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ESSENTIAL OIL AND COMPOSITION OF ANISE (*PIMPINELLA ANISUM* L.) WITH VARYING SEED RATES AND ROW SPACING

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Abstract

Two year study was carried out to explore the effect of seed rate and row spacing on the fruit yield, essential oil yield and composition of aniseed. The study factors included seed rate (6 g, 12 g, 24 g/10 m^2) and row spacing (15 cm, 25 cm, 37.5 cm. A significantly higher fruit yield was produced at narrow row spacing of 15 cm among treatments. Wider row spacing produced markedly higher essential oil than narrow row spacing. Essential oil accumulation decreased as planting densities increased. The major constituent of anise oil was *trans*-anethole (82.1%) followed by γ-himachalene (7.0%). The quality parameters including estragol, γ-himachalene and *trans*-anethole were significantly affected by different row spacing. Plant grown at 37.5 cm row spacing accumulated the highest estragol and *trans*-anethole concentration among the row spacing treatments. It can be concluded that higher plant density and wider row spacing increased the disease infestation and lodging cultivar Enza Zaden in current study exhibited high concentration trans-anethole in essential oil composition therefore is a good quality chemotype.

Keywords: Essential oil, *Pimpinella anisum*, *Trans*-anethole, Seed rate, Row spacing

Introduction

Anise (*Pimpinella anisum* L.), is an annual important spice and medicinal plant belonging to the family of *Apiaceae*, and native to Mediterranean region. Medicinal plants contain substances that could be used for therapeutic purposes (Hussain *et al*., 2014: Al-Juhaimi, 2014). Recently, this spice plant has drawn more consideration of consumers due to the antimicrobial, antifungal, insecticidal, and antioxidtaive effect of this herb on human health (Özcan & Chalchat, 2006; Tirapelli *et al*., 2007). The essential oil is characterized by carminative, mild expectorant, diuretic, antiseptic as well as antispasmodic effects (Bown 2001; Kreydiyyeh *et al*., 2003). Anise fruits contain 1.5-5.0% essential oil with *trans*-anethole, a phenylpropanoid, as predominant component (Tabanca *et al*., 2005). In addition, the essential oil of the anise fruits contains also small quantity of estragol, anisaldehyde, γ-himachalene and *cis*-anethole (Omidbaigi *et al*., 2003; Rodrigues *et al*., 2003). In European countries consumption of anise fruits is more than its production so the amount of imported anise fruits reached about 2000 t in 2004. Among other countries Germany remains the largest spice importer of anise (Rapisarda, 2004). This stimulates the cultivation of anise in European countries including Germany.

The yield of aniseed may noticeably vary depending on ecological conditions such as temperature, precipitation and soil fertility. Previous studies showed the effects of row spacing, water supply, fertilization, sowing time, sowing density on anise seed yield and quality were studied under field and greenhouse conditions (Maheshwari *et al*., 1989; Zethab-Salmasi *et al*., 2001; Tuncturk & Yildirim, 2006). The cultivation of anise in Germany is rather limited due to problems such as poor establishment of plant stand in the spring and lower yield in autumn.

Plant spacing and seed rate are important factors in determining the microenvironment in the anise field. The optimization of these factors can lead to a higher yield in the crop by favorably affecting the absorption of nutrients and exposure of the plant to the light. The, current study was carried out to explore the effect of seed rate and row spacing on the fruit yield, essential oil yield and composition of aniseed.

Materials and Methods

Experimental conditions and crop management: Two years experiments were carried out at experimental station Giessen (50°47´N and 8°61´E), Germany Topographically the Giessen experimental site is usually even with homogenous soils rich in clay contents. The row spacing experiments were laid out as RCBD design with split plot arrangement having four replications. Row spacing experiments were sown on April 1st in both years. Cv. Enza Zaden was planted in 1.5 x 7.0 m^2 plots. Row spacing was allocated to main plots and seed rate to sub plots. The study factors are included seed rate (6 g, 12 g, 24 g/10 m^2) and row spacing (15 cm, 25 cm, 37.5 cm). Weed control and fungal control was carried out by application of the herbicide Bandur (Aclonifen) 4 L/ha and Foliar application of Ridomil Gold (Mancozeb + Metalaxyl-M) (2 kg/ha) respectively. The weather condition data is presented in Table 1.

Prior to harvesting plant height was measured with yardstick. These yields determine components were calculated from each plant of two middle rows and averages of all plants worked out. TFW was obtained by counting of twice 1000-fruits of each sample using an automated seed counter (Contador). 20 grams each of the samples were weighted out. The harvest was made by a combine at the time of full ripeness of the fruits.

The infection of fungal pathogens like *Cercospora malkoffii* may be affected plant growth and development under humid climate conditions. Hence, fungal disease-severity on anise plants was recorded by grading 1-9 (1: without infection, 9: whole plants are infected. Visual fungal disease incidence was assessed for each plot fortnightly from Giessen experimental station. Lodging was also estimated similar way by grading 1-9 (1: erect plants, 9: whole plants are lodged). Lodging data was collected from each plot at full maturity before harvesting of anise plants.

Table 1. Air temperature (AT) in °C and precipitation (PS) in mm from March to September, and the long-term average (last 25 years), Giessen 2008-2009.

Months	2008				2009			
	AT °C	LAT °C	PS mm	LPS Mm	AT °C	LAT °C	PS mm	LPS mm
April	5.7	8.4	61	41	9.7	8.4	46	41
May	16.7	12.9	50	58	15.1	12.9	82	58
June	13.4	16.0	60	62	11.8	16.0	73	62
July	20.5	17.8	43	66	18.5	17.8	77	66
August	18.6	17.2	70	59	18.5	17.2	44	59
September	9.7	13.7	75	50	11.4	13.7	39	50
Sum			**359**	**336**			**360**	**336**
Mean	**14.1**	**14.3**			**14.2**	**14.3**		

AT: Air temperature (°C), LAT: Long term air temperature (°C), PS: Precipitation sum (mm), LPS: Long term precipitation average (mm)

Essential oil extraction: Essential oil was quantified gravimetrically by hydro-distillation using a Clevenger-type apparatus according to the European Pharmacopoeia (Anon., 2000). Each sample was analyzed in two replications and the average was used for statistical analyses. The obtained essential oil was stored at 4 ^{o}C for further laboratory analyses. The anise essential oil components were identified and quantified by means of GC and GC-MS. A Varian CP-3800 gas chromatography equipped with flame ionization detector (GC-FID) was used.

GC-MS: Besides the analysis of main components of anise essential oil by GC, GC-MS was used for the analysis of other components of anise essential oil, which were present in minor quantity. For identification of these components Kovat's retention indices were calculated by linear interpolation between bracketing n-alkanes (C8-C24; Alfa Aesar Karlsruhe, Germany)

Statistical analyses: Statistical analysis of the data was carried out by using statistical program PIAF Stat (Planning information analysis program for field trials) for checking the significance of the different treatments, whereas LSD at 5% probability level (p 0.05) was used to compare the differences between the treatments. Correlation analysis was performed by PASW (version 18) according to Pearson and Spearman's rho methods. The standard deviations (SD) were calculated by using Microsoft Excel.

Results and Discussion

Data regarding *Cercospora* leaf blight presented in Fig. 1. Disease infestation was lower in 2008 compared to 2009. Lower level of disease was observed in plants grown at wider row spacing compared to narrow row spacing in 2008. On the contrary, higher disease infection was recorded from plants grown under wider row spacing compared to narrow row spacing during 2009. It might be due to higher lodging observed at wider row spacing treatments which created favorable condition for fungus infestation. We found that disease level of *Cercospora malkoffii* increased as plant densities increased (Fig. 1) which might be due to the increased relative humidity within the canopy and increase the duration of leaf wetness by reducing air movement and sun light penetration which increased disease level. Increasing trend of lodging was observed as distance between the row increased. Highest lodging was recorded from plant grown at wider row spacing and higher plant densities (Fig. 2).

The results revealed that plant height of anise at maturity was not affected by row spacing treatments in both seasons. A decreasing trend of plant height was noticed with increasing plant densities (Table 2). A Significant higher 1000-fruit weight was attained at 37.5 cm row spacing and plant density of 189 plants m^{-2} respectively in 2008. Due to the higher competition at higher plant density, anise plant obtained limited nutrition, water uptake and light uptake for development which reduced formation of the fruit yield components and yield. This conclusion is in accordance with findings of Yan *et al*., (2011) who reported that higher sowing rates resulted higher plant density which reduced the yield components significantly. Different row spacing had significant effect on the fruit yield anise in 2008; opposite to that fruit yield was not affected by row spacing and plant densities during 2009. Maximum fruit yield was recorded at narrow row spacing of 15 cm row compared to wider row spacing (table 2). In both years, higher fruit yield was produced by narrow row spacing of 15 cm compared to wider row spacings. The reduced fruit yield at wider row spacing might be due the increased competition among individual plants for available resources (water and nutrients). These results are in good agreement with previous work (Maheshwari *et al*., 1989) suggesting that higher seed yield was noticed at narrow row spacing. In observation of Kizil *et al*., (2008), lower row distances produced higher seed yield compared to wider row distances in cumin (*Cuminum cyminum* L.).

Essential oil concentration was affected by plant densities and row spacing. Wider row spacing produced markedly higher essential oil (Table 3) which might be due to competition between the plants for available resources such as nutrients and water which caused stressful conditions and increased essential oil accumulation. These findings confirm the conclusion that the amount of essential oil can be increased under drought conditions (Zehtab-Salmasi *et al*., 2001, Azizi *et al*., 2009). In addition, higher essential oil concentration was produced in 2009 compared to 2008. Experimental data showed that essential oil accumulation decreased as planting densities increased (Table 3).

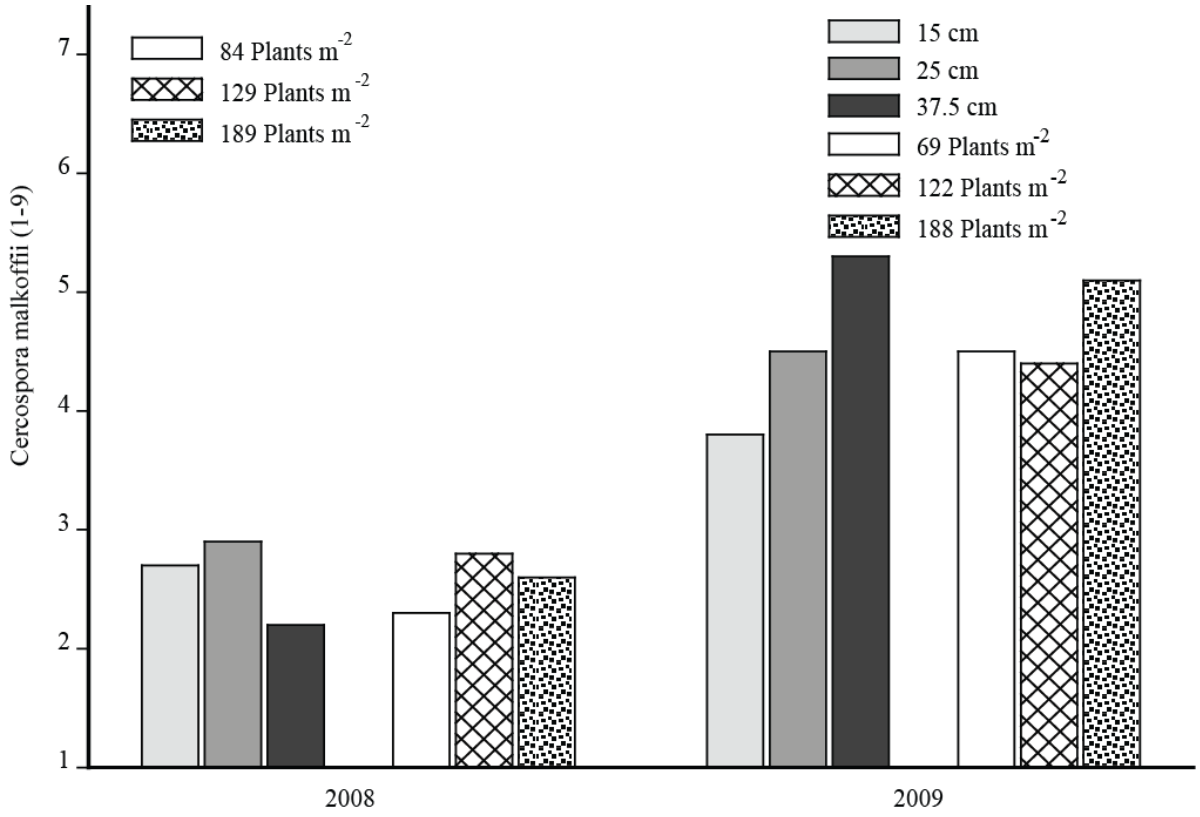

Fig. 1. Effect of row spacing and plant densities on *Cercospora malkoffii* (1-9) on anise 2008-09.

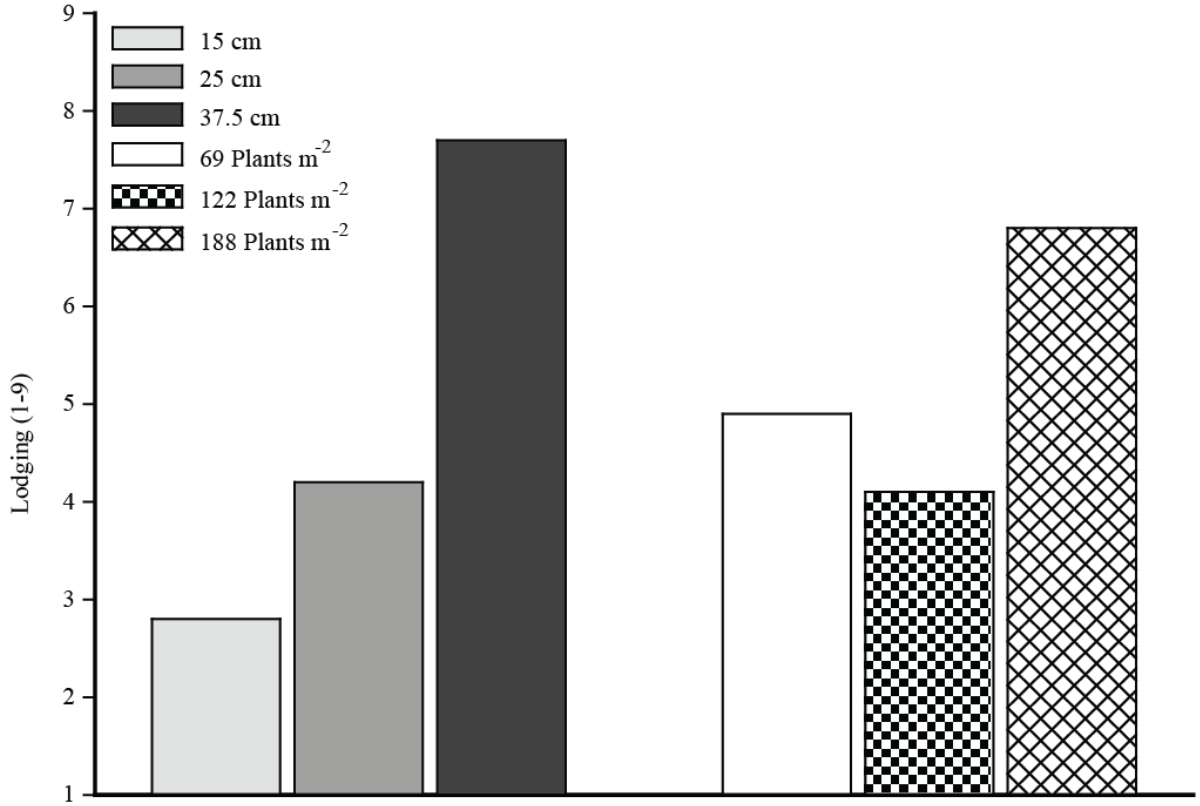

Fig. 2. Effect of row spacing and plant densities on lodging (1-9) in anise during 2009.

Table 2. Effect of row spacing (RS) and planting densities (PD) on plant height (PH) (cm), 1000-fruit weight (TFW) (g) and fruit yield (FY) (t/ha) of anise (*Pimpinella anisum* L.) at experimental station Giessen 2008-09.

Treatment	2008			2009		
	PH cm	TFW g	FY t/ha	PH cm	TFW g	FY t/ha
Row spacing						
15 cm	47a	2.63b	0.83a	62a	3.20a	0.68a
25 cm	47a	2.62b	0.72b	63a	3.00a	0.59a
37.5 cm	46a	2.78a	0.30c	63a	3.07a	0.55a
Sowing rates						
84	48a	2.70a	0.63a	66a	3.03a	0.59a
129	46a	2.57b	0.59a	62b	3.23a	0.65a
189	45a	2.76a	0.63a	61b	3.00a	0.58a
LSD 5%						
RS	ns	0.1	0.09	ns	ns	ns
PD	ns	0.1	ns	2.2	ns	ns
CV X PD	ns	ns	ns	ns	ns	ns

Significant differences were measured by the least significant differences (LSD) at $p < 0.05$ and indicated by different letters

Table 3. Effect of row spacing (RS) and planting densities (PD) on essential oil (EO) (%) and essential oil yield (EOY) (kg/ha) of anise (*Pimpinella anisum* L.) at experimental station Giessen 2008-09.

Treatment	2008		2009	
	EO %	EOY Kg/ha	EO %	EOY Kg/ha
Row spacing				
15 cm	2.83ab	23.6a	3.24b	22.3a
25 cm	3.00a	21.4a	3.43b	20.3a
37.5 cm	2.80b	8.6b	3.73a	20.7a
Sowing rates				
84	3.07a	19.2a	3.53a	20.8a
129	2.80a	16.7a	3.59a	23.5a
189	2.77a	17.6a	3.27b	19.0a
LSD 5%				
RS	0.2	3.2	0.2	ns
PD	ns	ns	0.2	ns
CV X PD	ns	ns	ns	ns

Significant differences were measured by the least significant differences (LSD) at $p<0.05$ and indicated by different letters

Significant differences were exhibited regarding essential oil yield from different row spacing treatments in 2008 whereas no affect was noticed under different row spacing and as well plant densities in 2009. We found that essential oil yields of anise varied between 8.6 and 23.6 kg/ha from different used treatments (Table 3). A significantly higher essential oil yield was recorded at 15 cm row compared to other row spacings (Table 3). The 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 similar range of 10 to 24 kg/ha (Zehtab-Salmasi *et al*., 2001; Tuncturk & Yildirim, 2006). Essential oil of anise fruits contains mainly phenylpropanoids and sesquiterpenoid hydrocarbons. It is of interest to note the presence of *trans*-anethole more than 80% in very high percentage, which was distinctive of *Pimpinella anisum* L. In current investigations the main compound of the anise fruit essential oil is *trans*-anethole followed by γ-himachalene and estragol. These findings are in accordance with previous experiments with anise (Özcan & Chalchat, 2006; Orav *et al*., 2008; Yan *et al*., 2011).

Estragol (methylchavicol) and γ-himachalene were found in all essential oil samples varied from 0.38 to 0.61% and 5.4 to 7.4 respectively in current study is in accordance with previous work (Orav *et al*., 2008; Yan *et al*., 2011). The quality parameters including estragol, γ-himachalene and *trans*-anethole were significantly affected by different row spacing. Plant grown at 37.5 cm row spacing accumulated the highest estragol and *trans*-anethole concentration among the row spacing treatments (Table 4). It can be assumed that under wider row spacing anise plants were heavily logged due to competition between plants which induced favorable conditions for fungal infection by *Cercospora malkoffii*. Environmental stresses, such as pathogen attack, UV-irradiation, high light impact, wounding, nutrient deficiencies and herbicide treatment often increase the accumulation of phenylpropanoids (Dixon & Paiva, 1995). Moreover, the activity of PAL (phenylalanine ammonia-lyase) enzyme which is responsible for the synthesis for phenolic and phenylpropanoids compounds increased with fungal infection. Fungal invasion triggers the transcription of messenger RNA that code for PAL, thus enhanced the amount of PAL in plant, which then stimulate the synthesis of phenolic compounds (Logemann *et al*., 1995; Taiz & Zeiger, 2002). Furthermore, we found an increase in level of *trans*-anethole while, γ-himachalene concentrations were decreased. Khorshidi *et al*., (2009) reported that percentage of *trans*-anethole and estragol was affected as spaces between the fennel plants change. A significantly higher concentration of estragol was determined at lower planting density compared to higher planting densities during 2009 (Table 4). A total of 17 components identified in the essential of cultivar Enza Zaden (Table 5). The major constituent of anise oil was *trans*-anethole (82.1%) followed by γ-himachalene (7.0%). The minor constituent in the essential oil of cultivar Enza Zaden were estragol, *cis* anethole, elemene, beta elemene, α-himachalene, γ-himachalene, α-amorphane, (E)-methylisoeugenol, α-zingiberene, β-himachalene, α-muurolene, β-bisabolene, beta-sesquiphellandrene, spathulenol and α-cadinol.

Table 4. Effect of different row spacing (RS) and planting densities (PD) on estragol (ES) (%), gamma-himachalene (GH) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at experimental station Giessen 2008-09.

Treatment	2008			2009		
	ES %	GH %	TA %	ES %	GH %	TA %
Row spacing						
15 cm	0.57a	6.3a	90.5b	0.55ab	7.3ab	88.8ab
25 cm	0.52b	6.1a	90.6ab	0.57a	7.4a	88.6b
37.5 cm	0.61a	5.6b	91.3a	0.52b	6.9b	89.4a
Sowing rates						
84	0.57a	5.9a	90.9a	0.59a	7.3a	88.4b
129	0.57a	6.0a	90.6a	0.54b	7.2a	88.9ab
189	0.56a	6.0a	90.9a	0.51b	7.0a	89.4a
LSD 5%						
RS	0.04	0.3	0.5	0.03	0.4	0.7
PD	ns	ns	ns	0.03	ns	0.7
CV X PD	ns	ns	ns	ns	ns	ns

Significant differences were measured by the least significant differences (LSD) at $p<0.05$ and indicated by different letters

Table 5. Chemical composition (%) in essential oil of cultivar Enza Zaden analyzed by GC-MS

No.	Components	Kovat 's retention index	%
1.	Estragol	1197	0.33
2.	*Cis* anethole	1252	0.14
3.	*trans*-anethole	1287	82.1
4.	Elemene (delta)	1333	0.45
5.	Beta elemene	1388	0.08
6.	α-himachalene	1449	0.71
7.	γ-himachalene	1478	7.0
8.	α-amorphane	1482	0.15
9.	(E)-Methylisoeugenol	1489	0.14
10.	α-zingiberene	1493	0.77
11.	β-himachalene	1499	0.44
12.	α-muurolene	1502	0.15
13.	β-bisabolene	1506	0.38
14.	Beta-sesquiphellandrene	1522	0.05
15.	Spathulenol	1580	0.04
16.	Unknown	1629	0.05
17.	α-cadinol	1651	0.08
18.	Unknown	1831	5.95
19.	Unknown	1886	0.92
No. of identified compound		19	100

*KI: Kovat´ s retention index

Conclusion

It can be concluded that higher plant density and wider row spacing increased the disease infestation and lodging. Results showed that higher essential oil was synthesized in wider row spacing and at lower plant densities. Two consecutive years study showed that plants grown under 15 cm row spacing with plant density ranging from 129 to 189 plants m^{-2} gave highest fruit yield due to reduced competition among plants. Cultivar Enza Zaden in current study exhibited high concentration trans-anethole in essential oil composition therefore is a good quality chemotype, however more cultivar should be included in further experiments.

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