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# Bio-priming influence on medicinal plants germination and growth

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**Abstract**

This study examines the effect of Nitragin concentrations on germination and early growth in laboratory condition. Different dosage of Nitragin (2, 3 and 4 cc) were applied on dill, fennel, cumin, calendula. Laboratory experiments were conducted in Islamic Azad University of Tabriz in completely randomized design (CRD) with three replicates. Results showed that the influence of various Nitragin treatments on germination percentage and crop growth rate were significant. Nitragin with concentration 4 cc had the most positive effect on germination percentage and crop growth rate in dill. Nitragin did not be impressive on germination percentage in fennel, but Nitragin (4 cc) increased crop growth rate. Nitragin concentrations stimulated germination percentage with 2 cc on calendula and 3 cc on cumin.

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**Introduction**

Seed priming is done with biochemical, biological and biophysical methods (Ashraf *et al.,* 2003). Many recent researches suggested that seed priming might be a useful way for better germination, seedling growth, establishment and yield (Tajabakhsh *et al.,* 2004; Sharafzadeh *et al.,* 2006). In the priming process, the seed is incited to start cell division and then will be dried. If again the seeds absorb water, it will start emergence from the dried situation (Shahba *et al.,* 2008). Good seed germination behavior is important for horticulture and agriculture. Uneven or poor germination and subsequently in homogeneous seedling growth can lead to great financial losses, by e.g., reduced possibilities for mechanization, or lower prices of in homogeneous plant batches (Ghiyasi *et al.,* 2008 a) seed priming can increase speed and uniformity of germination (Ghiyasi *et al.,* 2008 b). The past century was an age of advanced chemical application in agriculture and negative effects on food products and on the environment is commonly known. Therefore, many scientists believe that this century will be the age of biological and biophysical methods application. Using biological and biophysical methods would increase the plant growth, yield and quality. This technique protects plant against diseases and pests and decreases the use of fertilizers and pesticides. So the farmers can reach a crop with more quality and quantity with expensing less time, cost and effort. In this situation the knowledge of the biological and biophysical priming and relevance of plant growth and physical properties appear to be an important factor in the sustainable agriculture (Hoseini *et al.,* 2013a). The inoculation of seeds or seedlings with micro-organisms has been adopted as a method of modifying microbial populations around crop plants to promote both development and yield. *Azotobacter chroococcum* was originally used a seed inoculants because it was thought that its fixation of dinitrogen would provide a significant input to the Nitrogen economy of plants (Cooper, 1959). Biological seed priming methods can lead to better germination and establishment in many crops such as maize, wheat, rice, canola (Basra *et al.,* 2005; Ghiyasi *et al.,* 2008 a, b). Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Mahfouz and Sharaf-Eldin, 2007). Seed germination and seedling emergence are influenced by several factors, these being mainly the seed, the environment, and various mechanical factors. The environment provides the basic requirements of light, heat, oxygen and moisture. The mechanical factors provide such aspects of the planting configuration as row spacing, seed placement distance, depth of sowing, seed rate, and degree of seed-soil contact. These may also modify the environmental factors (Montemayor, 1995). Badran and Safwat (2004) and El-Ghadban *et al.* (2006) found that fennel responded to bio-fertilizer by increasing growth and oil yield and changing the chemical composition. These results are in agreement with those of Gad (2001) for fennel (*Foeniculum vulgare*) and dill (*Anethum graveolens*), who reported that biofertilizers on these plants increased growth and yield. Viable seed is capable of producing new plant under both favorable and unfavorable climatic conditions. Emergence of the seed lot having high vigor is more uniform and due to that it forms more vigorous seedlings which in turn provide better stand and establishment (Baleševi.-Tubi *et al.,* 2007). The aim of this study, the effect of inoculation with manure on some biological traits in vitro Nitragin and study the use of dill Nitragin in agriculture in order to achieve the goals of sustainable agriculture. Yousry *et al.* (2003) found that inoculation of pea (*Pisum sativum*) plants with *Bacillus megatherium* increased plant dry matter by 10.9%, while the combined application of *B. megatherium* and P-fertilizer increased dry matter by 19.7%. The aim is to study the effects of biological seed priming on germination and yield of dill, fennel, cumin and calendula in separate laboratory experiments.

**Materials and methods**

Laboratory experiments were carried out at the Research Station of the Islamic Azad University,

Tabriz Branch, Iran. Treatments are different concentrations of Nitragin (2, 3 and 4 cc) that applied on dill, fennel, cumin and calendula. This study was done on different medicinal plants on separate experiments. So, all plants compared with control (distilled water). The method used in the laboratory is CRD (Completely Randomized Design) with three replications. Nitragin (Azotobactin) is water-soluble and active fluid that contains bacteria *Azotobacter spp*. ،*Azospirillum spp*., *Pseodomonas spp*. and the number of viable cells (Okon, 2002). At the beginning of the experiment the seeds were disinfected in hypochlorite-Na 5% solution for 3 minutes. Then seeds were transferred to aseptic dishes, and solutions were added to dishes and remained for two hours. In order to paste Nitragin solution to seeds, starch and sugar solution were coated on seeds. Then inoculated with Nitragin concentrations were studied. Then the seeds were denatured from solutions and were dried in room temperature, and then in laboratory with use of special paper and pure water, 50 seeds were cultured in every petri-dish and were put in germinator with 19 $^{oc}$ temperature for 10 days and were counted daily. In various stages of Petri-dish visit, if necessary, distilled water was added to the wet filter paper. Finally, germination percentage (GP) was computed as the cumulative number of germinated seeds with normal radicles. Germination percentage was calculated by using the equation 1 and Crop growth rate (CGR) was calculated by using the equation 2.

$GP = \sum n / N \times 100$ Eq. (1)

Where, $n$: number of germinated seeds at each counting, $N$: total seeds

$CGR = \sum N / \sum (T \times N)$ Eq. (2)

Where, $N$: number of seeds germinated at 10$^{th}$ day, $T$: number of tests to begin counting the days and times.

The statistical analysis was done with MSTAT-C software. The means of the treatments was compared using the least significant difference test at $P < 0.05$ by LSD method.

**Results and discussion**

Dill *(Anethum graveolens L.)*

Table 1 indicates that there are significant differences between Nitragin concentrations and dill *(Anethum graveolens L.)* seeds. Dill seeds behaved differently by bio-priming methods in all studied characters. Figure 1 shows how the priming increased the germination percentage in dill. Analysis of variance of the laboratory data indicated that, the germination percentage was significantly affected by Nitragin concentrations. The highest germination percentage was recorded for 4 cc Nitragin (63 %). After that 2 cc has the most GP with 60% germination percentage (Fig. 1).

**Table 1.** Analysis of variance for percent of germination traits.

| SV | df | dill | fennel | calendula |
|---|---|---|---|---|
| Treatment | 3 | 135.62* | 491* | 1063.04** |
| Error | 8 | 5.18 | 70 | 22.92 |
| CV (%) | _ | 12.81 | 10.52 | 10.54 |

*, **, mean significant at 5% and 1% probability levels.

Table 2 shows the effect of different bio-priming concentrations on crop growth rate (CGR). All treatments increased CGR on dil. The most CGR was shown on 4cc on dill. Also, the lowest CGR occurred in seeds treated with distilled water (Fig. 2).

**Table 2.** Analysis of variance for crop growth rate traits.

| SV | df | dill | Fennel | Calendula |
|---|---|---|---|---|
| Treatment | 3 | 652.77* | 8.86** | 637.83** |
| Error | 8 | 47.91 | 0.63 | 9.21 |
| C.V (%) | _ | 14.08 | 11.54 | 16.97 |

*, **, mean significant at 5% and 1% probability levels.

Investigated the effects of pre-sowing seed treatments with bacteria *brasilense A*. on the germination (11%), emergence and seedling establishment (16.5%) of medicinal plants had positive results (Bhadauria *et al.,* 2000). The results with the findings Sorial and colleagues (1992) are also consistent.

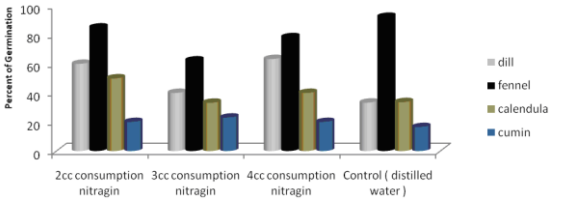


**Fig. 1.** Effect of Nitragin concentrations on medicinal plants Germination Percentage.

These results are in agreement with those of Gad (2001) for fennel (*Foeniculum vulgare*) and dill (*Anethum graveolens*), who reported that biofertilizers on these plants increased growth and yield. Amin (1997), who studied coriander (*Coriandrum sativum*), fennel (*Feoniculum vulgare*), and caraway (*Carum carvi*), showed that the growth was influenced by seed inoculation (*Azotobacter* and *Azospirillum*) with a half dose of inorganic fertilizer. Plant growth was nearly equal to that obtained when the plants were fertilized with a full dose of inorganic fertilizer. Tehlan *et al.* (2004) reported that plant growth and seed yield of fennel varied according to the strain applied (Mahfouz and Sharaf-Eldin, 2007).

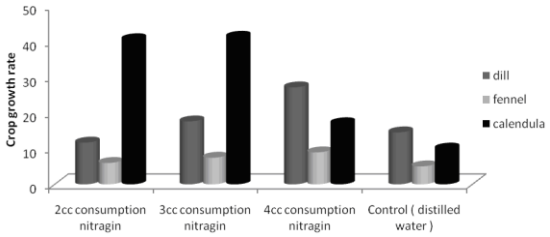


**Fig. 2.** Effect of Nitragin concentrations on medicinal plants Crop Growth Rate.

Fennel *(Foeniculum vulgare L.)*

Analysis of variance revealed that there was significant difference between bio-primed treatments on fennel *(Foeniculum vulgare L.)* (Table 1). Germination percentage (GP) responded positively and significantly to bio-priming methods. Control germination percentage on fennel is higher than treated seeds. So, different concentrations of Nitragin (2, 3 and 4 cc) had negative effect on germination percentage in fennel. Highest germination percentage (GP) was noted in control and 2 cc with 92% and 85% respectively (Fig. 1).

Table 2 indicates that there are significance differences within treatments in crop growth rate. Maximum crop growth rate (CGR) was depicted in 4cc (8.97 $g.m^{-2}.day^{-1}$) and minimum crop growth rate (CGR) was noted in control (5 $g.m^{-2}.day^{-1}$) (Fig. 2).Crop growth rate in 4 cc Nitragin was 1.79 times greater than control. After that other treatments like 3 cc Nitragin with 7.47 $g.m^{-2}.day^{-1}$ affected positively on CGP (Fig. 2).

Results in the survey showed that fertilizing fennel plants using different strains of biofertilizers amended with a half dose of N, P, and K increased plant height, number of branches, plant fresh weight, plant dry weight, and fruit yield compared with the 50% and 100% NPK treatments. The largest amount of growth and yield occurred with50%NPKand a mixture of strains *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megatherium*, but the lowest amount of growth and yield occurred with the 50% NPK treatment. These results may be due to the role of *Azotobacter* and *Azospirillum* in nitrogen fixation. In addition, they provide growth promoting substances such as indole acetic acid and gibberellins. The phosphate-dissolving bacteria (PDB) *B. megatherium* also may have increased the availability of phosphorus, and it may have increasing the uptake of trace elements(Mahfouz and Sharaf-Eldin, 2007).

Cumin *(Cuminum cyminum L.)*

Table 1 indicates that there are significant differences between Nitragin concentrations and cumin seeds. Figure 1 shows how the priming had increased the germination percentage in cumin *(Cuminum cyminum L.)*. Treatments with 3 cc Nitragin was effected the seeds more than other ones. After that the most seed germination percentage was observed in the case of 2 and 4 cc Nitragin with 20% germination.

Rezai *et al* (2005) said that, seed treatment with azotobacter caused increasing in germination percentage and on cumin.

Calendula *(Calendula officinalis L.)*

Analysis of variance of the laboratory data indicated that, the germination percentage was significantly affected by priming methods (Table 1). The highest germination percentage was recorded for seeds primed with control (68.33%). After that 2 cc has the most GP with 50% (Figure 1).

Table 2 exhibits the data pertaining to germination rate of calendula seed. The difference between primed and non-primed seed for CGR was statistically significant. The seed treatments in 3 cc increased the CGR more than other treatments with 39.31 $g.m^{-2}.day^{-1}$. CGR on control had tangible difference on comparison with Nitragin concentrations (Fig. 2).

**Conclusion**

Using biological priming methods (bio-priming) like Nitragin would increase the plant growth, yield and quality. Taking advantage from beneficial aspects of biological priming methods (bio-priming) with no environmental pollution, this technique protects the plant against diseases and pests and decreases the use of fertilizers and pesticides. So the farmers can reach a crop with more quality and quantity with expensing less time, cost and effort. In addition, different biological priming methods (bio-priming) help to reinforcement and support sustainable agriculture.

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