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Research Note

Effect of method of seed treatment with plant derived smoke solutions on germination and seedling growth of milk thistle (*Silybum marianum* L.)

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Summary

This study highlights the effects of plant derived smoke on seed germination and seedling growth of milk thistle (*Silybum marianum* L.). Aqueous smoke extract in different concentrations (0, 1: 250, 1:100, 1:10 and 1:5) and two seed treatment methods, namely direct exposure of seeds to smoke solutions for 1 h, and sowing the seeds in a perlite, coco peat mixture (1:1 v/v) saturated with smoke solutions, were applied to milk thistle seeds. Smoke saturated perlite, coco peat mixture significantly increased the germination and seedling growth parameters of milk thistle including germination percentage, germination speed, seedling length, and vigour index compared to direct treatment of seeds. In the direct treatment method, only a low concentration of aqueous smoke extract (1:250) was comparable with perlite, coco peat mixture and significantly promoted all of the mentioned traits.

Experimental and discussion

Silybum marianum is an annual or biennial plant of the Asteraceae family, native to Southern Europe through to Asia. The seeds of this species show little dormancy requirement (Sindel, 1991) and germination rates are higher in older seeds (Groves and Kaye, 1989). Plant derived smoke, either as a liquid or an aerosol have been used as a germination stimulant and to promote seedling vigour in a wide range of wild and cultivated plants (Light and Van Staden, 2004). Much of the recent research on this topic has focused on forest species (Tigabu *et al.*, 2007; Gomez-Gonzalez *et al.*, 2008) and several agriculture and horticulture plants (Sparg *et al.*, 2006; Van Staden *et al.*, 2006; Ahmed *et al.*, 2006; Kulkarni *et al.*, 2006, 2008, 2010). Only limited research has been conducted on indigenous medicinal plants (Sparg *et al.*, 2005). Successful cultivation of medicinal plants is dependent on efficient cultivation of these species. The application of smoke may assist in establishing healthy and vigorous seedlings for cultivation of medicinal plants. In the recent studies, different methods of seed treatment with plant derived smoke have been used (Sparg *et al.*, 2005; Sparg *et al.*, 2006; Jain *et al.*, 2006).

Perlite and coco peat provide a suitable bed for seed germination due to their properties of moisture maintenance and good nutrient absorption. In this study, the effect of a moistened perlite, coco peat mixture (1:1 v/v) with different concentrations of smoke extracts on seed germination and seedling growth of milk thistle was investigated and compared to direct treatment of seeds with smoke solutions.

Seeds of milk thistle were purchased from Pakanbazr's Seed Company (Isfahan, Iran) and stored at room temperature in aluminum foil packets until experimentation. Smoke solution was prepared by continuously bubbling smoke from the vegetative material of a medicinal plant, *Tanacetum parthenium*. (Asteraceae) in a 50 l metal drum, through 2000 ml distilled water for 45 min. (Baxter and Vanstaden, 1994). Neutral fraction of smoke water was prepared by method of Flematti *et al.* (2007). One milliliter of this fraction was added to 5, 10, 100 and 250 ml (v/v) of distilled water to make smoke extract solutions. Smoke from *Tanacetum parthenium* was used since preliminary experiments has shown positive effects on germination of *Sanguisorba minor* and *Pimpinella anisum* (data in press).

Germination experiments were carried out in 9 cm Petri dishes lined with one layer of filter paper or filled with a perlite, coco peat mixture (1:1 v/v). Seeds of milk thistle were surface sterilized in 2.5% sodium hypochlorite for 10 min and then rinsed with distilled water. For the smoke treatment, seeds were either soaked in smoke solutions for 1 h at room temperature (direct exposure) or were sown in perlite, coco peat mixture (1:1 v/v) saturated with different concentrations of aqueous smoke extract. Control seeds were soaked directly with distilled water or sown in perlite, coco peat mixture (1:1 v/v) saturated with distilled water. Following the exposure of seeds to smoke water, the soaked seeds were allowed to dry before sowing. Each experiment was replicated five times with 20 seeds per Petri dish. Petri dishes were incubated at 25 ± 0.5°C under a 16:8 h light/dark photoperiod. Germination was recorded daily from 4 to18 days after treatment, after which the number of germinated seeds, shoot length, radicle length and fresh weights of seedlings were recorded and germination speed, vigour index and root/shoot length ratio were calculated. Germination speed was calculated using the equation: $R = \Sigma ni/di$ where, R is the germination speed, d_i is number of days after the first computation of germinated seeds and n_i is number of germinated seeds in each day (Maguire, 1962). The vigour index of 14-day-old seedlings was calculated as VI = (shoot length + root length) × percentage germination (Dhindwal *et al.*, 1991).

A factorial experiment based on a completely randomized design with 5 replications was used in this study. Two procedures of seed treatment were considered as the first factor while the concentrations of aqueous smoke extract with 5 levels (0, 1:250, 1:100, 1:10 and 1:5) was the second factor. The experiment was run twice. SPSS statistical package (version 16, SPSS Inc., an IBM Company, USA) was used to analyze the data of this study.

Interaction effects of seed treatment methods with different concentrations of smoke extract were significant ($P < 0.05$) for all of the seed germination and seedling growth parameters except for seedling weight. Treating the milk thistle seeds with smoke dilutions using a perlite, coco peat method stimulated both germination and the seedling growth parameters including: germination percentage (figure 1A), germination speed (figure 1B),

vigour index (figure 1C) and seedling length (figure 1D) compared to direct treatment of seeds. Smoke extract dilutions of 1:250 and 1:100 in perlite, coco peat resulted in the highest germination (figure 1A), germination speed (figure 1B) and vigour index (figure 1C) compared to other treatment. The direct exposure of the seeds to smoke solutions at these concentrations also resulted in improved germination and vigour (figure 1 A-C) although the effects were less than in the perlite, coco peat mixture. Application of smoke extract at dilutions of 1:5, 1:10, and 1:100 with the perlite, coco peat method and at 1:250 in both methods significantly ($P < 0.05$) enhanced seedling length (figure 1D), shoot and root length (figure 2) of milk thistle seedlings compared to direct treatment of seeds with smoke solutions.

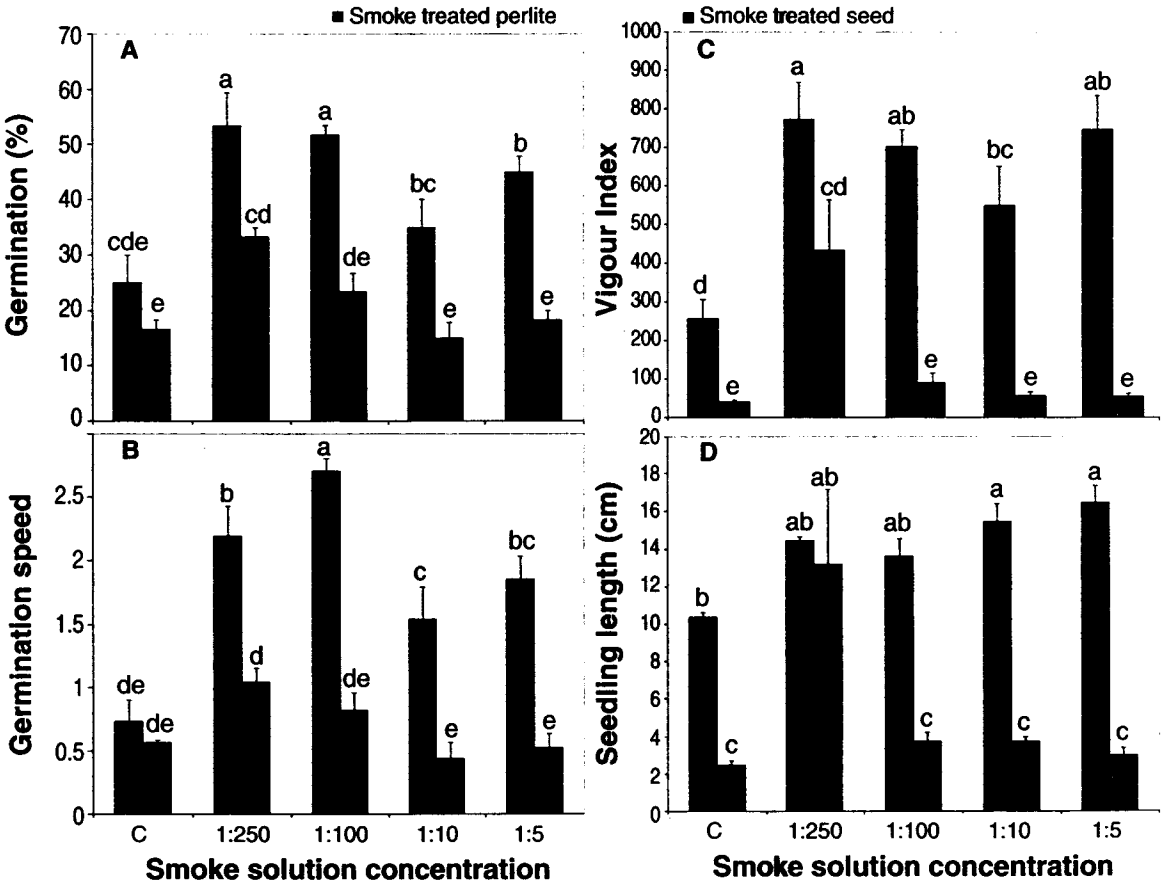

Figure 1. Effect of different concentrations of smoke extracts on seed germination and seedling growth parameters of *S. marianum*. Bars with the same letter(s) are not significantly different from each other at a 5% level.

In recent years, many researchers have studied the effect of plant derived smoke as aerosol smoke (Sparg *et al.*, 2006; Gomez-Gonzalez *et al.*, 2008), filter paper moistened with smoke solutions (Van Staden *et al.*, 2006; Ghebrehiwot *et al.*, 2009) and soaking the seeds in smoke solutions or butenolide (Senaratna *et al.*, 1999; Jain and Van Staden, 2007). This study shows that application of smoke extracts by a perlite, coco peat mixture can improve germination and seedling growth of milk thistle seeds. The physical nature

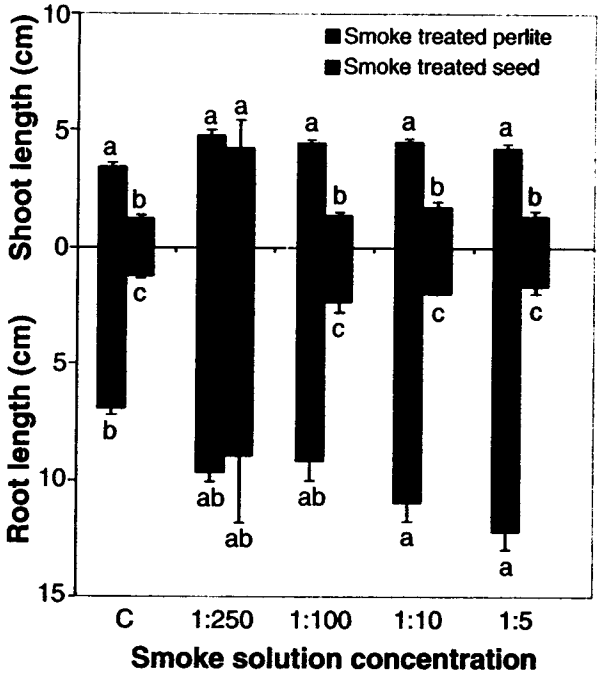

Figure 2. Effect of different concentrations of smoke extracts on shoot and root length of *S. marianum*. Bars with the same letter(s) are not significantly different from each other at a 5% level.

of the perlite, coco peat mixture may mean that it maintains the aqueous smoke extract on its surface and exposes seeds to smoke solutions gradually, and as a result reduce the detrimental effects of high concentrations of smoke extracts on seed germination and seedling growth. Detrimental effects of smoke solutions on seed germination and seedling growth parameters were evident at high concentrations (1:5, 1:10 and 1:100) in perlite, coco peat mixture. Furthermore the significant enhancement of germination and growth following direct exposure of seeds to 1:250 dilution of smoke solution compared to other dilutions also implied a negative effect at the higher concentrations. Light and co-workers (2002) showed detrimental effect of higher concentrations of smoke-water on seed germination and seedling growth of Grand Rapids lettuce. They found that the exposure of lettuce seeds to the smoke cue for a minimum period is a conditional requirement for smoke-induced germination. This implies that there is a requirement for a threshold level of the active component in smoke in the embryonic axis of the seed. At a high concentration of smoke water (1:100), the minimum period for smoke induced germination was 30 min while at a low concentration (1:1000), this minimum period was about 4h (Light *et al*, 2002). The treatment of the lettuce seeds with 1:100 dilution of the smoke extract for 24 h usually resulted to a very low germination response.

Finally, the findings of this study suggested that in previous studies where many species have not responded to smoke treatments, these species may show some response through the perlite, coco peat method of smoke treatment.

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