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Effects of pyridoxine and different levels of nitrogen on qualitative and quantitative yield of *Physalis alkekeng*

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Abstract

Alkekengi (*Physalis alkekengi*) is a medicinal plant from Solanaceae family which contains some important alkaloid compounds including Physalin. To evaluate the effect of pyridoxine and different levels of nitrogen fertilizer on yield of the alkekengi medicinal plant used the randomized complete block design with three nitrogen levels (included total application of 100 (control), 150 and 200 kg N ha⁻¹) in the main plots and three pyridoxine treatment included 0 (control), 0.02% and 0.04% in subplots in three replications. Results of this study shows that both pyridoxine and nitrogen treatments have significant positive effects on yield of alkekengi. According to the results, 0.04% pyridoxine and 150 kg N ha⁻¹ treatment resulted in maximum values for yield, as compared to other treatments. The lowest yield was obtained by application of 100 kg N ha⁻¹ and no pyridoxine (control treatment).

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Introduction

In recent years, an increasing interest has emerged in the use of medicinal plants and replacing them with synthetic drugs. Global statistics also show that about 50% of introduced drugs to the world markets have active ingredient of natural origin. In some countries, this statistics has even reached up to 90%. Nutrition plays an important role in vegetative growth, quantity and quality of active ingredients of medicinal plants. Information about the effects and properties of medicinal plants has been gradually quoted over the time, mixed with ethnic customs and traditions and eventually provided for contemporary generation (Omid Beigi, 2009).

Nowadays, everybody knows the importance of medicinal plants and Millions of people are working in cultivation, harvesting, processing and other aspects of these plants (Zare Zadeh *et al.*, 1997). Alkekengi is an herbaceous plant with height of 20 to 60 cm which grows in calcareous land and farms of most parts of Europe such as France, Germany, Italy, and some parts of Asia such as Japan, Iran, and North America. Alkekengi's fruits are the part that used for therapeutic purposes, though its leaves can also be used. In Iran, this plant is scattered in different areas such as Kermanshah, northern parts, etc (Zargari, 1996). The most important pharmaceutical compound of Alkekengi is an alkaloid called physalin that accumulates in fruit and leaves (liendley, 1983).

Also, the leaves, stem and calyx of this plant are among bitter tonic and blood purifier medicines (Vagujfalivi, 1968). Increased production of alkaloid active ingredients in plant of Solanaceae family is closely related to nitrogen consumption. In other words, the alkaloids will be produce at desirable level by addition of moderate amounts of nitrogen (Thomson and Witt, 1987). The most common methods of nitrogen consumption in industrialized agriculture is the use of synthetic fertilizers such as urea. For the first time, a German scientist named Meissner, referred alkaloids as nitrogen materials with alkaline properties which can produce salts in

acidic conditions. Known alkaloid that found in plants include several thousand alkaloids (Zare Zadeh *et al.*, 1997). Countries around the world including America, Russia, China, India, Japan, Germany, Colombia and Iran have conducted research on various species of the genus *physalis* sp. However, less research has been done for cultivation improvement of this plant, in particular the impact of nitrogen fertilizer and animal manure on the alkaloid active ingredient have not been fully investigated. In this regard, following researches can be mentioned.

Prasad *et al.*, (1985) investigated the effects of fertilizer (nitrogen - phosphorus - potassium) on yield and fruit quality of *P. peruviana*. they concluded that consumption of 100 kg of nitrogen along with 80 kg phosphorus and 80 kg potash fertilizers per ha produces the highest yield. Percent fruit juice was increased with increased use of fertilizers, K, P, and N. The same trend was observed in the levels of vitamin C (Matsuura *et al.*, 1969).

Zare Zadeh *et al.*, (1997) examined changes in total alkaloids of alkekengi plants in response to different levels of nitrogen application. They concluded that addition of nitrogen fertilizer to the soil increases alkaloids content of leaves, fruits and roots and maximum increase is correspond to 100, 150 and 200 kg N ha⁻¹ treatments.

Axelius (1991) examined different cultivation methods for alkekengi under greenhouse and field conditions. He briefly described the properties of alkekengi fruits and tried to use interspecific hybridization for breeding of this plant. However, only hybridization between (*P. peruviana*) and (*P. pruinosa*) species was partially successful and resulted in production of few hybrid seeds. Sorouraddin *et al.*, (2007) studied different procedures for extraction, separation and identification of physalins from alkekengi plants grown in Azerbaijan. They found that continuous extraction with acetone gives the best results in terms of amount of crude extract. Thomson and Witt (1987),

by investigating the effects of light and temperature on seed germination of some *Physalis* species concluded that seeds of *P. angulata* at constant temperatures of 5 and 10 °C were not capable of germination both in light and in darkness. So that light and darkness conditions at 30 °C in all or part of the day was necessary to achieve maximum germination. Seeds of perennial species, *P. virginia*, were more sensitive to temperature. Seeds of this species were able to germinate at 5, 10 or 20 °C in the presence of light or darkness. Maximum germination occurred at periodic temperatures of 30/10 °C (Prasad *et al.*, 1985).

Matsuura *et al.*, (1969) in studying bitter principles of *physalis alkekengi* and identification of physalin structure, found that physalins are 13, 14-seco-16, 24-cyclo-steroids compounds which first extracted from leaves of this plant. Also, in various research studies conducted since then, several Physalins were identified in *Physalis* species and named Physalin A to T. Kawaie *et al.*, (1987) in their investigation of a new type of Physalin, identified the structure of Physalin L and M isolated from *physalis alkekengi* var. fracheti. Their results showed that some of these compounds have anti-cancer properties against some tumors within or outside the body.

Talei *et al.*, (2004) investigated the effect of steroid alkaloids of *Saccharum officinarum*, *Oenanthe fistulosa* and *Physalis alkekengi* on a number of Gram-positive and Gram-negative bacteria and concluded that *Physalis alkekengi* alkaloids have bacteriostatic and bactericidal affects only on *Bacillus* and *Escherichia coli*.

Mousavi *et al.*, (2008), by studying the effect of environmental factors on seed germination of *P. divaricata*, showed that, seed germination percentage of this plant reduced with the increase or decrease in temperature above the optimum temperature of 30 °C. Increased concentration of fruit aqueous extract over 30%, significantly decreased seed germination of this annual plant. This indicates delayed seed germination in the presence of high

concentrations of fruit mucilage which seems to ensure germination and growth of this plant during long growing season.

Since the major goal of growing *Physalis alkekengi* is to increase the quantity and quality of its product, in this study we attempt to investigate the effect pyridoxine chemical as well as different levels of nitrogen on qualitative and quantitative performance of this medicinal plant.

Material and methods

Plant materials and experimental design

Alkekengi seeds were purchased from Pakan Seed Company, a prestigious center for the production of medicinal plants seed, on May 2010 and were potted at educational greenhouse of Islamic Azad University, Saveh branch. This study was conducted as a factorial experiment in randomized complete block design in three replications.

Method

The first factor included nitrogen fertilizer at three levels of 100 (control), 150 and 200 kg N ha⁻¹ and the second factor included pyridoxine application at three concentrations of 0, 0.02% and 0.04%. 27 pots were assigned to each replicate. Plastic pots with a diameter of 15 cm were used for seed planting. In order to prevent non-uniform germination of seeds, double the required amount of seeds were sown in each pot (5 seed per pot). Plants were thinned at 2 to 4 leaves stage. Seeds were planted at a depth of 1 to 2 cm of soil surface. Irrigation was regularly performed based on requirement and the amount of soil moisture.

The pots were placed in controlled conditions of heat and light, with day and night temperatures around 30 and 20 °C, respectively and a photoperiod of 16 hours light and 8 hours of darkness. All maintenance practices, including irrigation, pest and disease control were carried out according to need and results of previous researches conducted on this plant.

Pre-sowing seed treatments with pyridoxine were applied in controlled laboratory condition of Saveh

Islamic Azad University. For this purpose, the required amount of pyridoxine was measured by a digital balance with an accuracy of one milligram and then reached to the desired volume in a flat-bottom flask with distilled water. Each 0.02% and 0.04% pyridoxine treatments were provided in separate containers. Seeds were treated with pyridoxine solutions for eight hours. Distilled water soaked seeds for eight hours were treated as control treatment. Soaked seeds, were planted in pots within a short time. for nitrogen treatments, N was supplied using 46% urea fertilizer in three different levels, including n1: 100 kg N ha⁻¹ (control), n2; 150 kg N ha⁻¹ and n3: 200 kg N ha⁻¹. Nitrogen fertilizer was applied at two stages, once at 4 fully expanded leaves stage, and again at 6 to 8 fully expanded leaves stage. At each time, half of the desired amount of nitrogen (as urea 46%) was dissolved in half a liter of water and added to the each pot after weed controlling.

Observations dates were as follows: seedlings emergence, 2010; weeds observation, 2010, main leaves emergence (2-leaf stage), weed control, removal of excess plantlets and putting two plantlets in each pot, 2010; 4-leaf stage and the first application of fertilizer, 2010; 6 to 8 leaf stage and the second application of fertilizer, 2010, Weed control, plants thinning and early flowering observation, 2010, fruit maturity between 1to 2010 and final harvest, 2010.

One plant of each pot was selected randomly and evaluated for following measurements. For measuring fresh weight, plants were cut from the bottom and after cleaning and separating of waste materials, fresh weight of different organs (root, stem and leaves) were calculated using a balance with accuracy of centigram. Then each part was separately placed in cardboard bags with number of pot and replication. Bags were transferred to a shad place under the air flow and their content were removed and dried in the shade to prevent loss of alkaloid substances. In each sample, number of lateral shoots, number of leaves, plant height and number of fruits per plant were recorded separately.

Extraction of physalins

Lipids of dry materials of each sample were extracted using a Soxhlet extractor twice, and each time with 250 ml of normal hexane in 5 days. The lipid free powder was then dried for two days under the hood. The dry matter, was treated two times with acetone during 5 days and followed by solvent evaporation in a rotary, the crude extract of physalins was obtained (hotyin, 1968). Crude extract was added to saturated solution of lead acetate. After about 2 hours, active carbon was added and the solution was filtered immediately. The resulted solution was extracted two times with 240 ml of chloroform in a separation funnel. physalins were obtained after separating the organic phase and evaporation of the solvent (Makino et al., 1992). Physalins values for each sample are listed in the result section.

One and two-dim

ensional Thin layer chromatography (TLC) was performed in small plates of 5×2 and 5×5 cm with chloroform: methanol (9:1 ratio) and ethyl acetate: benzene (7:3 ratio) as solvent. Plates were immersed in Para-Anisaldehyde and then heated till appearance of purple spots of physalins (kawaie, 1987). All data were analyzed using the SAS software and mean separation was performed with Duncan's multiple range tests at 1% probability.

Results

Seed pyridoxine-priming treatments and different nitrogen levels

Results of the variance analysis showed that the effect of seed pyridoxine-priming treatments and different nitrogen levels were significant for plant height ($p < 0.01$) (Table 1). Maximum and minimum height was observed in 150 kg N ha⁻¹ and control treatments, respectively. Also, plants treated with 0.04% pyridoxine had the maximum height while control treatment resulted in lowest height. Analysis of pyridoxine × nitrogen levels interaction showed that the highest plant height was related to 200 kg N ha⁻¹ and 0.04% of pyridoxine (n3p3) treatment and the lowest height was correspond to 100 kg N ha⁻¹ and no pyridoxine (n1p1) treatment. Main effects of pyridoxine and nitrogen application were not

significant for number of lateral shoots, and there was no significant pyridoxine \times nitrogen levels interaction. However, 150 kg N ha⁻¹ treatment had the most impact by producing the highest number of lateral shoots while 200 kg N ha⁻¹ treatment produce the lowest number. The most and lowest impacts of pyridoxine treatment was observed for 0.04% and 0 concentrations, respectively. Also, result of pyridoxine \times nitrogen levels interaction demonstrated that the most number of lateral shoots was related to

150 kg N ha⁻¹ and 0.04% of pyridoxine (n2p3) treatment and the lowest number was belong to 100 kg N ha⁻¹ and no pyridoxine (n1p1) treatment. These results indicate that applying 150 kg N ha⁻¹ and 0.04% of pyridoxine has the greatest effect on the number of lateral shoots. Leaf dry weight was significantly affected by different levels of nitrogen fertilizer and pyridoxine ($p < 0.01$), but there was no significant pyridoxine \times nitrogen levels interaction (Table 1).

Table 1. Analysis of variance for the effects of nitrogen fertilizer and pyridoxine application on yield quality and quantity of *Physalis*.

Source of Variation	Plant Height	Number of Lateral Shoot	Leaf Dry Weight	Stem Dry Weight	Fruit Dry Weight	Plant Dry Weight	Root Dry Weight	Fruit Physalins Content	Leaf Physalins Content
Replication	3.72*	0.73 ^{ns}	0.15 ^{ns}	0.25 ^{ns}	0.16 ^{ns}	0.43 ^{ns}	0.12 ^{ns}	0.0004 ^{ns}	0.00002 ^{ns}
Nitrogen (N)	29.71**	1.05 ^{ns}	3.06**	0.79**	4.59**	22.83**	1.28*	0.0073**	0.0045**
Pyridoxine (P)	23.06**	0.13 ^{ns}	1.59**	0.24 ^{ns}	1.32 ^{ns}	8.1*	3.12**	0.0011**	0.0006**
N \times P	0.97 ^{ns}	0.32 ^{ns}	0.14 ^{ns}	0.03 ^{ns}	0.53 ^{ns}	0.69 ^{ns}	0.16 ^{ns}	0.0002 ^{ns}	0.0002*
Cv	2.48	24.14	4.75	9.27	28.03	6.88	4.48	5.3	4.5

Note: * and ** denote significance at $p < 0.05$ and $p < 0.01$, respectively. ns: non significance

Table 2. Mean comparison for the effects of nitrogen fertilizer and pyridoxine application on yield quality and quantity of *Physalis*.

Nitrogen \times Pyridoxine	Plant Height	Number of Lateral Shoot	Leaf Dry Weight	Stem Dry Weight	Fruit Dry Weight	Plant Dry Weight	Root Dry Weight	Fruit Physalins Content	Leaf Physalins Content
n ₁ p ₁	27.96 c	3.33 a	5.35 b	2.07 b	1.23 b	8.68 b	5.33 b	0.205 b	0.183 b
n ₁ p ₂	30.18 b	3.00 a	6.08 b	2.12 b	1.09 b	9.29 b	6.05 ab	0.226 b	0.203 b
n ₁ p ₃	30.76 b	2.50 b	6.55 ab	2.27 b	1.62 b	10.45 b	6.35 ab	0.245 ab	0.220 b
n ₂ p ₁	31.13 b	3.33 a	6.79 ab	2.49 b	1.86b	11.15 ab	6.06 b	0.267 ab	0.234 ab
n ₂ p ₂	32.33 ab	3.66 a	6.94 a	2.70 ab	2.80 b	12.44 ab	6.51 ab	0.282 a	0.244 a
n ₂ p ₃	34.47 a	3.50 a	7.57 a	2.98 a	3.38 a	13.93 a	7.29 a	0.283 a	0.251 a
n ₃ p ₁	31.75 b	3.00 a	6.66 ab	2.31 b	1.48 b	10.46 b	5.58 b	0.263 ab	0.235 ab
n ₃ p ₂	32.25 ab	2.50 b	6.67 ab	2.62 ab	2.04 ab	11.69 ab	6.83 a	0.273 a	0.236 ab
n ₃ p ₃	35.05 a	3.00 a	2.64 ab	2.64 ab	2.42 ab	11.74 ab	6.89 a	0.273 a	0.237 ab

Note: within a column, means followed by the same letter are not significantly different at the 0.01 level of probability by Duncan's multiple range tests.

Table 3. Mean comparison for the effects of nitrogen fertilizer application on yield quality and quantity of *Physalis*.

Nitrogen levels Pyridoxine	Plant Height	Number of Lateral Shoot	Leaf Dry Weight	Stem Dry Weight	Fruit Dry Weight	Plant Dry Weight	Root Dry Weight	Fruit Physalins Content	Leaf Physalins Content
Control n1(100 kg N ha ⁻¹)	29.75 b	2.9 a	6.05 c	2.17 b	1.34 c	9.57 C	5.95 b	0.227 b	0.204 B
n2 (150 kg N ha ⁻¹)	32.83 a	3.5 a	6.82 b	2.75 a	2.75 a	6.69 A	6.69 a	0.278 a	0.244 A
n3 (200 kg N ha ⁻¹)	33.52 a	2.85 a	7.15 a	2.54 a	1.99b	6.49 A	6.49 a	0.270 b	0.236 A

Note: within a column, means followed by the same letter are not significantly different at the 0.01 level of probability by Duncan's multiple range tests

Table 4. Mean comparison for the effects of pyridoxine application on yield quality and quantity of *Physalis*.

Pyridoxine Levels	Plant Height	Number of Lateral Shoot	Leaf Dry Weight	Stem Dry Weight	Fruit Dry Weight	Plant Dry Weight	Root Dry Weight	Fruit Physalins Content	Leaf Physalins Content
P1(0)- control	30.10 c	3.25 a	6.22 c	2.29 b	1.53 b	10.04 c	5.66 b	0.243 b	0.216 b
P2 (0.02%)	31.5 b	3.12 a	6.55 b	2.47 ab	2.06 ab	11.08 b	6.43 a	0.258 a	0.226 a
P3 (0.04%)	33.42 a	3.00 a	7.05 a	2.62 a	2.37 a	12.05 a	6.82 a	0.266 a	0.235 a

Note: within a column, means followed by the same letter are not significantly different at the 0.01 level of probability by Duncan's multiple range tests.

Extraction of physalins

The highest leaf dry weight was corresponded to 200 kg N ha⁻¹ treatment and the least amount was related to control (100 kg N ha⁻¹) treatment. Application of 0.04% pyridoxine resulted in the highest leaf dry weight while control treatment (no pyridoxine) produced the least amount. As it is shown in table 2, the greatest amount of leaf dry weight was related to n2p3 interaction (150 kg N ha⁻¹ and 0.04% of pyridoxine) and the lowest weight was for n1p1 interaction (100 kg N ha⁻¹ and no pyridoxine). Based on results, different levels of nitrogen application significantly affect stem dry weight, while there was no significant impact for different levels of pyridoxine ($p < 0.01$). No significant difference was observed for pyridoxine × nitrogen levels interaction. Maximum stem dry weight was related to 150 kg N ha⁻¹ treatment and the lowest weight was observed for control treatment (100 kg N ha⁻¹). Compare to other

levels of pyridoxine, 0.04% pyridoxine treatment resulted in the highest stem dry weight. Results for pyridoxine × nitrogen levels interactions showed the most shoot dry weight was belong to n2p3 interaction (150 kg N ha⁻¹ and 0.04% of pyridoxine) and the lowest weight was related to n1p1 interaction (100 kg N ha⁻¹ and no pyridoxine).

Analysis of variance for fruit dry weight, showed that fruit dry weight was significantly affected by different levels of nitrogen fertilizer, but there was no significant impact for pyridoxine levels at 1% probability. Also, no significant difference was observed for pyridoxine × nitrogen levels interactions (Table 1). Maximum and minimum fruit dry weight was observed in 150 kg N ha⁻¹ and control treatments, respectively. Among plants treated with different concentrations of pyridoxine, those treated with 0.04% concentration had the maximum fruit dry

weight. results of pyridoxine \times nitrogen levels interaction showed that the highest fruit dry weight was related to 150 kg N ha⁻¹ and 0.04% of pyridoxine (n2p3) treatment and the lowest weight was correspond to 100 kg N ha⁻¹ and no pyridoxine (n1p1) treatment.

The effect different pyridoxine and nitrogen levels were significant for root dry weight at 1% and 5% level of probability, respectively. However, pyridoxine \times nitrogen levels interaction effect was not significant for this trait. The most amount of root dry weight was related to n2 (150 kg N ha⁻¹) treatment and the lowest amount were related to control (100 kg N ha⁻¹) treatment. The concentration of 0.04% pyridoxine resulted in the highest root dry weight, while control treatment (no pyridoxine) produced the least amount. The greatest amount of root dry weight was related to n2p3 (150 kg N ha⁻¹ and 0.04% of pyridoxine) treatment and the lowest weight were obtained in n1p1 interaction (100 kg N ha⁻¹ and no pyridoxine) treatment. Percent of fruit physalins was significantly affected by different levels of nitrogen fertilizer and pyridoxine ($p < 0.01$) and significant pyridoxine \times nitrogen levels interactions were observed for this trait (Table 1). Among different levels of nitrogen fertilizer, 100 kg N ha⁻¹ treatment showed the most impact on percent of fruit physalins whereas the least effect was related to control treatment. Application of 0.04% pyridoxine led to the highest percent of fruit physalins as compared to other pyridoxine concentrations. Results for pyridoxine \times nitrogen levels interactions showed the most physalins was produced in fruits of n2p3 (150 kg N ha⁻¹ and 0.04% of pyridoxine) treatment and the lowest percent was produced in n1p1 (100 kg N ha⁻¹ and no pyridoxine) treatment.

According to the results of table 1, percent of leaf physalins was significantly affected by different pyridoxine and nitrogen levels at 1% probability. Pyridoxine \times nitrogen levels interactions was significant for this trait at 5% probability. The highest and the lowest percent of leaf physalins was related to n2 (150 kg N ha⁻¹) and control (100 kg N ha⁻¹)

treatments, respectively. Among different levels of pyridoxine, 0.04% concentration resulted in the most percent of leaf physalins. The most effective of pyridoxine \times nitrogen levels interaction for percent of leaf physalins was related to 150 kg N ha⁻¹ and 0.04% of pyridoxine (n2p3) treatment and the least effective interaction was correspond to 100 kg N ha⁻¹ and no pyridoxine (n1p1) treatment.

Discussion

Pyridoxine application could promote root growth which consequently enhance nutrient uptake from soil. This in turn leads to improved plants' vegetative and reproductive growth, as well as increased amount of active ingredients, essences and yield of medicinal plants (Prasad *et al.*, 1985). In Solanaceae family species, alkaloid substances are produced in roots which then transmitted to aerial parts. Furthermore, soil nutrition such as nitrogen and phosphorus, enhance active ingredients and essences of medicinal plants (Houshmandfar and Eradatmand, 2011). According to the average production of leaf, stem height, width and depth of the root, etc, it could be concluded that in all cases the maximum performance is achieved with the application of nitrogen fertilizer (Zare Zadeh *et al.*, 1997; Pournaghi and Eradatmand, 2008).

Conclusion

In general, results of this study indicate that nitrogen fertilizer mostly increased shoot growth (leaves and stems) and had lower effect on root growth. On the other hand, seed inoculation with pyridoxine enhanced plant rooting. Several studies conducted about seed treatment with pyridoxine, demonstrate increased uptake of nitrogen and phosphorus as a function of pyridoxine application (Khan *et al.*, 1996., Samiullah *et al.*, 1992).

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