Effect of Nitrogen and Phosphorus Levels on Yield, Concentration, Physical and Chemical Properties of Dill Seed Oil

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ABSTRACT

To test the effect of 4 levels of nitrogen (i.e. 0, 45, 90 and 135 Kg N ha^{-1}) as urea (46% N) and 3 levels of phosphorus (i.e. 0, 17.5 and 35 Kg P ha^{-1}) as triple superphosphate (21.8% P) on yield and concentration of dill (*Anethum graveolens* L. local cultivar) seed oil this experiment was carried out during winter season of 1999 - 2000 at the experimental field of Agriculture College, Abu-Ghraib.

Both fertilizers were applied in two equal splits, first at seeds sowing and the second was added one month after emergence. Dried and ground seed samples were subjected to water distillation for extraction of volatile oils

Result indicated that fertilization of dill plants with 90 Kg N + 35 Kg P ha^{-1} produced highest oil yield (32.19 ℓ ha^{-1}) and concentration (3.60%) with better quality. GLC analysis indicated that dill seed oil contain 27 volatile compounds, 15 were identified and the major constituents were Carvone, Limonene and α-Phellandrene. Nitrogen and phosphorus fertilization increased the concentration of all identified constituents of dill seed oil.

Nitrogen and phosphorus fertilization increased yield and concentration of dill seed oil. Moreover, physical properties of the oil were also improved by N and P fertilization. GLC analysis showed that Carvone, Limonene and α-Phellandrene are the major constituents of dill seed oil and could be increased by N and P application .

Key words : Volatile compounds, Carvone, Limonene, α-Phellandrene.

الخلاصـــــة

بهدف إختبار تأثير اربعة مستويات من النتروجين (صفر 0 , 45 , 90 , 135 كلغم N/ هكتار) على هيئة يوريا (46% N) وثلاثة مستويات من الفسفور (صفر 0 و 17.5 كلغم P/ هكتار) على هيئة سوبر فوسفات ثلاثي (21.8 % P) في حاصل وتركيز زيت بذور الشبنت (*Anethum graveolens* L.) الصنف المحلي نفذت هذه التجربة خلال الموسم الشتوي لعام 1999-2000 في حقول كلية الزراعة / ابو غريب. اضيف كلا السمادين بدفعتين متساويتين, الاولى عند البذار والثانية بعد مرور شهر من الإنبات. عينات من البذور الجافة تم إستخلاص الزيوت الطيارة منها بطريقة التقطير البخاري. اوضحت النتائج أن تسميد نباتات الشبنت بـ (90 كلغم N + 35 كلغم P)/ هكتار ادى الى إعطاء أعلى حاصل زيت (32.19 لتر / هكتار) وأعلى تركيز 3.60 % و بنوعية جيدة. تحليل المركبات الطيارة بوساطة جهاز GLC أوضحت أن زيت الشبنت يحتوي على 27 زيت طيار , أمكن تشخيص 15 منها حيث كان الكارفون و الليمونين والالفا- فيلاندرين تمثل اكبر نسبة منها. وظهر ايضاً ان إضافة النتروجين والفسفور أدت إلى زيادة جميع مكونات الزيت المشخصة. نستنتج من نتائج هذه الدراسة أن تسميد نباتات الشبنت بالنتروجين والفسفور يزيد من حاصل زيت البذور ويحسن من نوعيته. نتائج تحليل الـ GLCأوضحت أن زيوت الكارفون والليمونين والالفا فيلاندرين هي المكونات الرئيسية لزيت بذور الشبنت .

INTRODUCTION

Dill (*Anethum graveolens* L.) belong to Umbelliferae is one of nutritional and medicinal vegetables [1]. Dried leaves and ground seeds are used as flavour additives to the scald meat and cheeses [2], and to pickle cucumbers to improve taste and maintain good quality [3]. Dill seed oil is used for medical purposes as carminative, in flatulence of children, antispasmodic, stop children's stomac pain,

and aid lactation [4]. It has also been used for lowering blood hypertension and blood sugar level in diabetics [5].

Dill herb is used for lowring blood cholesterol [4]. Carvone a volatile oil is one of the major constituents of dill oil was found to inhibit spouting in stored potatoes and had an antifungal activity against potato tuber diseases [6].Dill seed may contain 4% of oil [7] with essential oil (chiefly consisting of carvone, limonine and terpens), fatty oils, proteins, tannins, resins and mineral salts [4].

Dill seed oil production is sensitive to an environmental and agronomical practices. Halva *et al* [8] noticed an increases in oil concentration in dill plants by 6 times under full light conditions

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(100%) as compared to those exposed to only 30% of the full light intensity. Growth stage also effect oil concentration, where it was the highest in dill herb when seeds were at milk stage and decreased as seeds matured [9].

Nitrogen fertilization increased seed oil yield and changed it chemical constituents [10]. However, levels of N fertilization higher than 120 Kg N ha^{-1} decreased oil concentration in dill seeds [11]. Singh and Mahey [12] also found that maximum seed oil concentration was reached when dill plants were fertilized with 120 Kg N + 17.5 Kg P ha^{-1}.

Dill oil density usually is lower than the density of water so it floating as oily layer on water surface during extraction [13], and affected by sowing dates but not by N or P fertilization, whereas, refractive index of the oil was increased by N and P fertilizers [11].

The objective of the present work was to examine the interactive effect of nitrogen and phosphorus application on dill seed oil yield, concentration and volatile oil constituents.

MATERIALS and METHODS

Experiment layout

A field experiment was carried out during the winter season of 1999-2000 at the experimental field of Horticulture Department, Agriculture College University of Baghdad. Dill seeds (local cultivar) were sown on 1st of October 1999 in rows 15cm appart in plots (2.5 m^2) at a rate of 2.5 Kg seeds ha^{-1}. Treatments included 4 levels of nitrogen as urea (46% N) (0, 45, 90 and 135 Kg N ha^{-1} referred to as N_0, N_1, N_2 and N_3 respectively), and 3 levels of phosphorus as triple superphosphate (21.8% P) (0, 17.5 and 34 Kg P ha^{-1} referred to as P_0, P_1 and P_2 respectively). Fertilizers were applied in two equal splits, first on seed sowing and the second was applied one month after emergence. Fruits were collected progressively when the colour turned brownish yellow and air dried on 15th of May 2000.

Oil Extraction

Ground seeds were water distillated according to British pharmacopoeia as described by ASTA [14]. Clevenger apparatus was connected to rotary evaporator flask where 25g of seeds powder were distillated with 250 ml of distilled water, and distillation continued for 2 hr [15].

Oil extract was transferred to a separatory funnel, and 30ml of diethyl ether ($C_4H_{10}O$) was added. The mixture was hand shacked and left to settle, a layer of oil and diethyl ether floated on the top of the extract, collected and this procedure was repeated three times for each sample. 3 – 5 g of calcium sulfate were added to the oil – ether mixture to absorb remaining water droplets. Ether was evaporated in rotary evaporator under vacuum at 37 c°, the oil transferred to dark brown color vials and stored in refrigerator (4 ± 1 c°).

Physical Properties of Dill Seed Oil

Extracted seed oil physical properties of different treatment samples were determined according to Guenther [16] those included specific gravity and density. Refractive index was determined by Abbe Refractometer (Abbe Type Universal, Haensch, Schmidt Co.). Determination of physical properties were performed at 20 c°.

Chemical Analysis

Chemical analysis of dill seed oil constituent was performed by Gas-liquid chromatography (Ge-9A Model, Shimazu, Osaka, Japan) by direct injection onto a 30m × 0.25mm column packed with 5% polydiphenyl siloxane and 95% dimethyl siloxane on SPB-5. Separation conditions were as follows: carrier as (helium) at flow rate = 25 ml min^{-1}, injection port temperature, detector (Flame ionization detector, FID) temperature, and oven temperature was 220 c˚. Oil constituents were identified as compared to a number of essential oils standards.

Statistical Design and Analysi

A split-plot experiment was used where nitrogen levels were represented by main plots and phosphorus levels as sub-plots with three replicates. Results were subjected to the analysis of variance and least significant differences (LSD P = 0.05) was used to compare the difference between treatments [17].

RESULTS and DISCUSSION

Oil yield and content of dill seeds

Yield and concentration of dill seeds oil were increased markedly by increasing nitrogen levels up to 90 Kg N ha^{-1} (N_2) (Table 1). The percent increases in oil yield was 114.5% whereas for oil concentration was only 49.4% when comparing N_2 to N_0. These increases may accounted for the increases in vegetative growth mainly leaf area which may reflected on dry matter and secondary metabolites production [10,18]. However, increasing nitrogen levels up to 135 Kg ha^{-1} resulted in a significant reduction in both yield and concentration of oil in dill seeds. These results may suggest that optimum level of nitrogen fertilization for highest yield and concentration of dill seed oil is 90 Kg ha^{-1}. Singh and Randhawa [18] had also found that fertilization of dill plant with this same level of nitrogen produced the highest yield and concentration of oil in the seeds, and increasing the N level up to 120 Kg ha^{-1} caused a significant reduction in both yield and concentration of the seeds oil.

Increasing phosphorus level, from P_0 to P_2 had also increased yield and concentration of dill seeds oil but to lesser extent as compared to the effects of nitrogen levels increases, where the percent increases were 13.7% and 4.8% for the oil yield and concentration respectively. These differences in the effects between N and P fertilization could be attributed to the magnitude effect of either nutrient in vegetative growth. The interaction was significant and the highest oil yield (32.19 ℓ ha^{-1}) and concentration (3.6%) was produced when dill plants were fertilized with 90 Kg N ha^{-1} + 35 Kg P ha^{-1}. Similar effects of N + K on dill seeds oil yield and content was found by Singh and Mahey [12].

Physical Properties of the Dill Seed Oil

Nitrogen application to dill plants had no marked effects on seed oil specific gravity, density and refractive index (Table 1). Increasing phosphorus level also was not effective in oil specific gravity and density, while refractive index was slightly increased (but significant) as phosphorus level increased.

Table 1. Effect of nitrogen and phosphorus levels on dill seed oil yield, concentration and physical properties.

Fertilizer Level	Seed oil yield (ℓ ha^{-1})	Seed oil content (%)	Oil specific gravity	Oil density (mg µ ℓ^{-1})	Refractive index
N levels N_0	14.28	2.39	0.933	0.873	1.487
N_1	25.42	3.19	0.942	0.880	1.488
N_2	30.63	3.57	0.943	0.881	1.488
N_3	22.08	2.69	0.940	0.877	1.489
$L.S.D_{0.05}$	1.715	0.004	n.s	n.s	n.s
P levels P_0	21.47	2.89	0.937	0.876	1.486
P_1	23.41	2.97	0.944	0.882	1.487
P_2	24.42	3.03	0.938	0.877	1.489
$L.S.D_{0.05}$	1.485	0.004	n.s	n.s	0.001
Interaction					
N_0P_0	13.72	2.36	0.921	0.861	1.485
N_0P_1	14.26	2.40	0.947	0.885	1.487
N_0P_2	14.84	2.43	0.933	0.872	1.489
N_1P_0	22.26	3.06	0.948	0.887	1.486
N_1P_1	25.77	3.18	0.942	0.881	1.488
N_1P_2	28.24	3.33	0.934	0.873	1.489
N_2P_0	28.79	3.53	0.934	0.874	1.486
N_2P_1	30.90	3.57	0.950	0.887	1.488
N_2P_2	32.19	3.60	0.945	0.888	1.489
N_3P_0	21.12	2.61	0.941	0.880	1.486
N_3P_1	22.71	2.71	0.938	0.877	1.487
N_3P_2	22.42	2.74	0.942	0.880	1.488
$L.S.D_{0.05}$	5.50	0.008	0.014	0.013	n.s

However, the interaction effect of N and P fertilization was significant and the highest specific gravity and density of the oil was noticed when plants were fertilization with N_2P_1 and N_2P_2 respectively, whereas refractive index was not affected significantly by the interaction treatments. Changes in physical properties of the oil by N and P fertilization could be due to the effect of both nutrients on oil oxygenous compounds such as Carvone, Linalool, Eugenol, α-Tujone and Citronellol [19]. Positive correlation was found between phosphorus content of the vegetative parts and oil specific gravity (r = 0.433*), density (r = 0.425*) and refractive index (r = 0.784**). These relationships could be explained on the basis of the effect of phosphorus on essential oil solid constituents (oxygenous compounds) [19]. These findings are in accordance with Randhawa *et al* [11] who concluded that physical properties of dill seed oil is not sensitive to N or P fertilization probably because of the difference in cultivars used and/or predominant environmental conditions.

Essential oils with high physical properties especially refractive index and density regarded as high quality.

Seed Oil Constituents Analysis by GLC

Analysis of dill seed oil showed that it contained 27 volatile oil components, 15 were identified and the rest were not because of lack of standards. These 15 compounds could be classified to three groups according to their concentrations, those are high (Carvone, Limonene, and α-Phellandrene), medium (Champhene, Linalool, Citronellol, Myrcene, Myrisiticin, Terpenolene, Terpene and Caryophyllene), and low (α-Thujone, β-Cymene, α-Pinene and Eugenol) (Table 2). Phellandrene and to some extent Limonene (monocyclic terpenic compounds) are the main constituents for odor and flavor of dill seed oil [16].

Increasing nitrogen levels increased the concentration of all identified volatile compounds. However, these increases were not linear as nitrogen level increased, where the concentration of all oil constituents were notably dropped when nitrogen application rose up to 135 Kg ha^{-1} (N_3). Concentration reduction could be attributed to the production of other metabolites such as pigments, hormones ……. etc., in addition to the dilution effect.

Increasing phosphorus levels gradually increased the concentrations of all identified volatile compounds of dill seed oil. Comparing the effect of N and P levels concentrations of Carvone, Limonene and α-Phellandrene, Which are the major constituents, phosphorus was more effective than nitrogen, where the percent increases due the N_2 or P_2 were 17.30% and 49.35% , 59.79% and 230.22% , and 225.63% and 378.81% for the three compounds respectively. However, Camphene, Terpenolene and Myrcene (medium concentration group) and β-Cymene and α-Pinene (low concentration group) had been affected drastically by increasing nitrogen level to 90 Kg ha^{-1} (N_2) as compared to the effect of increased level of phosphorus to 35 Kg ha^{-1} (P_2), where the percent increases were 225.29% and 91.12%, 345.77% and 57.86%, 182.62% and 147.44%, 1921.28% and 77.08%, and 968.25% and 118.61% respectively. These variations could be due to the rule of either nutrient in metabolism and synthesis of these compounds. . These results reveal that those nutrients may have different mode of action in the biosynthesis of these compounds.

Table 2. Effect of nitrogen and phosphorus levels on dill seed oil constituents concentration (mg Kg seed^{-1})

Compounds	Nitrogen levels (Kg N ha^{-1})				Phosphorus levels (Kg P ha^{-1})		
	N_0	N_1	N_2	N_3	P_0	P_1	P_2
Carvone	396.6	404.7	465.2	446.4	325.2	475.0	485.7
Limonene	68.55	92.73	109.5	77.15	35.37	108.8	116.8
α-Phellandrene	54.11	133.4	176.2	134.5	35.63	167.4	170.6
Camphene	24.79	53.55	80.64	58.64	37.96	52.70	72.55
Linalool	32.14	46.21	57.92	48.75	19.48	52.60	66.64
Eugenol	1.777	3.257	4.360	2.220	1.290	3.700	3.78
α-Thujone	0.790	1.280	1.650	0.996	0.640	1.430	1.520
Citronellol	9.740	9.910	16.36	9.530	0.460	13.56	20.14
Myrcene	8.230	12.02	23.26	14.32	8.390	15.37	20.76
β-Cymene	0.987	11.50	19.95	10.01	7.33	11.58	12.98
Mysrisiticin	7.470	11.81	15.05	11.08	9.190	12.10	12.77
α-Pinene	0.630	3.820	6.730	5.08	2.490	3.970	5.740
Terpenolene	3.780	7.650	16.85	14.26	8.020	11.23	12.66
Terpinene	15.86	24.04	25.04	17.78	15.60	23.36	24.00
Caryophyllene	19.57	29.53	42.09	30.72	5.82	30.26	55.43

Concentration of most identified seed oil constituents were the highest when dill plants fertilized with 90 Kg N + 35 Kg P ha-1 (Table 3). These results could be due to the optimum balance of N and P fertilization for increasing the concentration of seed oil constituents.

Table 3. Interactive effect of nitrogen and phosphorus levels on dill seed oil constituents concentration (mg Kg seeds^{-1})

Compounds	N_0			N_1			N_2			N_3		
	P_0	P_1	P_2	P_0	P_1	P_2	P_0	P_1	P_2	P_0	P_1	P_2
Carvone	235.6	469.1	485.2	263.1	468.4	482.5	371.2	509.0	515.5	431.0	453.6	454.6
Limonene	24.6	78.2	102.9	45.5	117.2	115.5	44.9	137.6	146.0	26.5	102.1	102.9
α-Phellandrene	26.8	75.0	60.5	42.5	166.9	190.8	32.0	243.0	253.6	41.3	184.6	177.6
Camphene	12.6	23.8	38.0	38.5	56.6	65.5	58.3	88.9	144.8	42.4	41.6	92.0
Linalool	11.5	25.5	59.5	27.8	42.1	68.7	21.5	76.7	75.6	17.4	66.2	62.8
Eugenol	0.791	1.95	2.89	1.52	4.78	3.48	1.77	5.59	5.73	1.10	2.51	3.05
α-Thujone	0.405	0.856	1.14	0.646	1.65	1.50	0.907	1.84	2.22	0.394	1.37	1.23
Citronellol	0.415	8.88	19.9	0.539	8.81	20.4	0.519	22.4	26.2	0.371	14.2	14.1
Myrcene	1.21	6.86	16.6	7.33	12.3	16.5	18.4	24.9	26.5	6.66	17.5	18.9
β-Cymene	0.227	1.25	1.49	9.42	11.9	13.3	14.2	22.4	23.3	5.37	10.8	13.9
Mysrisiticin	6.51	7.38	8.55	11.0	11.2	13.3	11.3	17.2	16.7	8.02	12.6	12.6
α-Pinene	0.375	0.658	0.870	2.42	4.22	4.82	3.63	6.59	9.98	3.56	4.42	7.29
Terpenolene	2.61	4.04	4.71	4.74	6.95	11.3	10.6	17.6	17.9	9.67	16.3	16.8
Terpinene	13.4	16.2	22.0	19.0	26.3	26.8	15.4	32.2	27.5	14.6	18.8	19.6
Caryophyllene	0.586	14.7	43.5	2.41	17.1	69.4	6.33	53.1	66.8	14.0	36.1	42.1

CONCLUSIONS

Nitrogen and phosphorus fertilization increased yield and concentration of dill seed oil. Moreover, physical properties of the oil were also improved by N and P fertilization. GLC analysis showed that Carvone, Limonene and α-Phellandrene are the major constituents of dill seed oil and could be increased by N and P application .

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