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Research Article

POSITIVE RESPONSE OF ESSENTIAL OIL PERCENTAGE AND ITS YIELD TO SEED PRE-TREATMENT WITH SOME OF MAJOR MICRO-ELEMENTS
CASE STUDY: *ANETHUM GRAVEOLENS* AS A MEDICINAL PLANT

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ABSTRACT
In order to study effects of seed priming by zinc and manganese on germination and yield components of dill (*Anethum graveolens*) an experiment was conducted in Tabriz, Iran. The priming solution concentrations chosen for the tests were 0.5%, 1%, 1.5% and 2% Zn ($ZnSO_4 \cdot H_2O$) or Mn ($MnSO_4 \cdot 3H_2O$), and 1.5% Zn+1% Mn. The lowest seedling dry weight was recorded in 2% Mn. Similarly, the seedling vigour index in Mn-primed seeds was restricted, when Mn concentration increased more than 1%. The highest seed yield was recorded in 1.5% Zn + 1% Mn, 1.5% Zn, 1% and 1.5% Mn solutions, which produced nearly 20%, 18%, 14% and 13% greater yield than control, respectively, but yield reduction was found when the seeds were primed in higher concentrations. Seed essential oil among treatments ranged from 3.14% in 1.5% to 2.90% in 0.5% Mn solutions. In field experiment, it is obvious from the recorded data that there was a positive response to seed priming with Zn and Mn, regarding to essential oil yield of dill.

Keywords: *Essential Oil, Nutrient Seed Priming, Seedling Dry Weight*

INTRODUCTION
Dill (*Anethum graveolens* L.) is native to south-west Asia or south-east Europe, and has been cultivated since ancient times (Bailer *et al*., 2001). It is used as a vegetable, a carminative, an aromatic and an antispasmodic (Hornok, 1992; Sharma, 2004). Crop productivity in developing world faces several constraints. One of the major crop productivity constraints in the world is the unavailability of crop nutrients in appropriate amount and form to crops (Hussain *et al*., 2012).
The roles of both macro and micronutrients are crucial in crop nutrition and thus important for achieving higher yields (Arif *et al*., 2006). Most of Iranian soils are deficient in these nutrients, and must be supplemented through proper crop nutrients management. Among the factors responsible for low yield are high weed infestations, imbalance use of fertilizers, improper plant protection cover and sub optimum plant population. Sub optimum plant population generally results from poor and erratic germination (Hussain *et al*., 2012). Micronutrients are required in very small quantities (Abd El-Wahab and Mohamed, 2011).
There are three methods of micronutrients application in crops: soil fertilization, foliar sprays and seed treatment (Johnson *et al*., 2005). Each method has the potential to affect plant micronutrient nutrition. Using enriched seeds by micronutrient priming is found to be as a better strategy for overcoming micronutrient deficiencies (Harris *et al*., 1999; Musakhandov, 1984). In nutrient priming, seeds are pre-treated (primed) in solutions containing the limiting nutrient instead of being soaked simply in water (Ajouri *et al*., 2004). Rapid and uniform field emergence is two essential prerequisites to increase yield, quality and ultimately profits in annual crops (Parera and Cantliffe, 2005). Seed priming has been shown to enhancing seed germination speed (Ryan and Young, 2006), decreasing time between sowing and emergence and improving seedling vigour (Harris, 1996), better stand establishment (Arif *et al*., 2005; Ali *et al*., 2007; Diniz *et al*., 2009) and increasing yield (Rengel and Graham 1995a, b; Yilmaz *et al*., 1998). Evidence suggests that sowing seeds enriched in micronutrients is also agronomically beneficial

(Rerkasem *et al*., 2009; Welch, 1986). Priming seeds in solutions of macro and micronutrients have been shown to improve yield of wheat (Wilhelm *et al*., 1988) and forage legumes (Sherrell, 1984), but the potential to damage the seed and inhibit germination by priming at high nutrient concentrations has also been reported (Roberts, 1948). Recently, Harris *et al*., (2007) showed that enhancing Zn seed content by priming seeds with solutions of $ZnSO_4$ was highly cost effective in increasing maize yield.

Micronutrients deficiencies and their impacts on crop yields are widely reported in various parts of the world (Singh, 2007). Zinc deficiency is common in soils throughout the developed and developing world (White and Zasoski, 1999). Especially during the early growth stages, a lack of Zn retards seedling growth, rendering the young plantlets particularly sensitive to the frequently encountered dry spells (Bort *et al*., 2011). But, in less developed countries use of seed nutrient priming methods is much less common. As much as 54% of soils in Iran are Zn deficient whereas crops showed significant responses to Zn fertilization in nearly 80% field experiments due to widespread hidden hunger. Soil fertilization with Zn as ZnSO4 is widely recommended in Iran but farmers do not use it as a soil additive to any great extent. Fewer than 10% of farmers in Iran use zinc fertilizer (Malakouti *et al*., 2009). Also, manganese deficiency is a problem in sandy soils, which suffer from low organic matter content (Obrador *et al*., 2007).

Multiple micronutrient deficiencies (Zn, Mn, Cu, B, Mo) occur in soils of the Iran and are becoming more prevalent as cropping intensity increases (Malakouti *et al*., 2009). Seeds incorporation with micronutrients has been investigated in a great number of plant species, in order to obtain higher productivities and lower costs (Diniz *et al*., 2009). In Iran, dill is the fourth important medicinal plant after saffron (*Crocus sativus* L.), cumin (*Cuminum cyminum* L.) and fennel (*Foeniculum vulgare* Mill.). During 2010, it was shown on 320,000 ha in Iran, with total seed production of almost 150,000 tones. An important constraint that influences on the field emergence and yield of dill under arid and semi-arid conditions is less availability of certain micronutrients (Omidbaigi, 2007). The main object of this study was to evaluate effects of seed priming by zinc and manganese on germination and yield components of dill.

MATERIALS AND METHODS

The laboratory seed priming test were done with dill (*Anethum graveolens*), using solutions at four concentrations of zinc and manganese. There were three replications of each solution concentrations/microelements combination, plus three replications of an unprimed control. Seeds were soaked in the required aqueous solutions of fertilizer grade $ZnSO_4{\cdot}H_2O$ (Zn 35%, S 12%) or $MnSO_4{\cdot}3H_2O$ (Mn 26%, S 15%). The priming solution concentrations chosen for the tests were 0.5%, 1%, 1.5% and 2% Zn or Mn, i.e., 14.5, 29, 43.5 and 58 g Zn L^{-1} or 19, 38.5, 58 and 77 g Mn L^{-1}, respectively, that have been recommended by Water and Soil Institute, Iran, besides, an optimum seed treatment with combined 1.5% Zn+1% Mn. Each treatment involved weighing approximately 10 g of the seed in a plastic cup, adding 20 mL of the priming solution (sufficient to submerge seeds), and allowing the seed/solution mixture to sit covered with plastic for 12 hours. The soaking treatment was followed by rinsing three times in distilled water to remove excess salts from the seed coat, as described by Johnson *et al*., (2005). A part of primed seeds was dried in an oven at 70^{o}C. The materials were ground to powder in a Wiley micro-mill with stainless steel blades and a 40-mesh sieve. Samples were digested in a HNO_3-$HClO_3$ acid mixture (10 mL HNO_3 + 4mL $HClO_3$) then Zn and Mn were determined at 214 nm wavelength using an AAnalyst-200 Perkins-Elmer atomic absorption spectrophotometer (Issac and Kerber, 1971) (Table 1). For each replicate, 25 seeds from each priming treatment were placed for a germination test in a Petri dish containing Whatman paper No. 1 that had been thoroughly moistened with water. The Petri dishes were placed in germinator at 25±1 °C temperature, and germination was checked once a day for 10 days. The paper in the Petri dishes was changed once after the first 24 hours (in case toxic amounts of micronutrients, which might inhibit seedling growth after germination, were leached from the seed coat into the paper during the first day). The recorded data were final germination percentage, seedling biomass and seedling vigour index.

Final germination percentage (GP) was calculated as the cumulative number of germinated seeds with normal radicles.

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Seedling vigour index (SVI) was calculated according to Abdul-Baki and Anderson (1973):
GP= SDW ×SVI
Where, SDW is seedling dry weight.
Field experiment was carried out at the Research Station of Islamic Azad University, Tabriz, Iran during 2011. Tabriz is located in the north-west of Iran; the climate is semi-arid and cold and average annual precipitation is 270 mm. The soil was sandy-loam with EC of 0.72 ds m^{-1}, pH of 7.9, Zn and Mn content of 1 ppm and 3 ppm, respectively. The research area was ploughed in October 2010 and again in March 2011. Then, after the application of 10 g/m^2 each of urea and ammonium phosphate and 5 g/m^2 potassium sulfate based on soil analysis, the site was harrowed to prepare the seed bed.
Plots were arranged in a randomized complete block design with four replications. Treatment plot size was 4×3 m with four rows of dill, and with harvested area 6 m^2. Primed seeds were sown in beds on 26th April at about 2 cm depth. The experiment was hand-weeded as needed. Fruits were harvested at the half-yellow stage. The essential oil from dill fruits was isolated by hydro distillation for 3 hours using Clevenger's apparatus based on method of Guenther (1992). The following data were recorded: number of umbels per plant, thousand seed weight (g), seed yield (g/m^2), essential oil (%) and essential oil yield.
All data were statistically analyzed as a factorial experiment using MSTAT-C software. The means of the treatments were compared using the least significant difference test at a significant level of 95%.

RESULTS AND DISCUSSION

The effects of Zn and Mn concentrations from 0.5% to 2% on the germination percentage of dill are presented in Figure 1. There was a significant ($P<0.01$) interaction between micronutrient and its concentrations on final percent germination. Also, there were significant main effects of micronutrient ($P<0.05$) and concentration ($P<0.01$).

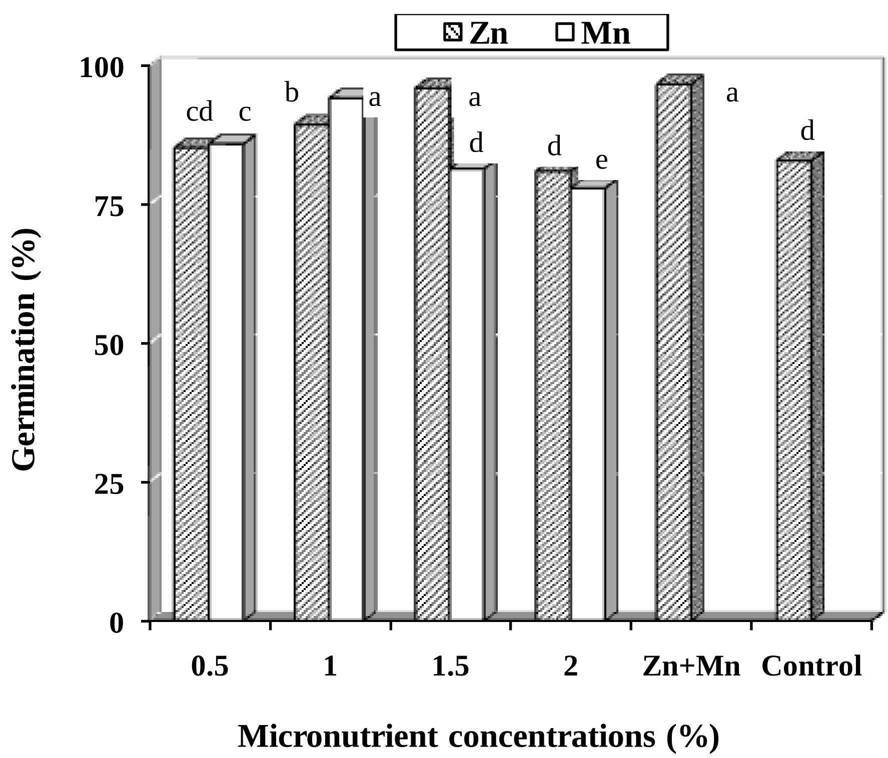

Figure 1: Germination percentage of dill seeds as affected by micronutrient seed priming

Priming at the concentrations of 1%; 1.5% Zn, 1% Mn, or 1.5% Zn+1% Mn for 12 hours increased the rate of germination of dill seeds compared to an unprimed control (Figure 1). The mean final germination

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from primed seeds with Zn and Mn were 88% and 85%, respectively, but only 83% from non-primed seeds (Figure 1). After 10 days, dill seeds had higher germination percentage in all solutions as compared with control, except for a 2% reduction in seed germination in 2% Zn solution and a 1.5% and 5% reduction in germination in those seeds primed with 1.5% and 2% Mn solutions, respectively.

In laboratory experiment, regarding the seedling dry weight (SDW) (Table 2), the effect of seed treatments were significant with $ZnSO_4$ and $MnSO_4$, solely or in combination. At 10 days after sowing, the maximum SDW (0.025 g/plant) was noticed in a determined Zn + Mn solutions and was followed by 1.5% Zn and 1% Mn (0.022 and 0.020 g/plant, respectively). The lowest value of SDW was recorded in 2% Mn (0.012 g/plant) and control (0.013 g/plant), as compared with other primed seeds. Dill SDW was inhibited by the highest Zn concentration, and by the two highest concentrations of Mn. In this experiment, seedlings from primed seeds with 0.5% Zn had statistically similar SDW with 2% Zn, also was not significantly difference between control and seeds primed in 2% Mn.

Table 1: Effect of the concentrations of Zn and Mn in the priming solution on the concentrations of Zn and Mn in primed dill seeds

Micronutrients	Concentrations in Priming solution (%)	Seeds before priming (mg kg^{-1})	Seeds after priming (mg kg^{-1})
Zn	0.5	10.2	100.9
	1		110.9
	1.5		130.4
	2		131.0
Mn	0.5	7.9	80.3
	1		100.0
	1.5		100.9
	2		110.3

Table 2: Mean comparisons of interaction of microelements and their concentrations on some studied variables in dill

	Concentrations (%)	SDW (g/plant)	SVI	Number of umbels per plant	Thousand seed weight (g)	Essential oil (%)
Zn	0.5	0.016 cd	1.36 de	34 bc	1.2 b	2.92 ab
	1	0.018 c	1.61 c	37 a	1.2 b	3.00 ab
	1.5	0.022 b	2.11 b	36 ab	1.8 a	3.11 ab
	2	0.015 de	1.22 ef	31 d	1.2 b	3.00 ab
Mn	0.5	0.016 cd	1.38 cde	33 cd	1.4 b	2.90 b
	1	0.021 b	1.51 cd	35 abc	1.7 a	3.10 ab
	1.5	0.015 de	1.22 ef	31 d	1.3 b	3.14 a
	2	0.012 e	0.89 g	31 d	0.9 c	3.09 ab
Zn+ Mn	1.5+1	0.025 a	2.42 a	36 ab	1. 9 a	3.09 ab
Control	-	0.013 e	1.08 f	33 cd	1.2 b	2.99 ab
F test	-	**	**	**	*	*
CV (%)	-	19.80	15.89	12.79	14.86	11.87

SDW and SVI mean seedling dry weight and seedling vigour index, respectively.

Regarding seedling vigour index (SVI), there was a significant positive response to seed priming with Zn, Mn and Zn+Mn relative to no priming. The data show that averaged SVI can be increased by seed priming with Zn and Mn up to 1.58 and 1.25, respectively, against control. In the present study, seeds primed in 1.5% Zn had higher SVI (2.11), but a further increase in the Zn concentration of the solution did not further increase in SVI. Similarly, the SVI in Mn-primed seeds was restricted, when Mn concentration increased more than 1% (Table 2).

The effect of seed nutrient priming in Zn and Mn concentrations from 1% to 2% was evaluated for yield and yield components. In case of umbel number per plant of dill (Table 2), the effect of seed treatments was highly significant. Treatments with dilute concentrations of Zn and Mn gave a significant increase over control. On-farm seed priming with 1% and 1.5% Zn before sowing, produced averaged 36.5 umbels per plant, which was 11% higher than control. The increase value in those seeds treated with 1% Mn was 6%, as compared to control. Umbel number per plant of dill tolerated 6% reduction in treatments enriched with the highest concentration of Zn and the two highest concentrations of Mn, in compared with unprimed check. Maximum increase in thousand seed weight (TSW) (nearly 46%) obtained from 1.5% Zn and 1% Mn, as compared to check plots (Table 2).

Seed yield was markedly influenced by the treatments. The highest seed yield was recorded in 12 hours seed soaking treatments in 1.5% Zn + 1% Mn, 1.5% Zn, 1% and 1.5% Mn solutions, which produced nearly 20%, 18%, 14% and 13% greater yield than control, respectively, but after that the yield reduction was found. When seeds primed in the highest concentration of Mn for 12 hours, dill statistically produced seed yield similar to unprimed treatment (Figure 2). Seed essential oil among treatments ranged from 3.14% in 1.5% to 2.90% in 0.5% Mn solutions. Nevertheless, there was a small non-significant profit (about 0.15%), when the seeds were enriched with the 1.5% Mn solution. In field experiment, it is obvious from the recorded data that there was a positive response to seed priming with Zn and Mn, regarding essential oil yield (EOY) of dill. All the seed treatments gave a significant increase in EOY, over control.

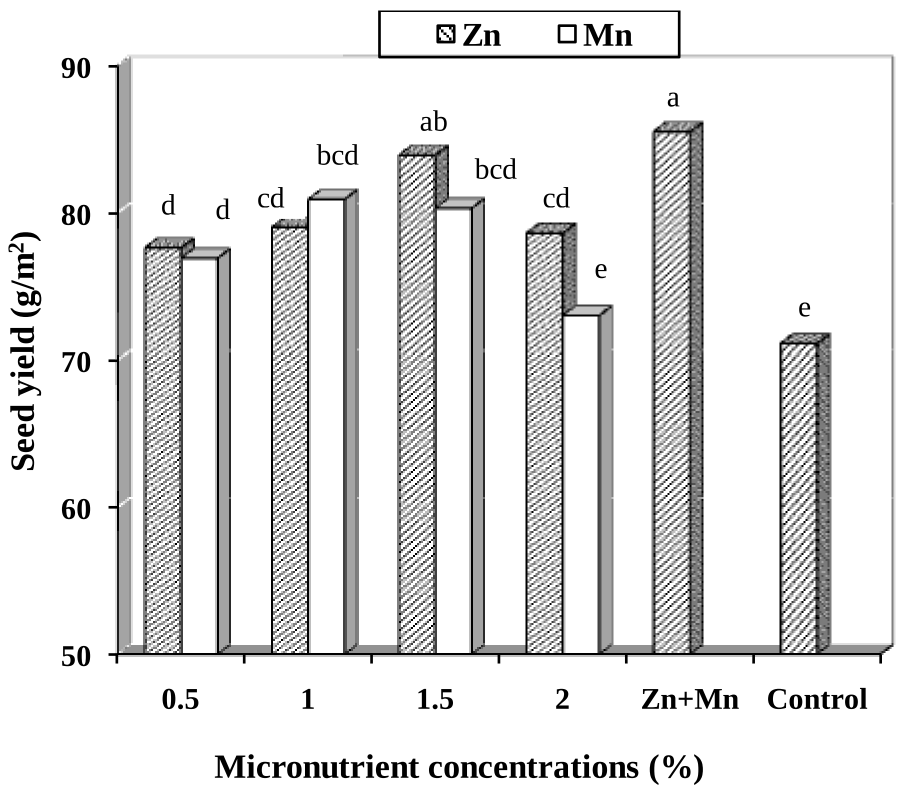

Figure 2: Seed yield of dill as affected by micronutrient seed priming

It was noted that seed treatment with microelements studied, solely or in combination with each other gave beneficial effects on germination in laboratory condition. The general beneficial effect of priming on emergence is in consistent with reports of farmers' perceptions of the effects of priming on some medicinal plants such as cumin and marigold (*Calendula officinalis* L.). There are many published studies comparing germination speed of different crops under nutrient priming over non-primed seeds. In our study, the presence of Zn+Mn in priming solution increased total germination up to nearly 97%. In on-station trials of priming, Harris *et al.*, (1999) reported that priming decreased time to 50% emergence by about 12 hours, which reflected the effect of priming on the time taken to germination. Concentrations exceeding 1.5% Zn and 1% Mn negatively affected germination. Similarly, Ajouri *et al.*, (2004) showed that concentrations exceeding 50 mM Zn significantly reduced germination rate of barley seeds to below the level of unprimed seeds. The lowest germination percentage was recorded for treatment of sweet pepper (*Capsicum annum* L.) with micronutrients at the highest dosage by Diniz *et al.*, (2009). In another experiment conducted by Deering and Young (2006), annual grasses germinated quickly than the unprimed seeds, with nearly 100% germination. Also, Arshad Ullah *et al.*, (2012) reported that the maximum field emergence of Raya (*Brassica carinata* L.) 75.5% was noted in $ZnSO_4$ followed by $MnSO_4$ treatment showing 72.8%.

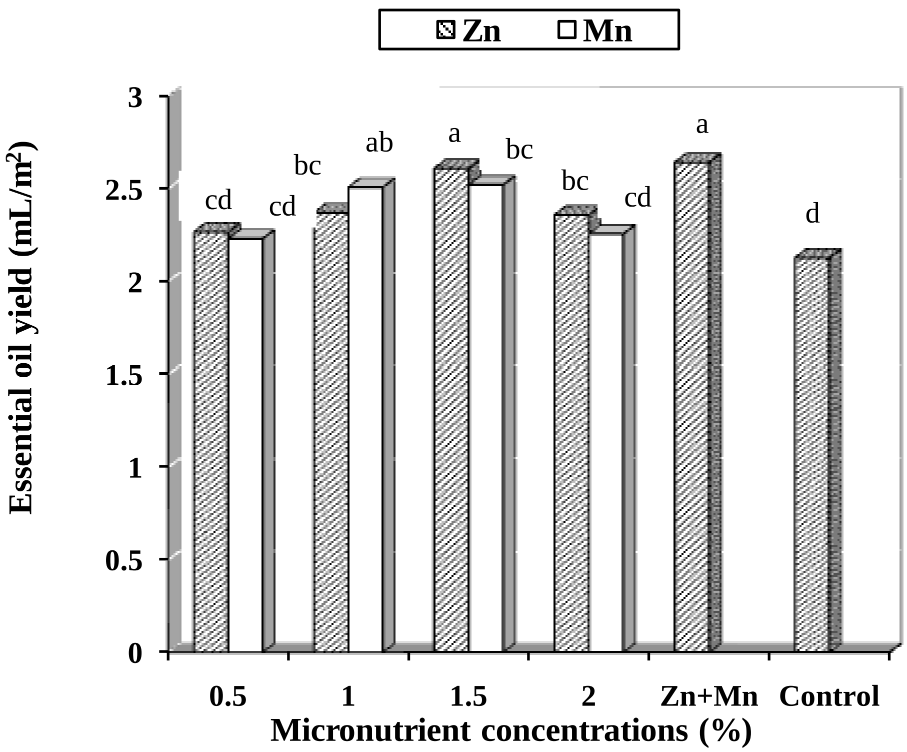

Figure 3: Essential oil yield of dill as affected by micronutrient seed priming.

Seedlings grown from high-Zn seeds produced more biomass, enabling the plants to take up soil water more efficiently in later growth stages. These results are supported by Arshad Ullah *et al.*, (2012) findings on Raya, who reported that regarding early growth of seedlings the effect of seed treatments was highly significant with $ZnSO_4$ treatment giving the maximum values for root and shoot fresh and dry weights, and treatment with $ZnSO_4$ and $MnSO_4$ gave a significant increase over control.

Tabrizian and Osareh (2007) stressed the important of the rapid crop establishment and a vigorous early growth for the performance of medicinal plants with high seedling vigour being essential for the crops competitiveness for water, light, and nutrients during the cold season. In the present study, it is revealed that seed priming with $ZnSO_4$ and $MnSO_4$ may give better results on SVI of dill, that is in agreement with findings of Rahnemaye Badr and Soltani (2006) on cumin, that the seedlings from treated seeds with Mn+Zn salt solutions had 18.5% higher SVI over check plots. Also, Louzada and Vieira (2005) verified that the application of very high doses of micronutrients in bean seeds caused an increase of abnormal and deceased seedlings due to the toxic action of the micronutrients. Based on Harris *et al.,* (2007) study, maize seedlings from seeds primed with either 1% or 2% Zn were significantly heavier and taller at 14 days after sowing than seedlings from non-primed seeds. Also, significant differences have been observed in dry matter accumulation and SVI of *Catharanthus roseus* L. between primed and non-primed seeds (Karthikeyan *et al*., 2007).

Inspection of the data on thousand seed weight (TSW) revealed that the use of micronutrients studied in seeds has shown great potential for improvement of the TSW. Ali *et al.,* (2007) resulted that when wheat and maize seeds were primed in 2% Zn, an increase in TSW and grain yield was observed as compared to control. Increased grain yield in dill is associated with increases in SDW under nutrient priming. Important role of Zn and Mn on growth and yield of *A. graveolens* has been confirmed (Nagiub *et al*., 1998). The results of our study indicated that micronutrient enriched seed could increase yield in micronutrient deficient soil and the Zn and Mn were responsible for higher yield, which is in agreement with those reported by Tabrizian and Osareh on marigold (2007) and Singh (2007) on ground-nut in Zn deficient soils. Whereas, higher doses of Zn and Mn micronutrient solutions injured the seedling establishment was the cause of yield reduction under treatment.

Nutrient seed priming makes the seed rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). The resulting improved stand can reportedly increase drought tolerance, reduce pest damage and ultimately increase crop yield (Harris *et al*., 1999). These results are in line with that of Harris *et al.,* (2000), who reported higher grain yield for seed priming as compared with control.

Overall, the treatments applied to the seeds did not provided expressive increases in the essence percentage, when compared to the control. Enriched seeds in 1.5% Mn solution had significantly 0.24% higher essential oil than primed seeds with 0.5% Mn. Significant increase in EOY of dill due to seed priming treatments relative to not-priming was expected. Because, the higher EOY was produced in primed seeds by a determined Zn + Mn solution, followed by Zn (1.5%) and Mn (1% and 1.5%), that had higher seed yield or essential oil percentage. These increases in yield may only indirectly reflect increases in final emergence from primed seed as farmers in those trials filled gaps in the crop stand by re-sowing (farmers reported that primed crops required less gap-filling, so they cost less to establish). However, priming would be expected to increase yield indirectly through an effect on the crop stand because even stands give higher yields than stands with uneven times of emergence (Wade and Meinke, 1994).

Several authors have described positive responses to seed priming with micronutrients in many crops in the world and the data from this study confirm that simple 'on-farm' seed priming with Zn + Mn is an effective way to improve seed and essential oil yield of dill. Nutrient priming, on the other hand, can be done at any time (e.g., during the dry season with no field activities) and does not require other inputs than the nutrients. This is important for resource-poor farmers as it is a low-cost technology and requires few external inputs. In addition, however, we have shown here that priming seeds in a determined Zn + Mn solution results in a further increase of crop yield over control.

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