



Effect of Biological and Chemical Nitrogen Fertilizers on Yield, Yield Components and essential oil content of German Chamomile (*Matricaria chamomilla* L.) in Shahr-e-Ray Region

Mohammad Kariminejad* and Alireza Pazoki**

*The member of Young Researcher's and Elite Club, Yadegar - e- Imam Khomeini (RAH) Shahr-e-ray Branch, Islamic Azad University, Tehran, IRAN

**Department of Agronomy and Plant breeding, Yadegar - e- Imam Khomeini (RAH) shahre-ray Branch, Islamic Azad University, Tehran, IRAN.

(Corresponding author: Alireza Pazoki)

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ABSTRACT: In order to evaluate the variations of some morphological traits, yield, seed yield components and essential oil yield of German Chamomile under different application levels of nitrogen (N) and biofertilizers, a split plot experiment laid out in a randomized complete blocks design with three replications in Shar-e-Ray (Gale No) region. Chemical N fertilizer application in three levels (0, 65 and 130 Kg/Ha) was considered as the main factor and biofertilizers application (Control, Nitroxin, Barvar-2 phosphate and a mixture of both) as the secondary factor. Variance analysis results showed that there was no significant relationship between Chamomile Harvest Index and N fertilizer application. Also, the interaction effect of N and biofertilizers was not significant on Chamomile seed and essential oil yields. The rise in N application led to a significant increase in plant height, Capitol fresh and dried yield, seed and essential yields. More ever, the application of biofertilizers, especially Nitroxin, increased the vegetative growth and essence percentage. The evaluation of interaction effects indicated the influential role of biofertilizers, along with high levels N fertilizers application, in Chamomile higher essence percentage and yield, since the highest essence yield (6.76 Kg/Ha) was obtained from the treatment that was concurrently fertilized with Nitroxin and Barvar-2 phosphate biofertilizers at the second N fertilization (N2B4) level. According to the results, it seems that concurrent application of biofertilizers and N not only increases vegetative growth and essence yield, but also gradually decreases chemical fertilizer consumption which is a prerequisite of Chamomile organic production.

Key words: Biofertilizer, Nitrogen, Essential oil, German chamomile (*Matricaria chamomilla* L.)

INTRODUCTION

German Chamomile is a perennial herb which belongs to Asteraceae (Franke & Schilcher, 2005). According to the reports, it is originated from central Asia and Mediterranean regions. This plant is widely spread in Iran, from West to East and from North to the center of the country. It has a direct stem and depending on the growth climatic conditions, its height varies from 30 to 80 cm. Also, the diameter of the flowers varies from 0.6 to 1.6 cm and rarely reaches 2.2 cm. flowers are hermaphrodite and the plant itself is a self-pollinated one (Bernath, 2000). Chamomile has various compounds. For example, Paulsen *et al* (2002) identified 10 chemical compounds in Chamomile essence, among them, Camphor, Chrysanthenyl acetate, and Camphene can be considered as the most important compounds. The aim of medicinal plant commercial production is to obtain more biomass per area along with higher amounts of active ingredients. Fertilizers are among the most significant factors which affect the production of such plants and the above-mentioned ingredients may positively or negatively respond to

fertilizer application. Realizing such an issue requires conducting several nutritional studies (Dufault *et al.*, 2003). In this regard, Okut and Drum (2005), in a study trying to evaluate the effects of different application levels of N fertilizer (0-90 Kg/Ha), found out that the number of umbrellas per plant and seed yield in a treatment fertilized with 30 Kg/Ha N, compared to other treatments, increased significantly.

Literally, the term "Bio stimulants" refers to a set of compounds which stimulate living (Thomas *et al.*, 2009). Generally, bio stimulants stimulate metabolism and metabolic processes in order to increase the quantitative and qualitative growth as well as the efficiency of plants (Starck, 2005). Fallahi *et al* (2008) in an experiment showed that the application of Nitroxin and phosphate solubilizing bacteria (PSB) (increased the fresh and dried yield since the highest yield (8600 gr/ha) was obtained from PSB applied treatment. Furthermore, the effects of biofertilizers on medicinal Chamomiles and Marigold were studied in another experiment in Cuba.

Results suggested that the application of these fertilizers increased Marigold flower yield and medicinal quality, but despite increasing Chamomile flower yield, had no significant effect on its medicinal quality (Sanches *et al.*, 2005).

MATERIALS AND METHODS

This research was conducted in 2013 crop planting season in Chal Tarkhan village, Ghale No region, Shahr-e-Ray district. It was a split plot experiment laid out in a randomized complete blocks design with three replications. Different levels of N chemical fertilizers (0, 65, and 135 kg/ha of pure N) were considered as the main factors and different levels of biofertilizers (not applying, Nitroxin, Barvar-2 phosphate biofertilizers, and Nitroxin + Barvar-2 phosphate biofertilizers) as the secondary factors of the study. Nitroxin which contained a set of most active N fixing bacterial strains (including *Azotobacter* sp. and *Azospirillum* sp.) was mixed with seeds and applied at 2 lit/ha. Also, a phosphate Biofertilizer, commercially named "Barvar-2" was used to inoculate seeds and then, was applied at 100 gr/ha an hour before sowing. It should be noted that seeds from a local German Chamomile strain was used in this research and sowed 1 cm below soil surface. After tilling, several plots with 4m length and 2.5m width were designed, each of which contained 5 sowing rows which were 30cm apart and the distance between the plants was considered to be 10 cm (33.33 plants/m²).

To achieve a greater level of accuracy, the first and last rows of every plot as well as the 50cm from the beginning and the end of each row were considered as margins. In order to determine plant height and number of capitols per plant, 7 plants were randomly sampled and these two traits were measured. Seed yield, capitol fresh and dried biological yield were also determined the same way. Capitol Harvest Index (HI) was computed using the following equation:

$$\text{Seed Harvest Index} = (\text{Seed Yield/Biological Yield}) \times 100$$

Finally, the dry flowers of each treatment and replication were separately carried to the laboratory and their essence percentage was measured. Through water distilling procedure and using Clevenger apparatus, essence was extracted from 50gr Chamomile dry capitol powder. Essence yield was computed using the following equation:

$$\text{Essence Yield} = (\text{Capitol Essence Percentage} \times \text{Capitol Yield}) / 100$$

SAS software package was used to analyze the obtained data and their means were compared using Duncan Test at a significance level of 5% and finally, diagrams were drawn using Excel software.

RESULT AND DISCUSSION

The results showed that concurrent application of bio stimulants and chemical N fertilizers had a significant effect on stem height, number of capitols per plant, capitol fresh and dry weight, seed yield, biological yield, essence percentage, and essence yield at a

significance level of 1%, while only the application of bio stimulants significantly affected harvest index. Furthermore, the interaction effects of the experimental factors had significant effects on several plant traits but seed yield and essence yield were not significantly affected (Table 1).

A. Stem Height and Number of Capitols per Plant

Results suggested an incremental trend between N consumption and two traits, namely, stem height and number of capitols per plant, since the highest plant (58.2 cm) and the highest number of capitols per plant (174.82) belonged to N3 treatment which was fertilized with 130 Kg/ha N (Table 2). Essentially, the increased stem height could be attributed to intensifying effect of N on vegetative growth and cell division in plant's organs, especially the stem. More ever, mean comparisons indicated the intensifying effect of biofertilizers on the above mentioned traits, since the concurrent application of Nitroxin and Barvar-2 phosphate (B4) biofertilizers increased the height by 15.32% and the number of capitols per plant by 21.70%, compared to the non-fertilized treatment. In a research, studying a species of Mesquite, it was observed that *Rhizobium* bacteria increased stem height and plant biomass, compared to the control (Ghassemi *et al.*, 2007). Also, the study of interaction effects suggested the rise in plant height and number of capitols per plant due to the concurrent application of Nitroxin and Barvar-2 phosphate biofertilizers at the third N application level N3B4, (Table 3). It seems that biological fertilizers have increased the height and yield through improving the availability of nutrients. According to Sanchez *et al* (2005), the application of biofertilizers increases the number of Chamomile's flowers.

B. Capitol Fresh and Dry Yield

Results of mean comparisons revealed the increasing effect of applying 130 Kg/ha N (N3 treatment) by 77.99% of fresh weight and 78.93% of dry yield, compared to the control (Table 2). In a pot test, applying 1.2 gr/pot more N fertilizer increased Chamomile's flower yield, while applying higher amounts of this fertilizer had no significant effect on yield (Emongor *et al.*, 2008). More ever, in another study it has been shown that higher application of chemical N fertilizer significantly increased Chamomile's dry flower yield (Anonymous, 2007). The evaluation of biofertilizers also revealed that applying a mixture of Nitroxin and Barvar-2 phosphate biofertilizers, compared to the control, increased capitol fresh and dry yield by 14.5% and 12.80% respectively (Table 2). Based on these results, it can be concluded that due to the optimum application of the nutrients in N3B4 treatment, the number of flowers per plant at each clipping was more than other plots and led to a higher capitol fresh and dry yield.

Table 1: Variance analysis of the effect of nitrogen fertilizer levels and biofertilizers application on the evaluated traits in German Chamomile.

S.O.V	df	Plant Height	Number of Capitols per Plant	Capitol fresh Weight	Capitol Dry Weight	Seed Yield	Biological Yield	Harvest Index	Essence Percentage	Essence Yield
Replication	2	14.96	42.98	9163.99	407.17	177.87	276830.74	1.15	0.012	0.556
Nitrogen Fertilizer (N)	2	960.3**	23220.50**	6553051.62**	839195.70**	6319.37**	37322585.21**	0.77 ^{ns}	0.128**	41.77**
E(a)	4	4.28	30.26	1331.35	85.58	16.28	301638.13	1.32	0.002	0.105
Biofertilizers (B)	3	116.24**	1070.32**	217537.78**	17052.72**	145.24**	7931038.92**	42.49**	0.01**	1.77**
Interaction Effect (N×B)	6	16.34**	66.08*	12790.38**	2297.11**	4.22 ^{ns}	404831.31**	0.91**	0.002**	0.06 ^{ns}
E(b)	18	3.46	17.57	591.87	49.34	5.98	8164.7	0.16	0.0004	0.044
CV (%)	-	13.68	13.26	10.88	14.82	12.55	11.56	12.70	9.44	9.88

ns, *, and ** refer to non-significant and significant at the statistical level of 0.05 and 0.01, respectively.

Table 2: Mean comparisons of different nitrogen fertilizer levels and biofertilizers application on the evaluated traits in German Chamomile.

Treatment	Plant Height (Cm)	Number of Capitols per Plant	Capitol fresh Weight (Kg/ha)	Capitol Dry Weight (Kg/ha)	Seed Yield (Kg/ha)	Biological Yield (Kg/ha)	Harvest Index (%)	Essence Percentage (%)	Essence Yield (Kg/ha)
N Fertilizer (N)	-	-	-	-	-	-	-	-	-
N₁= 0 kg/ha	40.71 c	87.32 c	2061.40 c	639.97 c	74.95 c	4289.5 c	15.11 a	0.37 b	2.42 c
N₂=65 kg/ha	52.75 b	123.14 b	2615.04 b	756.86 b	92.26 b	5279.2 b	14.68 a	0.57 a	4.32 b
N₃=130 kg/ha	58.20 a	174.82 a	3524.97 a	1145.13 a	120.42 a	7716.2 a	15.13 a	0.53 a	6.15 a
Biofertilizers (B)	-	-	-	-	-	-	-	-	-
B₁=Control	46.45 c	113.90 c	2515.29 d	795.96 d	90.77 c	4387.20 d	18.14 a	0.44 b	3.67 c
B₂=Nitroxin	53.56 a	126.57 b	2743.72 c	832.09 c	99.76 a	6210.62 b	13.50 c	0.51 a	4.36 b
B₃=Phosphate (Barvar 2)	48.62 b	134.62 a	2799.96 b	863.32 b	94.68 b	5974.61 c	14.45 b	0.50 a	4.43 b
B₄=Nitroxin+ Phosphate (Barvar 2)	53.57 a	138.62 a	2876.25 a	897.90 a	98.30 a	6474.15 a	13.78 c	0.51 a	4.72 a

In a column, according to the results of Duncan Test, those means with at least one common letter do not have a significant difference at the statistical level of 0.05.

Table 3: Mean comparisons of interaction effects of different nitrogen fertilizer levels and biofertilizers application on the evaluated traits in German Chamomile.

Treatment	Plant Height (cm)	Number of Capitols per Plant	Capitol Fresh Weight (Kg/ha)	Capitol Dry Weight (Kg/ha)	Seed Yield (Kg/ha)	Biological Yield (Kg/ha)	Harvest Index (%)	Essence Percentage (%)	Essence Yield (Kg/ha)
Interaction Effects (N×B)	-	-	-	-	-	-	-	-	-
N ₁ B ₁	37.83 f	78.86 h	1826.72 j	610.2 k	71.40 g	3429.83 h	17.80 b	0.28 e	1.76 g
N ₁ B ₂	41.64 e	82.20 h	2110.45 i	623.6 j	77.53 f	4371.93 f	14.26 cd	0.39 d	2.48 f
N ₁ B ₃	40.76 ef	95.00 g	2142.85 hi	655.1 i	73.90 fg	4434.98 f	14.77 c	0.41 d	2.71 f
N ₁ B ₄	42.63 e	93.23 g	2165.58 h	671.0 h	77.00 f	4921.22 e	13.63 ef	0.40 d	2.72 f
N ₂ B ₁	47.36 d	108.8 f	2459.00 g	719.3 g	86.50 e	3873.25 g	18.57 a	0.51 c	3.69 e
N ₂ B ₂	58.53 ab	120.9 e	2636.26 f	747.1 f	96.63 d	5752.12 c	12.98 g	0.58 ab	6.16 b
N ₂ B ₃	47.86 d	127.9 de	2667.15 ef	775.8 e	90.33 e	5588.45 d	13.88 def	0.58 ab	6.06 b
N ₂ B ₄	57.23 bc	135.0 d	2697.75 e	785.2 e	95.60 d	5902.94 c	13.30 fg	0.59 a	6.76 a
N ₃ B ₁	54.16 c	154.1 c	3260.14 d	1058.35 d	114.4 c	5858.50 c	18.06 ab	0.52 c	5.58 c
N ₃ B ₂	60.56 ab	176.6 b	3484.45 c	1125.59 c	125.1 a	8507.79 a	13.25 fg	0.55 ab	4.40 d
N ₃ B ₃	57.23 bc	181.0 ab	3589.87 b	1159.09 b	119.8 b	7900.40 b	14.71 c	0.52 c	4.51 d
N ₃ B ₄	60.83 a	187.6 a	3765.41 a	1237.47 a	122.3 ab	8598.27 a	14.41 cd	0.54 bc	4.68 d

In every column, according to the results of Duncan Test, those means with at least one common letter do not have a significant difference at the statistical level of 0.05.

N₁: the Control (not applying N fertilizers); N₂: 65 Kg/Ha N fertilizer; N₃: 130 Kg/Ha N fertilizer; B₁: the control (not applying biofertilizers); B₂: applying Nitroxin;

B₃: applying Barvar-2 phosphate fertilizer; B₄: applying a mixture of Nitroxin and Barvar-2

In a study conducted to evaluate the effects of *Bacillus* phosphate solubilizing biofertilizers on Squash, it was observed that total protein amount and vegetative growth including stem length and diameter and capitol dry yield have increased as a result of the application of these fertilizers (Abou El-yazeid *et al.*, 2007). In another study focusing on the effects of Cyanobacteria biofertilizers on Atriplex it was found that the bacteria has increased growth parameters, including stem diameter and number of leaves as well as flower fresh and dry yield (Abraham *et al.*, 2007). These results were consistent with those of Dufault *et al* (2003).

C. Seed and Biological Yield

Nutritional and fertilizer management is a key factor to the success of medicinal plant cultivation. In this research, it was found that the application of 130 Kg/Ha N fertilizer had a better gain than the control and increased biological and seed yields. According to Bernath (2000), increasing 40-50 Kg/Ha N in the form of dressing application led to a higher vegetative yield and a higher total biomass. He also added that under favorable conditions, crop yield would increase 0.7-1 ton in the first year and may reach 2-3 tons in the following years. Furthermore, biofertilizers significantly increased the seed yield and dry weight of the stalks, since the highest biological yield was obtained from B4 treatment and the highest seed yield was observed in B4 and B2 with 99.76 Kg/Ha and 98.30 Kg/Ha, respectively (Table 2). Studying the interaction effects on Biological yield suggested the priority of N3B4 treatment over all other treatments, although no significant difference was observed between this treatment and N3B2. However, the highest seed yield (125.1 Kg/Ha) was obtained from N3B2 treatment (Table 3). Several studies have shown that the application of biofertilizers led to a higher absorption of nutrients which, in turn, improved the quantitative growth, biological yield, and seed yield (Afzal *et al.*, 2005; Khalid *et al.*, 2004).

D. Capitol Harvest Index

Harvest index (HI) is defined as the ratio of the salable portion (economical portion) to the total dry weight (biological yield) and in this study, Capitol yield is considered as the economical portion, so the focus of the research is on capitol yield. Mean comparisons of the data indicated that there is no significant difference among all N application levels. Also, mean comparisons of the effect of biofertilizers highlighted the priority of the control (18.14%) over other treatments (Table 2). The application of biofertilizers and high amounts of N did not enhance HI, but led to its reduction, since at different levels of N application, the highest HI was obtained from those treatments which were not fertilized with biofertilizers (N1B1, N2B1, and N3B1; Table 3). To account for such a result, it can be asserted that those treatments which had lower total biomass got a higher HI. Thus, in B4 and

B2, although a higher capitol dry yield was observed, it seems that vegetative growth has been more stimulated than reproductive growth and this led to a lower HI. So, HI cannot be regarded as a suitable criterion for measuring German Chamomile yield.

E. Essence Yield and Percentage

The highest essence percentage was obtained from a treatment fertilized with 65 Kg/Ha N, while increasing the applied N3 had no significant effect on this trait (Table 2). Based on the results of Johri *et al* (1992), applying 60 Kg/Ha N produced the best and the highest amount of essence, while increasing the amount of applied N, despite increasing the production costs, did not significantly enhanced the quantity and quality of the essence. In this research, the highest amount of essence (averagely, 0.51%) was obtained from the treatment which was concurrently fertilized with Nitroxin and Barvar-2 phosphate biofertilizers (B4). However, this treatment had no significant difference with B2 (fertilized with Nitroxin) and B3 (fertilized with Barvar-2). Studying the interaction effects on Chamomile essence yield showed that applying bio stimulants along with N fertilizers enhanced the efficiency of N and decreased the amount of consumed N from 130 Kg/Ha to 65 Kg/Ha (Table 3). In a study it was found that the application of *Azotobacter*, *Azospirillum*, and *Bacillus* biofertilizers increased the vegetative growth, fresh and dry weight, and the essence percentage of medicinal Chamomile (Mahfouz and Sharaf-Eldin, 2007). Mean comparisons of the data suggested the higher essence yield in a treatment which was fertilized with 130 Kg/Ha N fertilizer (averagely, 6.15 Kg/Ha). The second highest essence yield was obtained from a treatment which was concurrently fertilized with Nitroxin and Barvar-2 phosphate biofertilizers (averagely, 4.72 Kg/Ha). Applying bacteria (including *Azotobacter*, *Azospirillum*, and *Pseudomonas*) as biofertilizers enhanced the efficiency of N and P fertilizers and as a result, improved the growth and increased the yield (Roesty *et al.*, 2006). Therefore, inoculating seeds with biofertilizers significantly increased the quantity and percentage of Chamomile essence which is consistent with the findings of Khalil (2006) on Fleawort and those of Karla (2003) on Mint. Results of fertilizers interaction effects on Chamomile essence yield highlighted the priority of N2B4 treatment (fertilized with 65 Kg/Ha N along with Nitroxin and Barvar-2 phosphate biofertilizers) over other treatments (with an average 6.76 Kg/Ha essence produced). Emonger *et al* (2006) in a research conducted on medicinal German Chamomile found that the application of N fertilizers would increase the essence percentage of the flower and the essence yield. They concluded that applying more than 100 Kg/Ha N fertilizers had a negative effect on essence yield.

CONCLUSION

The application of chemical N fertilizers has favorable effects on several plant traits such as plant height, Capitulum weight, and vegetative body, since with the rise in the amount of applied N fertilizer, the above-mentioned traits increased significantly, compared to the control. However, the application of biofertilizers enhanced the quantitative and qualitative yield of German Chamomile, since B2 and B4 treatments (Nitroxin and a mixture of Nitroxin and Barvar-2 phosphate) were the best ones in regard of seed yield and essence yield. It should be noted that evaluating the interaction effects suggested the relatively equal rise of the studied traits in N2 (65 Kg/Ha along with biofertilizers) and N3 (135 Kg/Ha along with biofertilizers).

Seemingly, the use of bio stimulants not only has desirable effects on Chamomile yield, but also enhances the efficiency of N Fertilizer consumption which can be introduced as an alternative to Chemical fertilizers in sustainable medicinal Chamomile cultivation.

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