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EFFECTS OF DIFFERENT NUTRITIONAL SYSTEMS ON SEED GERMINATION AND EARLY SEEDLING GROWTH IN MEDICINAL PUMPKIN (*CUCURBITA PEPO* L.)

D. ZAREI[1], G. SHABANI[2,*], M. REZA CHAICHI[3], M. KHANAHMADI[1], A. AKBARABADI[1]

*E-mail: shabani.ghobad@yahoo.com

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ABSTRACT. This study was carried out to determine the effect of different nutritional systems (chemical, biological and integrated) on germination and seedling growth in medicinal pumpkin (*Cucurbita pepo* L.). The statistical design was a randomized complete block design with four replications. Four levels of different fertilizing systems including chemical (T1), biological (a combination of nitrogen bacteria, *Azospirillum brasilense* and *Glomus mosseae*) (T2) and integrated fertilizing systems (biological fertilizer + 50% chemical fertilizer) (T3), and control (without fertilizer) (T0), were employed. The results indicated that the maximum seed germination was 95% and the highest seed germination rate with 30.4 per day was observed in the intergraded nutritional treatment. The experimental results showed that all nutritional treatments had positive effects on seed germination compared to control. The highest level of germination percentage with 95% and the highest rate of germination with 30.4 seeds per day were obtained in integrated nutritional treatment. However, the integrated nutritional system required more time to demonstrate its positive effect on the growth and yield of medicinal pumpkin compared to chemical system. The results of present experiment indicated that integrated nutritional treatment had the greatest positive impact on germination characteristics in medicinal pumpkin. Designing and developing such nutritional systems can guarantee and facilitate the achievement of long-term objectives of sustainable agriculture.

Key words: Nutritional systems (chemical, biological and integrated); Germination characteristic.

[1] Department of Medicinal Plant, Kermanshah Branch, Academic Center for Education, Culture and Research, Iran
[2] Young Researchers and Elite Club, Malayer Branch, Islamic Azad University, Malayer, Iran
[3] College of Agriculture, California State Polytechnic University, Pomona, USA

INTRODUCTION

Medicinal pumpkin (*Cucurbita* pepo L.) is one the most important annual oil seed plants from cucurbitaceous family. It is native to tropical and subtropical regions of the world (Gong *et al.,* 2012; Lim, 2012; Esteras *et al.,* 2012). The high percentage of protein and oil in pumpkin seed, reflecting the importance and the nutritional value of the product, have been reported in literature (Ryan *et al.,* 2007; Mohamed *et al.,* 2009).

Better performance of plants due to inoculation by plant growth promoting bacterial in terms of germination, seedling growth, nutrient uptake, and yield has been reported in tomatoes (Al-Karaki, 2000) and other crops (Sharifi *et al.,* 2007; Ruiz-Lozano and Azcon, 2000). Dileep Kumar *et al.* (2001) showed that the inoculation of pea seeds with *Pseudomonas* sp. lead to higher stems, root length and dry weight compared to control treatment. Migahed *et al.* (2004) reporded that seed inoculation by *Azotobacter* alone or by a combination with other bio-fertilizers such as *Bacillus* and *Azospirillum* significantly improved growth properties of *Apium graveolens*. Other studies, also confirmed the positive effects of root inoculation of herbs like dill and cumin with two Mycorrhiza strains, on shoot dry weight (Kapoor *et al.,* 2002). Amiri *et al*. (2013) in their study on fennel showed that bio and organic fertilizers application can be a proper approach to produce better quality seed in an organic farming system resulting in improving growth and germination properties. Mahfouz and Sharaf Eldin (2007), investigated the effects of *Azotobacter*, *Pseudomonas* and *Azospirillum* on fennel and showed that using these bio-fertilizer treatments lead to increased height and dry weight of the plant.

The ever growing importance of medicinal plants in human health and safety accounts for a considerable efforts for improving the qualitative and quantitative properties of such plants. The purpose of this study was to investigate the germination properties of paper skin medicinal pumpkin seeds under different nutritional systems (chemical, biological and integrated).

MATERIALS AND METHODS

This experiment was conducted in Academic Center for Education, Culture and Research (ACECR) Laboratory, Kermanshah, Iran, during 2013 growing season. The coordinates of the site was 34 17’N, and 47, 0’E. The elevation of the site was 1300 m above the sea level. The experimental treatments on parental plants consisted of four levels of different fertilizing systems including chemical (N, P, K fertilizers according to soil test) (T1), biological (a combination of nitrogen fixing bacteria of *Azospirillum brasilense* and *Glomus mosseae*) (T2) and integrated fertilizing system (biological fertilizer+50% chemical fertilizer) (T3) and control (no fertilizer application) (T0).

The primary seeds in corresponding biological and integrated treatments were inoculated in a plastic bag and mixed slowly with a biological fertilizer in room temperature. The amount of the chemical fertilizers (amonium phosphate and urea) in chemical nutrition and integrated treatments were calculated based on soil test (Shabani *et al.,* 2012). After plants reached physiological maturity, all the seed from different fertilizing treatments were harvested and kept at room temperature before being transferred to laboratory for germination tests.

The germinator was set at 25°C. Petri dishes with 9×9 cm dimensions were used to perform the experiment in laboratory. Two layers of filter papers (at the bottom and the top of the seeds) were placed in Petri dishes and then 5 mm of distilled water was added.

Each Petri dish contained 10 seeds from each treatment and was replicated three times. The number of germinated seeds was counted and recorded on daily basis up to 14 days (Amiri *et al.,* 2012). After fourteenth days, the number and length of plumule along with the root length were measured by transparent ruler. To determine the dry weight of plumules and roots, the samples were placed in an electrical oven at 72˚C for 48 hours. The dry weight of seedlings were measured by a digital scale with 0.001 g precision. The percentage of germinated seed was measured after 14 days. The rate of germination was calculated by the following equation (Salehzade *et al.*, 2009):

$$X1/Y1 + (X2 – X1)/Y2 + \ldots + (Xn – Xn\text{-}1)\ Yn,$$

where Xn is the germinated seeds percentage of nth counting and Yn is the number of days from beginning of cultivation to the nth counting time. The data obtained was analyzed by SAS 9.1 statistical software. The Duncan test with probability level of 1% was used to compare the means.

RESULTS AND DISCUSSION

The percentage and rate of germination

The results of ANOVA showed that there is a significant difference among the treatments in germination percent and rate (*Table 1*). The integrated nutritional treatment had the highest germination percentage (95%), compared to control with only 20% of germination. The seeds obtained from chemical and integrated nutrition treatments had significantly higher germination rate compared to other treatments and control (*Table 2*).

Plant growth promoting bacteria species of *Pseudomonas* sp., *Azotobacter* sp., *Azospirillum* sp. and *Bacillus* sp. have been reporded for their positive effects on germination of different crop plants (Rajaee *et al.,* 2007). The seed germination enhancement can be as a result of the increasing of cytokenin which stimulates cell division (Tajik *et al*., 2009). Krishna *et al*. (2008) have recorded that utilizing bio-fertilizers such as *Azospirillum* sp., phosphate solubilizing bacteria and their combination in plants such as *Ocimum sanctum* and *Withania somniferum* improved germination percentage and rate. Moreover, the results of Wu *et al*. (2005) indicated that application of growth promoting bacteria, improve water and nutrition absorption in plants. So, the higher seed germination percentage and rate under chemical and integrated nutritional systems could be probably explained by the higher amount of food reserves in seeds compared to other treatments.

Length of rootlet and plumule

The results of ANOVA of the length of plumule data and rootlet showed a significant difference among nutritional treatments (*Table 1*). Seeds from integrated nutritional treatment had the greatest impact on rootlet length with 7.5 cm and had a significant superiority compared to other treatments. The lowest rootlet length of 3.7 cm belonged to biological nutritional treatment. Plumule and rootlet length followed the same trend among different nutritional systems. The highest length of the plumule belonged to integrated nutritional treatment with 1.9 cm longer than control treatment with the lowest plumule length (*Table 2*).

Growth promoting bacteria enhance plant growth by stimulating the plant growth substances production such as auxin, gibberellin and cytokenin hormones (Ravikumar *et al.,* 2004). Dileep Kumar *et al.* (2001) showed that inoculation of pea seeds with *Pseudomonas* lead to increased plant height, root length and dry weight, compared to control treatments. It is reported that bacteria can contain growth regulating substances such as auxin, gibberellin and cytokinin resulting in the improvement of seed germination characteristics including plumule elongation (Amiri *et al*., 2013). Arancon *et al*. (2004) reported in their study that increased activity of microorganisms and increased holding capacity of nutrient elements resulted in improved plant growth characteristics. Ilbas and Sahin (2005) also reported inoculated soybean stem elongation by *Arbuscular mycorrhizal* fungus and *Glomus fasciculatum* bacteria inoculation. Research has shown that bacteria belonging to the genus *Pseudomonas* had the ability to consume aminocyclopropane-1-carboxylate by creating the enzyme aminocyclopropane-1-carboxylate deaminase. Bacteria that consume aminocyclopropane-1-carboxylate cause the elongation of roots through reducing the concentration of ethylene within the plant and converting it into nitrogen sources (Cartieaux *et al.,* 2003).

Wet and dry weight of rootlet and plumule

The ANOVA results showed a significant difference among the treatments in terms of wet and dry weight of rootlet and plumule (*Table 1*). Seeds from integrated nutritional treatment had significantly the highest wet (0.24 g) and dry weights (0.03 g). Control treatment had the lowest wet weight of rootlet, which was 0.1 g lower than integrated treatment. The lowest dry weights of rootlet were observed in the biological nutritional system and control treatments, which were 0.02 and 0.1 g lower than integrated and chemical treatments, respectively (*Table 2*).

The highest wet weight of plumule was observed in the seeds from integrated nutritional treatment (1.9 g) that was 0.1 and 0.2 g more than the chemical and biological systems, respectively. The seeds from control treatment had significantly the lowest wet weight of plumule.

The highest dry weight of plumule was observed in the seeds from integrated nutritional treatment (0.24 g) that was 0.1 g more than control treatment having the lowest dry weight of plumule (*Table 2*). Our results supports other research involving application of bio-fertilizers. Ravikumar *et al*. (2004) reported that *Azotobacter* caused the highest dry weight of rootlet and plumule by creating growth promoting hormones and various siderophores and also through increasing the solubility of phosphorus. Migahed *et al*. (2004) also indicated that using *Azotobacter* alone or combined with other biological fertilizers such as *Azospirillum* and *Bacillus* resulted in improved growth characteristics of wild celery including its dry weight. Biofertilizers can improve growth characteristics by creating plant hormones such as indole acetic acid (IAA) (Amiri *et al.,* 2013). Shaalan (2005) reported that the seeds treated by bio- fertilizers such as *Azospirillum, Azotobacter* and *Pseudomonas*, improved the growth characteristics and dry weight of medicinal plants.

Traits correlation analysis

Correlation analysis of the traits related to the seed germination tests is shown in *Table 3*. There was a significant negative correlation between plumule dry weight and germination percentage (r=-0.980**).

Table 1 - ANOVA result table for characteristics

S.O.V.	df	M.S.							
		Germination (%)	Germination rate (no.day-1)	Length of rootlet (cm)	Wet weight of rootlet (g)	Dry weight of rootlet (g)	Length of plumule (cm)	Wet weight of plumule (g)	Dry weight of plumule (g)
R	3	12.50^{ns}	0.49^{ns}	0.47^{ns}	0.001^{ns}	0.00001^{ns}	0.06^{ns}	0.01^{ns}	0.001^{ns}
Nutrition	3	291.66**	23.60**	9.15**	0.008**	0.00004**	2.60**	0.29**	0.004**
Error	9	24.72	1.18	0.19	0.001	0.00001	0.08	0.02	0.001
C.V.		15.37	10.62	9.51	7.36	4.11	8.21	9.16	7.05

ns - non significant; *significant at p≤0.05; **significant at p≤0.01

Table 2 - The main effects of treatments on the traits

S.O.V.	M.S.							
	Germination (%)	Germination rate (no.day-1)	Length of rootlet (cm)	Wet weight of rootlet (g)	Dry weight of rootlet (g)	Length of plumule (cm)	Wet weight of plumule (g)	Dry weight of plumule (g)
T0	75 b	25.2 b	5.1 b	0.15 c	0.01 c	2.9 c	1.36 b	0.14 b
T1	85 ab	29.6 a	5.2 b	0.2 b	0.02 b	4.1 b	1.8 a	0.2 ab
T2	80 ab	27.1 ab	3.7 c	0.14 c	0.01 bc	4.1 b	1.7 a	0.15 c
T3	95 a	30.4 a	7.4 a	0.24 a	0.03 a	4.8 a	1.9 a	0.24 a

[a]T0 to T3: different nutritional systems; [b]Means with the same letter in each column are not significantly different at 5% probability level.

Tabel 3 - Simple correlation coefficients of different nutritional systems effects on germination and seedling growth in medicinal pumpkin (*Cucurbita pepo* L.)

Characteristics	Germination (%)	Length of rootlet	Length of plumule	Wet weight of rootlet	Wet weight of plumule	Dry weight of rootlet	Dry weight of plumule	Germination rate
Germination	1							
Length of rootlet	-0.29 ns	1						
Length of plumule	0.87 ns	0.44 ns	1					
Wet weight of rootlet	0.06. ns	-0.43 ns	-0.25 ns	1				
Wet weight of plumule	- 0.18. ns	-0.49 ns	-0.40 ns	0.95 *	1			
Dry weight of rootlet	- 0.24. ns	- 0.28 ns	- 0.53 ns	0.95*	0.97 *	1		
Dry weight of plumule	- 0.98.**	0.18 ns	- 0.76 ns	- 0.15 ns	0.11 ns	0.13 ns	1	
Germination rate	0.80. ns	- 0.09 ns	0.93*	-0.52 ns	-0.69 ns	-0.76 ns	-0.71 ns	1

ns - non significant; *significant at $p \leq 0.05$; **significant at $p \leq 0.01$

There was a significant positive correlation between germination speed and plumule length (r=0.930*). The rootlet wet weight was positively correlated with plumule wet weight (r=0.956*) and rootlet dry weight (r=0.950*). There was also a significant positive correlation between plumule wet weight and rootlet dry weight (r=0.973*).

The negative correlation between plumule dry weight and germination percentage could mean that with increasing germination percentage, a higher number of seeds would germinate, that consume higher portion of available moisture and occupy more growth space. As a result, plumule growth and the amount of absorbed moisture would decrease, which ultimately leads to reduced dry weight. The positive correlation between germination speed and plumule length indicates that more rapid germination means an early start in plant growth, and consequently provide more time for growth and results in longer plumule.

Positive relationship between rootlet wet weight and rootlet dry weight is obvious, because dry weight was obtained after water had been evaporated from the rootlet tissue. But the higher rootlet weight means it has higher number of cells and there is higher amount of moisture in them and it also helps in the absorption of moisture by plumule. That is probably why the rootlet wet weight and the plumule dry weight was positively correlated. The plumule wet and rootlet dry weights were positively correlated for the same reason.

CONCLUSION AND RECOMMENDATIONS

The results of present experiment indicated that the nutritional treatments used on parental plants had a positive impact on germination characteristics of produced seeds, compared to the control treatment (no fertilizer application). Integrated nutritional treatment had the greatest positive impact on germination characteristics in medicinal pumpkin. While reducing the application of chemical fertilizer, inoculation of seeds with growth promoting bacteria in integrated nutritional system, improved germination characteristics and reduced environmental hazards. Designing and developing such nutritional systems can guarantee and facilitate the achievement of long-term objectives of sustainable agriculture.

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