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Research Article

The effect of nitrogen and phosphorous quantities on the major components of Matricaria recutita L. including chamazulene and apigenin 7- glycoside

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ABSTRACT

In order to investigate the effect of nitrogen (0, 50, 100 and150) and phosphorous (triple super phosphate (TSP)) (0, 75, 150 and 225 kg/ha) quantities on the quantities of major components in Matricaria recutita L. including chamazulene and apigenin 7-glycoside, a factorial experiment with a randomized complete block design (RCB) with three replications was carried out at theAgricultural Research Station of The University of Guilan, Iran. The results of this research showed that chamazulene increases with an increase in the quantities of nitrogen and phosphorous. The greatest chamazulene quantity was observed in the treatment with a nitrogen level of 150 and phosphorous level of 225 kg/ha with a quantity of 88.1% and the highest quantity of apigenin 7-glycoside was observed in a treatment with a nitrogen level of 50kg/ha and no phosphorous with a quantity of 0.088mg/ml. In terms of the increase of apigenin 7-glycoside, a consumption of up to 150kg/ha for nitrogen and phosphorous is recommended. For more than 150kg/ha, except for the treatment with a nitrogen level of 150 and phosphorous level of 225kg/ha, the quantity of apigenin 7-glycoside was decreased.

INTRODUCTION

Matricaria recutita L. is one of the earliest medicinal plants known by mankind (8). It is introduced as a medicinal plant in all valid pharmacopoeia and the healing properties of its flowers are investigated (1). It has also been one of the most important economic plants over the past decades due to its applications in pharmaceutical, food and health industries (28). Generally, the performance of the plant is affected by various factors such as genotype, environmental, and cultural practicesand the right selection of these factors will result in the proper performance of this plant. The correct use of chemical fertilizers causes soil fertilitywhich will have a very important effect on the production and increase of active ingredients (7).

The active ingredient in the Matricaria recutita L. is of essential oil type with various quantities in flowers and genotype, ecological habitat, production management and post-harvest processes (27, 29).

The main constituents in the essential oil of Matricaria recutita L. are: chamazulene, bisabolol, bisabololoxide A and B, Farnesene, and another part of its flowers' active ingredients are flavonoids with their main constituents including apigenin, apigenin glycoside (15, 17). Vitamin C, coumarin, mucilaginous materials and pectin compounds (22).

The essential oil of fresh Matricaria recutita L. has a blue ink color due to the existence of chamazulene (azulene) (27). The quantity of essential oil in Matricaria recutita L. varies from 0.5-0.9% on average which changes with the formation and development of flower (30). The essential oil in the lower parts of the tubular florets are formed as spherical droplets in the bag and secretory duct schizogene which are created with the creation of a gap in the intermembrane space of cells (2, 4).12 to 20% of the essential oil of Matricaria recutita L. consist of chamazulene. Chamazuleneis a sesquiterpene which is created from the "Prochamazulene" precursor (Matricine) exposed to heat (2). In the leaf and stem of chamazulene, there is about 0.9% of essential oil which lacks chamazulene (17).

Chamazulene exists as a prochamazulene precursor or matricinewhich changes into chamazulene when exposed to heat. Therefore, the only way to get chamazulene is through water distillation or steam and the extraction of the essential oil of Matricaria recutita L. through chemical methods will lack chamazulene (5). The color of the essential oil depends on the quantity of the chamazulene in it. The more the quantity of the chamazulene, the more dark blue the color of the essential oil will be (20, 21). The quantity of the chamazulene depends on the developmental stage of the flowers and simultaneous with the flower development, its quantity increases and reaches its peak in the second flowering stage (32). After this stage, the quantity of the chamazulene significantly decreases (17).

The quantity of the chamazulene in the flowers in the morning is higher than other parts of the day and there is also a weak correlation between the quantity of the azulene and the total essential oil extracted (16). In an experiment, it was observed that the varieties of the Tetraploid have higher chamazulene quantities compared to diploid varieties.

The correct and appropriate use of essential mineral elements have a very important yet different role in the development of medicinal plants and the product performance and highly affect the quantity and quality of their activeingredient. Of the most important nutrients that can be added to soil are nitrogen fertilizers, phosphate and potash(1, 3).

One of the essential elements requirement by a plant is nitrogen. It enables the plant to develop faster and produce a higher photosynthesis level and also increases the production of secondary metabolites (sesquiterpene) (14, 13, 9, 35).In a study, the effects of various levels of nitrogen (0, 20, 40 and 60kg/ha) were examined in the culturing of Matricaria recutita L. and it was discovered that with the increase of the use of nitrogen from 0 to 60kg/ha, the performances of the flower and essential oil increase and reach their peak at 60kg/ha (18).The beneficial effect of nitrogen on the performance of Matricaria recutita L. essential oil,plays an important role in the development and division of new cells containing essential oil. Nitrogen may, due to the fact that it increases the proportion of carbohydrate accumulation to gibberellin and auxin concentration in Matricaria recutita L., enhance the performance of the essential oil. Also, nitrogen plays an important role in the biosynthesis of the essential oil and active ingredients (25).

In 2003, the effect of various levels of nitrogen (at 75, 150 and 225kg of pure nitrogen per hectare) on Matricaria recutita L. was examined. The increase of nitrogen up to 150 to 225kg, improved all the properties but this result was not significant for the dry weight of Matricaria recutita L. Also, the greatest amount of the essential oil was obtained from 150kg nitrogen per hectare (6).

The results from the study of Lechamu (2001) performed on the development and performance of Matricaria recutita L. shows that with increased nitrogen, flavonoids and essential oils in flowers increase and the bush height and number of flowers are significantly affected by the planting season and increase with the increase of nitrogen (26). According to a report comparing the application of 100kg/ha of nitrogenwith a control Matricaria recutita L., it was discovered that nitrogen causes an increase of 3 to 6% of the dry matter in the Matricaria recutita L. (14).Franz (1983) in European climate observed that nutritional treatments affect the quantity and quality of the essential oil. He acquired the incremental effect of nutrition on the performance of Matricaria recutita L. (19). Prazna and Bernat (1993), by doing some research on mint showed that a shortage of nitrogen is accompanied by the decrease in the fresh weight and essential oil, and that the use of nitrogen in the farm increases the level of the essential oil (31).Sing (1992) showed that safflower seed yield increased significantly with increasing levels of consumable nitrogen (33). Sing Dalip (1994) showed that the use of nitrogen fertilizer increases the safflower, number of flowers per plant and quantity of seeds in the plant (34).

In a study, the effects of various levels of nitrogen on Matricaria recutita L. were investigated and it was observed that nitrogen significantly increases the weight of the dry flower at 5% essential oil performance per weight unit, but in above 150kg, the essential oil is significantly decreased. Decreased essential oil performance is due to the toxicity of ammonium ions. Nitrogen at 5%, significantly increases the quantity of chamazulene and alpha bisabolol in the essential oil. At above 150kg, the quantity of chamazulene significantly decreases but even up to 225kg, the quantity of alpha bisabolol continues to increase. Nitrogen significantly decreases the quantity of alpha bisabolol oxide A and B. Also in this research, the quantity of the farnesene existing in the essential oil of Matricaria recutita L. increased due to the consumption of nitrogen but this increase was not significant (23). The effects of phosphorous on plants varied so much and it generally has a significant role in the combination of proteins, fat production, cell division and energy exchange of different organs (12).

In a research, it was discovered that the use of nitrogen and phosphorus fertilizers, enhance the performance of the flower and essential oil of the Matricaria recutita L. (89). Researchers have also found out that the use of nitrogen and phosphorus fertilizers increase the quantity of the essential oil whereas potash fertilizersdecrease it (83).

According to some fertilization and implantation experiments in Hungary, Matricaria recutita L. in heavy soils or semi-heavy soils provided good yields even without fertilization. Uniform and low fertilization, increase the flower yield and in poor soils, a quantity of 20-30kg/ha of P_2O_5 can be used. Also, in erosive sandy soil with poor nutrition, 40 to 60kg/ha of P_2O_5 must be used to acquire an appropriate product (22).

In a study, the results from the effect of the two factors of nitrogen and phosphorous each of which were investigated at 4 levels, showed that 67.5kg/ha of phosphorous fertilizer has the best average yield (83).

In another study the effects of urea fertilizer at three levels (0, 40, 80 kg/ha) and triple superphosphate at three levels (0, 30, 60 kg/ha) on the performance and essential oil production of Matricaria recutita L. were investigated. The results showed that there was a significant difference between the performances of the treatments of phosphate fertilizers and the highest performance belonged to the 60kg/ha phosphate with a production of 716.24 flowers and 18.17grams per bush. A significant difference was observed between different treatments in terms of the quantity of the essential oil, and in general, in terms of the performance and percentage of the essential oil, a consumption of 60kg of phosphate per hectare is recommended (10).

In another experiment, it was observed that in order to preserve the plant under suitable conditions in a one-time planting, from the second year, the annual quantity of the P_2O_5 fertilizer required is 60-70kg per hectare (22).

MATERIALS AND METHODS

The experiment was performed in the agricultural year of 2009 at theAgricultural Research Station of The University of Guilan, Iran. It is located at 7 meters above the sea level at a latitude of 49 degrees and 36 minutes east and latitude of 37 degrees and 16 minutes north. Guilan province, according to the meteorological division, is part of the semi-warm Mediterranean regions with hot summers and mild winters.

According to a 10 year average, the annual rainfall of the experiment location is 1441mm and its annual temperature is 16.8°C.

In order to specify the soil properties at the experiment location, samples were taken from a depth range of 0-30cm of the tilled soil. The soil properties of the research location are provided in table 1.

Table 1: Chemical and physical properties of the soil

5.9	Soil acidity
56.7	Soil electrical conductivity (mmhos/cm)
2.5	Real Specific Gravity
16	Sand (percent)
36	Clay (percent)
48	Silt (percent)
0.15	Nitrogen (percent)
2.62	Carbon (percent)
27.54	Soil moisture content
55.99	Porosity percent
1.246	Bulk density
15.2	absorbable phosphorus(mg per kg)
Loam, salty clay	Texture type

The agricultural stages of this experiment were carried out on Matricaria recutita L. at theAgricultural Research Station of The University of Guilan from the spring of 2009 to the summer of 2009 as a factorial experiment with a randomized complete block design (RCB) with three replications.In this experiment, the first factor included the application levels of nitrogen (urea) at four levels (0, 50, 100 and 150) and the second factor was the application levels of phosphorous (TSP) at the four levels of 0, 75, 150 and 225 kilograms per hectare (figures 1-3). First, for soil analyses, samples were collected up to 30cm deep into the ground of the experiment location. The soil properties of the experiment location are given in table 1. After the soil analyses, the ground was prepared and the seeds were planted inside experimental plots with dimensions of 2×1 square meters on the 25th of June with row spacing of 25cm and inter-row spacing of 35cm. The nitrogen fertilizers were applied on three stages. The first stage was simultaneous with the transfer of the seeds to the main ground when the plant is at the stage of growing two leaves. The second stage, included adding the nitrogen fertilizer when the plant was at the stage of growing 3 to 4 leaves. The third stage, included adding the nitrogen fertilizer almost a month after the planting. The addition of the phosphorous fertilizer was done in one stage simultaneous with the transfer of the seeds to the main ground.

After the buds were opened, the flowers were picked and dried using special papers in the dark in open air and then the essential oil extraction of the dried Matricaria recutita L. was performed in the Laboratory of Plant Protection, Department of Agriculture, University of Guilan, with the use of water distillation via an essential oil extraction device (i.e. Clevenger) during 2 hours through the European Pharmacopoeia method. The extracted essential oils were stored in a refrigerator until being measured with a spectrophotometer. The measurement of the quantity of the chamazulene in the essential oils extracted was performed by the spectrophotometer.

At this stage, to determine the quantity of the chamazulene in the essential oil extracted via the above method using dichloromethane, we transferred the essential oil to a 10ml volumetric flask and increased it to 10ml using dichloromethane and at the end of the absorption (E), the resulting solution was measured at 6.3nm wavelength by a 1cm KOT. Before reading the samples' absorptions, the spectrophotometer was first calibrated using pure solvent dichloromethane.

The chamazulene percentage was calculated using the equation below:

$$\text{chamazulene percentage} = \frac{30 \times 10 \times E \times 184.3}{E \times 1000}$$

Determining the quantity of the apigenin 7-glycoside

Measuring the quantity of the apigenin 7-glycoside via an HPLC device based on the United States Pharmacopeia (USP). The flow rate of the mobile phase was set to 1ml per minute. The chemical standard used for the injection was taken from Flooka a US company, and all the experiment stages were carried out in the laboratory of the research center of the Institute of Medicinal Plants, Karaj.After the sample solution was prepared, 15ml of the standard solution and 15ml of the sample solution were injected into a high performance chromatography device.

According to the calibration curve of Krebs, the quantity of the apigenin 7-glycoside in the sample was calculated using the formula below:

$$x = \frac{y}{10}$$

y: area below the curve belongs to the apigenin 7-glycoside in the sample

x: the concentration of the apigenin 7-glycoside in the sample

This design was used in a factorial way with randomized complete block design (RCB) with three replications. The statistical model of the design is as follows:

$Y_{ijk} = \mu + R_i + B_k + AB_{jk} + e_{ijk}$

The resulting data were analyzed using an SAS software. The Tukey test was used for the comparison of the means at 5% and also the software of Excel was used to draw the diagrams.

RESULTS

The results from the data show that different levels of nitrogen and phosphorous have different effects on the quantity of the chamazulene in the essential oil of Matricaria recutita L. (diagram 1).In this study, it was observed that the nitrogen level of 150 and phosphorous of 225kg/ha, have the highest chamazulene in the essential oil with a quantity of 88.1% and the treatments lacking nitrogen which included the use of 150 and 225kg of phosphorous per hectare and the treatment with a nitrogen level of 50 and phosphorous of 225kg/ha with a quantity of 31.1%, had the lowest quantity of chamazulene. Investigating the effect of different levels of phosphorous in each nitrogen level (diagram 1) showed that at the first level of nitrogen (no use of nitrogen), the best level of phosphorous belonged to the one where 75kg/ha of phosphorous were used. At the second level of nitrogen (use of 50kg/ha of nitrogen), the best level of phosphorous belonged to the one with 75kg/ha of phosphorous. At the third level of nitrogen (use of 100kg/ha of nitrogen), the best level of phosphorous belonged to the one with 75kg/ha of phosphorous.At the fourth level of nitrogen (use of 150kg/ha of nitrogen), the best level of phosphorous belonged to the one with 225kg/ha of phosphorous.As is shown in diagram 1, with increased levels of nitrogen and phosphorous, the quantity of chamazulene increases. Since the quantity of chamazulene is one of the most important factors in the determination of the quality of the essential oil, therefore, in the range of the phosphorous and nitrogen under study, it increases the quality of the essential oil (7). In a study, the effects of different levels of nitrogen (0, 75, 150 and 225kg/ha) in Matricaria recutita L. were investigated and it was observed that the increase of nitrogen, significantly increases the quantity of chamazulene and alpha bisabolol in the essential oil, but when more than 150kg/ha are used, the quantity of the chamazulene significantly decreases (23).Given the direct relationship between the performance of the essential oil and the chamazulene quantity in it, it is observed that the proper increase of the fertilizers of nitrogen and phosphorous and the proportion of these two elements (N/P) enhances the performance and percentage of the essential oil and increases the chamazulene quantity in the essential oil.The results form a study by Farahani et al. (2004) performed to investigate the most appropriate level of nitrogen and phosphate fertilizers, these fertilizers each with 4 levels including (0, 45, 90 and 135) and (0, 22.5, 45 and 67.5) kg/ha were examined on the performance and percentage of the Matricaria recutita L. The results showed that the best level was the one with 135kg/ha of nitrogen and 67.5kg/ha of phosphate (11). The results of the present research are in alignment with the results of that study.

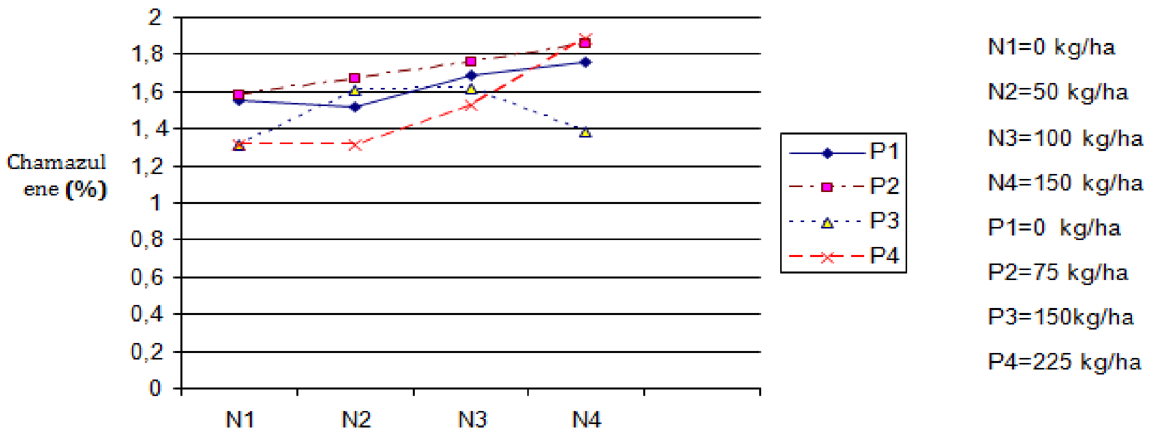

Diagram 1: the effects of different levels of nitrogen and phosphorous on chamazulene (%)

Apigenin 7-glycoside

The results showed that different levels of nitrogen and phosphorous, affect the quantity of apigenin 7-glycoside in the essential oil of Matricaria recutita L. (diagram 2). Investigations showed that the treatment with a nitrogen level of 50kg/ha with no use of phosphorous has the highest quantity of apigenin 7-glycoside with a quantity of 0.088mg/ml and the lowest quantity of the apigenin 7-glycoside belonged to the treatment with no use of nitrogen and aphosphorous level of 225kg/ha with a quantity of 0.052.Investigating the effect of different levels of phosphorous in each of the nitrogen levels showed that at the first nitrogen level (no use of nitrogen), the best phosphorous level belonged to the case with no use of phosphorous. At the second nitrogen level (use of 50kg/ha of nitrogen), the best phosphorous level belonged to the case with no use of phosphorous. At the third level of nitrogen (use of 100kg/ha of nitrogen), the best phosphorous level belonged to the use of 75kg/ha of phosphorous. At the fourth nitrogen level (use of 150kg/ha of nitrogen), the best phosphorous level belonged to the use of 225kg/ha of phosphorous (diagram 2). Since the quantity of apigenin is directly related to the medicinal quality of Matricaria recutita L. the effect of using appropriate quantities of nitrogen and phosphorous on the quantity of apigenin, indicates that the proper management and selection of nitrogen and phosphorous quantities can play an important role in the increase of the medicinal value of the product.

In the present research, given the fact that the highest quantity of apigenin 7-glycoside was obtained from the treatment with a nitrogen level of 50 and no use of phosphorous with a quantity of 0.088mg/ml, and the treatment with a nitrogen level of 100 and phosphorous level of 75kg/ha with a quantity of 0.083mg/ml comes next with a small difference in the concentration level, also, given the effect of the nitrogen to phosphorous proportion (N/P) which affects the increase of the content of the essential oil (36), it can be concluded that in terms of the quantity to increase apigenin 7-glycoside, a consumption of up to 150kg/ha of nitrogen and phosphorous is recommended, and for more than 150kg/ha of phosphorous, except for the treatment with a nitrogen level of 150 and phosphorous level of 225kg/ha, the quantity of the apigenin 7-glycoside has decreased. The results from Lechamoo (2001) on the development and performance of Matricaria recutita L. show that with increased levels of nitrogen, the quantities of nitrogen, flavonoids and essential oils in the flowers increase (83).

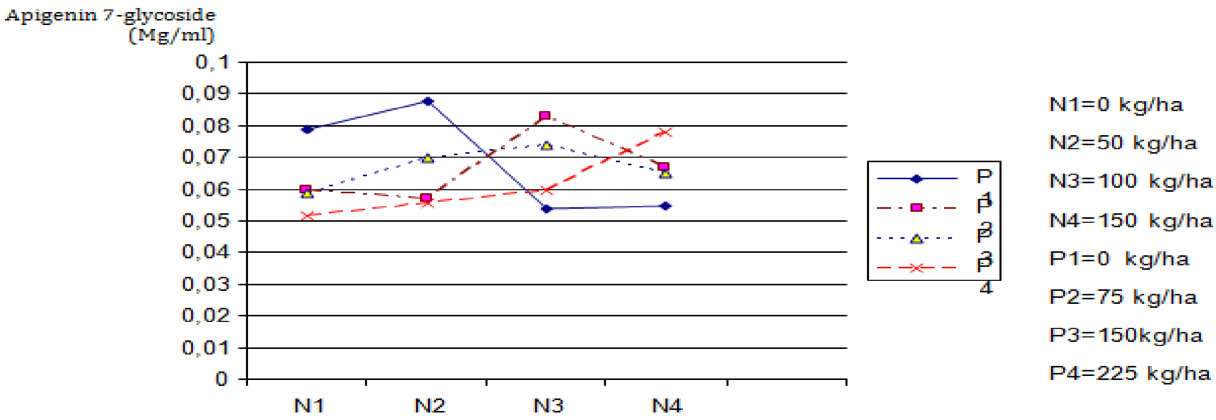

Diagram 2. The effect of different levels of nitrogen and phosphorous on apigenin 7-glycoside (mg/ml)

DISCUSSION

Also the highest quantity of chamazulene was obtained from the treatment with a nitrogen level of 150 and phosphorous level of 225kg/ha (N_4P_4 treatment) with a quantity of 88.1%. The highest quantity of apigenin 7-glycoside was obtained in the treatment with a nitrogen level of 50kg/ha with no use of phosphorous (N_2P_1 treatment) with a quantity of 0.088mg/ml.

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