nm (Radjabian *et al*., 2008).

Statistical Analysis

The data were analyzed by using one way ANOVA. Experiment on seed priming was laid out in Completely Randomized Design. Treatment means were compared by using Least Significantly Difference (LSD) test at $P \leq 5\%$.

Results

Effect of seed Priming on Emergence Potential and Growth Attributes of Milk Thistle

Seed priming treatments improved emergence potential of milk thistle seeds by reducing mean emergence time (MET) and increasing final emergence percentage (FEP) compared with the control (Table 1). Lowest value for time taken to 50% emergence was observed in KCl treated seed. All priming treatments significantly reduced mean emergence time of seeds. Maximum FEP was recorded for MLE treated seeds. Similarly higher emergence index was recorded in seeds primed with KCl followed by MLE and humic acid as compared to hydroprimed and untreated seeds.

Seed invigoration strategies also significantly affected some of growth related attributes (Table 2). Priming with KCl and humic acid maximally improved number of leaves and flowers per plant grown in the pot while, number of fruit bearing branches and pods per plant were not significantly improved with the application of seed priming treatments (Table 2).

Extraction of Silymarin

The extraction conditions such as temperature and extraction time for maximum extraction were optimized. Efficiency of extraction technique was evaluated by quantification of silymarin. Microwave-assisted extraction technique was found effective for the extraction of silymarin contents from *Silybum marianum* and maximum yield (11.2%) was obtained with solvent methanol (Table 3). The percentage yield of extract was greater when extracted through maceration with shaking (7.8%) in comparison with maceration without shaking (5.8%). The yield was gradually increased with time from 24-48 h; however, there is no significant difference in yield from 36 to 48 h. So, optimum time for better yield was considered as 36 h. Better yield in term of % yield was found in maceration with shaking than maceration without shaking, while methanol was found relatively better solvent as compared to ethanol.

The yield of ethanolic extract was 6.7% and 3.45% with Soxhlet and reflux techniques respectively. In this way the Soxhlet technique was proved better than reflux. The yield with Soxhlet extraction after pretreatment of seeds with sodium hydroxide, sulphuric acid and sodium bicarbonate as defatting solvent was 6.5%. Finally it was inferred that the yield of silymarin extract was same with Soxhlet, either the seeds were defatted with n hexane or pretreated with inorganic acid/bases.

Quantification of Silymarin

Maximum contents of silymarin were observed in methanolic extract prepared by Microwave-assisted (55 mg g^{-1}) followed by Soxhlet (27 mg g^{-1}), Reflux (18.7 mg g^{-1}, maceration with shaking (17.7 mg g^{-1}) extraction procedures (Table 4). Amongst the different extracting solvents, the methanolic extract showed higher amount of silymarin, so HPLC analysis was carried out only with methanolic extracts. The concentrations of silymarin detected in various extracts of milk thistle seed have been shown in Table 5. Results of HPLC analysis revealed that maximum concentration (1813.3 mg g^{-1}) of silymarin was observed in extract of microwave-assisted extraction technique followed by Soxhlet technique (1656.5 mg g^{-1}). Silymarin content was 1419.9 and 281.55 mg g^{-1} in extracts obtained through maceration with and without shaking, respectively. Less content of silymarin (968.12 mg g^{-1}) was noted in the extract processed through reflux technique as compared to maceration with shaking (1419.9 mg g^{-1}) method. Content of silymarin was 722.8 mg g^{-1} in extract prepared by Soxhlet after pretreatment of seeds with acids and base (Fig. 1).

Discussion

As milk thistle is among top selling herbs in the world (Nasiri *et al.*, 2014), in Pakistan its demand is increasing a lot due to uncontrolled spread of hepatitis. However, lack of synchronized crop stand results in lower yield of this important plant species due to genetic and environmental factors (Foley and Fennimore, 1998; Heidary *et al.*, 2014).

Table 1: Influence of seed priming treatments on emergence potential of milk thistle seeds

Priming treatments	Final emergence (%)	Mean emergence time (days)	Time taken to 50% emergence (days)	Emergence index
Control	86	16.42 a	12.22 a	5.29 b
Hydropriming	90	15.28 b	10.00 ab	7.22 a
Osmopriming with KCl	90	15.36 b	9.37 b	7.30 a
Osmopriming with Humic acid	90	15.63 b	10.52 ab	6.76 ab
Priming with MLE	94	15.54 b	10.27 ab	7.14 a
Critical value for comparison	13.32	0.62	2.77	1.60

Table 2: Effect of seed priming treatments on growth attributes of Milk Thistle

Priming treatments	No. of fruit bearing branches	Number of Flowers	Number of leaves	Pods per plants
Control	4.8	4.2	16.8 ab	6.2
Hydropriming	4.6	5.2	16.4 ab	6.2
Priming with KCl	4.4	6	18 a	5.8
Priming with Humic acid	4.8	6.2	18.6 a	6.6
Priming with MLE	4.4	5.2	14.6 b	6
Critical value for comparison	1.28	2.36	2.82	1.8

Table 3: Percentage yield of different extracts

Techniques	Solvent	Time	Percent yield
Maceration with shaking	Ethanol	24	2.2
		36 h	6.4
		48 h	6.3
	Methanol	24 h	6.4
		36 h	7.7
		48 h	7.8
Maceration without shaking	Ethanol	24 h	4.1
		36 h	5.8
		48 h	5.9
	Methanol	24 h	3.2
		36 h	5.8
		48 h	5.3
Reflux	Methanol	1 h	4.13
	Ethanol	1 h	3.45
Soxhlet without pretreatment of seed	Ethanol	6 h	6.7
	Methanol	1 min	11.2
Microwave		2 min	10
	Ethanol	1 min	9.6
		2 min	9.2
Soxhlet with pretreatment	Sodium hydroxide, Sulphuric acid, sodium bicarbonate	24 h	6
		36 h	6.5
		48 h	6.3

Table 4: Concentration of Silymarin in extracts

Techniques	Solvent	Time (h)	Concentration(mg g^{-1})
Maceration with shaking	Ethanol	24 h	9.2
		36 h	10.2
		48h	10.2
	Methanol	24 h	14.4
		36 h	17.7
		48 h	16
Maceration without shaking	Ethanol	24 h	6
		36 h	7
		48 h	10.2
	Methanol	24 h	6
		36 h	7
		48 h	7
Reflux	Ethanol	1 h	14.4
	Methanol	1 h	18.7
Soxhlet without pretreatment of seeds	Ethanol	6 h	22.9
Microwave assisted extraction	Ethanol	1 min	56.6
		2 min	53.4
	Methanol	1 min	57.6
		2 min	55.6
Soxhlet With Pretreatment of seeds	Sodium hydroxide, sulphuric acid, sodium bicarbonate	24	21.9
		36	27
		48	26

Table 5: Silymarin content in milk thistle seed quantified by HPLC

Extraction techniques	Concentration (mg g^{-1})
Microwave assisted extraction	1813.3
Soxhlet after n-hexane defatting of seeds	1656.5
Soxhlet after pretreatment of seeds	722.8
Maceration without shaking	93.8
Maceration with shaking	1419.9
Reflux	968.12

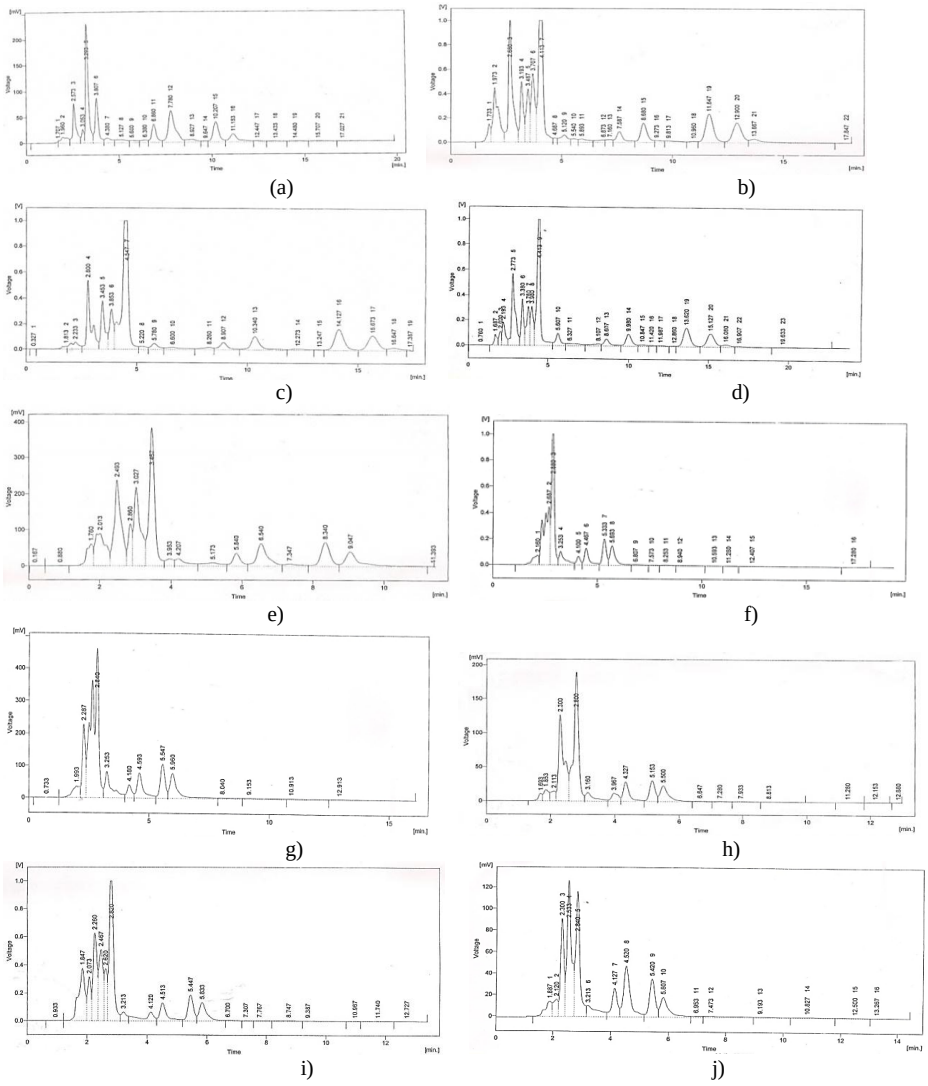

Fig. 1: HPLC chromatograms of seed extracts of milk thistle showing silymarin contents
a). Standard, b) extract prepared with microwave assisted extraction c) Extract prepared by soxhlet, d) Extract prepared by mercerization with shaking, e). Extract prepared by reflux, f & g. Extract prepared by soxhlet after pretreatment, h & i). Extract prepared by maceration without shaking, j) Extract prepared by maceration with shaking

Seed priming not only improve stand establishment by reducing emergence time and harmonize germination but also help in widening the temperature range required for germination which ultimately leads to higher crop yield of many horticultural and agronomic crops (Basra *et al.*, 2011; Afzal *et al.*, 2015). In the current study, priming with KCl followed by MLE and humic acid maximally improved seed performance in terms of reduction in mean emergence time with increased final emergence and emergence index compared with the control. The reason for this improved stand establishment is that MLE is rich in zeatin, nutrients and vitamins which might transfer to the growing embryo during the priming germination *sensu stricto* phase (Afzal *et al.*, 2012a). Powell *et al.* (2000) also pointed out that activation of metabolic repair during priming resulted in improved quality of primed seeds. Thus faster production of

germination metabolites and better macromolecular repair was responsible for early emergence in primed seeds (Basra *et al*., 2011). Similarly, Jowkar *et al*. (2012) concluded that faster germination in primed seeds of milk thistle was due to enhanced activities of antioxidant enzymes and higher soluble protein levels in the seed. Other researchers have also declared the beneficial effects of priming for improvement of emergence potential and stand establishment of milk thistle seeds under abiotic stresses (Parmoon *et al*., 2013; Nasiri *et al.*, 2014). Improved early seedling growth, nutrient partitioning and higher chlorophyll contents are important manifestations of seed priming with Ca and K salts (Afzal *et al.*, 2012b). Therefore, an increased number of leaves per plant in present study (Table 2) are due to early emergence time and rate of milk thistle seeds.

Microwaves assisted extraction was established as best method for maximum yield of silymarin. Spectrophotometric quantification and. HPLC analysis (Table 5; Fig. 1) revealed the highest content of silymarin in methanolic extract that was prepared by microwave assisted extraction. Microwave radiations may reduce the extraction time and relatively lesser solvent is needed for extraction. Therefore researchers have been attracted more towards microwave assisted extraction (Radjabian *et al.*, 2008; Rafiee *et al*., 2011; Zhang *et al.*, 2011; Wong Paz *et al.*, 2014). Microwave extraction is potential alternative of conventional liquid solvent extraction methods (Yemis and Mazza, 2012). It can be inferred that there was non-significant difference of both solvents methanol and ethanol regarding silymarin extraction potential from seeds of milk thistle. The conventional methods like reflux, Soxhlet and maceration are frequently applied for extraction of plant bioactive components (Spigno *et al.*, 2007; Elwekeel *et al*., 2013). In soxhlet extraction fresh solvent can interact with sample repeatedly. Extraction of silymarin with Soxhlet is required two processes in which first seed is defatted with *n*-hexane and then extracted with other solvents like ethanol or methanol etc. (Radjabian *et al*., 2008). Maceration is very simple method in which sample is soaked in solvent for a long time.

Solvent extraction is commonly used for extraction and isolation of silymarin flavonoids from milk thistle. An extracting solvent is selected according to the polarity of bioactive compound, cost and safety. Various solvents like water, ethanol, methanol and ethyl acetate have been reported for extraction of Silymarin (Wallac *et al*., 2008; Xin *et al*., 2008; Tan *et al*., 2013). Methanol extracted significantly high silymarin contents. However, ethanol is also a good solvent that can be used for extraction of silymarin as its extraction potential is very close to methanol in some procedures. Owing to less toxicity and more environment friendly nature, the ethanol can be preferred for the extraction of silymarin. HPLC quantification of silymarin proved that after microwave assisted extraction, Soxhlet technique exhibited better results. Ethanolic extract of milk thistle prepared by Soxhlet technique contained good quantity of silymarin. Extraction with maceration showed yield almost similar to Soxhlet technique but HPLC analysis confirmed that silymarin contents were much higher in the extract prepared by Soxhlet extraction *n*-hexane is the most commonly used solvent for defatting seeds. In this study, efforts have been made to replace the *n*-hexane with aqueous solution of acids and base (Subramaniam *et al*., 2008). For this purpose seeds were pretreated with NaOH (1.2%), H_2SO_4 (1.5%) and $NaHCO_3$ (2%) at various time intervals. Similar contents of silymarin, extracted after *n*-hexane defatting and pretreatment of seeds with solution of acid and base, revealed that *n*-hexane may be replaced with aqueous solution of acids/bases. But HPLC analysis explored that silymarin contents are relatively less in the extracts prepared after pretreatment of seeds with acid/base as compared with *n*-hexane defatting. Therefore, more studies are to be conducted to confirm that either *n*-hexane may be replaced with aqueous solution of acids/bases or not.

Conclusion

It can be concluded from both studies, that seed priming substantially improved emergence, stand establishment and growth of milk thistle. Furthermore, microwaves assisted and Soxhlet extractions were proved better as compared to the other adapted procedures regarding percent yield and silymarin contents.

Acknowledgement

The authors are highly thankful to Endowment Fund Secretariat, University of Agriculture, Faisalabad, Pakistan for providing financial support under a Project No. TT- 04-12).

References

Afzal, I., M.A. Noor, M.A. Bakhtavar, A. Ahmad and Z. Haq, 2015. Improvement of spring maize (*Zea mays*) performance through physical and physiological seed enhancements. *Seed Sci. Technol*., 43: 1–12

Afzal, I., B. Hussain, S.M.A. Basra and H. Rehman, 2012a. Priming with MLE reduces imbibitional chilling injury in spring maize. *Seed Sci. Technol.*, 40: 271–276

Afzal, I., A. Butt, H. Rehman, S.M.A. Basra and A. Afzal, 2012b. Alleviation of salt stress in fine aromatic rice by seed priming. *Aust. J. Crop Sci*., 6: 1401–1407

Basra, S.M.A., M.N. Iftikhar and I. Afzal, 2011. Potential of moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *Int. J. Agric. Biol*., 13: 1006–1010

Alvarez, J.F., S.N. Wallace, D.J. Carrier and E.C. Clausen, 2003. Extraction of nutraceuticals from milk thistle: Part I. Hot water extraction. *J. Appl. Biochem. Biotechnol*., 105: 881–889

Aslam, S., N. Jahan, S. Ali and K.U. Rahman, 2012. An innovative microwave-assisted extraction and antioxidant potential of polyphenols from different parts of *Ocimum basilicum*. *J. Med. Plants Res.*, 6: 2150–2159

Dibert, K., E. Cros and J. Andrieu, 1989. Solvent extraction of oil and chlorogenic acid from Mani, S., S. Jaya and R. Vadivambal. 2007. Optimization of solvent extraction of Moringa *(Moringa Oleifera)* seed kernel oil using response surface methodology. *J. Food Bioprod. Process*, 85: 328–335

Elwekeel, A., A. Elfishawy and S. AbouZid, 2013. Silymarin content in Silybum marianum fruits at different maturity stages. *J. Med. Plants Res*., 7: 1665–1669

Foley, M.E. and S. A. Fennimore, 1998. Genetic basis for seed dormancy. *Seed Sci. Res.*, 8: 173–182

Fuchs, E.C., R. Weyhenmeyer and O.H. Weiner, 1997. Effects of silibinin and of a synthetic analogue on isolated rat hepatic stellate cells and myofi broblasts. *J. Arzneimittel Forschung-Drug Res.*, 47: 1383–1387

Heidari, Z., B. Kamkar and J. Masoud Sinaki, 2014. Determination of Cardinal Temperatures of Milk Thistle (*Silybum marianum* L.) Germination. *Adv. Plants Agric. Res.*, 1: 00027

Jowkar, M., A. Ghanbari, F. Moradi and M. Heidari, 2012. Alterations in seed vigor and antioxidant enzymes activities in *Silybum marianum* under seed priming with KNO_3. *J. Med. Plants Res*., 6: 1176–1180

Mani, S., S. Jaya and R. Vadivambal, 2007. Optimization of solvent extraction of Moringa (*Moringa Oleifera*) seed kernel oil using response surface methodology. *J. Food Bioproducts Process*, 85: 328–335

Nasiri, Y., P. Feyzi and A. Javanmard, 2014. Effects of Hydro and Hormonal Seed Priming on Seed Germination of Milk Thistle under Saline Stress Condition. *Not. Sci. Biol.*, 6: 374–380

Pradhan, S.C. and C. Girish, 2006. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Ind. J. Med. Res.*, 124: 491–504

Parmoon, G., S.A. Moosavi, H. Akbari and A. Ebadi, 2015. Quantifying cardinal temperatures and thermal time required for germination of *Silybum marianum* seed. *Plant J*., 3: 145–151

Parmoon, G., A. Ebadi, S. Jahanbaksh and M. Davari, 2013. The Effect of Seed Priming and Accelerated Aging on Germination and Physiochemical Changes in Milk Thistle (*Silybum marianum*). *Not Sci. Biol.*, 5: 204–211

Powell, A.A., L.J. Yule, H. Jing, S.P.C. Groot, R.J. Bino and H.W. Pritchard, 2000. The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equations. *J. Exp. Bot*., 51: 2031–2043

Qato, D., C. Alexander, R. Conti, M. Johnson, P. Schumm and S. Lindau, 2008. Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *J. Amer. Med. Assoc.*, 300: 2867–2878

Radjabian, T., S. Rezazadeh and H.F. Huseini, 2008. Analysis of silymarin components in the seed extracts in some milk thistle ecotype from Iran by HPLC. *Ira. J. Sci. Technol.*, 32: 141–146

Rafiee, Z., S.M. Jafari, M. Alami and M. Khomeiri, 2011. Microwave-Assisted Extraction of Phenolic Compounds From Olive Leaves; A Comparison With Maceration. *J. Anim. Plant Sci.*, 21: 738–745

Ramasamy, K. and R. Agarwal, 2008. Multitargeted therapy of cancer by silymarin mini-review. *J. Cancer Lett.*, 269: 352–362

Roy, S., G. Gagan and R. Agarwal, 2012. Silibinin prevents ultraviolet B radiation-induced epidermal damages in JB6 cells and mouse skin in a p53-GADD45α-dependent manner. *J. Carcinogenesis*, 33: 629–636

Schuumann, J., J. Prock, A.K. Kiemer, A.M. Vollmar, R. Bang and G. Tiegs, 2003. Silibinin protects mice from T cell-dependent liver injury. *J. Hepat.*, 39: 333–340

Spigno, G., L. Tramelli and D.M. De Faveri, 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng*., 81: 200–208

Subramaniam, S., K. Vaughn, D.J. Carrier and E.C. Clausen, 2008. Pretreatment of milk thistle seed to increase the silymarin yield: An alternative to petroleum ether defatting. *J. Bioresour. Technol.*, 99: 2501–2506

Tan, M.C., C.P. Tan and C.W. Ho, 2013. Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. *Int. Food Res. J*., 20: 3117–3123

Tyagi, A., N. Bhatia, M.S. Condon, M.C. Bosland, C. Agarwal and R. Agarwal, 2002. Antiproliferative and apoptotic effects of silibinin in rat prostate cancer cells. *Prostate J*., 53: 211–217

Wallace, S.N., D.J. Carrier and E.C. Clausen, 2005. Batch Solvent Extraction of Flavanolignans fromMilk Thistle (*Silybum marianum* L. Gaertner.). *Phytochem. Anal*., 16: 7–16

Windauer, L., A. Altuna and R.B. Arnold, 2007. Hydrotime analysis of Lesquerella fendleri seed germination responses to priming treatments. *Indust. Crop Prod.*, 25: 70–74

Wong-Paz, J.E., J.C. Contreras-Esquivel, D. Muñiz-Marquez, R. Belmares, R. Rodriguez, P. Flores and C.N. Aguilar, 2014. Microwave-assisted extraction of phenolic Antioxidants from semiarid plants. *Amer. J. Agric. Biol. Sci.*, 9: 299–310

Yemis, O. and G. Mazza, 2012. Optimization of furfural and 5-hydroxymethylfurfural production from wheat straw by a microwave-assisted process. *Bioresour. Technol*., 109: 215–223

Xin, W., Z. Xianzhe and L. Chenghai, 2008. Optimization of microwave-assisted extraction of Silymarin from milk thistle seeds. *Int. J. Agric. Biol. Eng.*, 1: 75–81

Zhang, L., Y. Wang, D. Wu, M. Xu and J. Chen, 2011. Microwave-Assisted Extraction of Polyphenols from *Camellia oleifera* Fruit Hull. *Molecules*, 16: 4428–443

(Received 17 April 2015; Accepted 31 December 2015)