Pak. J. Agri. Sci., Vol. 52(2), 349-357; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

EFFECT OF ROW SPACING AND SEED RATE ON FRUIT YIELD, ESSENTIAL OIL AND COMPOSITION OF ANISE (*Pimpinella anisum* L.)

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This research was conducted to compare the relative advantages of different row spacing (15, 25 and 37.5 cm) and seed rates (6, 12 and 24 kgha^{-1}) in anise cultivation in terms of fruit yield and quality characteristics. A two factor experiment was conducted in a randomized complete block design in split plot arrangement comprising four replicates. Results revealed that plant height, number of primary branches per plant, umbels per plant, 1000-fruit weight and fruit yield were not significantly affected by various row spacing in both seasons. However, plants grown under wider row spacing of 37.5 cm resulted in significantly higher fruit number and fruit weight per plant in both years. Plants grown with lesser seed rate produced higher yield-contributing parameters including number of primary branches, umbels, fruits and fruit weight per plant. With all the treatments essential oil concentration reached a maximum of 2.84 to 3.05%. A qualitative study showed that the effect of plant density and row spacing had no effect on the percentage of trans-anethole, γ-himachalene and estragol in anise essential oil during 2008. In contrast, row spacing and seed rate had a significant effect on the percentages of trans-anethole, γ-himachalene and estragol during 2009. This effect might have been caused by different levels of infection at different row spacing and planting plant densities in 2009 with *Passalora malkoffii*.
Keywords: *Pimpinella anisum,* spacing, seed rate, *Passalora malkoffii,* fruit yield, Trans-anethole

INTRODUCTION

Anise (*Pimpinella anisum* L.) is an annual spice and medicinal plant belonging to the family *Apiaceae* and native to Mediterranean region. Today, anise fruits are an important natural raw material, which is used in the pharmaceutical, perfumery, food and cosmetic industries. Recently, this spice plant has attained more attention of consumers due to the antimicrobial, antifungal, insecticidal, and antioxidative effects on human health (Gulcin *et al*., 2003; Knio *et al*., 2008; Tepe *et al*., 2006). The drug as well as the essential oil is characterised by carminative, mild expectorant, diuretic, antiseptic and antispasmodic properties (Bown, 2001). Anise fruits contain around 1.5 to 5.0% essential oil mainly composed of volatile phenylpropanoids like trans-anethole (more than 90% of the total essential oil content) followed by γ-himachalene, methyl chavicol (estragol), anisaldehyde, β-himachalene and α-zingiberene (Orav*et al*., 2008; Tabanca *et al*., 2005). Effects of row spacing, water supply, fertilisation, sowing time and sowing density on anise seed yield and quality were studied under field conditions by Ahmad *et al*. (2004), Figueiredo *et al*. (2008) and Tuncturk and Yildirim (2006). They also reported that the major component in essential oil composition is trans-enethole γ-himachalene, methyl chavicol (estragol), anisaldehyde, β-himachalene and α-zingiberene.

In an earlier study, it was observed that fruit yield showed a large variation, which was associated with the severity of the fungal disease named "anise blight", which is caused by the fungal pathogen *Passalora malkoffii* (Erzurum *et al*., 2005; Ullah *et al*., 2013).

Anise is a warm climate crop, yet to be adapted to the temperate climate prevailing in Germany. Because of its sensitivity to low temperatures, the sowing of anise in Germany cannot be carried out in early spring (Ullah and Honermeier, 2013). The major factors affecting plant growth and secondary metabolites of anise under field or greenhouse conditions include sowing date, plant density, water supply and harvesting time (Awad *et al*., 2005; Tuncturk and Yildirim, 2006). Delayed sowing under warmer conditions in spring may lead to shortening of the growing cycle, which decreases the amount of UV radiation intercepted by the crops and may reduce the formation of reproductive organs. Delayed sowing results in reduced vegetative growth and immediate response to photoperiod, which leads to reduced plant length and number of umbels and decreased fruit yield (Zolleh *et al*., 2009; Mirshekari *et al*., 2011). Differences in the chemical composition of anise due to genetic and environmental factors have been reported in several studies (Orav *et al*. 2008; Yan *et al*. 2011).

However, the effect of some other agronomical practices on fruit yield and essential oil concentration are yet to be explored. Two readily manipulatable agronomic variables that are required for optimal cultivation are sowing rate and row spacing, both of which are considered to affect the fruit yield and essential oil concentration of anise.

This study, therefore, was designed to investigate the effects of row spacing on anise plant development by changing its micro-climate by using different plant growth strategies to reduce infection level and increase anise yield and quality. As the higher plant densities negatively affect the yield and yield components, using an appropriate plant density is necessary for maximum utility of existing environmental factors like water, air, light and nutrients. Due to slow growth of anise plants, a higher planting density can benefit the development of anise plants and achieve higher fruit yield. For that reason, field experiments were carried out to determine the effect of different row spacing and seed rate on fruit yield and quality of anise plants under ecological conditions of Germany.

MATERIALS AND METHODS

***Experimental design and site description*:** Two-year field experiments were carried out at the research station of the Institute of Agronomy and Plant Breeding I (Justus-Liebig University Giessen, Germany) in Gross–Gerau, Germany (49°45´N and 8°29´E, 90.7 m above sea level, mean air temperature: 9.4°C, mean precipitation: 590 mm per year). The soils are described as slightly loamy to loamy sand consistency, characterised by the following parameters: 5% clay content (0-30 cm), 6.4 pH, and <100 mm (0-100 cm) available field capacity. Soil is characterized by P: 9.2, 12.5 mg/100 g, K: 12.9, 13.5 mg/100 g and Mg: 2.0, 2.4 mg/100 g of soil during 2008 and 2009, respectively. Before seeding, fields were disked and harrowed and fertilizer was added to provide 31 kg/ha P, 174 kg/ha K (in 2008) and 24 kg/ha P, 134 kg/ha K (in 2009) in accordance with soil analysis and the fertilizer requirement of the crop. After germination of anise 40 kg N/ha was applied to meet the nitrogen requirement of the crop in each successive year. Anise was sown on 1st April in both years comprising of three seed rates (1= 6 g/10 m^2, 2 = 12 g/10 m^2, 3 = 24 g/10 m^2) along with three different row spacing (1= 15 cm, 2 = 25 cm, 3 = 37.5 cm). Row spacing was assigned to the main plots and seed rate to sub plots. The plot size was kept as 10 m^2. The experiments were laid out as a randomized complete block design (RCBD) in split plot arrangements having four replicates. The anise cultivar was purchased from Enza Zaden Deutschland GmbH & Co. KG for both experimental years. During the whole growing season, anise plants were irrigated three times (10, 20 and 20 mm) in 2008 and two times (20 and 20 mm) in 2009 according to irrigation requirements. Weeds were controlled by herbicide Bandur, (ai: aclonifen) @ 3 L/ha application as well as manually. Disease was controlled using the fungicides Ridomil Gold (ai: Metalaxl-M) @ 2 kg/ha and Score (ai: Difenoconazole,) @ 0.4 l/ha in 2008 (one time) and in 2009 (two times). Monthly average air temperature and sum of precipitation for the growing seasons of 2008-2009 at experimental station Gross-Gerau are presented in Figure 1.

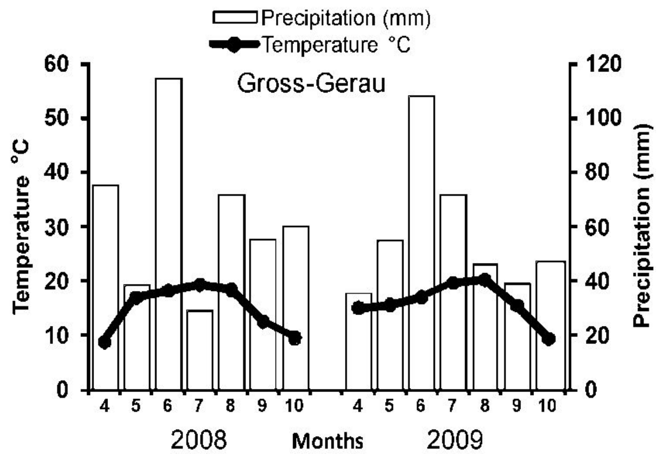

Figure 1. Monthly average air temperature (°C) and monthly sum of precipitation (mm) for the growing seasons of 2008-09 at experimental station Gross-Gerau. The numbers on x-axis indicate the names of months i.e. 4 = April, 5 = May, 6 = June, 7 = July, 8 = August, 9 = September and 10 = October.

***Disease and lodging estimation*:** Fungal disease severity on anise plants was estimated by grading 1-9 (1: without infection, 9: whole plants are infected) for each plot fortnightly. Lodging was estimated from each plot at full maturity before harvesting of anise plants by grading 1-9 (1: erect plants, 9: whole plants are lodged). The plants were harvested with combine harvester at the time of full ripeness of the fruits.

***Morphological and yield components*:** To study the morphological parameters, samples of anise plants were taken from two rows (2 m length) in the middle of the each plot. With these plant samples, the following yield components were determined: number of primary branches plant^{-1} (PBP), number of umbels plant^{-1} (UNP), number of fruits plant^{-1} (FNP), fruit weight plant^{-1} (FWP) and 1000-fruit weight (TFW). Prior to harvesting, plant height was measured with a yardstick. Dry matter content of the sample was determined after drying at 40°C for two days.

***Extraction of essential oil*:** Essential oil was quantified gravimetrically by hydro-distillation using a Clevenger-type apparatus according to the European Pharmacopoeia (2000). Each sample was analyzed two times and the average of both was used for further statistic analyses. The obtained essential oil was stored at 4°C for further lab analyses.

***GC and GC-MS analyses*:** The components of the essential oil were analyzed by GC-FID (Varian CP 3800), capillary column DB-5 (30m x 0.25 mm and 0.25 µm coating thickness). Helium was employed as the carrier gas with a flow rate of 1.1 ml/min. The temperature was programmed from 60 (5 min) to 250°C with a ramp rate of 5°C/min and a final hold time of 10 min. Injector and detector were maintained at 260 and 280°C, respectively. The sample (1µl) was injected with 1:50 split ratio by an autosampler (Varian 8200 CX). The percentage of individual components was computed from peak areas. Response factors of detector and FID normalisation were considered for data processing.

The identification of the components was carried out by GC-MS (Varian 3900 GC, Varian Saturn 2100T ion trap mass detector), capillary column VF-5ms (30 m x 0.25 mm, 0.25 µm coating thickness). Helium was used as a carrier with a flow rate of 1.1 ml/min. Ionisation was realised by electron impact at 70 eV, electron multiplier 2200 V, ion source temperature 230°C and transfer line temperature 240°C. Mass spectral data were acquired in the scan mode in the m/z range 35-450. The identification of components based on comparison of Kovat's retentation indices and mass spectra in corresponding data libraries (Adam, 1995; Ozcan and Chalchat, 2006) and mass spectra libraries (Weily 90 and NIST 98). Kovat's retentation indices were calculated from the GC by linear interpolation between bracketing n-alkanes (C8-C24; Alfa Aesar Karlsruhe, Germany). The most important components *trans*-anethole and estragol were further identified by co-injection of authentic standards (Roth, Karlsruhe, Germany).

***Statistical analyses*:** ANOVA of the Data was calculated by using statistical program SAS. Means were compared based on t-test by least significance difference (LSD), at 5% probability level. Correlation analysis was performed by SPSS (version-18) to determine the relationship among the characters according to Pearson and Spearman's rho method.

RESULTS

***Fruit yield and Morphology*:** Anise seedlings emerged (approximately more than 75% germination) 24 and 20 days after sowing for the corresponding treatments in 2008 and 2009. In 2009, seedlings emerged earlier due to higher temperatures during seed germination. Data presented in Table 1 shows that applied seed rates led to different levels of plant densities determined after germination with 209, 333 and 499 plants and 294, 537 and 707 plants m^{-2} respectively in the years 2008 and 2009. There were clear differences between both years with a higher level of planting densities in 2009. Lower germination rate was observed under wider row spacing of 37.5 cm during both years. Furthermore, it was observed that anise plants were infected with the fungal pathogen *Passalora malkoffii* in a range from 3.3 to 7.3 and 3.3 to 6.4 (level), respectively in 2008 and 2009, which led to brown-coloured leaf spots. Higher fungal infection was assessed in 2008 compared with that of 2009. In 2009, at a plant density of 294 plants m^{-2} showed lower fungal infection in comparison with that of 707 plants m^{-2} (data not shown). A similar trend of disease infection was found regarding plant densities in 2008. Plants grown under a wider row spacing of 37.5 cm caused lower infection level compared to those grown in row spacing of 15 cm. Plant height at maturity was not affected by different row spacing and seed rate in 2008. A row spacing of 37.5 cm showed a significantly greater plant height compared to other row spacing during 2009. A plant density of 294 plants m^{-2} exhibited significantly taller plants whereas the lowest plant height was recorded at a planting density of 707 plants m^{-2} (Table 1). Plant density significantly differed with the number of primary branches and umbels per plant in both seasons whereas these yield components showed no significant variation by different row spacing treatments (Table 1). The lower plant densities of 209 plants m^{-2} and

Table 1. Effect of row spacing and seed rate on number of plants, plant height, primary branches per plant (PBP) and umbel number per plant (UNP) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau 2008-09.

Treatment	2008	2009	2008	2009	2008	2009	2008	2009
	Plants m^{-2}		PH (No.)		PBP (No.)		UNP (No.)	
Row Spacing								
15 cm	415	562	43.7^a	60.8^c	2.8^a	2.8^a	3.9^a	4.1^a
25 cm	342	520	45.1^a	64.0^b	3.3^a	2.9^a	4.6^a	4.3^a
37.5 cm	284	456	45.7^a	67.3^a	3.4^a	3.0^a	4.9^a	4.7^a
$LSD^{0.05}$	-	-	ns	2.17	ns	ns	ns	ns
Plant Density								
6 g	209	294	46.5^a	65.4^a	4.2^a	3.8^a	5.8^a	5.7^a
12 g	333	537	44.4^a	64.6^a	2.9^b	2.8^b	4.2^b	4.1^b
24 g	499	707	43.5^a	62.1^b	2.3^b	2.1^c	3.5^b	3.2^c
$LSD^{0.05}$	-	-	ns	2.17	0.8	0.4	1.0	0.8

Different letter indicate the significant difference ($p \leq 0.05$) among treatments

Table 2. Effect of row spacing (RS) and seed rate on fruit number per plant (FNP), fruit weight per plant (FWP) and essential oil yield (EOY) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau 2008-09.

Treatment	2008	2009	2008	2009	2008	2009	2008	2009
	Plants m^{-2}		FNP (No.)		FWP (g)		EOY (Kg ha^{-1})	
Row Spacing								
15 cm	415	562	55^{b}	51^{b}	0.16^{b}	0.14^{b}	13.5^{a}	29.3^{a}
25 cm	342	520	76^{ab}	82^{ab}	0.21^{ab}	0.21^{ab}	14.3^{a}	28.0^{a}
37.5 cm	284	456	99^{a}	97^{a}	0.27^{a}	0.28^{a}	14.6^{a}	27.0^{a}
$LSD^{0.05}$	-	-	31	36	0.1	0.1	ns	ns
			Plant Density					
6 g	209	294	116^{a}	127^{a}	0.33^{a}	0.38^{a}	15.7^{a}	34.1^{a}
12 g	333	537	64^{b}	61^{b}	0.17^{b}	0.17^{b}	14.2^{a}	28.5^{b}
24 g	499	707	50^{b}	41^{b}	0.14b	0.09^{b}	12.5^{a}	21.8^{c}
$LSD^{0.05}$	12	21	31	36	0.1	0.1	ns	2.6

Different letter indicate the significant difference ($p \leq 0.05$) among treatments

294 plants m^{-2} produced significantly higher numbers of PBP and UNP compared to higher plant densities in each year (Table 1). The highest number of PBP and UNP was recorded at a row spacing of 37.5 cm, while the smallest number of these components was attained at the narrow row spacing of 15 cm in both seasons. Number of fruits (FNP) and fruit weight per plant (FWP) was considerably affected by row spacing as well as by planting densities in both years (Table 2). The lower seed rate had a significantly higher FNP compared to higher seed rates (Table 2). Fruit weight per plant ranged from 0.14 to 0.33 g and 0.09 to 0.38 g during 2008 and 2009, respectively (Table 2). In various row spacing, 37.5 cm gave the maximum FNP and FWP compared to other row spacing. Higher FNP and FWP were recorded at a lower plant density and wider row spacing.

Seed rate had no significant effect on TFW in 2008 whereas a significant difference was observed in 2009. In 2009, 294 plants m^{-2} produced a significantly higher TFW while the lowest was observed at a plant density of 707 plants m^{-2} (Fig. 2). Fruit yield of anise was significantly affected by different plant densities but not by row spacing. Generally, the fruit yield in the year 2009 was superior to those in the year 2008 (Fig. 3). Averaged over the years, the highest fruit yield of 1.13 t/ha was obtained from the planting densities of 294 plants m^{-2}, while the lowest fruit yield of 0.43 t/ha was attained at planting densities of 499 plants m^{-2}.

***Content and yield of essential oil*:** The essential oil concentration of anise fruit was not significantly influenced by seed rate and row spacing (Fig. 4). In 2009, a tendency of higher essential oil concentration was observed at lower seed rate and narrow row spacing (Fig. 4). During 2009, essential oil yield was affect significantly by different seed rates. A significantly higher essential oil yield ha^{-1} was achieved at a lower seed rate compared to higher seed rate (Table. 2).

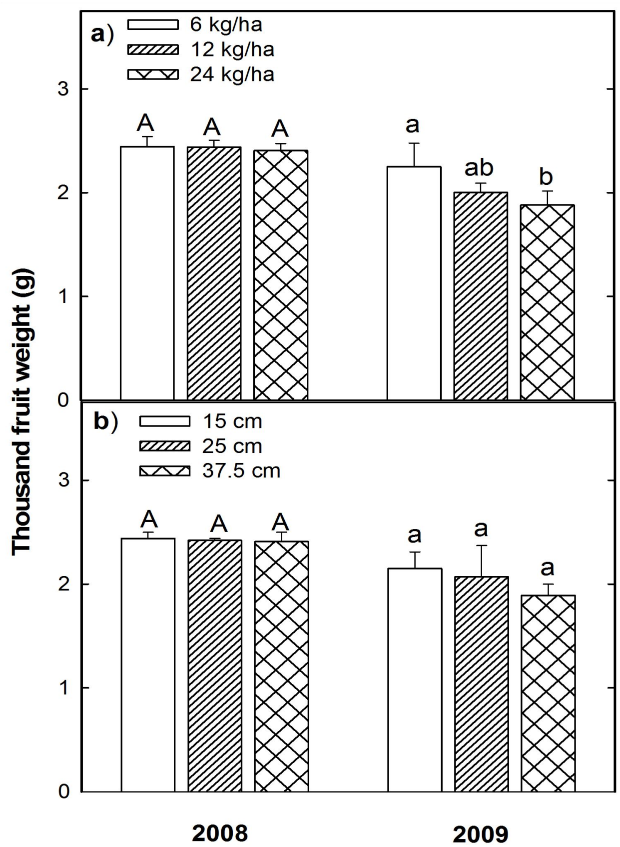

Figure 2. Effect of different seed rates (a) and row spacing (b) on thousand fruit weight of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

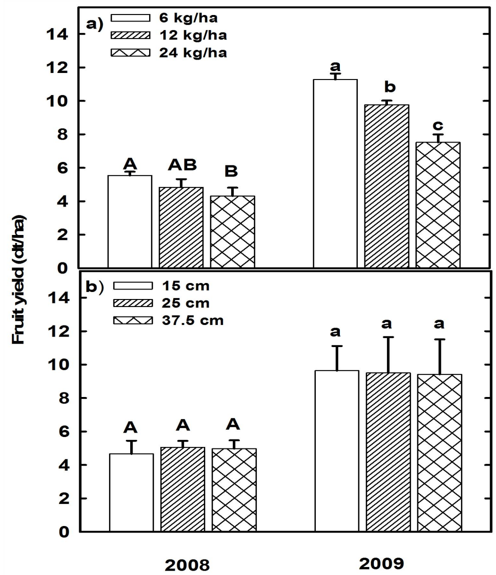

Figure 3. Effect of different seed rate (a) and row spacing (b) on fruit yield of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

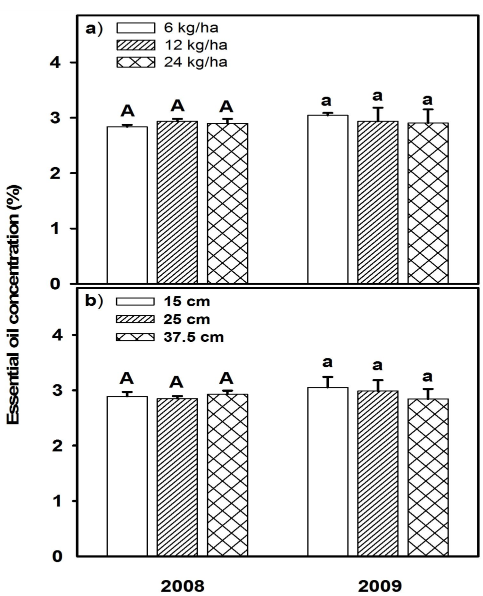

Figure 4. Effect of different seed rate (a) and row spacing (b) on essential oil concentration of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

Chemical composition of essential oil: In total, 19 components were identified by GC/MS from essential oil of anise fruits. The essential oil of anise was made up largely of phenylpropanoid. Beside phenylpropanoids, γ-himachalene (7%), cis-anethole (0.14%), elemene delta (0.45%), α-himachalene (0.71%) and β-himachalene (0.44%) are the other components identified in the anise essential oil (Table 3).

Table 3. Mean values of components of anise essential oil analyzed by GC-MS.

Components	Kovat 's retention index	%
Estragol	1197	0.33
Cis anethole	1252	0.14
trans-anethole	1287	82.1
Elemene (delta)	1333	0.45
Beta elemene	1388	0.08
α-Himachalene	1449	0.71
γ-Himachalene	1478	7.00
α-amorphane	1482	0.15
(E)-Methylisoeugenol	1489	0.14
α-Zingiberene	1493	0.77
β-Himachalene	1499	0.44
α-Muurolene	1502	0.15
β-Bisabolene	1506	0.38
Beta-Sesquiphellandrene	1522	0.05
Spathulenol	1580	0.04
Unknown	1629	0.05
α-cadinol	1651	0.08
Unknown	1831	5.95
Unknown	1886	0.92
No.of identified compound	19	100

A quantitative study showed that seed rate and row spacing had no effect on the percentage of trans-anethole in 2008. In contrast, a significant variation was evident for trans-anethole among treatments in 2009. Trans-anethole, found to be the main component of the anise oil, varied from 91.1 to 91.9% in both years. The compound estragol (methyl chavicol), which is considered as a quality-relevant component, was not influenced by the treatments in 2008. However, a significant variation was found in 2009 regarding seed rate. Averaged over the years, the concentration of estragol in the essential oil varied from 0.38 to 0.54%. In addition, estragol percentage was lower in fruit samples of 2008 than those in 2009. An increasing trend of estragol concentration was observed as seed rate increased during 2009 (Fig. 5). A significantly higher concentration of estragol (0.54%) was determined at a plant density of 707 plants m^{-2} whereas a significantly lower value (0.49%) was observed at a plant density of 294 plants m^{-2} in 2009 (Fig. 5). The γ-Himachalene content in the essential oil of anise varied from 5.4 to 5.7% in both years. γ-Himachalene was not influenced by the treatments in 2008. However, a clear effect of plant density and row spacing was observed during

2009. Significantly higher concentrations of γ-himachalene (5.7 and 5.7%) were produced at a plant density of 707 plants m^{-2} and row spacing of 37.5 cm (Fig. 6).

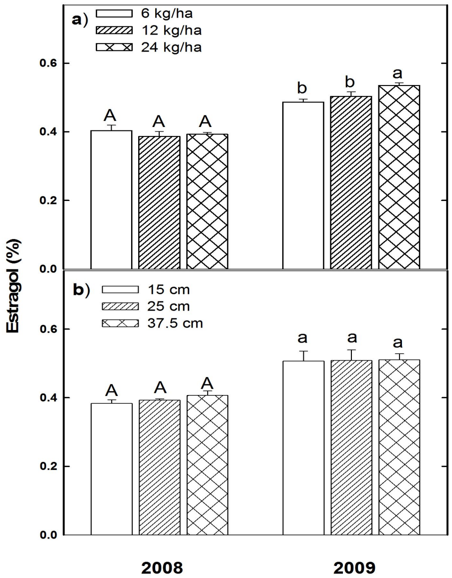

Figure 5. Effect of different seed rate (a) and row spacing (b) on estragol concentration of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

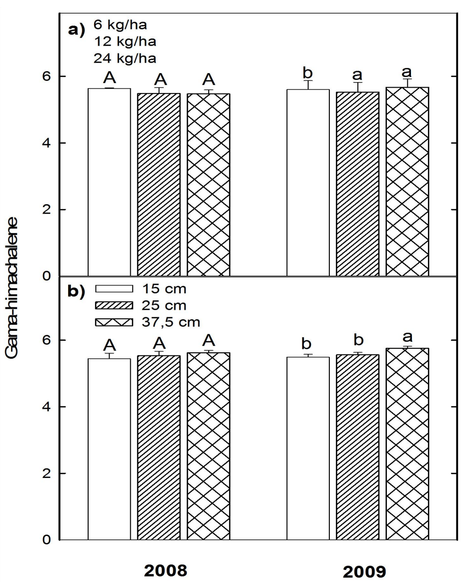

Figure 6. Effect of different seed rate (a) and row spacing (b) on γ-Himachalene concentration of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

A significant variation was evident for trans-anethole among treatments in 2009. In 2009, a narrow row spacing of 15 cm had a significantly higher concentration of trans-anethole (91.4%) whereas the lowest concentration of 91.1% was gained at a wider row spacing of 37.5 cm (Fig. 7). Plant densities of 294 and 537 plants m^{-2} produced significantly higher concentrations of trans-anethole (91.3 and 91.4% respectively) in comparison with a plant density of 707 plants m^{-2} (Fig. 7).

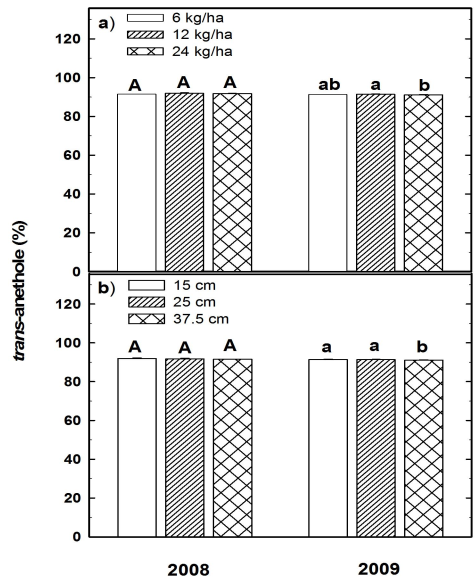

Figure 7. Effect of different seed rate (a) and row spacing (b) on the percentage of trans-anethole of anise at experimental station Gross-Gerau 2008-09. Different letters indicate significant differences ($p \leq 0.05$) among treatments.

DISCUSSION

In 2008, a much lower fruit yield was observed compared to 2009. The reason for this differences may be due to disease infection of anise plants caused by the fungus *Passalora malkoffii* (infection level: 7.0 to 7.3 in 2008 and 5.2 to 6.4 in 2009), which infects all above-parts of the plants, including leaves, flowers, stems and fruits (Ullah *et al.*, 2013).

In current trials, the fruit yield of anise was not affected by different row spacing whereas plant density had a pronounced effect in both seasons in which fruit yields varied between 0.43 and 1.13 t ha^{-1}. A plant density of 294 plants m^{-2} led to 127 fruits and a fruit weight of 0.38 g per plant, which was reduced to 41 fruits and 0.09 g fruit weight per plant under the maximal plant density of 707 plants m^{-2} in 2009. It seems that under current field conditions, anise fruit yield is determined not by the plant density per unit area alone, but more strongly by plants with more branches, umbels, fruits and fruit weight per plant. Current study indicate that higher plant densities obtained limited nutrition and water uptake for anise plant development, which reduced formation of the fruit yield components and fruit yield. This conclusion is in accordance with findings of Yan *et al*. (2011) who reported that higher sowing rates resulted in higher plant density, which reduced the number of branches and number of umbels as well as fruit number and fruit weight per plant significantly. Maheshwari *et al*. (1989) carried out field experiments with anise and reported that higher seed yield was attained at a narrow row spacing of 15 cm in comparison with wider row spacing of 45 cm.

Overall, a higher germination rate was observed in 2009, which can be attributed to higher air temperature (15°C) and lower precipitation (36 mm) during the germination period (sowing-emergence) of anise. In 2008, low temperature (8.8°C) during the germination period might be a reason of low germination rate because optimum soil temperature for anise seed germination is from 18 to 21°C (Peter, 2001).

Lower soil temperature of 6°C induces depressed anise plant growth (Weiss, 2002).

In the current study, the number of primary branches, and umbels per plant was higher at lower plant densities compared with narrow specify the density level densities. It can be supposed that the aniseed plant stand is characterised by a plasticity of yield component formation within a range of 200 to 300 plants m^{-2}. These results are in agreement with the findings that higher seed rates resulted in a higher plant density but a reduced number of branches and umbels per plant (Tuncturk and Yildirim, 2006; Yan *et al*., 2011). It can be explained that at a lower plant density, anise plants show efficient use of available water, light and nutrient while at a higher plant density, there is competition among plants which leads to lower yield contributing-components.

Higher number of branches and umbel $plant^{-1}$ was recorded in wider row spacing of 37.5 cm. The current results are in line with findings of Kizil *et al*. (2008) who reported that wider row spacing produced the maximum number of branches and umbels per plant, while the least number of these yield components were obtained from a close row distance. It can be concluded from the current study that better growth of anise plants occurs at wider row spacing, which is attributed to the better use of water, nutrients and UV radiation resulting in higher photosynthesis, which ultimately leads to higher yield components. We found a significantly higher number of fruits and fruit weight per plant at lower seed rate and wider row spacing in both seasons. The present results are in accordance with the findings of Yan *et al*. (2011) who reported that with increasing plant density the fruit number per plant decreased significantly.

In the current study, a higher seed rate resulted in higher plant densities but reduced yield components of anise. It can be supposed that more space availability in case of lower plant density might have increased the root and plant spread, eventually utilized the resources such as water, nutrients, and light very effectively which increased yield components.

A tendency of higher essential oil contents were accumulated in lower plant densities compared with higher plant densities. Khorshidi *et al*. (2009) also reported the maximum essential oil percentage at the minimum plant density, whereas the minimum essential oil percentage was recorded with the maximum plant density.

In the current trials, the essential yield of anise varied from 12.5 to 34.1 kg/ha in both seasons. Essential oil yield is associated with fruit yield and essential oil content. Differences in these components directly affect essential oil yield. Yields of essential oil from anise have been reported in the range of 10 to 24 kg/ha (Tuncturk and Yildirim, 2006). The present results differ from other findings that showed an increase in essential oil yields by increasing the seed rates (Tuncturk and Yildirim, 2006). In the current study, a significant correlation was found between fruit yield ($r = 0.98$) and essential oil yield.

The essential oil of anise fruits contains mainly phenylpropanoids and sesquiterpenoid hydrocarbons. It is of interest to note the presence of trans-anethole at a very high percentage (>90%), which was distinctive of *Pimpinella anisum* L. In the current investigations, the main compound of the anise fruit essential oil was trans-anethole followed by γ-himachalene and estragol. These findings are in accordance with previous experiments with anise (Askari *et al*., 1988; Orav *et al*., 2008; Ozcan and Chalchat, 2006; Yan *et al*., 2011).

Estragol (methylchavicol) and γ-himachalene were found in all essential oil samples with 0.38 to 0.54 and 5.4 to 5.8%, respectively, in accordance with previous work (Orav *et al*., 2008; Yan *et al*., 2011). Further, it was observed that with increased in levels of trans-anethole, γ-himachalene concentrations were decreased. Khorshidi *et al*. (2009) reported that the percentage of trans-anethole and estragol was affected by space between the fennel plants.

A quantitative study showed that plant density and row spacing had no effect on the percentage of trans-anethole, γ-himachalene and estragol in 2008 but these oil components were affected in 2009. It can be supposed that the activity of phenylalanine ammonia-lyase (PAL) enzyme, which is responsible for the synthesis of phenolic and phenylpropanoids compounds, increased with fungal infection during 2009 in 15 cm row spacing. Fungal invasion triggers the transcription of messenger mRNA that codes for PAL, thus enhancing the amount of PAL in plants, which then stimulates the synthesis of phenolic compounds (Logemann, 1995; Taiz and Zeiger, 2002).

It can be assumed that the plant can modify the stress conditions influencing the plant metabolism. In narrow row spacing, plants produce a higher number of primary and secondary branches, which changed the micro-climate of the plants and is responsible for the higher infection of *Passalora malkoffii*. Thus, stress conditions influence the formation and the composition of secondary metabolites, especially the production of phenylpropanoids and phenolic compounds.

Conclusion: Two consecutive years study showed that plants grown under 15 cm row spacing with plant density ranging from 200 to 300 plants m^{-2} gave the highest fruit yield due to reduced competition among plants. The tested cultivar (Enza Zaden) showed a high essential oil percentage with more than 90% *t*rans-anethole and less than 1% estragol and is a good chemotype of anise cultivar.

Acknowledgments: The authors are thankful to Higher Education Commission (HEC) Pakistan and German Academic Exchange Service (DAAD) for financial support to complete this project. The authors also thank provided by Mr. Bernhard Hormann, Pia Doernfeld and Rosa Allerdings for technical assistance and precious suggestion of Dr. Feng Yan for valuable suggestions.

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