Fruit Yield and Quality of Anise (*Pimpinella anisum* L.) in Relation to Agronomic and Environmental Factors

HABIB ULLAH

A thesis submitted for the requirement of doctoral degree in agriculture from
Faculty of Agricultural and Nutritional Sciences, and Environmental Management
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Institute of Agronomy and Plant Breeding I
Justus Liebig University Giessen, Germany
Prof. Dr. Bernd Honermeier

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submitted by

HABIB ULLAH

Rahim Yar Khan, Pakistan

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Chairperson: Prof. Dr. Sven Schubert

Supervisor: Prof. Dr. Bernd Honermeier

Co-supervisor: Prof. Dr. Guenter Leithold

Examiner: Dozent Dr. Agr. Feng Yan

Examiner: Prof. Dr. Diedrich Steffens

Dedicated to my beloved parents

Ahmad Latif Ullah

Yasmeen Begum

May Allah, the Almighty bless my parents with good health and prosperous long lives and be source of prayers for me.

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Abbreviations and definitions

FAO	Food and agriculture organization of the united Nations
SFE	Supercritical fluid extraction
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
FID	Flame ionization detector
TA	*Trans*-anethole
ES	Estragol
GH	Gamma-himachalene
IPP	Isopentenyl diphosphate
MEP	Methylerythritol phosphate pathway
DPP	Dimethylallyldiphosphate
GPP	Geranyl diphosphate
FPP	Farnesyl diphosphate
RT	Retention time
PIAF	Planning information analysis program for field trials
SD	Standard deviations
DAB	Deutsches arzneibuch
P	Probability level
LSD	Least significant difference
GGPP	Geranylgeranyl diphosphate
PAL	Phenylalanine ammonia lyase
GG	Gross-Gerau
Gie	Giessen
EO	Essential oil
RS	Row spacing
CV	Cultivar
ST	Sowing time
PD	Plant density
AT	Air temperature
LAT	Long term air temperature
PS	Precipitation sum
LPS	Long term precipitation sum
RCBD	Randomized complete block design
EOY	Essential oil yield
FU	Fungicide
MG	Magnesium
N	Nitrogen
P	Phosphors
K	Potassium
TFW	Thousand fruit weight

1. Introduction

Anise (*Pimpinella anisum* L.), is an annual important spice and medicinal plant belonging to the family of *Apiaceae*, and native to Mediterranean region. Today, anise seeds are an important natural raw material which is used for pharmaceutics, perfumery, food and cosmetic industries (Ross, 2001). In 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 (Tunc and Sahinkaya, 1998, Gülcin et al. 2003; Özcan and Chalchat 2006, Tepe et al. 2006, Tirapelli et al. 2007). The world production of anise essential oil amounts to 40-50 tons per annum. The most significant importing countries of anise oil are the USA and France. Russia, Spain and Poland are among the largest producers of anise oil. There is no distillation of anise oil and no production of *trans*-anethol in many of the countries which cultivate the crop (Basher 1997, Yalcin 1988, Arctander 1960).

Table 1.1: World's top anise, badian (star anise), fennel and coriander producing countries (FAO Stat 2010)

Country	**Area harvested** (Hectares)	**Production** (tones)
Syria	55172	30829
India	373600	217300
Mexico	4500	35900
China	33700	42000
Iran	29300	31300
Bulgaria	46000	35000
Morocco	22000	23000
Egypt	26000	22000
Turkey	18135	13992
Tunisia	12500	9800
World	**704695**	**537801**

Food and Agricultural Organization of United Nations: Economic and Social Department: The Statistical Division.

The drug as well as the essential oil is characterized by carminative, mild expectorant, diuretic, antiseptic as well as antispasmodic effects (Bown 2001, Kreydiyyeh et al. 2003). Its fruits known as aniseed were used as traditional medicine in china as early as in the 5th century (Buchgraber et al. 1997). In addition to its medicinal value, its fruits and oil have been used in food industry, such as cookie, candy, toothpaste, liquor and in some alcoholic drinks like pernot, pastis, and anisette for flavorings. Also it is added in American tobacco products because of its aromatic characteristics (Sengul 1994, Ozguven 2001, Ozguven 2005). Because of distinct aromatic characteristics of essential oil, anise fruits widely used as spice in food

production such as bread and biscuits productions or production of alcoholic drinks (Hänsel et al. 1999). Anise fruits known also as aniseed 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 (Lawrence 1984, Askari et al. 1998, Omidbaigi et al. 2003, Rodrigues et al. 2003, Tabanca et al. 2006). 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.
Because of anise favors warm climatic conditions throughout the growing season it is cultivated particularly in subtropical regions (Reineccius 1994, Hänsel et al. 1999). The quality of anise is determined mainly by the essential oil content and its composition. For both quality parameters it is necessary to determine the environmental factors under which they give higher yields and better quality (Omidbaigi 2000). The yield may noticeably vary depending on ecological conditions such as temperature, precipitation and soil fertility. Previous studies showed that, 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, Awad et al. 2005, Tuncturk and 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. Because of its sensitivity to low temperatures the sowing of anise in Germany cannot be carried out in early spring. On the other hand delayed sowing under warmer conditions in spring may lead to shortening the growing cycle which decreases the amount of UV radiation intercepted by the crops which may reduce the formation of reproductive organs.

Seed rate has important effect on yield and yield components such as the number of branches, number of umbels, number of fruits per plant, fruit weight per plant and 1000-fruit weight. As the higher plant densities affect negatively the yield and yield component, so optimal seed rate is very important for maximum seed yield. Plant spacing is an important factor in determining the microenvironment in the anise field. The optimization of this factor can lead to a higher yield in the crop by favorably affecting the absorption of nutrients and exposure of the plant to the light.

Additionally, aniseed plants can be infected by several fungal pathogens observed under practice cultivation. One of the most important pathogens in anise cultivation in Germany is the fungus *Passolara malkoffii*. The symptoms of this infection are characterized by cylindrical light brown spots with dark veins and later the whole leaves can be colored brown. The infection starts at the lower parts of the plants at the underside of the leaves. Later the whole leaves, stems, flowers and seeds can be infected. The seeds get dark color which comes from stomata of the fungus which reduced the quality of seeds.

2. Hypothesis

Though aniseed plant is not native in Germany, the climate conditions in Germany are generally suitable for the cultivation of anise plant. The intention behind this study was to evaluate the cultivation strategies, under which anise produced higher fruit yield and essential oil accumulation. For that reason field experiments were carried out to determine the effect of different sowing times, row spacing, cultivars and planting densities as well as fungicides on fruit yield and quality of *Pimpinella anisum* L. under two different ecological conditions in Germany.

It was hypothesized that:

Early sowing time can benefit the establishment of anise yield and quality.

Higher sowing density can benefit the establishment of anise plants due to slow growth of anise plants.

Reduced row spacing and adaptive planting densities improve plant establishment and enhance fruit yield.

Fungicide application improves fruit yield as well as essential oil accumulation.

3. Literature review

3.1 Botany of Anise

Taxonomy of Pimpinella anisum L.

Anise is belonging to the family of *Apiaceae* (*Umbelliferae*) which consists 300-455 genera and 3000-3750 species distributed in the northern hemisphere (Rechinger 1972, Heywood 1999). Members of this family have alternate leaves, widening at the base into a sheath that clasps the stem. The stems of these family members are often furrowed. The compound flowers are determined in umbels. The rays of the main umbel produced a secondary umbel with the flower bearing pedicels. The flowers of this family have 5 petals and 5 stamens. The fruits form below where the petals and stamen originate. Fruits or seeds are in pairs, commonly conspicuously ribbed, and sometime winged.

The genus *Pimpinella* L. consist 150 species spread in Eurasia and Africa, more than 16 of which present in Europe. The family *Apiaceae* can be familiar by certain characters that are generally found in the group including the herbaceous nature of the family; the frequent occurrence of compound leaves; small flowers, with a small number of floral parts arranged in whorls and grouped in shaped inflorescences. The genus includes herbaceous annual, biannual, or perennial plants, usually with a fine hair covering. From medicinal and agricultural point of view, only few species are economically significance, these are including, *Pimpinella anisum* L., *P. major*, *P. saxifraga* L., *P. peregrina* L. and *P.diversifolia* L. (Kubeczka et al.1989, Merkel and Reichling 1990, Kisiel et al.1998, Rajeshwari et al. 2011).

Morphological characteristics

Anise plant reaches a maximum height of 30-70 cm with ternately pinnate leaves. Very small and white flowers are born in compound umbels which distributed into 7 to 15 rays. The leaves of anise plant at the basal part are simple, 1.3-5.1 cm long and shallowly lobed, while leaves top on the stems are feathery pinnate divided into numerous leaves (Chevallier 1996). The fruit of anise is pyriform or ovoid laterally compressed which 3-5 mm in length and 2-3 mm wide. The color of anise fruits is greyish-green to greyish-brown with a sweet smell. Every fruit contains two carpals both containing an aniseed. The seed is small and curved, about 0.5 long and greyish-brown. The pericarp is broadly ovoid, five ridged with short hairs and various vittae (Ross 2001). The essential oil is located in the schizogenic oil ducts of anise fruits, and shoots (Figueiredo et al. 2008).

3.2 Cultivation of anise

Anise (*Pimpinella anisum* L.) is a slow growing annual herb which is cultivated throughout the world. For cultivating of anise plant a warm, sunny and dry autumn is ideal to meet economical yield and high quality of essential oil. So cultivation of anise in the northern part of the world does not pay, because the fruits do not usually ripen and harvest is repeatedly poor. The anise plant grows well in light to medium weight loose, humus soil. The field must be free from the weeds however rich in nutrients and not too dry. The anise cultivated field should be protected from wind to save the plants from lodging (Heeger 1956, Ebert 1982, Poss 1991). The reported life zone for anise cultivation is 8 to 23 °C with 1000-1200 mm annually precipitation produce excellent crop and rain fall of 2000 mm is tolerated and a soil pH of cultivated field ranged from 6.3 to 7.3 (Simon et al. 1984). The temperature during the growing duration should be quite uniform without very hot periods, particularly following precipitation. When the fruits are near maturity, alternate wet and dry conditions change it to brown color. This reduces the quality of anise fruit and makes the harvesting difficult and ultimately reduced the fruit yield. Sow the seeds or fruits about ½ inches deep in the soil in rows 18-30 inches apart at the rate of one to two fruits per inch. Some growers of the European countries broadcast the seeds, but if weeds are present in field at the harvesting they will influence the market value of both fruits as well as oil quality. While broadcasting the seeds, it is very important that the cultivation field have been fallow and in clean culture the earlier season to eliminate the weeds. The harvesting of anise is difficult because the umbels mature progressively and fruits ripen unevenly within each umbel (Stephens, 1997).

Aniseed plants have indeterminate growth nature and under favorable growing conditions, they will continue producing one more umbel from the node just below the prior umbel. Each successive umbel is smaller in size and later maturing than the one before it. The umbels of anise plant ripen progressively and the fruits ripens unevenly within each umbel because of this harvesting of anise is very difficult. When 85-90 percent fruits begin to turn greyish green in colour, cut the tops of the anise plants along with the branches or pull out of the ground and tied in bundles.

The soaking of anise fruits over night prior to sowing in water at room temperature for rapid germination within 8-10 days is recommended. Within this duration two irrigations were provided in addition 3-4 more irrigations at an interval of 20 days (Maheshwari et al. 1989). Soaking of anise seeds or fruits in water prior to sowing improves germination and hence flourishing stand establishment. Drying process is the most critical step in soaking of aniseed. Aniseeds should be dried down to 1.5% moisture if they are planted within 10 days of planting, 12% moisture if they are planted within 11-21 days and 10% moisture if they are planted after 21 days (Holm and Slinkard, 2002). The 1000-fruit weight of the fruit lies between 1.07 and 3.43 g (Ipek et al. 2004, Tuncturk and Yildirm 2006). The seeds purity for sowing should be at least 90% and germination rate at least 70%. The germination ability of anise seeds decrease rapidly when using inadequate conditions of storage. The best seeds for cultivation will come from the previous year harvest (Heeger 1956, Ebert 1982).

In central Europe, the fruits are sown in open fields between the middle of April and the beginning of May. For the cultivation of anise 15 to 20 kg/ha of seeds and a distance of 20 to 30 cm between rows has proven to be successful in Europe. Because anise seeds germinate in dark, the fruits are sown 1.0 to 1.5 cm into the ground, pressed lightly with roller, and then covered with soil. After 2 to 3 weeks, depending on the weather conditions, the aniseed germinates. In subtropical cultivation areas, other times may be more favorable for its cultivation. In Turkey aniseed fruits are sown at the end of January or start of February. Even sowing at the end of November has produced positive fruit yields (Boshart 1942, Heeger 1956, Taysi et al. 1977, Fazecas et al. 1985).

The cultivation field of anise should be fertilized before sowing according to fertilizer requirement of anise crop. The amount of fertilizer depends on the nutrient uptake by the plants and minerals contribution of the soil. In practice, 80 to 100 K_2O kg/ha and 50 to 70 P_2O_5 kg/ha have confirmed to be favorable. Care should be taken during adding nitrogen to the anise field, because it can result in too-exuberant growth of weeds as well poor fruit setting and storing conditions. For cultivation of aniseed 20-30 kg N ha^{-1} given some weeks after germination has proved to be successful (Heeger 1956, Noack 1996).

Awad et al. (2005) demonstrated the effects of biological and nitrogenous fertilizers on quantity and quality of anise plants. The results proved inoculation with N_2 fixing bacteria (*Azotobacter chroococcum* and *Azospirillum Lipoferum*) with half doses of chemical nitrogen fertilizers affected the quantity and quality of anise plants. Ammonium nitrate and urea plus mixed cultures of N_2 fixing bacteria (*Azotobacter chroococcum* and *Azospirillum Lipoferum)* gave highest values of shoot dry weight, fruit yield and essential oil. On the other hand bio fertilizer and ammonium nitrate significantly enhanced shoot dry weight, fruit yield and essential oil compared with urea. Maheshwari et al. (1989) reported higher fruit yield, essential oil, *trans*-anethole was recorded by sowing anise on October 25 or November 5 in comparison with when seed sown on Nonmember 30. A more than 20% higher fruit yield, essential oil and *trans*-anethol was obtained by sowing aniseed broadcast or in 15 cm rows as compared to sowing it in 45 cm rows.

3.3 Weeds, insect, pests and diseases

Anise plant is very sensitive to weeds due to its nature of slow growth. The herbicide application in cultivation of anise depends on the actual concessions of the individual countries. In Germany, there are no concessions for herbicide application that could be used in cultivation of anise. Therefore, it is necessary to contact the reproductive official advisory board for plant protection. Fungus diseases and pests are likely to develop under unfavorable environmental conditions and heavy weed growth. Such conditions hamper and weaken the growth of anise.

In well cultivated and healthy anise stocks, disease and pests only seldom appear (Heeger 1956). *Plasmopara nivea* and *Puccinia pimpinellae* infect all above parts of the anise plant. In 2009, it was reported that rust disease caused by *Puccinia pimpinellae* Mart. has become one of the most common and destructive diseases of anise plants in Egypt (Ghoneem et al. 2009). It was assumed to be particular to anise among the members of the *Apiaceae* family. *Puccinia pimpinellae* is autoecious, microcyclic rust characterized by rust colored uredial pustules on the upper and lower leaf surfaces, being more predominant on the underside of the leaf. The rust infection extended to stem, flowering buds, inflorescence and fruits. Severe infection may cause leaves to curl upwards, dessicate, turn brown and drop prematurely. Flowering set, number of umbels and fruit size can be reduced if early infection is severe. It adversely affects the germination rate, quality and weight of anise fruits. *Puccinia pimpinellae* on anise plants was first reported in the USDA 1960. Recently, Reichling and Bomme (2004), in the UK and Ghoneem et al. (2009) in Egypt, have reported *P. pimpinellae* as the causative pathogen of anise rust.

Passalora blight of aniseed caused by *Passalora malkoffii*. U. Braun was the first reported in 1906 from samples collected from Sadovo, near Philippopel, Bulgaria, named as *Cercospora malkoffii*. Soon after, a disease under the similar name was reported from other countries where anise was grown. In turkey, it was first mentioned by Bremer, who gave a brief description of the casual agent and its symptoms. The generic name of the pathogen has been changed to *Passalora*. At the leaves of anise symptoms of *Passalora malkoffii* were observed. At the first stage of infection cylindrical light brown spots with dark veins are present on leaves. On the later stages whole leave can be colored brown. The infection with *Cercospora malkoffii* starts at the basal parts of the plants at the underside of the leaves. Later the leaves, stems, flowers and fruits will be infected. Infected flowers of anise get brown to black color. The seeds or fruits get dark color which comes from stroma of the fungus. The fungus can be transferred by fruits. Azoxystrobin, Chlorothalonil + Carbendazim and Flutriafol seed treatments at 0.04 g a.i kg^{-1} seed, 1.0 g + 4.5 g a.i. kg^{-1} seed and 0.015 g a.i. kg^{-1} seed reduced the disease by 92.5%, 89.6% and 36.2% in 2002 and by 78.9%, 75.8% and 41.2% in 2003, respectively. Three foliar applications of Azoxystrobin, Chlorothalonil + Carbendazim and flutriafol at the rates of 187.5 g a.i. ha^{-1}, 1500 g + 6750 g a.i. ha^{-1} and 31.3 g a.i ha^{-1} reduced disease incidence by 92.5%, 86.0% and 96.8% in 2002 and by 97.5%, 90.8% and 97.0% in 2003, respectively (Erzurum K. et al. 2005). Spring tails cause severe damage at the root collar. Caterpillars of different butterflies damage the leaves, and the caterpillar of the moth *Depressaria depresella* destroys the umbels of the anise plants.

Sometime plant louses appear (Heeger 1956). According to Schremmer, most Harpalini species feed on the *Apiaceae* fruits or seeds (Brandmayr and Brandmayr 1987). Carterus (Sabienus) *calydonious* females on the umbels collect the seeds of *Daucus gingidium*. Carterus have damaged on the cultivated plants, as a pest. This is the first proof about damaging of *carterus dama* on anise fruits. Some year's *C. dama* causes a serious damage on the all of the anise fields in Burdur province. It is recorded that pest cut the flowers clusters from the bottom and transport the seeds from flowering. Any chemical application cannot be performed due to honeybee activation at the flowering period of the crop. The honey producers pay a rent to anise producers in order to utilize their honeybees from the anise flowers. The pest caused confusion and crop losses. However, the pest population and damage have reduced recently (Kocak et al. 2007).

3.4 Essential oil

Essential oil of the genus *Pimpinella* is a complex mixture of various components that contain sesquiterpenes, phenolic compounds (C_6-C_3) and alkenes. The essential oil is located in schizogenetic oil ducts of fruits, shoots and roots. According to European Pharmacopoeia, anise fruits as drugs must have an essential oil concentration higher than 2% (European Pharmacopoeia 2000). It is also clear that the concentration of essential oil can significantly vary among anise fruits from different origins (Tabanca et al. 2005, Tabanca et al. 2006, Orav et al. 2008). The fruits of *pimpinella anisum* contain about 2 to 6% and the roots about 0.05% of an essential oil (Becker 1971, Lee et al. 1997). Samaller amounts of essential oil are found in leaves and stems. Anise oil is a colorless to pale yellow liquid with strong, sweet-spicy, licorice-like odor and a characteristic, sweet, aromatic flavor. Essential oil content ranged from 1 to 5 % was reported from anise fruits from different European countries (Orav et al. 2008). The concentration of anise essential oil depends not only on genetic resources but also on the development of anise fruits. It was observed that a significant change in essential oil concentration occurred during the development of anise fruit with the maximum at waxy stage (Zehtab-Salmasi et al. 2001, Omidbaigi et al. 2003). El-Hady (2005) reported that there was a significant increase in the essential oil percentage of anise induced by GA_3 treatment at 50 ppm (67.6% for first season and 66% for 2^{nd} season also at 75 ppm 55% for first season and 56% for 2^{nd} season). The same trend was observed with kinetin treatment at 50 ppm (67.6% for first season and 65 .5% for 2^{nd} season.

3.4.1 Chemical composition

More than 25.000 different terpenoids are known (Cortaeu et al. 2002). In plants terpenes play a board range of physiological and ecological roles including, plant primary metabolism, (plant hormones, phtol, the side chain of the photosynthetic pigment chlorophyll) and protection against herbivores and pathogens, attractants for pollinators and as allelopathic agent. Other economically important terpenes include cartenoid pigments, natural rubber and the essential oils.

The main component a phenylpropanoid (comprising 80 to 90% of the oil) and typical odor and flavor carrier of the fruit oil *of P. anisum* oil is *trans*-anethole (1-propenyl-4-methoxy benzene) (Tabanca et al. 2005, Orav et al. 2008). *Trans-anethole* is of medicinal importance because of its carminative and expectorant effect. The isomeric methylchavicol (estragol) accounts for 4% of the oil composition; it possesses an anise like smell but lacks the sweetish taste (Ernst 1989, Santos et al. 1998). Other substances found in anise oil are anisaldehyde, dianethole, anisketone, anisic acid, p-methoxy acetophenone, γ-himachalene and *cis*-anethole. The amount of *cis*-anethole which is toxic, in commercially available anise oil is usually below about 0.2 to 0.5%. The essential oil of the herb *of P.anisum* contain considerable amount of sesquiterpenes hydrocarbon such as germacrene-D, β- bisbolene, γ-himachalene, β-himachalene, α-zingiberene and ar-curcumene. The root oil is characterized by the presence of β-bisabolene, pregeijerene, and its cope-rearrangement product geijerene, two terpenoic hydrocarbons with 12 C-atoms, as well as by *trans*-epoxypseudoisoeugenol-2-methylbutyrate. Pregeijerene and geijerene were not detected in the anise fruit oil (Kubeczka et al. 1976, Santos et al. 1998).

In previous studies Rodrigues et al. (2003) extracted 3.1-10.6% essential oil by the supercritical fluidextraction (SFE) method. The oil contained anethole (90%), γ-himachalene (2-4%), *p*-anisaldehyde (1%), methylchavicol (0.9-1.5%), *cis*-pseudoisoeugenyl-2-methylbutyrate (3%) and *t*-pseudoisoeugenyl-2-methylbutyrate (1.3%) as the major constituents. Tabanca et al. (2006) analysed essential oils from 15 *Pimpinella* species by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques. A total of 140 compounds were identified, which included mono-, sesqui- and trinorsesquiterpenoids, propenylphenols and pseudoisoeugenols. Trinorsesquiterpenoids and phenylpropanoid are the chemical markers of the *Pimpinella* species. The essential oils obtained from *Pimpinella* roots share the same principal compound, epoxypseudoisoeugenyl-2-methybutyrate, at concentrations from 20 to 82.6%. El-Hady (2005) reported that spraying with GA_3 at 50 ppm gave an increment of anethole percentage (10.4%) concomitant with decrement of anisaldehyde percentage (68.5%) and slight effect on methylchavicol. The same trend was observed by the treatment of kinetin at 50 ppm, which gave an increment of anethole percentage (17.2%) concomitant with a decrement (63.6%) of anisaldehyde percentage and methylchavicol too. The major components of the essential oils from the hairy root cultures were *trans*-epoxypseudoisoeugenyl 2-methylbutyrate, geijerene, pregeijerene, zingiberene and *b*-bisabolene (Santos et al. 1998).

3.4.2 Biosynthesis of terpenes

The terpenes comprise the largest class of secondary products. The various substances of this class are normally insoluble in water. They are biosynthesized from acetyl Co A or glycolytic intermediates. All terpenes are originated from the union of five carbon elements (also called C_5 units) that have the branched carbon skeleton of isopentane. The essential structural elements of terpenes are sometimes called isoprene units. The terpenes are further distributed by the number of C_5 units they hold. Ten-carbon terpenes, which obtain two C_5 units, are called monoterpenes; 15-carbon terpenes (three C_5 units) are sesquiterpenes; and 20 carbon terpenes (four C_5 units) are diterpenes. Larger terpenes include triterpenes (30 carbon), tetraterpenes (40 carbon), and polyterpenoids ([C_5] n carbons, where $n > 8$). Terpenes are biosynthesized from primary metabolites in at least two different ways. In well studied **mevalonic acid pathway**, three molecules of acetyl-Co A are coupled together stepwise to form mevalonic acid. This key six-carbon intermediate is then pyrophosphorylated, decarboxylated, and dehydrated to yield isopentenyl diphosphate (**IPP**). IPP is the activated five-carbon bulding block of terpenes. IPP also can be formed from intermediates of glycolysis or the photosynthetic carbon reduction cycle via a separate set of reactions called the **methylerythritol phosphate (MEP) pathway** that operates in chloroplast and other in plastids (Lichtenthaler 1999). Glyceraldehyde-3-phosphate and two carbon atoms derived from pyruvate condense to form the five carbon intermediate 1-deoxy-D-xylulose-5-phosphate. After rearrangement and reduction of this intermediate to 2-C-methyl-D-erythritol 4-phosphate (MEP), it is eventually converted to IPP. Isopentenyl diphosphate and its isomer, dimethylallyldiphosphate (DPP), are the activated five-carbon building blocks of terpene biosynthesis that join together to form larger molecules. First IPP and DPP react to give geranyl diphosphate (GPP), the 10-carbon precursor of nearly all the monoterpenes. GPP can then link to another molecule of IPP to give the 15-carbon compound farnesyl diphosphate (FPP), the precursor of nearly all the sesquiterpenes. Addition of yet another molecule of IPP gives the 20-carbon compound geranylgeranyl diphosphate (GGPP), the precursor of the diterpenes. Finally, FPP and GGPP can dimerize to give the triterpenes (C_{30}) and the tetraterpenes (C_{40}), respectively.

The most abundant classes of secondary metabolites compounds in plants are derived from phenylalanine via the elimination of an ammonia molecule to form cinnamic acid. This reaction is catalyzed by **phenylalanine ammonia lyase** (**PAL**), perhaps the most studied enzyme in plant secondary metabolism. PAL is situated at a branch point between primary and secondary metabolism, so the reaction that it catalyzes is an important regulatory step in the formation of many metabolites compounds. Phenylpropanoid *trans*-anethole and estragol which are found in the essential oil of anise; also synthesized by shikimic acid pathway.

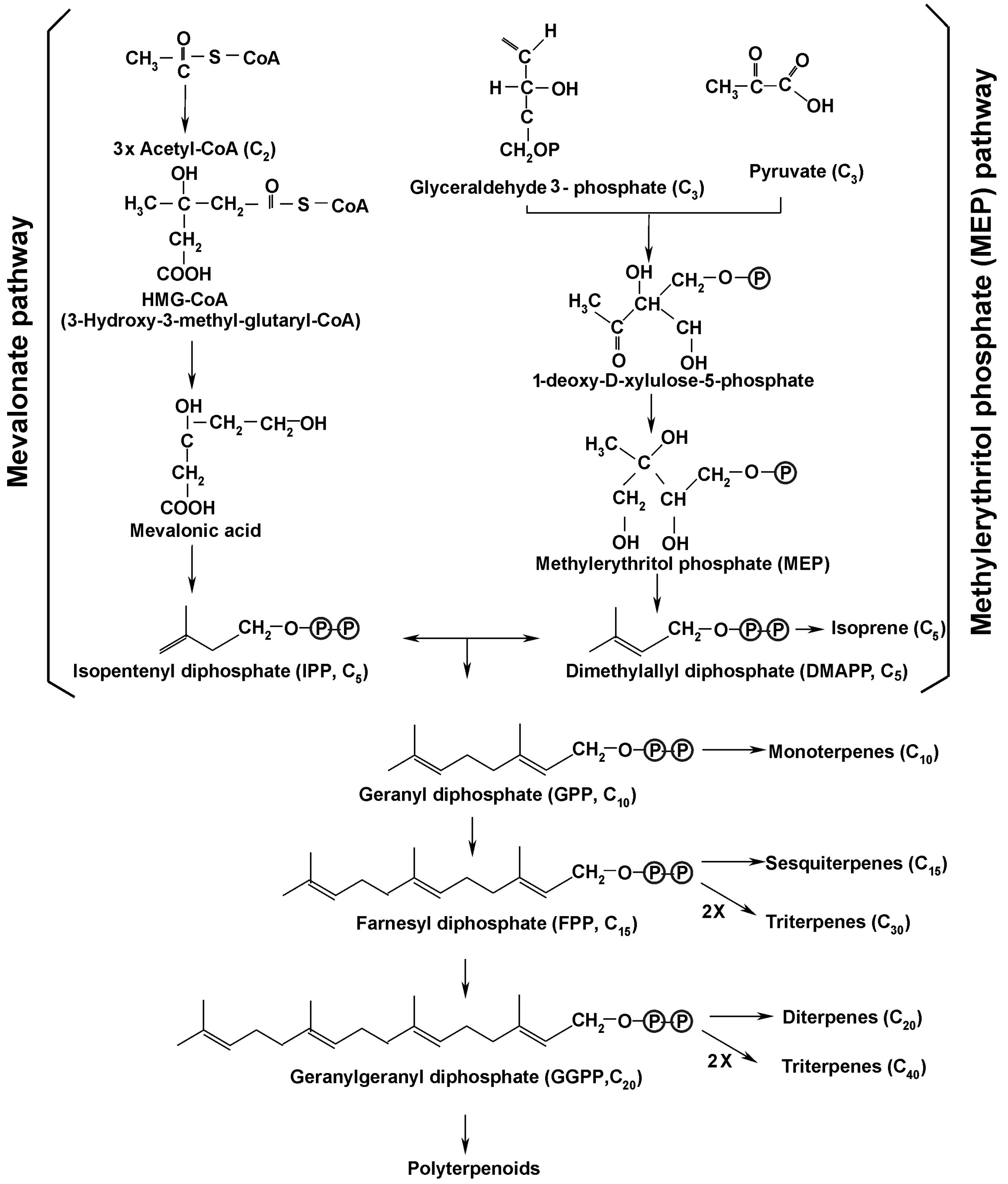


Fig 1.1: Outline of terpenes biosynthesis. The basic five carbon units of terpenes are synthesized by two different pathways. The phosphorylated intermediates, IPP and DMAPP, are combining to make 10-carbon, and larger terpenes (Taiz and Zeiger 2002).

3.5 Physiological effects and application of anise

Anise fruits and essential oils are extensively used for flavoring curries, soups, candies, cakes, breads, soups, non alcoholic beverages, and liqueurs such as anisette. Toothpastes and mouthwashes or dentifrices were common uses for anise (Harry 1963). It was also used to cover the flavor of unpleasant tasting medicines, as a flavor for some teas (Launert 1989), and in the preparation of various liqueurs. It is flavoring in pernod (Graves 1990), anisette (Leung and Foster, 1996, Wichtl 1994), ouzo (Greek aniseed spirit), and pastis, and it is an ingredient of Benedictine, boonekamp, Danziger Goldwasser, etc. (Wichtl, 1994). In India, aniseed is also used as mouth freshener, for flavoring some foods and in confectionaries.

Aniseed essential oil is very important component in perfumes and soaps and has been used in mouthwashes and skin creams (Harry 1963). It is also used as a constituent of potpourri (Back 1987), in which the crushed fruits can be used for their aroma (Bremness 1991) or simply for their look. Because of the traditional use of anise oils with licorice in sweets, the flavor of anise is often confused with that of licorice (Leung 1980). The use of anise tea will decrease skin oiliness (Heinemann 1988), and the fruits can be ground and added to face pack (Bremness 1991).

The leaves of anise plant can be used in salads. The fruits are used in Italy to flavor various pastries; in Germany they are used in breads and cakes; and in England, they are put in particular breads, in rye breads, and even in cheese (Sturtevant 1972). Anise and star anise essential oils are extensively used as flavoring ingredients in all main categories of foods including frozen dairy dessert, sweets (e.g., licorice confections), baked goods, gelatins, and puddings, as well as in meat products. The major maximums utilize levels for anise oil is about 0.06% (570 ppm) in alcoholic beverages and 0.07% (681 ppm) in sweets (Leung and Foster 1996).

Medicinal and pharmacological properties

The anise tea is used for children's flatulence, upper respiratory tract problems, and bronchial asthmatic attacks (Buchman 1987). The tisane tea is also used as expectorant (British herbal manufacturing Association 1996), as a cough suppressant (Fluck 1988). *Trans*-anethole (4-methoxyphenyl-1-propane), the major component of anise oil, is precursor that can produce 2, 5-dimethoxybenzaldehyde which is used in the synthesis of psychedelic drugs such as DOB (2, 5 dimethoxy-4-bromoamphetamine) (Waumans et al. 2006). Anise is useful in destroying body lice (Spoerke 1980), head lice, and itching insects (Buchman 1987) and the oil can used by itself (Hoffman 1991), which makes it helpful for pediculosis, the skin conditions caused by lice (Newall et al. 1996). It can also be used for scabies (Ody 1996), where it may be applied externally in an ointment base (Hoffman 1991).

Aniseed is used in aromatherapy to facilitate to ease difficulty in breathing (Price 1987). There is also thought to be an aphrodisiac (Wichtl 1994), though the action is unclear from any source. In several references it is said to be specifically a female aphrodisiac (Ody 1996), while in others it is said to enhance libido and alleviate symptoms of male climacteric (Leung and Foster, 1996). Aniseed used in folk medicine as an antispasmodic agent. Tirapelli et al. (2007) reported that ethanol: water 40:60 extract of aerial parts of anise 50 µg/ml inhibited acetylcholine induced contraction in rat smooth muscle. The *Pimpinella* species are used as food plants by larvae of some Lepidoptera species, including the lime-speck pug and wormwood pug.

Aniseed contains anti-inflammatory properties. Topical application of ethyl acetate and hexane extract of aniseed, at dose of 20 µl/animal formed an anti-inflammatory effecting mouse treated with 12-O-tetradecanoyl phorbol-13-acetate (Okuyama et al. 1995). Aqueous suspension of anise hold important cytoprotective and anti-ulcer activities against experimentally induced gastric lesions. Al-Mofleh et al. (2007) reported that in pylorus-ligated shay rats anise suspension reduced basal gastric acid secretion and acidity significantly and completely inhibited ruminal ulceration. The suspension replenished ethanol-induced depleted levels of gastric mucosal NP-SH and gastric wall mucus concentration significantly. The anti-ulcer effect of anise is possibly prostaglandin-mediated and or through its antisecretory and antioxidtaive properties. Pourgholami et al. (1999) demonstrated the anticonvulsant effect of essential oil of anise, used in the Iranian traditional medicine. This essential oil suppressed tonic convulsions induced by pentylenetetrazole or by maximal electroshock in male mice, and it is also elevated the threshold of pentylenetetrazole-induced clonic convulsions in mice.

Aniseed contains different levels of fungitoxicity. Aniseed fluid extract shows antimycotic activity against, *C. parapsilosis*, *Candida albicans C. tropicalis*, *C. krusei* and *C. pseudotropicalis* with MIC values between 17 and 20% (v/v). Extract of fruits of anise inhibits the growth of dermatophyte species (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis* and *M. gypseum*) with MIC values between 1.5 and 9% (v/v). The essential oil of anise shows strong antifungal activity against yeasts with MIC lower than 1.56% (v/v) and dermatophytes with MIC lower than 0.78% (v/v) Kosalec et al. (2005). Recent report indicated that anise essential oil found to be effective fungitoxicans for Aspergillus section *flavi* (Bluma et al. 2008). Shukla and Tripathi (1987) reported for the first time that *trans*-anethol from anise essential oil was found to be responsible for its antifungal activity.

The essential oil of *P. anisum* is very useful as both larvicidal and ovicidal against three mosquito species, *Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus*. The oil showed toxicity against 4th instar larvae of *A. stephensi* and *A. aegypti* with LD95 values of 115.7 µg/ml, whereas it was 149.7 µg/ml against

C. quinquefasciatus larvae (Prajapati et al. 2005). Recently, larvicidal activity of the essential oil against the seaside mosquito, Ochlerotatus caspius, has been reported by Knio et al. (2007). Essential oil from anise shows potent fumigant activity against the larvae of Lycoriella ingénue (Dufour). *Trans*-anethol, the chief constituent of the anise oil, was toxic with an LC 50 value of 0.20 µl/l (Park et al. 2006). Essential oils extracted from the fruits of anise exhibit significant repellency against the adult females of the mosquito, *C. pipiens* (Erler et al. 2006). Anise seed oil was found to be toxic to two greenhouse pests, the carmine spider mite, *Tetranychus cinnabarinus* (Boisd.) and cotton aphid *Aphis gossypii* Glov. In general, a minimum dose of 0.5 µl/l air and 2-3 days of exposure was required for 99% mortality (Tunc and Sahinkaya, 1998). The essential oil of *P.anisum* L. (doses of 108 to 135µl air) caused 95% mortality in *Tribolium confusum* Du val adults, *Sitophilus oryzae* L. adults, and Ephestia kuehniella Zeller last instars within an exposure period of 24 hours S. oryzae and 96 hours in E. kuehniella (Sarac and Tunc, 1995).

Mahady et al. (2005) reported that Methanol extract of *P. anisum* seeds is effective against the Gram-negative bacterium *Helicobacter pylori* at MIC of 100 µg/ml. This bacterium is recognized as the primary etiological factor associated with the development of gastritis and peptic ulcer disease. HP infections are also associated with chronic gastritis, gastric carcinoma and primary gastric B-cell lymphoma.

The antiviral activity of the essential oil of *P. anisum* has been tested against potato virus X, tobaco mosaic virus, and tobaco ring spot vitus on the hypersensitive host *Chenopodium amaranticolar*. At a concentration of 300 ppm, the essential oil totally inhibited the formation of local lesions (Shukla et al. 1989).

4. Materials and methods

4.1 Overview of field experiments

Three field trials (1. Cultivar/seed rate experiment, 2. Row spacing/seed rate experiment and 3. Fungicide experiment) were carried out in three successive seasons 2008, 2009 and 2010. For the experiments two research stations of the Institute of Agronomy and Plant Breeding in Giessen and in Gross Gerau which are characterized by different soil and climate conditions were used. An overview about the executed field experiments is given in table 4.1.

Table 4.1: Overview about the executed field experiments

<table>
<tr><th>Year</th><th>Location</th><th>Study factor</th><th>Treatments</th></tr>
<tr><td colspan="4">Cultivar/seed rate experiment</td></tr>
<tr><td>2008</td><td>GG + Gie</td><td rowspan="2">1. Cultivars (3)
2. Seed rate (3)
In combination with two sowing times.</td><td rowspan="2">3 x 3 = 9 treatments</td></tr>
<tr><td>2009</td><td>GG + Gie</td></tr>
<tr><td colspan="4">Row spacing/seed rate experiment</td></tr>
<tr><td>2008</td><td>GG + Gie</td><td rowspan="2">1. Row spacing (3)
2. Seed rate (3)</td><td rowspan="2">3 x 3 = 9 treatments</td></tr>
<tr><td>2009</td><td>GG + Gie</td></tr>
<tr><td colspan="4">Fungicide experiment</td></tr>
<tr><td>2009</td><td>GG</td><td rowspan="2">1. Fungicides
2. Cultivars</td><td rowspan="2">6 x 2 = 12 treatments</td></tr>
<tr><td>2010</td><td>GG</td></tr>
</table>

GG: Gross Gerau, Gie: Giessen

4.2 Soil conditions

Experimental station Gross-Gerau

The experimental station Gross-Gerau (49°45´N and 8°29´E) is situated in the upper Rhine valley with the river Main to the north, the river Rhine to the west and the Odenwald mountains to the east. Experimental station located 90 m to 145 m above sea level. The upper layer (0-25 cm) of the soil is crumb which contains sandy soil. The soils are weakly humic with low buffering capacity. The soils are hence best described as having a slightly loamy to loamy sand consistency. The pH of the soil is between 6.0 to 6.9. The soil number is in the range between 20 and 25. The field capacity is 100 cm for sandy soils at 115 mm. Irrigation was therefore an inevitable part of the experimental scheduling in Gross-Gerau during all three field experiments carried out in 2008, 2009 and 2010. The long-term average (LTV) of the annual air temperature is 9.4 °C; LTV of the annual precipitation is 590 mm. General characteristics of the experimental station Gross-Gerau are illustrated in table 4.2. The trial field used for anise cultivation in 2008 was previously sown with summer barley in 2007. That used for the 2009 experiments had winter rye in 2008 as pre crop. For the 2010 experiments the field used was pre-sown with summer barley in 2009.

Table 4.2: General characteristics of the experimental station Gross-Gerau

Factor	Value
Soil texture	sandy loam
Soil type	Haplic cambisols
Clay content	<5 %
Field capacity (100cm)	<115 mm
Height above NN	90-145 m
Long term average air temperature (1990-2009)	9.4°C
Long term average precipitation (1990-2009)	590 mm/year

NN= Normal null (the German standard for measuring height above sea level

Soil analyses

Nutrition contents and pH value of the soil for the experimental years are presented in table 4.3.

Table 4.3: Results of soil analysis (0 - 90cm) used for growing anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau 2008, 2009 and 2010

Nutrient	Unit	GG 2008	GG 2009	GG 2010
pH		6.4	6.5	6.9
P	mg $100g^{-1}$	7.5	11.0	10.6
K	mg $100g^{-1}$	12.5	13.3	10.8
Mg	mg $100g^{-1}$	1.8	2.4	3.0
B	mg $1000g^{-1}$	0.16	0.35	0.29
N-min	kg ha^{-1}	52.0	36.0	29.0
NO_3-N	mg/100 g	0.78	0.47	0.34
NH_4-N	mg/100 g	0.01	0.04	0.04

N-min, NO_3-N and NH_4-N in 0-90 cm soil depth

Fertilization

Before seeding, fields were disked and harrowed and fertilizers phosphorous and potash were applied to the soil as basal dose.

Table 4.4: Fertilizers applied at the seed bed preparation stage in Gross-Gerau 2008, 2009 and 2010

Type of Fertilizer	2008	2009	2010
	Dose kg/ha	Dose kg/ha	Dose kg/ha
Kalkammonsalpeter (NH_4NO_3 + $CaCO_3$) (N)	40	40	40
Thomaskali (P&K)	70 : 210	54 : 162	70 : 210

After germination of anise nitrogen in form of calcium ammonium nitrate 27% N ($CaCO_3$ + NH_4NO_3) were applied to meet the nitrogen requirement of the crop (table 4.4). Same amount of fertilizers were applied for all executed experiments in all years. An additional amount of fertilizer 60:200 kg Thomaskali (8% P & 15% K) and 60 kg N in form of calcium ammonium nitrate 27% N were applied to anise plants due to its slow and yellowish growth in fungicide experiments in 2010.

Irrigation schedule

In Gross Gerau anise plants were irrigated (2-4 times) with sprinkler irrigation system according to irrigation requirement of anise crop (table 4.5).

Table 4.5: Irrigation applied at different stages of anise at Gross-Gerau, 2008, 2009 and 2010

No. of irrigations	2008		2009		2010	
	Date	level	Date	level	Date	Level
1	May 12th	10 mm	May 27th	20 mm	April 4th	8 mm
2	May 27th	20 mm	June 3rd	20 mm	April 23rd	8 mm
3	July 9th	20 mm	-	-	June 28th	20 mm
4	-	-	-	-	July 9th -	20 mm

Plant protection

In all experimental years same type of herbicide and fungicides were applied to ensure a healthy anise crop stand. Weed control was carried out by application of Bandur (Aclonifen) as well as by hand weeding. First application of herbicide was carried out after seeding of aniseed and second application was after 15 days. Information's regarding these herbicides, fungicides and insecticides are listed below in table 4.6. First application of fungicides Mancozeb + Metalaxyl-M and Difenoconazol were applied 47 days after anise germination in row spacing and sowing time experiments and second application of these fungicides were applied after 15 days interval before flowering initiations. Only one application of Karate Zeon was carried out before flowering.

Table 4.6: Herbicide, fungicide and insecticide used for anise crop during 2008, 2009 and 2010 at experimental station Gross-Gerau

Plant protection	Trade mark	Active ingredients	Dose
Herbicides	Bandur	Aclonifen	3 L/ha
	Fusilade	Fluazifop	1L/ha
Fungicides	Ridomil-Gold	Mancozeb + Metalaxyl-M	2 kg/ha
	Score	Difenoconazol	0.4 L/ha
Insecticides	Karate Zeon	Lambda -cyhalothrin	75 ml/ha

Experimental station Giessen

The Giessen experimental station (50°47´N and 8°61´E) is situated in the valley of the Lahn River about 1° 12’ northward displacement. Topographically the Giessen experimental site is usually even with homogenous soils rich in clay contents. The fluvogenic (river side) soils are best described as having silty clay characteristics. The clay content in the topsoil is 28-33%. The pH value of the soil ranged from 6.0 to 6.4. In spite of high level of clay, the soils are characterized by lower field capacity with high dead water contents around (202mm/100cm). The average air temperature and precipitation were 9.0 °C and 650 mm/year respectively. The experimental field used in 2008 was previously sown with winter wheat and that used in 2009 was previously sown with summer barley.

Table 4.7: General characteristics of the experimental station Giessen

Factor	Value
Soil texture	Silty clay
Soil type	Stagnic fluvisol
Clay content	28-33 %
Field capacity (100cm)	123 mm
Height above NN	158 m
Long term average air temperature (1990-2009)	9.0°C
Long term average precipitation	650 mm/year

Soil type: According to World Reference Base for Soil Resources (WRB)

Soil analyses and fertilization

Soil pH was measured before the preparation of the seedbed in each successive year. Soil nutrient contents were evaluated from top soil to 90 cm deep. The results are presented in table 4.8.

Table 4.8: Results of soil analysis and fertilization used for growing anise (*Pimpinella anisum* L.) at experimental station Giessen, 2008-2009

Nutrient	Unit	Giessen 2008	Giessen 2009
pH		6.5	7.4
P	mg 100g-1	6.6	4.0
K	mg 100g-1	5.3	7.2
Mg	mg 100g-1	16.4	9.4
N-min	kg ha-1	52	42
Fertilization			
N	kg ha^{-1}	40	40
P	kg ha^{-1}	70	54
K	kg ha^{-1}	210	162

After germination of anise N (NH_4NO_3 + $CaCO_3$) was applied in each successive year. Before seeding, fields were disked, harrowed and fertilizer was added to provide phosphorous and potash as basal dose.

Plant protection

In both years, same herbicides and fungicides were applied to ensure a healthy anise crop stand. Weed control was carried out by application of the herbicide (Bandur, 4 L/ha) in both seasons. Foliar application of Ridomil Gold (Mancozeb + Metalaxyl-M, 2 kg/ha) and Juwel top (Kresoxim-methyl, 1 L/ha) were applied for fungal control, one application in 2008 and two applications were applied in 2009 after fortnightly. Only one application of herbicide was applied after seeding of anise crop.

4.3 Climate conditions

Experimental station Gross-Gerau 2008-2010

From both experimental stations, the effects of weather on the performances of the anise cultivars were also accessed by comparing meteorological results of each experimental year with the known long term averages for temperature (°C) and precipitation (mm). As it clear from table 4.9 that experimental station Gross-Gerau a generally warmer climate conditions as compared to Giessen. Data presented in table 4.9 indicate that, the air temperature (AT) with an average of 14.9 °C during cultivation period from April to October is slightly higher than LAT 14.5 °C (25 years).

Table 4.9: Air temperature (AT) in °C and precipitation (PS) in mm from April to October, and the long-term average (last 25 years), Gross-Gerau 2008, 2009 and 2010

Months	2008				2009				2010			
	AT	LAT	PS	LPS	AT	LAT	PS	LPS	AT	LAT	PS	LPS
	°C	°C	mm	mm	°C	°C	mm	mm	°C	°C	mm	mm
April	8.8	9.4	76	41	15.1	9.5	36	41	11.5	9.6	17	41
May	17.0	14.0	39	57	15.7	14.0	55	57	12.4	14.0	112	58
June	18.3	17.2	115	64	17.1	17.2	109	65	18.6	17.3	94	65
July	19.4	19.0	30	67	19.7	19.0	72	67	21.6	19.1	64	67
August	18.4	18.2	72	64	20.2	18.2	46	64	18.3	18.2	141	65
September	12.6	14.4	56	47	15.6	14.4	40	47	14.2	14.4	46	47
October	9.6	9.5	60	50	9.4	9.5	47	50	8.4	9.5	22	50
Sum			**448**	**390**			**405**	**391**			**496**	**393**
Mean	**14.9**	**14.5**			**16.2**	**14.5**			**15.0**	**14.5**		

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

In all years, July and August were the hottest months and corresponded with the period of anthesis as well as fruit formation stages. Like in 2008 May was the driest month and June the wettest. The precipitation sum (PS) of about 448 mm occurred during cultivation period of anise which is located near the LPS of 391 mm. During 2008, maximum rain fall of 115 mm occurred in June followed by 76 mm in April (table 4.9). Long term averages regarding precipitation and temperature remain constant in both years. In 2009, relatively higher air temperature of 15.1 °C was observed during April compared to 8.8 °C in 2008. Temperatures in April were above 10 °C suggesting good potentials for seed germination during 2009. During cultivation period 2009, air temperature (AT) of 16.2 °C was higher than long term air temperature (LAT) of 14.5 °C table 4.9. In 2010 July was the hottest month with temperature of 21.6 °C. During 2010 highest precipitation occurred during months of May (112 mm) and August (141 mm) respectively. Monthly sum of precipitation was higher than long term precipitation average during cultivation period of 2010.

Experimental station Giessen 2008-2009

In 2008, monthly air temperature of 14.1 °C was different from long term air temperature (LAT) in 25 years. The precipitation sum (PS) of the sixth months was 359 mm with close to LPS with 336 mm. During 2009, the average air temperature of 14.2 °C was recorded which is lower than LAT of 14.3. Similar trend of precipitation was observed in 2009 regarding monthly precipitation sum and long term precipitation sum (mm). It can be observed from table 4.10 that April has minimum air temperature of 5.7 °C and 9.7 °C respectively in 2008 and 2009. The temperature below 10 °C observed in April was an unfavorable sign for good germination potentials as is known for anise cultivation table 4.10.

Table 4.10: 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	LAT	PS	LPS	AT	LAT	PS	LPS
	°C	°C	mm	Mm	°C	°C	mm	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)

The highest temperature was observed during month of July in both years 20.5 and 18.5 °C respectively which might be suitable environmental conditions for essential oil accumulation. Highest precipitation was recorded during August and September in 2008, whereas in 2009 higher precipitation was noticed during May, June and July. In both years precipitation sum of the sixth months were higher as compared to long term precipitation sum (mm).

4.4 Description of the experiments

4.4.1 Cultivars/seed rate experiments in two sowing times

Design of the experiment

The experiments were carried out to investigate the effect of sowing times and plant density on fruit yield, and quality of three anise cultivars. The experiments were designed as randomized complete block design (RCBD) under factorial arrangements with four replications. Cultivars were planted in 1.5 x 7.0 m^2 plots maintaining eight rows with row spacing of 18.75 cm in both seasons. In sowing time experiments, cultivars were given less importance and were assigned in main plots, where as the more important factor seed rate was placed in sub plots. The experiments were combined with two sowing times (early sowing time and two weeks delayed sowing time). Two factors studied included seed rate (8 g, 15 g, 30 g/10 m^2) and cultivars (Enza Zaden, Pharmasaat, Hild Samen).

Study parameters

Plants from two middle rows of the plots were counted and plant density was calculated on plants m^{-2} basis (Fig. 4.2). Prior to harvesting plant height was measured with yardstick. Plant height was taken from the surface of the soil to the meristimatic part of leading stem recorded for 10 plants from each plot and average was computed. Number of primary branches, secondary branches and umbels per plant were counted from both experimental stations. For morphological analysis samples of anise plants were taken from two rows (2 m length) in middle of the plot. These yields determine components were calculated from each plant of two middle rows and averages of all plants worked out.
Data regarding fruit number and fruit weight per plant was taken from experimental station Gross-Gerau. The plants from two rows in middle of each plot were manually harvested and threshed with machines and fruits of two rows were counted by counter machine. Number of fruit per plant was obtained by dividing the total number of fruits by total number of plants. Fruits harvested from two middle rows were weighed after cleaning and fruit weight of individual plant was achieved by dividing the total number of plants. The thousand fruit weight (TFW) is an important yield component which has direct impact on final yield of crop. TFW was obtained by counting of twice 1000-fruits of each sample using an automated seed counter (Contador). The average of twice samples recorded as 1000-fruit weight. 20 grams

each of the samples were weighted out and put into a laboratory drying oven set at a constant temperature of 105 °C. The samples were intended to dry over a period of 48 hours. The harvest was made by a combine at the time of full ripeness of the fruits. Fruit yield was obtained at dt/ha 91% dry matter contents.

Fig. 4.2: Anise plants marked for further evaluation at experimental station Gross-Gerau

Disease severity

The infection of fungal pathogens like *Cercospora malkoffii* may be affected the plant growth and development under humid climate conditions. So fungal disease severity on anise plants was recorded by grading 1-9 (1: without infection, 9: whole plants are infected) (Fig. 4.3). Visual fungal disease incidence was assessed for each plot fortnightly from both experimental stations.

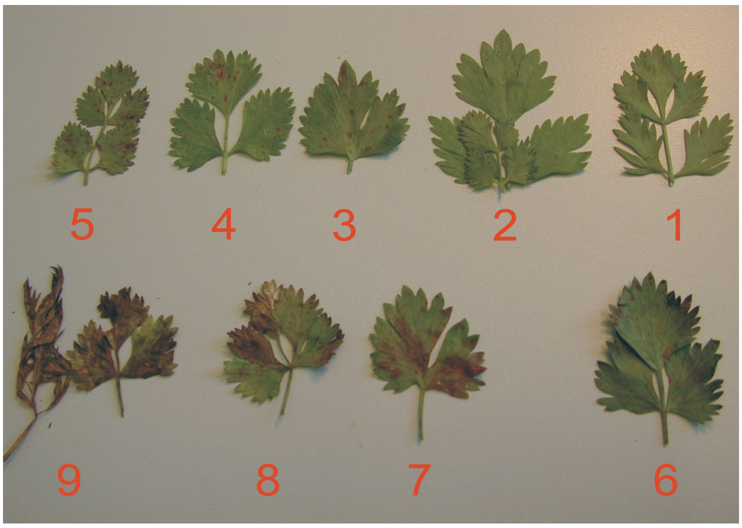


Fig. 4.3: Disease severity index on anise leaves (1-9)

Lodging was also estimated with 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. Lodging data was recorded from both experimental stations.

Fig. 4.4: Lodging of anise plants at experimental station Gross-Gerau 2009

4.4.2. Row spacing/seed rate experiment

Design of the experiment

The row spacing experiments were laid out as RCBD designed 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).

Study parameters

Plants from two middle rows of the plots were counted and plant density was calculated on plants m^{-2} basis (Fig. 4.2). Prior to harvesting plant height was measured with yardstick. Plant height was taken in centimeters from the surface of the soil to the meristimatic part of leading stem. The plant height was recorded for 10 plants from each plot and average was computed. Number of primary branches, secondary branches and umbels per plant were counted from experimental station Gross-Gerau. For morphological analysis samples of anise plants were taken from two rows (2 m length) in middle of the plot. These yields determine components were calculated from each plant of two middle rows and averages of all plants worked out.

Data regarding fruit number and fruit weight per plant was taken from experimental station Gross-Gerau. The plants from two rows in middle of each plot were manually harvested and threshed with machines and fruits of two rows were counted by counter machine. Number of fruits per plant was obtained by dividing the total number of fruits by total number of plants. Fruits harvested from two middle rows were weighed after cleaning and fruit weight of individual plant was achieved by dividing the total number of plants. The thousand fruit weight (TFW) is an important yield component which has direct impact on final yield of crop. TFW was obtained by counting of twice 1000-fruits of each sample using an automated seed counter (Contador). The average of twice samples recorded as 1000-fruit weight. 20 grams each of the samples were weighted out and put into a laboratory drying oven set at a constant temperature of 105 °C. The samples were intended to dry over a period of 48 hours. The harvest was made by a combine at the time of full ripeness of the fruits. Weight from each plot was recorded in (g). The recorded weight was then converted to dt/ha for statistical analysis. Fruit yield was obtained at dt/ha 91 % dry matter contents.

The infection of fungal pathogens like *Cercospora malkoffii* may be affected plant growth and development under humid climate conditions. So fungal disease severity on anise plants was recorded by grading 1-9 (1: without infection, 9: whole plants are infected) (Fig. 4.3). Visual fungal disease incidence was assessed for each plot fortnightly from both experimental stations. 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. Field evaluations were made from both experimental stations (Fig. 4.4).

4.4.3. Fungicide experiments

Design of the experiment

Fungicide experiments were conducted only at experimental research station Gross-Gerau during 2009-2010. The experiment was design as randomized complete block design (RCBD) under factorial arrangement with four replications. Cultivars (Enza Zaden, Pharmasaat) were planted in 1.5 x 7.0 m^2 plots maintaining eight rows with row spacing of 18.75 cm in both seasons. Fungicides were applied at different growth stages before flowering. Fungicides were allocated to sub plots and cultivars to main plots given less importance. The anise cultivars were sown on 1-04-2009, 23-03-2010 and harvested on 18-8-2009 and 10-8-2010 respectively. In 2009 fungicides were applied on 5-6-2009 and 19-6-2009 where as in 2010 fungicides were applied on 1-6-2010 and 15-6-2010 respectively. Classifications of these fungicides depending on mode of action are given below in table 4.12.

Table 4.11: Fungicidal treatments used in the field experiments with anise in Gross-Gerau 2009-2010

A. Fungicides			
No.	**Trade name**	**Active ingredient**	**Dose**
1	Ridomil Gold MZ	Mancozeb + Metalaxyl-M	2 kg/ha
2	Askon	Azoxystrobin + Difenoconazol	2 x 1.0 L/ha
3	Acrobat plus WG	Mancozeb + Diemethomorph	2 kg/ha
4	Previcur N	Propamocarp	3 L/ha
5	Aliette WG	Fosetyl	3 kg/ha
B. Cultivars			
1. Enza Zaden		2. Pharmasaat	

Table 4.12: Classification of the fungicides and their mode of action used during the course of the study

No	Trade name	Active ingredient	Mode of action	Chemical Group
1	Ridomil Gold MZ	Mancozeb	Inhibits cell division	Thiophthalimide
		Metalaxyl-M	Inhibit protein synthesis	Acylamino acids
2	Askon	Azoxystrobin	Inhibit mitochondrial resp.	Strobilurin
		Diefenoconazol	Inhibition of sterol syn.	Triazole
3	Acrobat Plus WG	Mancozeb	Disrupt fungal cell wall	Polymericicarbamate
		Dimethomorph	Inhibition of sterol syn.	Morpholine
4	Previcur M	Propamacarp	Disrupt fungal cell wall	Carbamate
5	Aliette WG	Fosetyl-Al	Plant defence stimulation	Organophosphorus

Study parameters

Plants from two middle rows of the plots were counted and plant density was calculated on plants m^{-2} basis (Fig. 4.2). Prior to harvesting plant height was measured with yardstick. Plant height was taken in centimeters from the surface of the soil to the meristimatic part of leading stem. The plant height was recorded for 10 plants from each plot and average was computed. Number of primary branches, secondary branches and umbels per plant were counted from both growing seasons. For morphological analysis samples of anise plants were taken from two rows (2 m length) in middle of the plot. These yields determine components were calculated from each plant of two middle rows and averages of all plants worked out.

Data regarding fruit number and fruit weight per plant was taken from both years. The plants from two rows in middle of each plot were manually harvested and threshed with machines and fruits of two rows were counted by counter machine. Number of fruits per plant was obtained by dividing the total number of fruits by total number of plants. Fruits harvested from two middle rows were weighed after cleaning and fruit weight of individual plant was achieved by dividing the total number of plants. The thousand fruit weight (TFW) is an important yield component which has direct impact on final yield of crop. TFW was obtained by counting of twice 1000-fruits of each sample using an automated seed counter (Contador). The average of twice samples recorded as 1000-fruit weight. 20 grams each of the samples were weighted out and put into a laboratory drying oven set at a constant temperature of 105 °C. The samples were intended to dry over a period of 48 hours. The harvest was made by a combine at the time of full ripeness of the fruits. Weight from each plot was recorded in (g). The recorded weight was then converted to dt/ha for statistical analysis. Fruit yield was obtained at dt/ha 91% dry matter contents.

The infection of fungal pathogens like *Cercospora malkoffii* may be affected plant growth and development under humid climate conditions. So fungal disease severity on anise plants was recorded by grading 1-9 (1: without infection, 9: whole plants are infected) (Fig. 4.2). Visual fungal disease incidence was assessed for each plot fortnightly in both years. 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 (Fig. 4.4).

4.5. Lab analysis

Water steam distillation

Essential oil was quantified by using a Clevenger apparatus according to European Pharmacopoeia (2000) (Fig. 4.5). Steam distillation is a special type of distillation for temperature sensitive materials like oils, resins and hydrocarbons etc. which are insoluble in water and may decompose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling points of the individual constituents. Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C, at atmospheric pressure. The grinding of the anise sample must take place immediately prior to distillation to oil to avoid losses by evaporation (Hartke and Mutschler 1986). The heat of the steam forces the tiny intercellular pockets that hold the essential oils to open and release them. The temperature must be high enough to open the pouches and not so high that it destroys the fruit material or burn the essential oil. As they are released, the tiny droplets of essential oil evaporate together with the steam molecule and travel through a tube into the part of the apparatus (Fig. 4.5, F-G) where cooling water is connected. Here essential oil is condensed and collected. The distillation of the anise samples were carried out at the pot experiment station Rauischholzhausen. After cleaning impurities the sample was crashed with labor miller (Janke & Kunkel, Staufen, Germany) for 1.5 min. Seven gram fruits sample were added in 500 ml round bottom flask. The prescribed volume of distilled water (H_2OD 200 ml) was added for distillation.

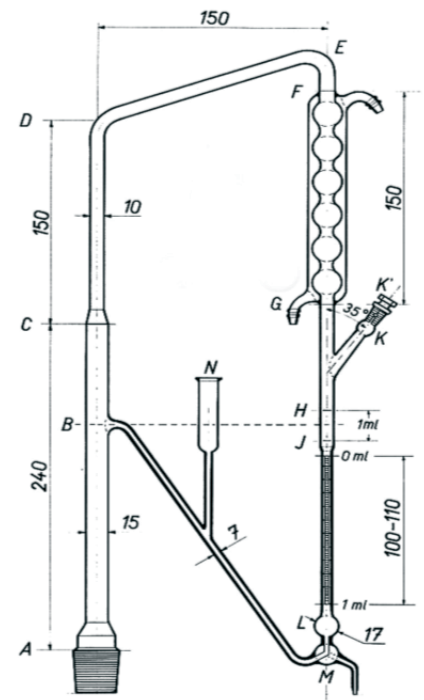


Fig 4.5: Neo-Clevenger type apparatus DAB 9 1989

Then, 0.5 ml of n-pentane (99% purity, Carl Roth GmbH & Co. KG, Karlsruhe) was added to the water column in the collection tube (Fig. 4.5, H). The content of the round bottom flask was heated to boiling and distilled for 2 h (European pharmacopoeia 2000).

The essential oil which was mixed with pentane was obtained after careful drainage of water from the tube. The mixture of essential oil and pentane was then collected with a glass vessel whose weight was determined before. For complete removal of the essential oil from the distillation apparatus about 1 ml pentane was added into the collection tube of the distillation apparatus and collected in the glass vessel. The pentane was evaporated overnight in an extractor hood. The weight of essential oil was gravimetrically determined according to following form:

$$\text{Essential oil \%} = \frac{\text{Weight of vessel + oil (g) – empty vessel (g)}}{\text{Weight of total sample used (g)}} \times 100$$

Fig. 4.6: Distillation apparatus (Neo-Clevenger) in Rauischholzhausen

Double analyses were carried out for each sample (chemical replication). When the variation of the double analyses was more than 10% of the mean, a third analysis was carried out. The obtained essential oil was kept at 4 ^{o}C for further analysis.

GC and GC-MS analysis

Following equipment was used: GC "Varian Chrompack CP-3800 (Varian GmbH Germany, Darmstadt), Saturn ® 2100 Benchtop GC/MS, 3900 gas chromatography (Varian, Germany GmbH, Darmstadt).

The chemicals used for GC and GC/MS were n-hexane (99% purity, Carl Roth GmbH & Co. KG, Karlsruhe), *trans*-anethole (99% purity, Carl Roth GmbH & Co. KG, Karlsruhe), methylchavicol (estragol) (> 98% purity, Carl Roth GmbH & Co. KG, Karlsruhe) and anisaldehyde (98% purity, Carl Roth GmbH & Co. KG, Karlsruhe).

Helium (purity 4.6, Air Liquid, Kassel), Hydrogen (purity 4.6, Air Liquid, Kassel) were used as carrier gas and fuel gas, respectively.

Preparation of standard solutions for the determination of essential oil components

The *trans*-anethole, estragol and anisaldehyde were purchased (Carl Roth GmbH & Co. KG, Karlsruhe) for the preparation of standard solutions for the determination of essential oil components. First of all stock solutions were prepared.

Table 4.13: Preparation of stock solutions

Component	Volume	Hexan µl
Trans-anethole	90 (pure anethole) µl	910
Estragol	20 (pure estragol) µl	980
Anisaldehyde	20 (pure anisaldehyde) µl	980

In further step the stock solutions were used for preparation of standard solution.

Table 4.14: Preparation of standard solution for the determination of essential oil components of anise

Component	Volume	Hexan
Trans-anethole	100 µl	850 µl
Estragol	25 µl	
Anisaldehyde	25 µl	

After pipetting of the stock solutions and hexane the solution was mixed thoroughly. The standard solution had following concentrations (relative in %) for each component of anise essential oil (table 4.14).

Table 4.15: Concentrations of essential oil components in the standard solution

Component	Concentration (%)
Trans-anethole	90
Estragol	5
Anisaldehyde	5

For the measurement of each components of anise essential oil the essential oil samples obtained by distillation were 100-fold diluted with hexane for GC or GC-MS analysis.

Gas-chromatography

The anise essential oil components were identified and quantified by means of GC and GC-MS in the laboratory of institute of crop science and plant breeding I. A Varian CP-3800 gas chromatography equipped with flame ionization detector (GC-FID) was used (Fig. 4.7). A capillary column DB-5 (30 m x 0.25 mm i.d. and 0.25 µm coating thickness) was used for the separation of individual components of the essential oil.

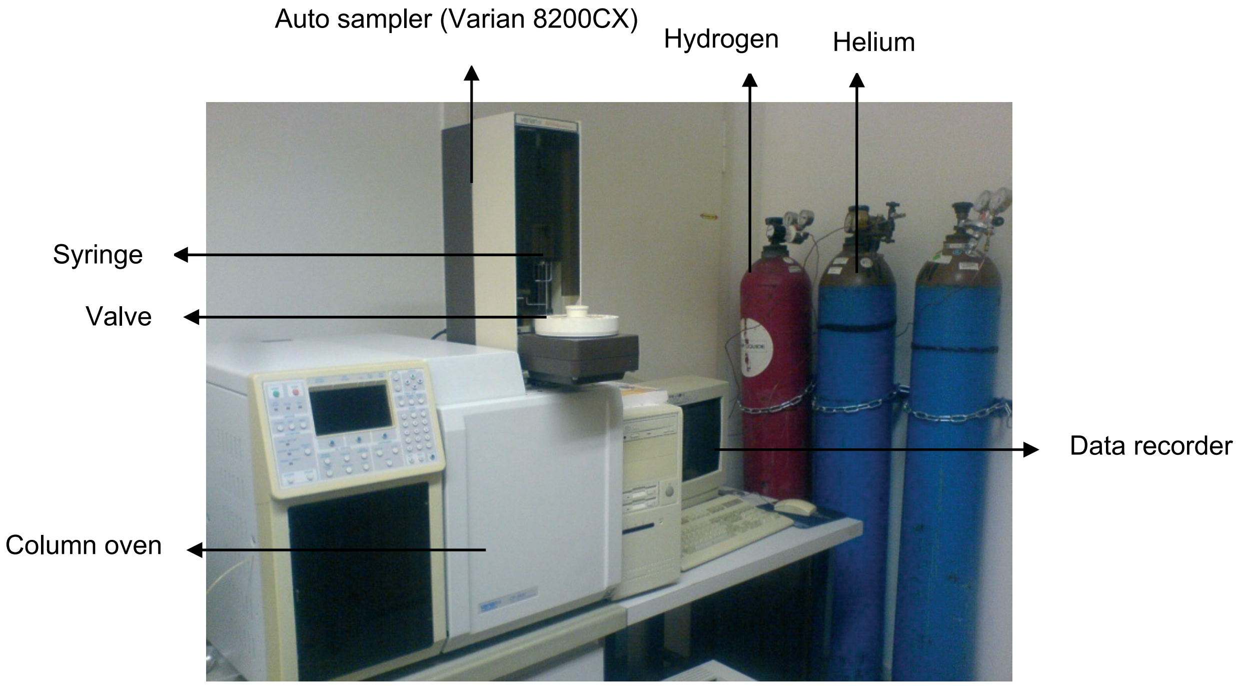


Fig. 4.7: Varian gas chromatography with flame ionization FID (CP 3800)

Helium gas was employed as the carrier gas with a flow rate of 1.1 ml/min. Temperature was programmed from 60 (5 min), to 250 °C with a ramp rate of 5 °C/min, followed by a final hold time of 10 min. The injector with 1:50 split ratio was maintained at 260 °C and detector at 280 °C, respectively. The sample of 1µl was injected by autosampler (Varian 8200CX). The percentage concentration of individual components was computed from peak areas. Response factors of detector and FID normalization were considered for data processing. For the measurement of anise essential oil components the standard solution was gas-chromatographyed. The result is presented in Fig. 4.8.

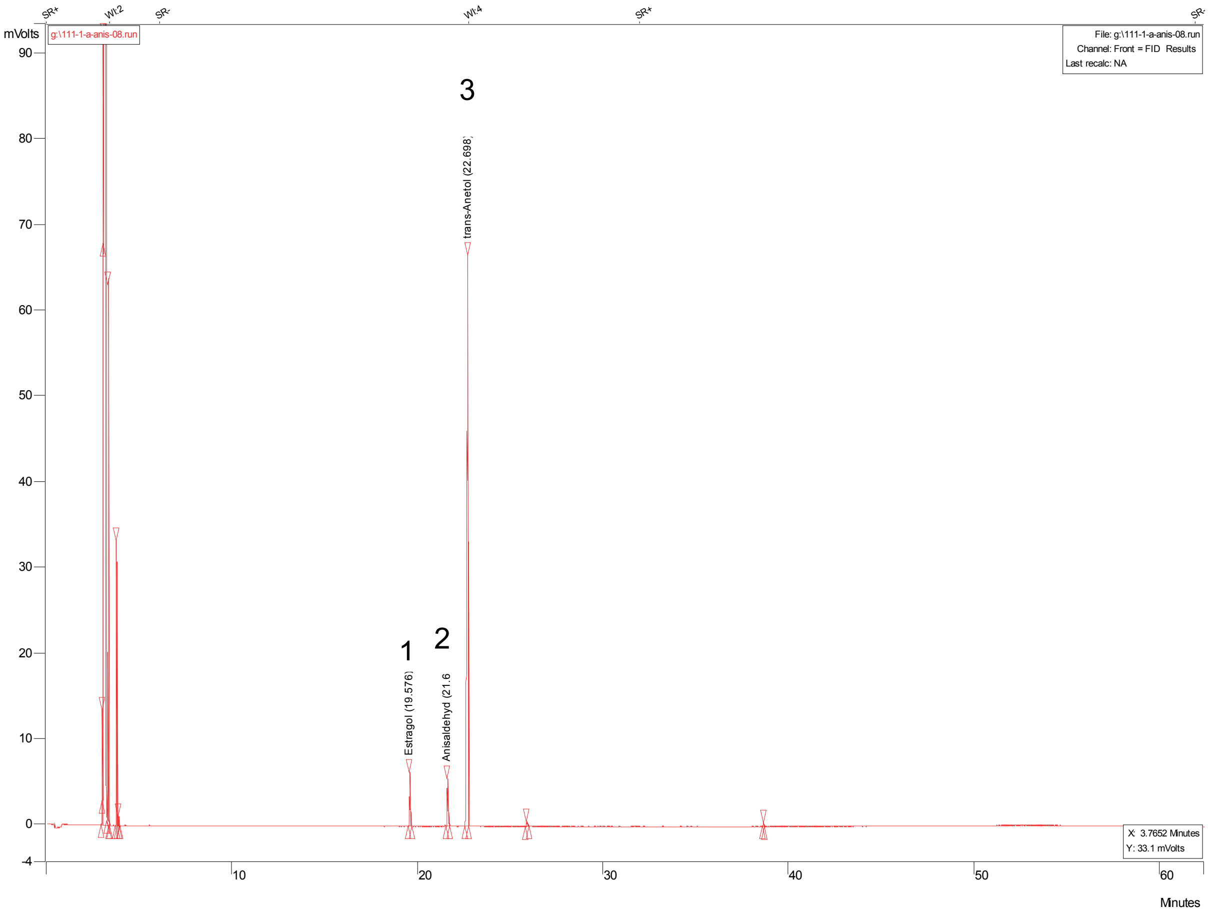


Fig. 4.8: Chromatography of standard substances showing estragol (1), anisaldehyde (2) and *trans*-anethole (3) and their retention time

The peaks appeared at the beginning of the chromatograph were conditioning peaks which were ignored for the calculations. It is evident from the Fig. 4.8 that estragol had a retention time (RT) of 19.612 min., anisaldehyde 21.660 min. and *trans*-anethole 22.806 min., respectively. The data in table 4.16 are the relative concentrations (%) of individual components of the standard solution prepared for the analysis of anise essential oil. It is also evident from the table 4.16 that there were small variations for each component. Therefore, a correction factor was calculated by using the results of standard solution:

Correction factor = Concentration detected / actual concentration

Table 4.16: Results of standard solution analyzed by gas-chromatography

Peak name	Retention time	Area count	Concentration (%)
Trans-anethole	22.806	820201	89.35
Estragol	19.612	43348	4.72
Anisaldehyde	21.660	46692	5.08

The results of the anise samples were then corrected by using the correction factor. This correction factor was estimated after every 30 anise samples. To optimize the accuracy of the measurement the total peak area of all essential oil components was adjusted to about 1000,000 counts by dilution.

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) as following:

$$RI_{x=} 100\ N_{\ +} 100 *[(RT- RT_N / RT_{N+1} - RT_N)]$$

X: required component
RT: retention time
$RT_{N:}$ is the retention time of the n-alkane with carbon number $_{\mathbf{N}}$
$RT_{N+1:}$ is the retention time of the n-alkane with carbon number $_{\mathbf{N+1}}$

The homologous series of alkanes was used as reference substance. The retention time of the desired substance can be placed between the retention times of two adjacent homologous alkanes which were already determined. The retention index is a good comparison to identify the samples of unknown substance. The most important components *trans*-anethole and estragol were further identified by co-injection of authentic standards (Roth, Karlsruhe, Germany).

A varian 3900 GC coupled with a Varian Saturn 2100T ion trap mass detector was employed for the analysis of essential oil of anise. A capillary column VF-5ms (30 m x 0.25 mm i.d. and 0.25 µm coating thickness) was used for separation of the components. Helium (99.99%) was used as carrier gas with a flow rate of 1.1 ml/min. Temperature was programmed from 60 (5 min), to 250 °C with a ramp rate of 5 °C/min, followed by a final hold time of 10 min. The injector with 1:50 split ratio was maintained at 260 °C and detector at 280 °C, respectively. The sample of 1µl was injected by autosampler (Varian CP-8400). Ionization was realized 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 of 35-450. A gas chromatogram and mass spectrometer of anise essential oil are given below in Fig. 4.10 and Fig. 4.11, respectively.

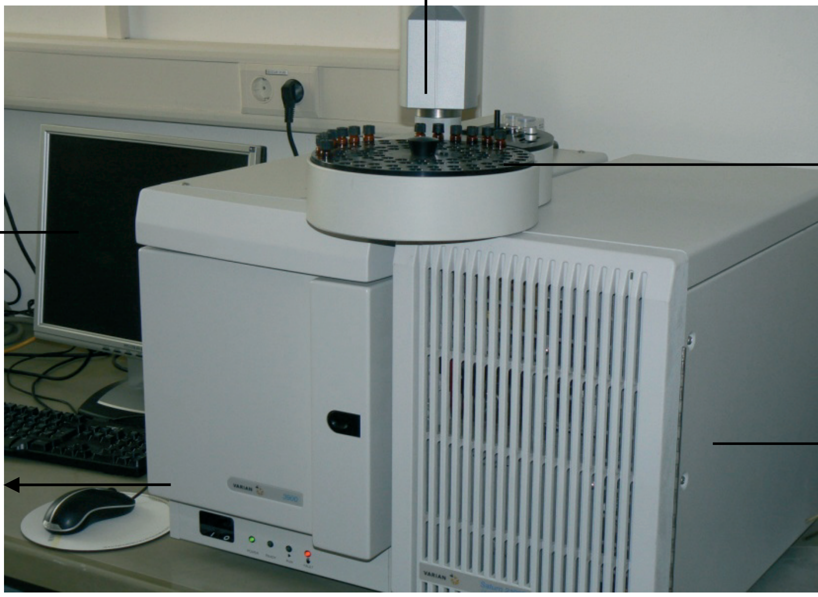


Fig. 4.9: Gas-chromatography- mass spectrometry (GC-MS)

The identification of anise essential oil components was achieved on the basis of comparison of Kovat's retention indices with those of literature data (Adams, 1995, Figueredo et al., 2006) and mass spectrometry by comparing mass spectra of the unknown peaks with those stored in the Wiley 90 and NIST 98 MS libraries.

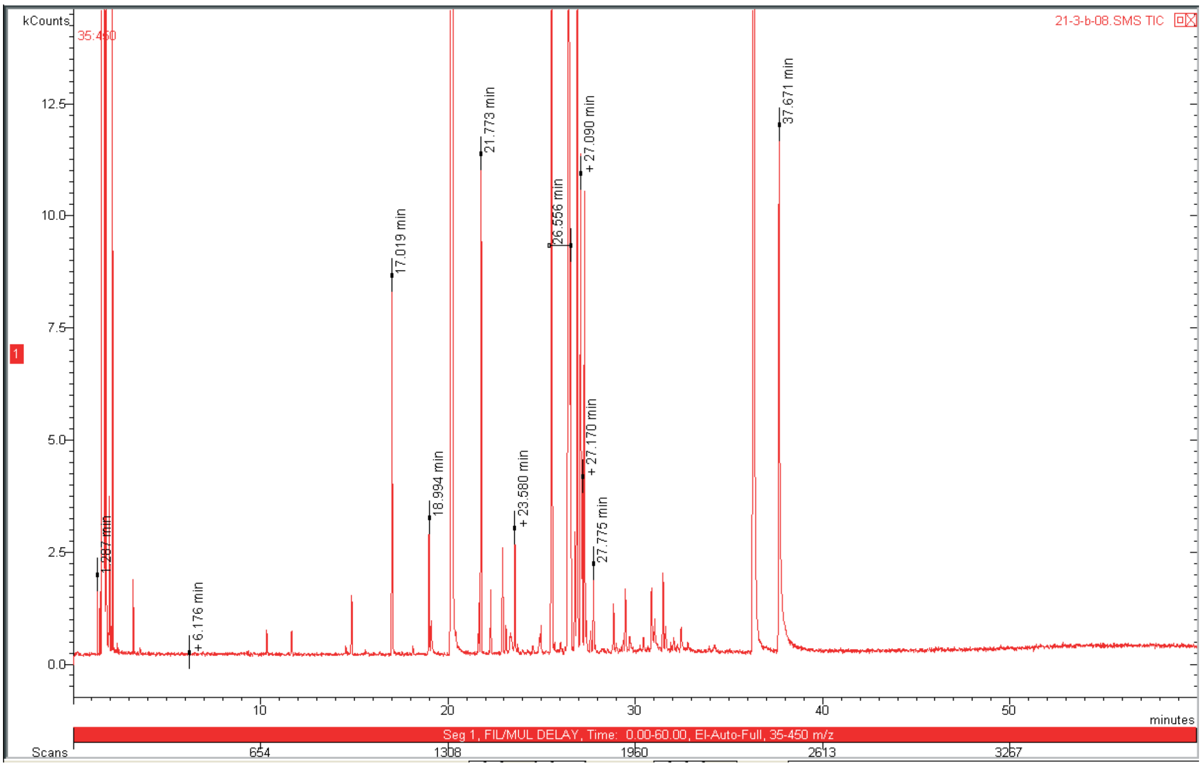


Fig. 4.10: A gas chromatogram of essential oil of anise fruits (*P. anisum*) of cultivar Enza Zaden

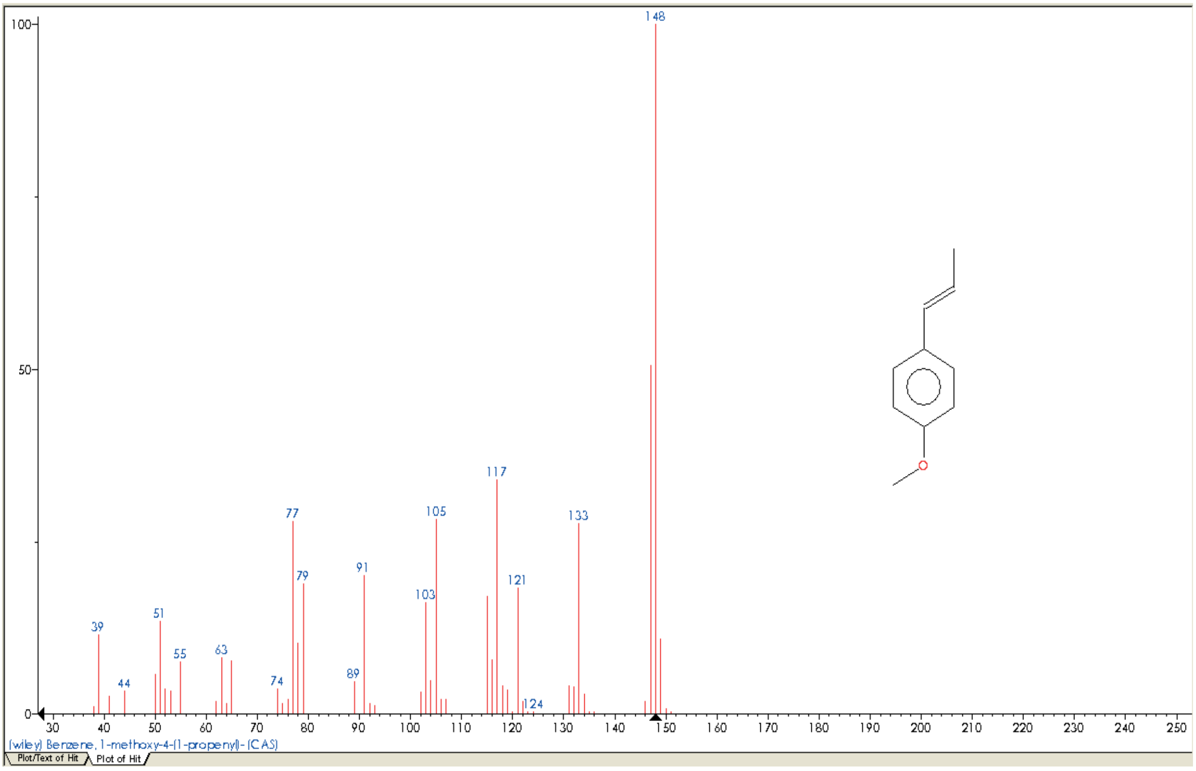


Fig. 4.11: A mass spectrometer of (E)-anethole, the main component of the essential oil of anise fruits (*P. anisum*)

4.6. 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) to determine the relationship among the characters according to Pearson and Spearman's rho methods. The standard deviations (SD) were calculated by using Microsoft Excel.

5. Results

5.1 Effect of different sowing times, plant densities and cultivars

5.1.1 Field experiment Gross-Gerau 2008

5.1.1.1 Disease and lodging assessment

A disease incidence of (*Cercospora malkoffii*) was recorded from both early and delayed sowing times. First disease evaluation was performed on 25-7-2008 at the 100% flowering stage. During this period severity of *Cercospora malkoffii* ranged from 2.4 to 5.9 (level) in early and delayed sowing times (Fig. 5.1). Higher disease level was noticed in narrow plant densities during this period of evaluation in both sowing times.

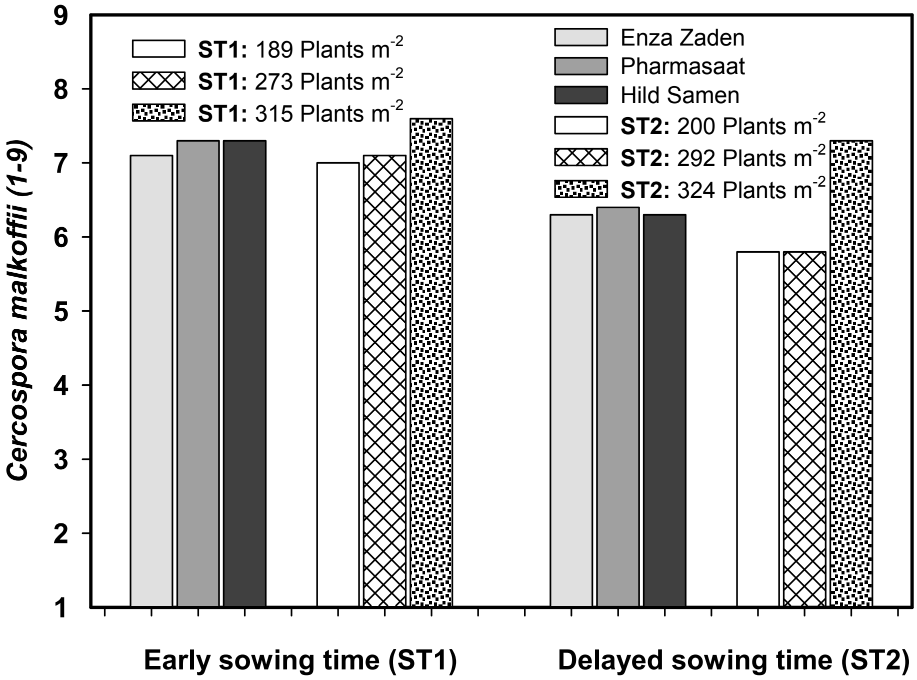


Fig. 5.1: Effect of cultivars and plant densities on *Cercospora malkoffii* (1-9) in early and delayed sowing of anise at experimental station Gross-Gerau 2008

At the flowering stage cv. Hild Samen was more susceptible to *Cercospora malkoffii* compared with other cultivars. Overall higher disease incidence was noticed in early sown anise plants compared with delayed sowing. Second disease scoring was performed at the full maturity stage (20-8-2008) of anise plants. In final evaluation diseased level reached from 5.8 to 7.6 in early and delayed sowing times (Fig. 5.1). Increasing trend of *Cercospora malkoffii* was noticed as planting densities increased. At the full maturity stage fungal infection varied from 5.8 to 7.3 and 7.0 to 7.6 in early and delayed sowing respectively. Similar disease level was observed in cv. Pharmasaat and cv. Hild Samen in early sowing time. Cv. Enza Zaden was less susceptible compared with other cultivars in both sowing times. Lodging rate was estimated at full maturity stage of anise before harvesting. Lodging was only observed in cv. Hild Samen and its value ranged from 3 to 4 (level 1-9) in early and delayed sowing times.

5.1.1.2 Growth and fruit yield parameters

The applied sowing rates led to plant densities (PD) after germination of 189, 273 and 315 plants m^{-2} as well as 200, 292 and 324 plants m^{-2} respectively in early and delayed sowing times (table 5.1). Comparable plant densities with similar levels could be established in both sowing times. The germination ability of the seeds of the used anise cultivars was different. Cv Hild Samen had lowest germination rate in comparison with the other both cultivars in both sowing times which were lower than 50% of the comparative cultivars.

Table 5.1: Effect of different cultivars (CV) and planting densities (PD) on plant height (PH) (cm), primary branches per plant (PBP), and secondary branches per plant (SBP) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2008

CV	PD	1st sowing time (1.4.2008)				2nd sowing time (17.4.2008)			
		Plants m^{-2}	PH	PBP	SBP	Plants m^{-2}	PH	PBP	SBP
			cm	no	no		cm	no	no
1		309	44 a	3.2 b	0.07 b	336	42 a	2.6 b	0.08 a
2		350	43 a	3.1 b	0.13 b	354	41 a	2.6 b	0.10 a
3		117	42 a	4.6 a	1.10 a	126	40 a	3.7 a	0.68 a
	1	189	44 a	4.4 a	0.53 a	200	41 a	3.6 a	0.42 a
	2	273	43 a	3.7 ab	0.12 a	292	41 a	2.9 ab	0.12 a
	3	315	42 a	2.8 b	0.65 a	324	41 a	2.4 b	0.32 a
LSD (5%)									
CV			ns	0.8	0.8		ns	0.7	ns
PD			ns	0.8	ns		ns	0.7	ns
CV x PD			ns	ns	ns		ns	1.3	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

The height of anise plant stand was around 40 to 44 cm in both sowing times. This field parameter was affected neither by cultivar nor by plant density (table 5.1). Delayed sowing resulted in decreasing trend of plant height. The number of primary branches per plant varied from 2.8 to 4.6 in first sowing and from 2.4 to 3.7 primary branches per plant in second sowing time (table 5.1). Data concerning secondary branches per plant (SBP) showed non significant difference among planting density treatments in both sowing times. However cv. Hild Samen attained significant higher number of SBP as compared to other cultivars in early sowing time. There were significant differences in number of primary branches per plant (PBP) among the treatments in early and delayed sowing times (table 5.1). In both sowing times plants with lowest density of 189 plants m^{-2} and 200 plants m^{-2} had the highest number of PBP with 4.4 and 3.6 respectively (table 5.1). Opposite to that highest plant density resulted in lowest number of primary branches of anise with only 2.8 (1st sowing) and 2.4 (2nd sowing). Due to lower germination rate cv. Hild Samen produced significant higher number of primary and secondary branches per plant as compared to other cultivars in both sowing times. Regarding number of primary branches per plant there was an interaction between PD and CV in second sowing time. The PD x CV interaction is characterized by deviated reactions of the cultivars on increased plant densities (Fig. 5.2). The cultivars Enza Zaden and Pharmasaat reduced their primary branches by increase of plant density whereas cv. Hild Samen showed no reaction on increased plant densities.

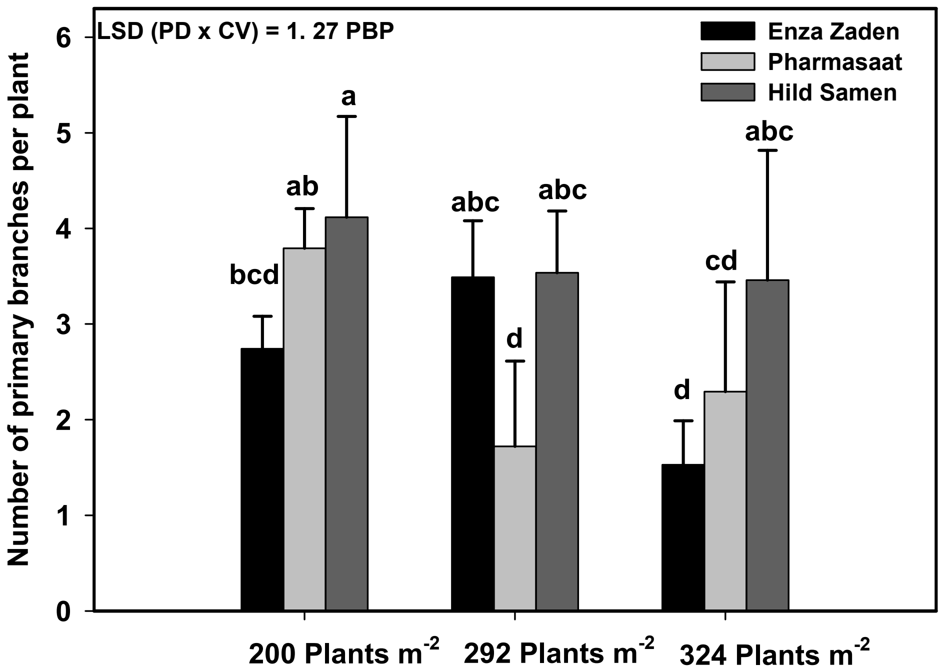


Fig. 5.2: Effect of different planting densities (PD) and cultivars (CV) on primary branches per plant in delayed sowing time of anise Gross-Gerau 2008

Data presented in table 5.2 reveal that all tested cultivars showed significant differences regarding umbel number per plant (UNP), fruit number per plant (FNP) and fruit weight per plant (FWP) in both sowing times. Cv. Hild Samen showed pronounced effect concerning these anise plant features as compared to other cultivars. Fruit yield components of cv. Hild Samen were 6.7 umbels (UNP), 152 fruits (FNP), 0.62 g fruit weight (FWP) and 5.4 umbels (UNP), 115 fruits (FNP), 0.33 g fruit weight (FWP) respectively for early and delayed sowing times. Under early sowing time plants with lowest plant density of 189 plants m^{-2} had the highest number of umbels and number of fruits per plant (table 5.2). Similar trend was observed in second sowing time with respect to plant density where UNP, FNP and FWP were reduced as plant density narrowed. In delayed sowing time plant density of 200 plants m^{-2} led to significant higher FNP with 118 fruits (p = 0.01) and higher fruit weight with 0.33 g per plant (p =

0.01) as compared to other treatments (table 5.2). These yield contributing features of anise were decreased as planting densities increased.

Table 5.2: Effect of different cultivars (CV) and planting densities (PD) on umbels number per plant (UNP), fruits number per plant (FNP) and fruit weight per plant (FWP) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2008

CV	PD	1st sowing time (1.4.2008)				2nd sowing time (17.4.2008)			
		Plants m^{-2}	UNP	FNP	FWP	Plants m^{-2}	UNP	FNP	FWP
			no	no	g		no	no	g
1		309	4.2 b	62 b	0.17 b	336	3.6 b	77 b	0.21 b
2		350	4.2 b	68 b	0.20 b	354	3.7 b	86 ab	0.23 b
3		117	6.7 a	152 a	0.67 a	126	5.4 a	115 a	0.33 a
	1	189	5.9 a	101 a	0.36 a	200	5.0 a	118 a	0.33 a
	2	273	4.8 ab	87 a	0.30 a	292	4.0 a	89 ab	0.24 ab
	3	315	4.4 b	95 a	0.38 a	324	3.7 a	70 b	0.19 b
LSD (5%)									
CV			1.2	70	0.4		1.1	32	0.09
PD			1.2	ns	ns		ns	32	0.09
CV x PD			ns	ns	ns		ns	55	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

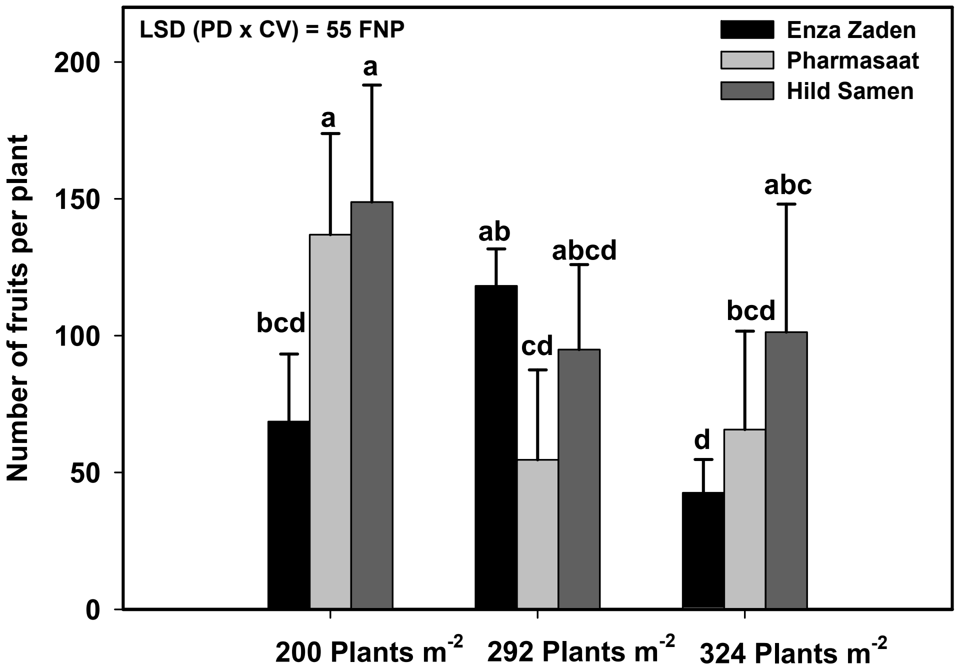


Fig. 5.3: Effect of different planting densities (PD) and cultivars (CV) on number of fruits per plant in delayed sowing time of anise Gross-Gerau 2008

There was an interaction between cultivars and plant density with respect to FNP of anise in second sowing time (Fig. 5.3). The interaction is explained by dissimilar response of cv. Hild Samen on increased plant density with respect to other cultivars. The cultivars Enza Zaden and Pharmasaat reduced their fruit number per plant (FNP) by increase of plant density whereas cv. Hild Samen showed no reaction on increased plant densities. Fruit weight has a direct impact on final fruit yield per area (per m^2 or per ha) of anise crop. For that reason it can be characterized as an important fruit yield component of anise crop. Higher the weight of fruit, greater will be the fruit yield. Thousand fruit weight (TFW) data indicate that this yield component was not affected by used treatments in early sowing time. On the other hand delayed sowing time showed significant difference regarding TFW. From both sowing times TFW varied between 2.30 to 2.51 g in 2008 (table 5.3). Cv. Hild Samen achieved significant higher TFW as compared with other cultivars in delayed sowing time. In early sowing time fruit yield of anise was affected by both factors cultivar as well as plant density (PD).

Table 5.3: Effect of different cultivars (CV) and planting densities (PD) on 1000-fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2008

CV	**PD**	**1st sowing time (1.4.2008)**			**2nd sowing time (17.4.2008)**		
		Plants m^{-2}	**TFW**	**FY**	Plants m^{-2}	**TFW**	**FY**
			g	dt/ha at 91 %		g	dt/ha at 91 %
1		309	2.40 a	4.5 a	336	2.30 b	5.5 a
2		350	2.40 a	4.6 a	354	2.32 b	5.4 a
3		117	2.50 a	2.4 b	126	2.51 a	3.4 b
	1	189	2.49 a	4.5 a	200	2.39 a	5.1 a
	2	273	2.38 a	3.7 b	292	2.33 a	4.9 a
	3	315	2.38 a	3.4 b	324	2.40 a	4.2 a
LSD (5%)							
CV			ns	0.7		0.1	0.7
PD			ns	0.7		ns	ns
CV x PD			ns	ns		ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Cv. Hild Samen had the lowest fruit yield caused by smallest PD of 117 plants m^{-2}. However also maximal PD of 315 plants m^{-2} led to significant lower fruit yield in early sowing time whereas in 2nd sowing time no PD effect was observed. In 2008 highest fruit yield of 5.1 dt/ha was obtained from delayed sowing time and the lowest value of 2.4 dt/ha was observed in early sowing time (table 5.3). The highest fruit yield of anise was related to the delayed sowing time in 2008, while in early sowing time anise plants were heavily infected by fungal disease which affects all above parts of the plants, including leaves, flowers, stems and seeds as well.

5.1.1.3 Content, yield and composition of essential oil

There were significant differences induced by cultivars within sowing times regarding essential oil concentration. Lowest concentration of essential oil was found by cv. Hild Samen in both sowing times. An interaction effect was observed with respect to essential oil concentration between used cultivars and planting densities (Fig. 5.4). Within all treatments essential oil concentration of aniseed reached a maximum level of 2.90% (2.30 to 2.90%) (table 5.4). Significant lower essential oil was synthesized by cv. Hild Samen as compared to other cultivars in second sowing time. The interaction is characterized by differ response of cv. Hild Samen on increased plant density in comparison with the other both cultivars. Cv. Enza Zaden and Pharmasaat had the same level of essential oil in low and medium plant density but reduced in highest plant density. Opposite to that cv. Hild Samen increased the content of essential oil by increasing the plant density from 189 to 273 plants m^{-2} (Fig. 5.4).

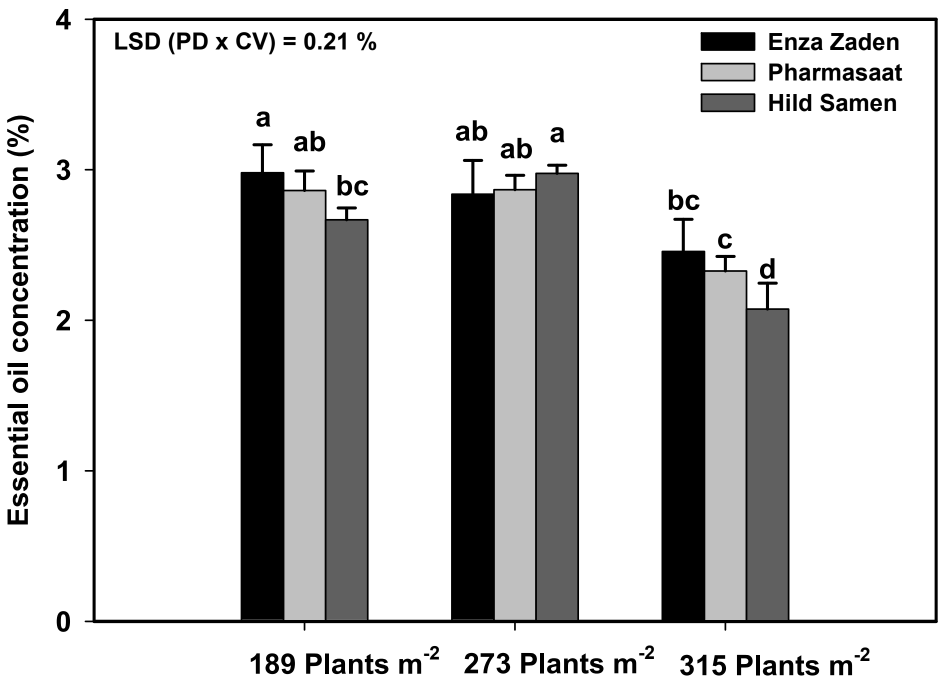


Fig. 5.4: Effect of different planting densities (PD) and cultivars (CV) on essential oil concentration (%) in early sowing time of anise at Gross-Gerau 2008

Essential oil yields (EOY) of anise varied between 6.4 and 15.5 kg/ha from different used treatments (table 5.4). In present study, EOY increased with the lower planting densities related to higher fruit yields. Significant differences were exhibited regarding EOY from tested cultivars and planting density in early sowing time. It could be observed that plant density did not show significant difference regarding EOY but cultivar clearly influenced the EOY in 2nd sowing time which related to lower fruit yield and essential oil concentration.

Table 5.4: Effect of different cultivars (CV) and planting densities (PD) on essential oil concentration (EO) (%) and essential oil yield (EOY) (kg/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2008

CV	PD	1st sowing time (1.4.2008)			2nd sowing time (17.4.2008)		
		Plants m^{-2}	EO	EOY	Plants m^{-2}	EO	EOY
			%	Kg/ha		%	Kg/ha
1		309	2.77 a	12.8 a	336	2.80 a	15.3 a
2		350	2.70 ab	12.3 a	354	2.90 a	15.5 a
3		117	2.60 b	6.4 b	126	2.30 b	7.7 b
	1	189	2.87a	12.8 a	200	2.57 a	13.6 a
	2	273	2.90 a	10.7 a	292	2.73 a	13.5 a
	3	315	2.30 b	7.9 b	324	2.70 a	11.5 a
LSD (5%)							
CV			0.1	2.0		0.2	1.9
PD			0.1	2.0		ns	ns
CV x PD			0.2	ns		ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

The main component of anise essential oil is the component *trans*-anethole which ranged from 92 to 97% (table 5.5).

Table 5.5: Effect of different cultivars (CV) and planting densities (PD) on estragol (ES) (%), gamma-himachalene (GA) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2008

CV	PD	1st sowing time (1.4.2008)				2nd sowing time (17.4.2008)			
		Plants m^{-2}	ES	GH	TA	Plants m^{-2}	ES	GH	TA
			%	%	%		%	%	%
1		309	0.50 b	5.4a	92.6 b	336	0.50 b	5.5a	92.3 b
2		350	0.47 b	5.3a	93.6 b	354	0.50 b	5.3a	92.0 b
3		117	1.07 a	2.4b	96.9 a	126	0.97 a	2.2b	96.2 a
	1	189	0.70 a	4.5a	94.2 a	200	0.67 a	4.5a	93.3 a
	2	273	0.67 a	4.3a	94.5 a	292	0.67 a	4.3ab	93.5 a
	3	315	0.67 a	4.3a	94.4 a	324	0.63 a	4.2b	93.6 a
LSD (5%)									
CV			0.05	0.2	0.45		0.04	0.2	0.3
PD			ns	ns	ns		ns	0.2	ns
CV x PD			ns	ns	ns		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Contrary to that the compound estragol was at a very low level of around 0.50 to 1.00% of total essential oil. Cv. Hild Samen led to significant higher concentration of estragol and *trans*-anethole 1.07, 0.97% and 96.9, 96.2% respectively in early and delayed sowing times (table 5.5). Plant density had no significant effect on estragol, γ-himachalene and *trans*-anethole concentration of essential oil in 2008. In present study second higher constituent of essential oil of anise fruits is γ-himachalene varied from 2.4 to 5.5% in early and delayed sowing times. Cv. Hild Samen induced significant lower concentration of γ-himachalene as compared to other cultivars. Cv. Enza Zaden exhibited higher concentration of γ-himachalene in both sowing times. It was observed that γ-himachalene decreased with an increase in plant density. Maximum values of γ-himachalene of 4.5% were found at a level of 200 plants m^{-2} and minimal γ-himachalene of 4.2% was observed at 324 plants m^{-2} in delayed sowing time (table 5.5). The compounds *trans*-anethole and estragol were not statistically affected by varying planting densities in both early and delayed sowing times (table 5.5).

5.1.2 Field experiment Gross-Gerau-2009

5.1.2.1 Disease and lodging assessment

In 2009 disease scoring was performed on 16-7-2009 and 29-7-2009 in early and delayed sowing times during the flowering stage. The severity of *Cercospora malkoffii* was ranged from 1.8 to 3.9 and 1.6 to 3.3 in early and delayed sowing time respectively during flowering period (Fig. 5.5). Higher disease infection was noticed with cv. Agri-Saaten compared with other cultivars.

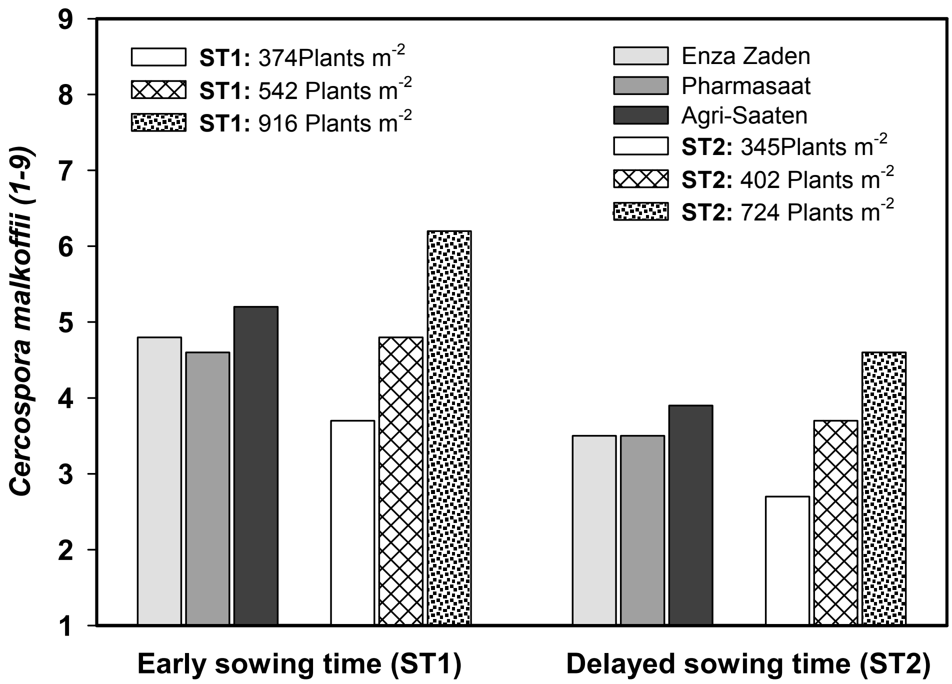


Fig. 5.5: Effect of cultivars and plant densities on *Cercospora malkoffii* (1-9) in early and delayed sowing of anise at experimental station Gross-Gerau 2009

In final disease assessment *Cercospora malkoffii* infection level reached from 3.7 to 6.2 and 2.7 to 4.6 in early and delayed sowing times respectively (Fig. 5.5). Increasing trend of *Cercospora malkoffii* was observes as planting densities increase in both sowing times. Higher infection level of 6.2 and 4.6 was observed in planting densities of 916 plants m^{-2} and 724 plants m^{-2} respectively in early and delayed sowing times. Lower infection rate was recorded in lower plant densities in both sowing times. Overall higher disease level was observed in early sowing time compared with delayed sowing time. Cv. Enza Zaden and cv. Pharmasaat had similar infection level in delayed sowing time (Fig. 5.5).

5.1.2.2 Growth and fruit yield parameters

Data presented in table 5.6 show that the applied sowing rates led to differ level of plant densities (PD) after germination of 374, 542 and 916 plants m^{-2} as well as 345, 402 and 724 plants m^{-2} respectively in early and delayed sowing times in 2009.

Table 5.6: Effect of different cultivars (CV) and planting densities (PD) on plant height (PH) (cm), primary branches per plant (PBP), and secondary branches per plant (SBP) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2009

CV	PD	1st sowing time (1.4.2009)				2nd sowing time (15.4.2009)			
		Plants m^{-2}	PH	PBP	SBP	Plants m^{-2}	PH	PBP	SBP
			cm	no	no		cm	no	no
1		656	54 a	2.5 a	0.09 a	517	47 a	2.1 a	0.05 a
2		526	54 a	2.8 a	0.13 a	387	49 a	2.2 a	0.11 a
3		650	53 a	2.3 a	0.10 a	568	48 a	2.3 a	0.07 a
	1	374	55 a	3.6 a	0.21a	345	48 a	2.5 a	0.12 a
	2	542	54 a	2.4 b	0.10 ab	402	49 a	2.5 a	0.07 a
	3	916	51 a	1.5 c	0.01 b	724	47 a	1.5 b	0.04 a
LSD (5%)									
CV			ns	ns	ns		ns	ns	ns
PD			ns	0.5	0.1		ns	0.7	ns
CV x PD			ns	ns	ns		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Agri-Saaten

Higher germination rate was observed in 1st sowing time in comparison with 2nd sowing time. Averaged over the years higher planting densities were found in 2009 as compared to 2008. The plant density of the used cultivars varied between 526 and 656 as well as between 387 and 568 plants m^{-2} in first and second sowing time. However it seems that cv. Pharmasaat is characterized by lower germination ability in comparison with the other two cultivars. It can be stated that anise plant height at maturity stage was not influenced significantly by the used cultivars and plating densities in both sowing times. However plant height of anise ranged from 47 to 55 cm in both sowing times (table 5.6). Maximum plant height of 55 cm was recorded in 1st sowing time whereas minimum plant height of 47 cm was found in 2nd sowing time. Independent of that a tendency of higher plants was observed in lower planting densities in both sowing times. Significant difference was noticed regarding primary branches per plant (PBP) in both sowing times. The number of primary branches per plant ranged from 1.5 to 3.6 and 1.5 to 2.5 in early and delayed sowing time respectively (table 5.6). Decreasing trend of PBP and SBP were observed in early and delayed sowing times as planting densities were increased. Significant but very lower number of secondary branches per plant affected by plant density was achieved in early sowing time.

Table 5.7: Effect of different cultivars (CV) and planting densities (PD) on umbels number per plant (UNP), fruits number per plant (FNP) and fruit weight per plant (FWP) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2009

CV	PD	1st sowing time (1.4.2009)				2nd sowing time (15.4.2009)			
		Plants m^{-2}	UNP	FNP	FWP	Plants m^{-2}	UNP	FNP	FWP
			no	no	g		cm	no	g
1		656	3.6 a	36 a	0.10 b	517	3.1 a	51 a	0.15 a
2		526	3.9 a	50 a	0.15 a	387	3.3 a	68 a	0.18 a
3		650	3.4 a	35 a	0.10 b	568	3.4 a	58 a	0.17 a
	1	374	4.8 a	71 a	0.22 a	345	3.7 a	70 a	0.21 a
	2	542	3.5 b	36 b	0.10 b	402	3.5 a	63 a	0.18 a
	3	916	2.1 c	13 c	0.03 c	724	2.6 b	37 a	0.10 a
LSD (5%)									
CV			ns	ns	0.04		ns	ns	ns
PD			0.5	14	0.04		0.7	ns	ns
CV x PD			ns	ns	ns		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Agri-Saaten

Data demonstrated in table 5.7 show that fruit yield contributing parameters including umbels number per plant (UNP), fruit number per plant (FNP) and fruit weight per plant (FWP) were significantly affected by treatments of planting densities in early sowing time. The planting densities of 374 plants m^{-2} achieved significant higher number of UNP (4.8), FNP (71) and FWP (0.22 g) whereas significant lower yield components were observed with planting densities of 916 plants m^{-2} UNP (2.1), FNP (13) and FWP (0.03 g) respectively in early sowing time which ultimately affect final fruit yield (table 5.7). Cv. Pharmasaat attained statistically higher fruit weight of 0.15 g ($p = 0.03$) as compared to other used cultivars in early sowing time (table 5.7). Overall cv. Pharmasaat was achieved maximum number of umbels per plants (UNP), fruit number per plants (FNP) and fruit weight per plant (FWP) from both sowing times. Planting densities showed remarkable variation regarding UNP in delayed sowing time where highest UNP were observed at planting densities of 374 plants m^{-2} (table 5.7). FNP and FWP were not affected by used cultivars and planting densities in delayed sowing time. There was no interaction between cultivars and plant density with respect to these yield contributing parameters of anise.

Table 5.8 shows the results of thousand fruit weight (TFW) and fruit yield (FY) of anise which were observed in Gross-Gerau 2009. It was found that there was a significant effect of planting densities on 1000-fruit weight (TFW) in early sowing time, where as non significant difference was observed in delayed sowing time. Within both sowing times it ranged from 1.8 to 2.5 g (table 5.8). The highest TFW of 2.5 g was obtained from the planting densities of 374 plants m^{-2}, while the lowest TFW of 1.8 g was noticed from the planting densities of 916 plants m^{-2} (table 5.8).

Table 5.8: Effect of different cultivars (CV) and planting densities (PD) on 1000-fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2009

CV	PD	1st sowing time (1.4.2009)			2nd sowing time (15.4.2009)		
		Plants m^{-2}	TFW	FY	Plants m^{-2}	TFW	FY
			g	dt/ha at 91%		g	dt/ha at 91%
1		656	2.1 a	6.3 a	517	2.2 a	6.1 a
2		526	2.2 a	7.4 a	387	2.4 a	7.3 a
3		650	2.2 a	6.2 a	568	2.2 a	6.6 a
	1	374	2.5 a	8.2 a	345	2.3 a	7.1 a
	2	542	2.2 b	6.7 b	402	2.3 a	7.0 a
	3	916	1.8 c	5.0 c	724	2.2 a	5.9 a
LSD (5%)							
CV			ns	ns		ns	ns
PD			0.15	1.1		ns	ns
CV x PD			ns	ns		ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Agri-Saaten

The different planting densities significantly affected the fruit yield (FY) in early sowing time but a non significant difference was observed in delayed sowing time. The lower PD resulted in the more PBP, UNP, and FNP and enhances the fruit yield. The highest FY of 8.2 dt/ha was obtained from 374 plants m^{-2} and the lowest value 5.0 dt/ha was observed in 916 plants m^{-2} in early sowing time (table 5.8). Non significant differences were noticed regarding 1000-fruit weight and fruit yield in delayed sowing time. A decreasing trend of fruit yield was noticed in both early and delayed sowing times as planting densities increased. Cultivars tested in our study showed non significant effect regarding 1000-fruit weight and fruit yield both sowing times. Independent of that cv. Pharmasaat led to higher anise fruit yield in early and delayed sowing times (table 5.8).

5.1.2.3 Content, yield and composition of essential oil

The essential oil (EO) concentration was not affected by different planting densities (PD) and used cultivars (CV). The EO concentration of anise fruits reached a level of around 3% (2.69 - 3.30%) for all treatments in both sowing times (table 5.9). However a higher concentration of anise essential oil was synthesized in plants of delayed sowing time which reflect the better environmental conditions at the time of essential oil accumulation. Cv. Enza Zaden accumulated higher concentration of essential oil in delayed sowing time. Overall higher essential oil concentration of anise was accumulated in 2009 as compared to 2008.

Table 5.9: Effect of different cultivars (CV) and planting densities (PD) on essential oil concentration (EO) (%) and essential oil yield (EOY) (kg/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2009

CV	PD	1st sowing time (1.4.2009)			2nd sowing time (15.4.2009)		
		Plants m^{-2}	EO %	EOY Kg/ha	Plants m^{-2}	EO %	EOY Kg/ha
1		656	2.74 a	17.6 a	517	3.23 a	18.3 a
2		526	2.70 a	19.9 a	387	3.03 a	23.2 a
3		650	2.79 a	17.0 a	568	3.05 a	20.0 a
	1	374	2.69 a	22.2 a	345	2.96 a	21.9 a
	2	542	2.81 a	18.7 b	402	3.30 a	21.5 a
	3	916	2.73 a	13.6 c	724	3.05 a	18.2 a
LSD (5%)							
CV			ns	ns		ns	ns
PD			ns	3.1		ns	ns
CV x PD			ns	ns		ns	ns

CV 1: Enza Zaden, CV 2: Pharmasaat, CV 3: Agri-Saaten

Data presented in table 5.9 show that anise essential oil yield (EOY) is characterized by significant differences regarding planting densities in early sowing time whereas no clear affect was found in delayed sowing time. Higher EOY was recorded in lower planting densities related to higher fruit yield. In present study EOY of anise varied between 13.6 to 23.2 kg/ha within both sowing times (table 5.9). Significant higher EOY of 22.2 kg ha^{-1} was obtained from lowest planting density of 374 plants m^{-2} and significant lower EOY of 13.6 kg ha^{-1} was attained from highest planting density of 916 plants m^{-2}. Cv. Pharmasaat had better performance because of higher essential oil yield in both sowing times.

Quantitative results showed that treatments regarding planting densities had no significant effect on concentrations of estragol, γ-himachalene and *trans*-anethole of anise essential oil from both sowing times (table 5.10). In current study tested cultivars were characterized by significant differences with respect to estragol, γ-himachalene and *trans*-anethol concentration in early and delayed sowing times (table 5.10). The percentage of *trans*-anethole in the essential oil of anise ranged from 92.3 to 93.3% in both sowing times (table 5.10). In present study, all essential oil samples contained low concentration of estragol which was at a very low level of around 0.37 to 0.52% in both sowing times. Cv. Enza Zaden contained significant lower concentration of 0.38% (p= 0.00) in 1st sowing time and 0.44% (p= 0.00) in 2nd sowing time of estragol (methyl chavicol) as compared to other cultivars in both sowing times (table 5.10). Contrary to that cv. Agri Saaten contained significant lower concentration of *trans*-anethole in early and delayed sowing time in comparison with other cultivars.

Table 5.10: Effect of different cultivars (CV) and planting densities (PD) on estragol (ES) (%), gamma-himachalene (GA) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Gross-Gerau 2009

CV	PD	1st sowing time (1.4.2009)				2nd sowing time (15.4.2009)			
		Plants m^{-2}	ES	GA	TA	Plants m^{-2}	ES	GA	TA
			%	%	%		%	%	%
1		656	0.38 b	4.8b	93.3 a	517	0.44 b	5.0b	92.8 a
2		526	0.42 a	4.9b	93.1 a	387	0.51 a	5.0b	92.9 a
3		650	0.43 a	5.2a	92.7 b	568	0.51 a	5.2a	92.3 b
	1	374	0.37 a	4.9a	93.2 a	345	0.47 a	5.1a	92.6 a
	2	542	0.42 a	5.1a	92.9 a	402	0.48 a	5.1a	92.7 a
	3	916	0.45 a	5.0a	93.0 a	724	0.52 a	5.1a	92.8 a
LSD ($5_{\%}$)									
CV			0.03	0.2	0.3		0.04	0.2	0.3
PD			ns	ns	ns		ns	ns	ns
CV X PD			ns	0.3	0.4		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Agri-Saaten

Increasing trend of estragol and *trans*-anethole concentrations were observed in delayed sowing time as planting densities narrowed. Cv. Agri-Saaten led to significant higher concentration of γ-himachalene with 5.2% in both sowing times as compared to other cultivars in 2009 (table 5.10). In early sowing time there was a CV x PD interaction regarding *trans*-anethole and γ-himachalene concentration, which is presented in figure 5.5 and figure 5.6. This interaction is caused by different reactions of the used cultivars on the applied planting densities. At the lowest level of planting densities (374 plants m^{-2}) cv. Enza Zaden had higher *trans*-anethole concentrations than the other both cultivars. At the medium level of PD (542 plants m^{-2}) cv. Agri-Saaten was significant lower than the other both cultivars. Contrary effect was observed at highest PD where no significant differences between all these cultivars were found (Fig.5.6 and 5.7).

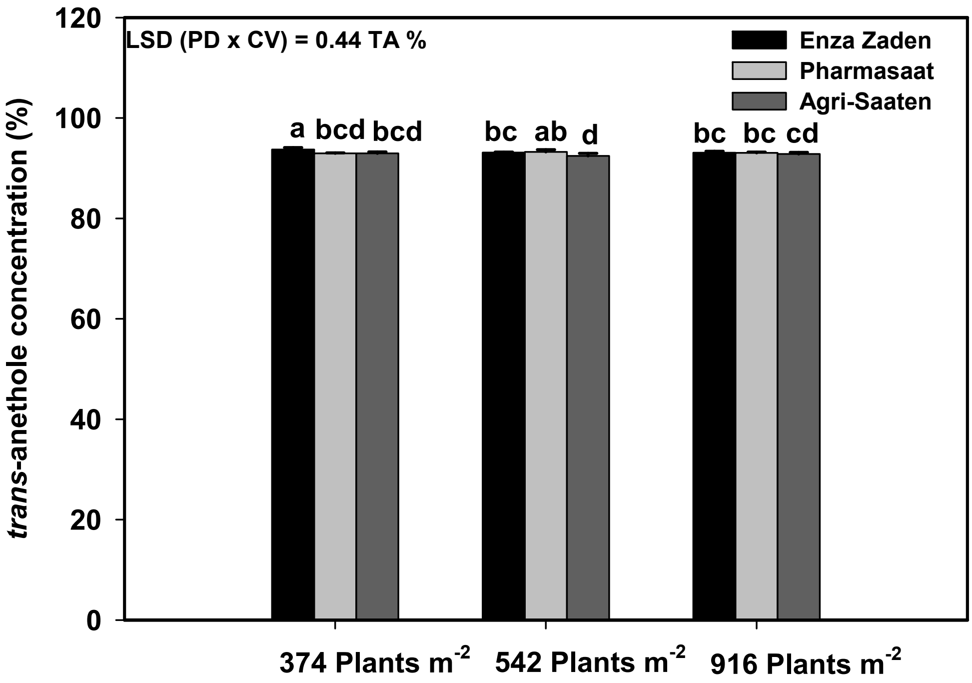


Fig. 5.6: Effect of different planting densities (PD) and cultivars (CV) on *trans*-anethole concentration (%) of anise in early sowing time at experimental station Gross-Gerau 2009

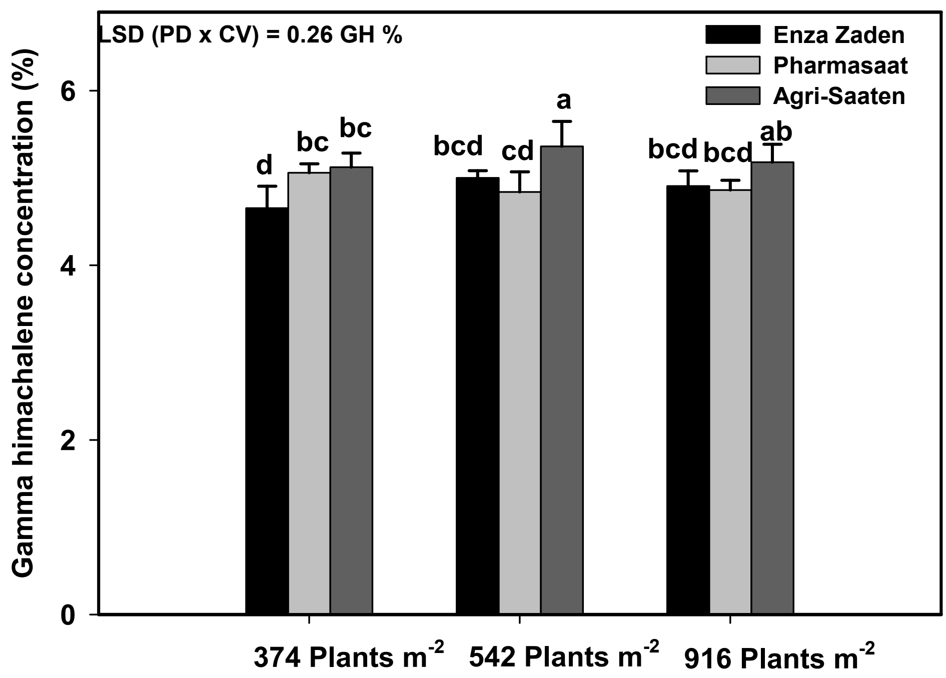


Fig. 5.7: Effect of different planting densities (PD) and cultivars (CV) on gamma-himachalene concentration (%) of anise in early sowing time at experimental station Gross-Gerau 2009

5.1.3 Field experiment Giessen 2008

5.1.3.1 Disease assessment

Overall higher *Cercospora malkoffii* infection level was noticed in anise plants sown in experimental station Gross-Gerau compared with Giessen. Severity of infection ranged from 2.7 to 4.4 in early and delayed sowing. Higher disease level was observed in early sowing time compared with delayed sowing (Fig. 5.8).

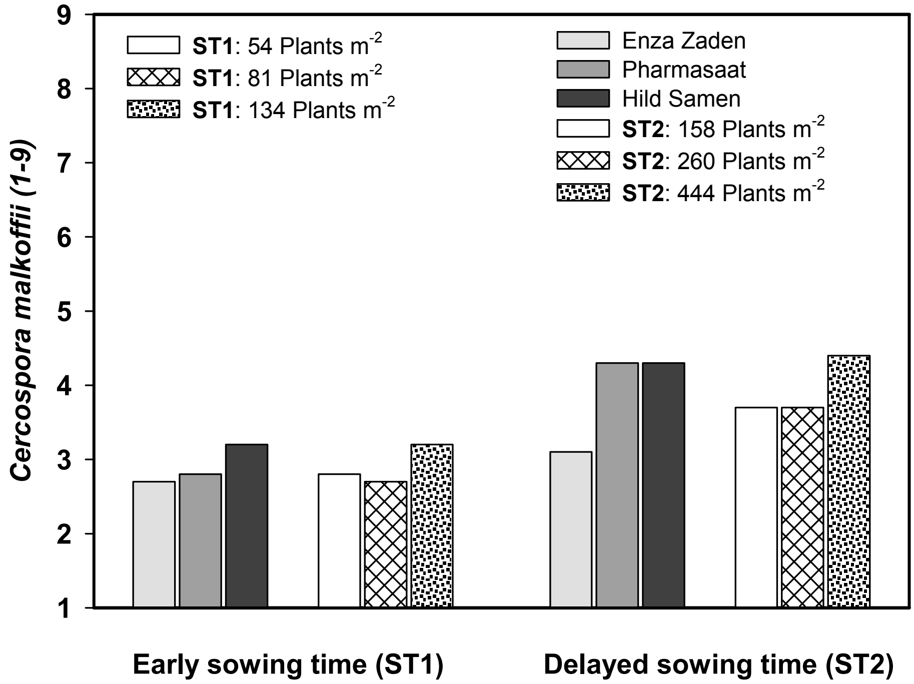


Fig. 5.8: Effect of cultivars and plant densities on *Cercospora malkoffii* (1-9) in early and delayed sowing of anise at experimental station Giessen 2008

Cv. Hild Samen was more susceptible compared with other cultivars. Higher disease level of 3.2 and 4.4 prevailed in plant densities of 134 plants m^{-2} and 444 plants m^{-2} respectively in early and delayed sowing times (Fig. 5.8). Higher disease level in delayed sowing might be a reason of higher level of plant densities compared with early sown anise plants. Cv. Hild Samen and cv. Pharmasaat had similar disease level in delayed sowing time.

5.1.3.2 Growth and fruit yield parameters

The applied sowing rates led to plant densities after germination of 54, 81 and 134 plants m^{-2} as well as 158, 260 and 444 plants m^{-2} respectively in early and delayed sowing times (table 5.11). Higher germination rate was observed in delayed sowing time. However the soil conditions in Giessen induced strong reduction of plant density compared with Gross-Gerau. In both stations there was a contrary effect of sowing rate on plant growth parameters as well as on fruit yield of anise. The germination ability of the seeds of the used anise cultivars was different in both sowing times. Cv. Hild Samen had lowest germination rate in comparison with the other both cultivars in early times which were lower than the comparative cultivars.

The plant height of anise plant stand was around 40 to 47 cm in early and delayed sowing times. Plant height of anise was not affected by cultivars as well as by plant density in early sowing time, where as cv. Hild Samen attained significant lower plant height of 40.1 cm in delayed sowing time (table 5.11). A trend of taller plant height was observed at lower plant densities in both sowing times. The number of primary branches and umbel number per plant showed significant differences among the treatments in early and three weak delayed sowing times. The number of primary branches per plant varied from 4.2 to 5.2 and 4.5 to 6.5 branches respectively in early and delayed sowing time (table 5.11). Similar trend regarding umbel number per plant was observed which ranged from 4.1 to 6.1 and 5.4 to 7.5 respectively in early and delayed sowing times (table 5.11).

Table 5.11: Effect of different cultivars (CV) and planting densities (PD) on plant height (PH) (cm), primary branches per plant (PBP), umbels number per plant (UNP) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2008

CV	PD	1st sowing time (1.04.2008)				2nd sowing time (23.04.2008)			
		Plants m^{-2}	PH	PB	UN	Plants m^{-2}	PH	PB	UN
			cm	no.	no.		cm	no.	no.
1		136	46.3a	5.1a	5.4a	380	45.3a	5.9a	6.9a
2		103	46.6a	4.9a	5.7a	348	42.5ab	5.5b	6.7a
3		31	43.3a	4.2b	4.1b	134	40.1b	4.9c	5.9b
	1	54	46.3a	5.2a	6.1a	158	43.3a	6.4a	7.5a
	2	81	45.2a	4.9a	4.9b	260	43.0a	5.4b	6.6b
	3	134	44.6a	4.2b	4.2c	444	41.7a	4.5c	5.4c
LSD 5%									
CV			ns	0.4	0.6		3.5	0.4	0.3
PD			ns	0.4	0.6		ns	0.4	0.3
CV x PD			ns	ns	ns		ns	0.7	0.6

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

The lowest plant density of 54 plants m^{-2} (early sowing) and 158 plants m^{-2} (delayed sowing) led to highest number of primary branches per plant (PBP) with 5.2 and 6.5 branches per plant respectively in early and delayed sowing time. Contrary to that lowest PBP 4.2 (1st sowing) and 4.5 (2nd sowing) of anise resulted from higher plant density of 134 plants m^{-2} and 444 plants m^{-2} respectively (table 5.11). Cultivar Hild Samen recorded significant lower number of primary branches and umbel number per plant in both sowing times. In delayed sowing time plant density of 158 plants m^{-2} had the highest number of PBP with 6.5 branches and UNP with 7.6 umbels as compared to other planting density treatments (table 5.11).

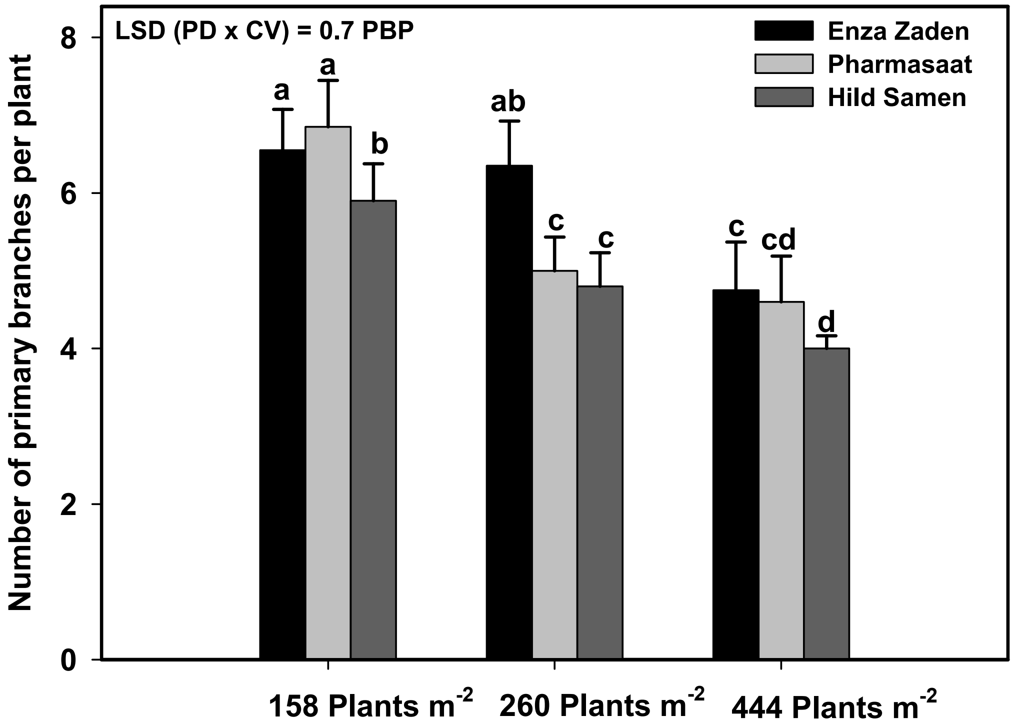


Fig. 5.9: Effect of different plant densities (PD) and cultivars (CV) on number of primary branches per plant in delayed sowing time of anise at experimental station Giessen 2008

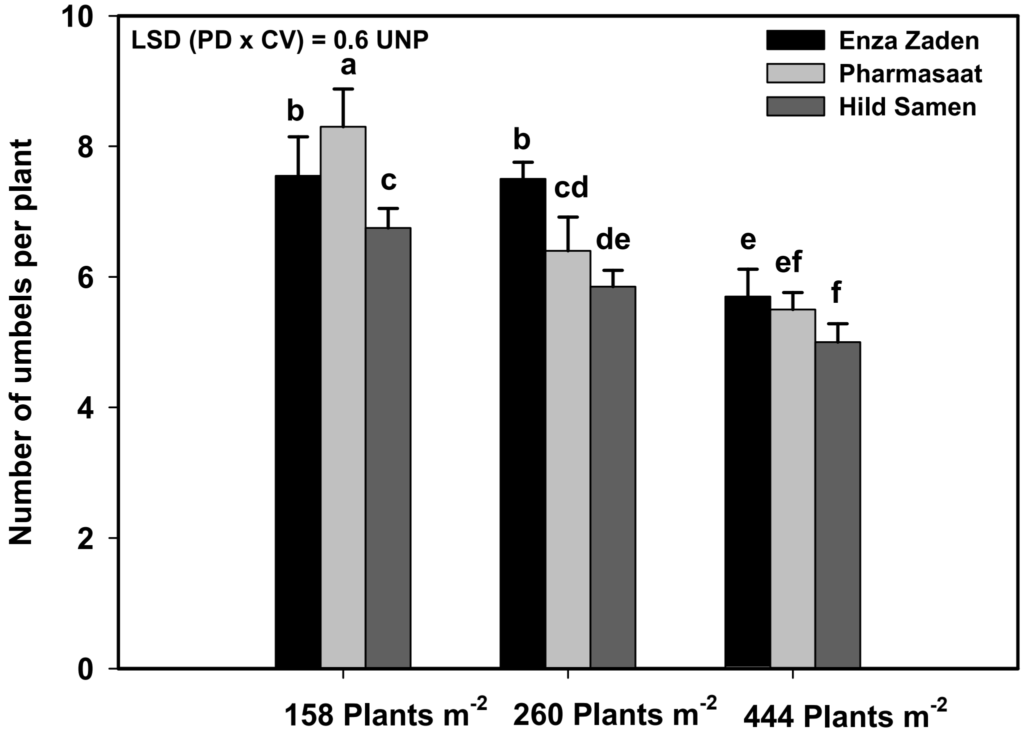


Fig. 5.10: Effect of different plant densities (PD) and cultivars (CV) on number of umbels per plant in delayed sowing time of anise at experimental station Giessen 2008

There was an interaction between cultivars and plant density with respect to primary branches and umbel number per plant of anise in second sowing time (Fig. 5.9, Fig. 5.10). Significant lower number of primary branches of 4.9 branches per plant and umbel number per plant with 5.9 umbels were attained by cv. Hild Samen in comparison with other used cultivars of anise. Narrow plant densities induced strong effect on these growth parameters of anise in both sowing times.

Data presented in table 5.12 show that there was a significant difference regarding thousand fruit weight (TFW) between used planting densities in early sowing time. In delayed sowing time contrary affect was noticed where cv. Hild Samen recorded significant lower 1000-fruit weight as compared to other used anise cultivars. However from both sowing times TFW varied between 2.73 and 3.30 g in 2008. Plant density of 134 plants m^{-2} achieved significant higher TFW of 2.94 g whereas significant lower value of 2.73 g was found under plant density of 81 plants m^{-2} in early sowing time (table 5.12).

Table 5.12: Effect of different cultivars (CV) and planting densities (PD) on thousand fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2008

CV	PD	1st sowing time (1.04.2008)			2nd sowing time (23.04.2008)		
		Plants m^{-2}	TFW	FY	Plants m^{-2}	TFW	FY
			g	dt/ha at 91%		g	dt/ha at 91%
1		136	2.78a	5.6a	380	2.83b	4.6a
2		103	2.80a	5.6a	348	2.73b	4.8a
3		31	2.90a	0.7b	134	3.30a	1.9b
	1	54	2.82ab	2.8b	158	3.03a	4.2a
	2	81	2.73b	4.6a	260	2.97a	4.0a
	3	134	2.94a	4.5a	444	2.87a	3.2b
LSD 5%							
CV			ns	1.2		0.2	0.6
PD			0.2	1.2		ns	0.6
CV x PD			ns	ns		ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Fruit yield (FY) of anise was affected by both factors cultivar as well as plant density (PD) in early and delayed sowing time. In early sowing time plant density of 81 plants m^{-2} led to significant higher fruit yield of 4.6 dt/ha, whereas significant lower 2.8 dt/ha fruit yield was observed under the plant density of 54 plants m^{-2} in early sowing time (table 5.12). Cultivar Hild Samen produced significant lower fruit yield in both sowing times. In delayed sowing time plant density of 158 plants m^{-2} was superior with fruit yield of 4.2 dt/ha, whereas lowest fruit yield of 3.2 dt/ha was noticed from plant density of 444 plants m^{-2} (table 5.12). Highest fruit yield of 4.8 dt/ha was produced by cv. Pharmasaat whereas lowest fruit yield was recorded by cv. Hild Samen (table 5.12). Decreasing trend of fruit yield was observed in delayed sowing time as planting densities increase.

5.1.3.3 Content, yield and composition of essential oil

There were significant differences regarding essential oil (EO) concentration among the used treatments in both early and delayed sowing times (table 5.13). The concentration of essential oil of aniseed within all treatments reached a maximum level of 3.67% (2.30 to 3.67%). Plant density of 54 plants m^{-2} accumulated significant higher amount of essential oil of 2.77%, whereas significant lower EO of 2.48% was synthesized by 81 plants m^{-2} in early sowing time (table 5.13). Lower plant densities led to higher concentration of essential oil in both sowing times. CV. Pharmasaat achieved significant higher EO in comparison with other used cultivars in both sowing times. Overall higher concentration of essential oil was synthesized by the plants in delayed sowing time which might be due to better environmental conditions during essential oil accumulation. Essential oil yield was clearly affected by plant density as well as by the used cultivars in both sowing times.

Table 5.13: Effect of different cultivars (CV) and planting densities (PD) on essential oil (EO) (%) and essential oil yield (EOY) (Kg/ha) (%) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2008

CV	PD	1st sowing time (1.04.2008)			2nd sowing time (23.04.2008)		
		Plants m^{-2}	EO	EOY	Plants m^{-2}	EO	EOY
			%	Kg/ha		%	Kg/ha
1		136	2.70a	15.0a	380	3.40b	15.8a
2		103	2.84a	15.8a	348	3.67a	17.9a
3		31	2.31b	1.6b	134	3.17b	6.0b
	1	54	2.77a	7.9b	158	3.50a	14.8a
	2	81	2.48b	12.1a	260	3.43a	14.0ab
	3	134	2.60ab	12.5a	444	3.30a	11.1b
LSD (5%)							
CV			0.2	3.2		0.2	3.4
PD			0.2	3.2		ns	3.4
CV x PD			ns	ns		ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Essential oil yields (EOY) of anise varied between 1.6 and 17.9 kg/ha from different used treatments (table 5.13). Essential oil yield is a trait which directly depended on essential oil percentage and fruit yield of anise. Cultivar Enza Zaden and Pharmasaat produced similar level of EOY, whereas cv. Hild Samen was attained significant lower essential oil yield in both sowing times.

The compound estragol which can be characterized as the chemical methyl chavicol considered as a relevant quality component varied from 0.60 to 1.41% from both sowing times (table 5.14). Quantitative study showed that plant density had no effect on the percentage of trans-anethole and estragol in anise essential oil in early sowing time. Cultivars showed pronounced effect with respect to γ-himachalene in both sowing times. Cv. Enza Zaden and Pharmasaat led to higher concentration of γ-himachalene whereas significant lower concentration was induced by cv. Hild Samen in early and delayed sowing times.

Table 5.14: Effect of different cultivars (CV) and planting densities (PD) on estragol (ES) (%), gamma-himachalene (GA) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2008

CV	PD	1st sowing time (1.04.2008)				2nd sowing time (23.04.2008)			
		Plants m^{-2}	ES	GH	TA	Plants m^{-2}	ES	GH	TA
			%	%	%		%	%	%
1		136	0.69b	6.2a	90.8b	380	0.60b	5.2a	92.4b
2		103	0.72b	6.2a	90.8b	348	0.62b	5.4a	91.9c
3		31	1.18a	2.7b	95.0a	134	1.41a	2.1b	95.6a
	1	54	0.83a	5.0a	92.0a	158	0.86a	4.2a	93.3ab
	2	81	0.85a	5.0a	92.3a	260	0.91a	4.4a	93.0b
	3	134	0.90a	5.0a	92.1a	444	0.87a	4.1a	93.6a
LSD (5%)									
CV			0.2	0.3	0.6		0.07	0.3	0.4
PD			ns	ns	ns		ns	ns	0.4
CV x PD			ns	ns	ns		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Gamma-himachalene of anise essential oil varied from 2.1 to 6.2% (table 5.14). Overall higher concentration was induced in early sowing time as compared to delayed sowing. Contrary to that a phenylpropanoid, *trans*-anethole influenced by plant density as well as by used cultivars. The *trans*-anethole content in the essential oil of anise ranged from 90 to 95.6% in early and delayed sowing times. Cv. Hild Samen led to significant higher concentration of estragol and *trans*-anethole with 1.18% and 1.41% and 95.0, 95.6% respectively in early and delayed sowing times (table 5.14). In delayed sowing time narrow plant density of 444 plants m^{-2} induced significant higher concentration of *trans*-anethole 93.6% as compared to other planting densities (table 5.14).

5.1.4 Field experiments Giessen 2009

5.1.4.1 Disease and lodging assessment

In 2009, the severity of *Cercospora malkoffii* was ranged from 2.8 to 4.9 in early sown anise plants, whereas it varied from 2.6 to 3.3 in delayed sowing time (Fig. 5.11). However relatively higher infection was recorded in early sowing plants. Similar disease trend was observed in all cultivars in early and delayed sowing times. Plant densities of 39 and 54 plants m^{-2} had low severity level of 2.6 and 2.0 respectively in early and delayed sowing time. Increasing trend of disease was noticed as plant densities increases. Highest disease level was recorded in narrow plant densities of 271 and 258 plants m^{-2} in early and delayed sowing plants.

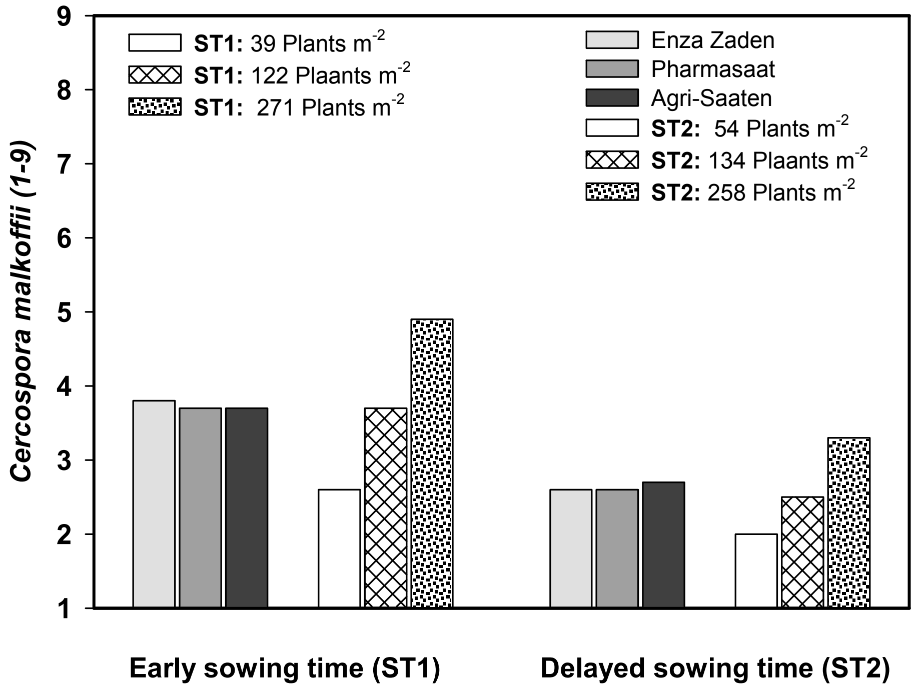


Fig. 5.11: Effect of cultivars and plant densities on *Cercospora malkoffii* (1-9) in early and delayed sowing of anise at experimental station Giessen 2009

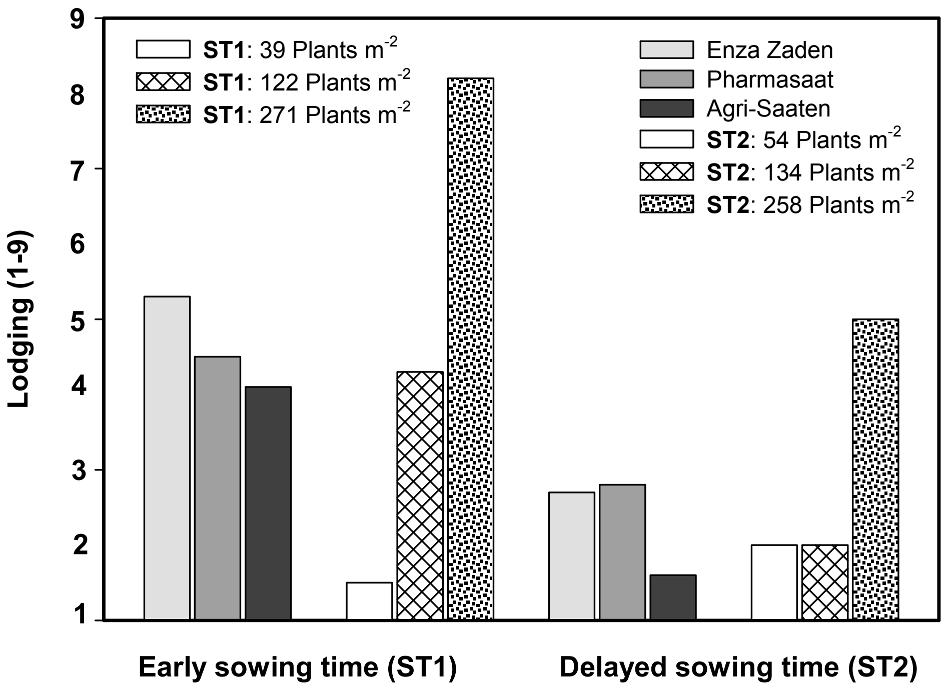


Fig. 5.12: Effect of cultivars and plant densities on lodging (1-9) in early and delayed sowing of anise at experimental station Giessen 2009

Narrow plant densities provide favorable conditions for spreading of infection especially with increasing the duration of leave wetness.

Lodging was estimated at full maturity of anise plants before harvesting, and its value ranged from 0.5 to 7.2 during this experiment (Fig. 5.12). Maximum lodging (7.2) was noted under the plant densities of 271 plants m^{-2} whereas lower level was noticed in plant densities of 39 plants m^{-2} (0.5) in early sowing time. Increasing trend of anise lodging was observed as plant density increase in early and delayed sowing times. Minimum lodging was noticed with cv. Agri-saaten in both sowing times. Cv. Enza Zaden was more affected by lodging as compared with other cultivars in early sowing time. Higher disease of *Cercospora malkoffii* was recorded where anise plants affected by lodging in both sowing times.

5.1.4.2 Growth and fruit yield parameters

Data presented in table 16 show that different sowing rates led to plant densities of 39, 122, 271 and 54, 134, 258 plants m^{-2} respectively in early and delayed sowing times (table 5.15). The soil condition in Giessen induced strong reduction of plant densities as compared to Gross-Gerau. Comparable plant densities with similar trend could be established in both sowing times.

Table 5.15: Effect of different cultivars (CV) and planting densities (PD) on plant height (PH) (cm), primary branches per plant (PB), umbels number per plant (UN), 1000-fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2009

CV	PD	1st sowing time (1.04.2009)				2nd sowing time (20.04.2009)					
		Plants m^{-2}	PH	TFW	FY	Plants m^{-2}	PH	PB	UN	TFW	FY
			cm	g	dt/ha		cm	no	no	g	dt/ha
1		156	65a	3.36a	10.2a	147	64a	7.0a	9.5a	2.77a	4.3a
2		118	67a	3.43a	10.3a	148	66a	7.5a	11.4a	2.79a	4.7a
3		157	65a	3.14a	10.5a	151	65a	6.9a	10.5a	2.66b	4.4a
	1	39	70a	3.30a	12.0a	54	69a	8.5a	14.8a	2.81a	4.4a
	2	122	65b	3.38a	9.9b	134	65a	7.2b	10.2b	2.78a	4.8a
	3	271	62c	3.25a	9.0b	258	62a	5.6c	6.2c	2.62b	4.2a
LSD 5%											
CV			ns	ns	ns		ns	ns	ns	0.11	ns
PD			2.0	ns	1.5		ns	0.7	2.2	0.11	ns
CV x PD			3.4	ns	ns		ns	ns	ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Height of anise plant stand was around 62 to 70 cm in early and delayed sowing times. Maximum plant height of 70 cm was obtained from 39 plants m^{-2} and the lowest value of 62 cm was observed from highest plant density of 271 plants m^{-2} in early sowing time (table 5.15). Plant height was not affected by used treatments in delayed sowing time. However a decreasing trend of plant height was noticed as plant densities increased. Cultivars used in our study showed similar level of plant height in both sowing times.

Interaction effect was observed regarding plant height in early sowing time (Fig. 5.13). Primary branches and umbel number per plant were significantly affected by various plant densities in delayed sowing time. The number of primary branches and umbels per plant varied from 5.6 to 8.5 and from 6.2 to 14.8 respectively in delayed sowing time (table 5.15). Under the second sowing date, plant density of 54 plants m^{-2} resulted in higher number of primary branches (8.5) and higher umbel number plant (14.8) in comparison with 258 plants m^{-2}. Primary branches and umbels per plant were not affected by the used cultivars. Data indicate that thousand fruit weight was not affected by used treatments in early sowing time, whereas thousand fruit weight (TFW) was affected by plant density as well as by used cultivars in delayed sowing time. However TFW of anise from both sowing times ranged between 2.62 and 3.43 g. Overall higher thousand fruit weight of anise was achieved under early sowing time as compared to delayed sowing. Plant density of 54 plants m^{-2} recorded significant higher TFW of 2.81 g as compared to other plant densities in delayed sowing time (table 5.15). Cultivars Pharmasaat and Enza Zaden produced significant higher TFW as cv. Hild Samen in delayed sowing time.

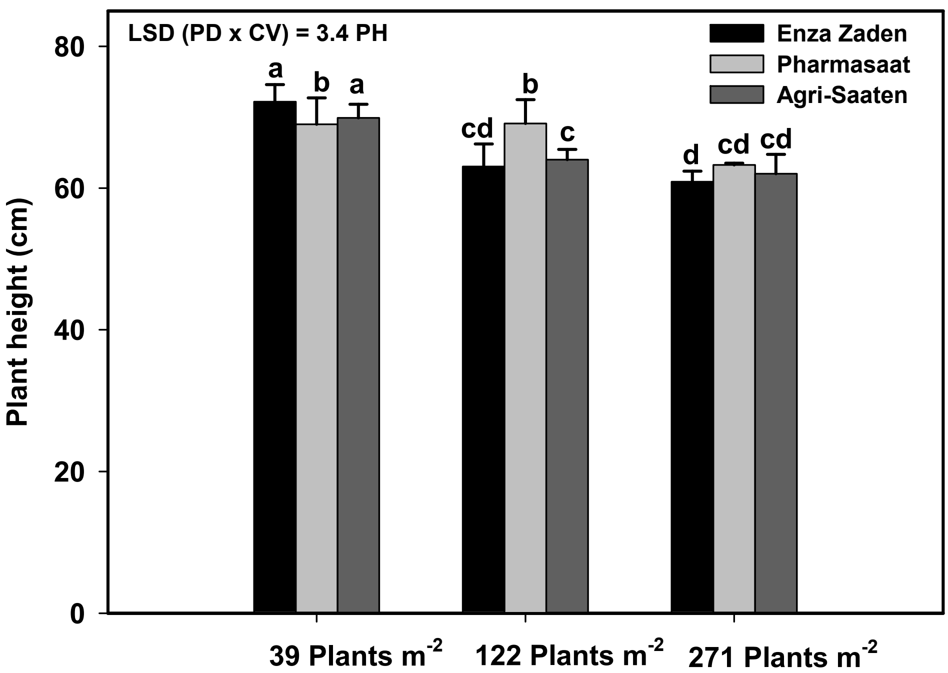


Fig. 5.13: Effect of different planting densities (PD) and cultivars (CV) on plant height (cm) in early sowing time of anise at experimental station Giessen 2009

Generally, the fruit yield of anise was higher in early sowing time than in delayed sowing time. Fruit yield of anise was strongly dependent on the sowing time indicating that delayed sowing reduced the fruit yield. Fruit yield of anise was significantly affected by different level of plant densities in early sowing time whereas no effect was found after delayed sowing of anise. In both sowing times fruit yield of anise ranged from 4.2 to 12 dt/ha (table 5.15). The present study showed that the fruit yield increased with lower planting densities of 39 plants m^{-2}, whereas it led to decline in fruit yield with planting densities of 271 plants m^{-2} (table 5.15). A trend of decrease in fruit yield was noticed as planting densities increased in 1st sowing time. Delayed sowing of anise resulted in insufficient vegetative growth and plant immediately responded to photoperiod so fruit yield contributing parameters reduced. Thousand fruit weight has a direct impact on final fruit yield of anise crop which was higher in early sowing time as compared to three weak delayed sowing times (table 5.15). Tested cultivars showed that there was no significant difference regarding fruit yield in both sowing times.

5.1.4.3 Content, yield and composition of essential oil

Data presented in table 5.16 shows that at experimental station Giessen in 2009 essential oil content accumulated by the anise seeds ranged from 3.25 to 3.58% for all treatments in both sowing times. No significant differences were observed in essential oil content extracted from the fruits of the different cultivars of anise in early sowing time. Cultivars showed significant differences regarding essential oil accumulation in delayed sowing time.

Table 5.16: Effect of different cultivars (CV) and planting densities (PD) on essential oil (EO) (%) and essential oil yield (EOY) (Kg/ha) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2009

CV	PD	1st sowing time (1.04.2009)			2nd sowing time (20.04.2009)		
		Plants m^{-2}	EO	EOY	Plants m^{-2}	EO	EOY
			%	Kg/ha		%	Kg/ha
1		156	3.42a	34.7a	147	3.58a	15.2a
2		118	3.33a	34.1a	148	3.25b	15.4a
3		157	3.42a	35.6a	151	3.55a	15.4a
	1	39	3.44a	41.0a	54	3.51a	15.5a
	2	122	3.27a	32.3b	134	3.51a	16.7a
	3	271	3.46a	31.1b	258	3.36a	14.0a
LSD (5%)							
CV			ns	ns		0.2	ns
PD			ns	4.1		ns	ns
CV x PD			ns	ns		0.4	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

Delayed sowing led to increased essential oil concentration which can be attributed to better weather conditions during the stage of oil synthesis. Cultivar Enza Zaden synthesized 3.58% EO which was significant higher as compared to other tested cultivars in 2nd sowing time (table 5.16). Overall cv. Pharmasaat accumulates lower concentration of essential oil in both sowing times. Higher concentration of essential was noticed in lower plant densities in delayed sowing time. Interaction effect was observed concerning essential oil percentage in delayed sowing time (Fig. 5.14).

Table 5.16 reveals that the main effect of all plant density treatments on essential oil yield was statistically significantly in early sowing time whereas no effect was noticed in second sowing time. In present study essential oil yield of anise was varied from 14 to 41 kg/ha in both sowing times (table 5.16). The essential oil yield directly depended on essential oil percentage and fruit yield. In present study, plant density of 39 plants m^{-2} achieved significant higher essential oil yield (EOY) of 41 kg/ha in comparison with other planting densities in early sowing time (table 5.16). Lower plant densities gained higher essential oil yield which relates to higher fruit yield. Essential oil yield was not affected by used cultivars in both sowing times.

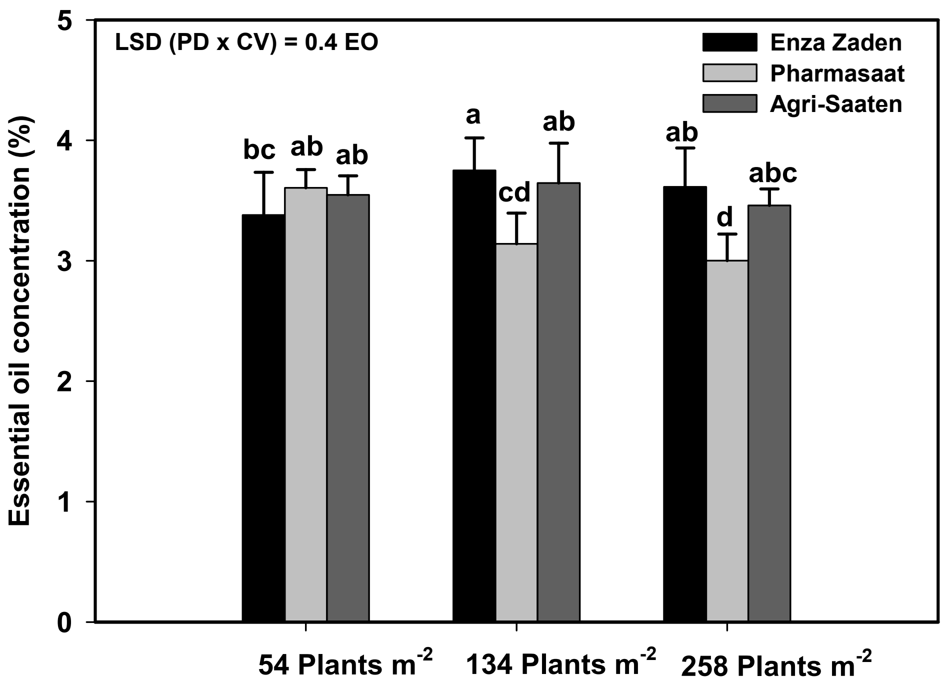


Fig. 5.14: Effect of different planting densities (PD) and cultivars (CV) on essential oil concentration (%) in delayed sowing time of anise at experimental station Giessen 2009

Table 5.17 illustrates that the variables plant density and cultivars create remarkable variation in estragol percentage in both sowing times. In present study, all essential oil samples contained low concentration of estragol which was at a very low level of around 0.46 to 0.72% in both sowing times. Cultivar Pharmasaat recorded significant higher concentration of estragol 0.55 and 0.72% respectively in early and delayed sowing time (table 5.17). Cv. Enza Zaden and cv. Agri Saaten had similar level of estragol concentration in both sowing times. In delayed sowing time estragol concentration was increased as plant density narrowed. Concentration of γ-himachalene in the essential oil of anise fruits ranged from 6.5 to 7.4% in both sowing times. Plant densities showed remarkable variation regarding γ-himachalene concentration in early sowing time. Plant density of 39 plants m^{-2} induced significant higher concentration of γ-himachalene 7.4%, whereas significant lower concentration of 6.5% was recorded by 271 plants m^{-2} (table 5.17). A decreasing trend of γ-himachalene was observed as planting densities narrowed in early sowing time. Opposite results found in delayed sowing time where cultivars showed pronounced effect on γ-himachalene concentration. Cv. Pharmasaat achieved higher concentration of gamma-himachalene as compared to other cultivars.

The major essential oil constituent in the fruits of anise was *trans*-anethole, comprising 90% of the essential oil. In early sowing time *trans*-anethole was affected by various plant density treatments. Increasing trend of *trans*-anethole was noticed as plant density increase in 1st sowing time. Plant density of 271 plants m^{-2} contains relatively higher concentrations of *trans*-anethole of 91.4% as compared to the plant density of 39 plants m^{-2} which obtain 89.8% *trans*-anethole in early sowing time (table 5.17). Opposite results were found in delayed sowing time where plant density had no remarkable variation regarding *trans*-anethole.

Table 5.17: Effect of different cultivars (CV) and planting densities (PD) on estragol (ES) (%) gamma-himachalene (GA) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at early and delayed sowing time in Giessen 2009

CV	PD	1st sowing time (1.04.2009)				2nd sowing time (20.04.2009)			
		Plants m^{-2}	ES	GH	TA	Plants m^{-2}	ES	GH	TA
			%	%	%		%	%	%
1		156	0.48a	7.0a	90.6a	147	0.65b	6.5b	90.0a
2		118	0.55a	7.1a	90.0a	148	0.72a	6.8a	89.5b
3		157	0.46b	6.9a	90.6a	151	0.65b	6.6ab	89.7ab
	1	39	0.54a	7.4a	89.8b	54	0.64b	6.6a	89.9a
	2	122	0.49b	7.2a	90.0a	134	0.67ab	6.7a	89.6a
	3	271	0.46b	6.5b	91.4a	258	0.70a	6.7a	89.7a
LSD (5%)									
CV			0.03	ns	ns		0.04	0.2	0.3
PD			0.03	0.3	0.7		0.04	ns	ns
CV x PD			ns	ns	ns		ns	ns	ns

CV1: Enza Zaden, CV2: Pharmasaat, CV3: Hild Samen

However in delayed sowing time cv. Enza Zaden produced significant higher percentage of *trans*-anethole in comparison with other used cultivars (table 5.17). Interaction effect was not observed regarding *trans*-anethole and estragol concentrations in both sowing times.

The list of the identified components and their quantity was analyzed by GC-MS presented in (table 5.18). As shown in the table, the number of identified compounds in the essential oil of the tested cultivars ranged from 13 to 19. The aroma of the anise essential oil is dominated by *trans*-anethole, which is the phenylpropanoid compound and present in concentration of 80-90% in tested cultivars. The higher concentration of *trans*-anethole was found in the essential oil of cultivar Hild Samen from both experimental station Giessen and Gross-Gerau.

Table 5.18: Chemical composition (%) in essential oil of three anise cultivars analyzed by GC-MS at two different experimental stations (means of 4 replications)

Compounds	Giessen				Gross-Gerau		
	*KI	Enza Zaden	Pharmasaat	Hild Samen	Enza Zaden	Pharmasaat	Hild Samen
Linalool	1098	0.0	0.0	0.2	0.0	0.0	0.0
Estragol	1197	0.32	0.38	0.49	0.35	0.36	0.76
Cis anethole	1252	0.10	0.13	0.06	0.18	0.17	0.07
Trans-anethole	1287	79.62	82.09	90.2	84.58	83.8	89.07
Elemene (delta)	1333	0.54	0.47	0.08	0.36	0.42	0.08
Cyclosativene	1367	0.0	0.1	0.0	0.0	0.0	0.0
Beta elemene	1388	0.12	0.12	0.0	0.05	0.11	0.0
α-himachalene	1449	0.86	0.76	0.13	0.57	0.62	0.15
γ-himachalene	1478	8.31	7.39	2.11	5.75	6.18	2.9
α-amorphane	1482	0.17	0.17	0.03	0.13	0.13	0.04
(E)-Methylisoeugenol	1489	0.14	0.12	0.12	0.14	0.15	0.07
α-zingiberene	1493	0.96	0.85	0.45	0.59	0.71	0.51
β-himachalene	1499	0.53	0.48	0.15	0.36	0.38	0.21
α-muurolene	1502	0.19	0.16	0.03	0.11	0.13	0.0
β-bisabolene	1506	0.5	0.43	0.21	0.27	0.31	0.29
Beta-sesquiphellandrene	1522	0.1	0.05	0.03	0	0.07	0.0
Spathulenol	1580	0.0	0.0	0.0	0.08	0.0	0.0
Unknown	1629	0.0	0.0	0.0	0.11	0.13	0.0
α-cadinol	1651	0.1	0.0	0.0	0.07	0.08	0.0
Unknown	1831	6.44	5.5	4.95	5.47	5.31	5.23
Unknown	1886	1.03	0.8	0.71	0.82	0.92	0.62
No. of identified compounds		17	17	16	18	18	13
Total		100	100	100	100	100	100

*KI: Kovat´ s retention index

The *cis*-isomer of anethole (Z)-anethole is known to be more toxic and present only 0.06 to 0.18%. Estragol, quality relevant component was found in all the samples of tested cultivars and varied from 0.32 to 0.76%. γ-himachalene concentration in the essential oil of anise fruits ranged from 2.1 to 8.3% in both station. The minor constituents in the essential of anise from current experiments were γ-himachalene (2-

8%), α-himachalene, estragol, *cis* anethole, elemene delta, α-amorphane, α-zingiberene, β-himachalene, α-muurolene and β-bisabolene (table 5.18).

5.2 Effect of different row spacing and plant densities

5.2.1 Field experiments Gross-Gerau 2008-2009

5.2.1.1 Disease assessment

Disease incidence of *Cercospora malkoffii* was assessed at different growth stages of anise plant development in both years. Severity of *Cercospora malkoffii* on anise plants ranged from 3.3 to 4.1 at fruiting stage whereas it varied from 7.0 to 7.3 at later stages of anise plants before harvesting (Fig. 5.15). At the both stages, higher infection rate was recorded under narrow plant densities. Minimum disease level was observed in lower plants densities. An increasing trend of disease was noticed as planting densities narrowed in both stages. Higher disease level was recorded where plants grown with 15 cm row spacing compared with 37.5 cm row spacing.

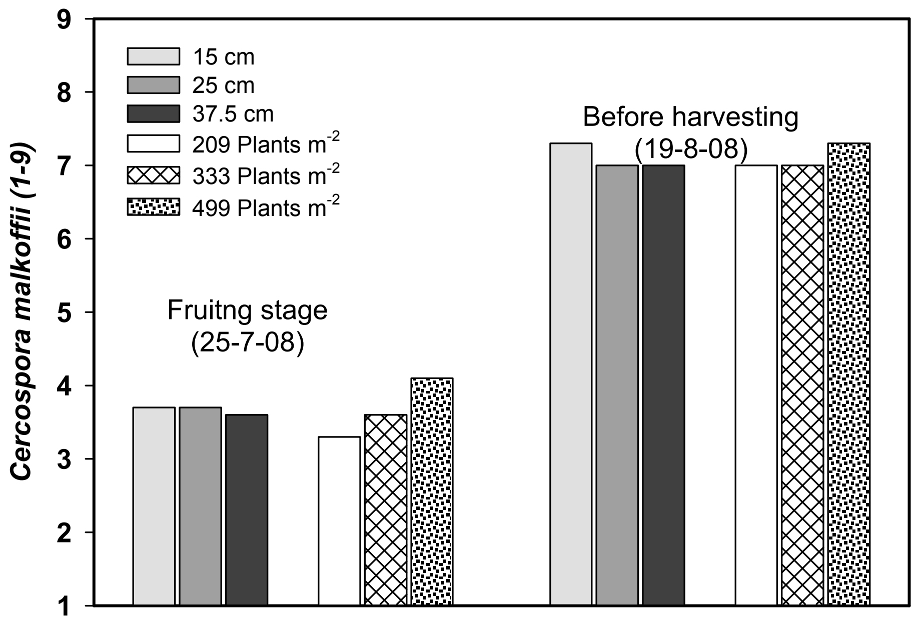


Fig. 5.15: Effect of row spacing and plant densities on *Cercospora malkoffii* (1-9) at two different stages of anise at experimental station Gross-Gerau 2008

In 2009, lower disease level was assessed compared with 2008 (Fig. 5.16). However disease scoring varied from 1.9 to 3.8 and 3.3 to 6.4 at fruiting stage and before harvesting respectively. Higher disease level 6.4 was assessed under the plant densities of 707 plant m^{-2} whereas lower disease rate 3.3 was noticed with plant densities of 294 plants m^{-2} in final evaluation before harvesting of anise. Decreasing trend of *Cercospora malkoffii* was recorded as distance between the rows increased.

However highest infection rate was recorded in 15 cm row spacing whereas lowest disease scoring was noticed in wider row spacing of 37.5 cm (Fig. 5.16).

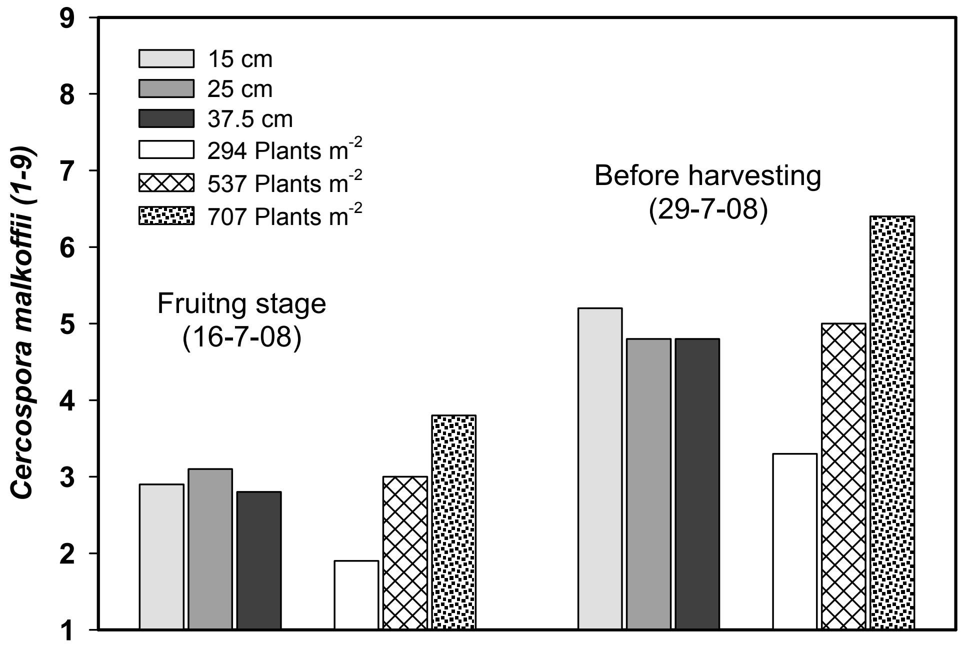


Fig. 5.16: Effect of row spacing and plant densities on *Cercospora malkoffii* (1-9) at two different stages of anise at experimental station Gross-Gerau 2009

5.2.1.2 Growth and fruit yield parameters

Data presented in table 5.19 show that applied sowing rates led to differ levels of plant densities (PD) determined after germination with 209, 333, 499 plants and 294, 537, 707 plants m^{-2} respectively in the years 2008 and 2009 (table 5.19). There were clear differences between both years with higher level of planting densities in 2009. Lower germination rate was observed under wider row spacing (37.5 cm) in both trial years. Comparable plant densities with similar trend could be established in both growing seasons regarding row spacing.

Plant height at maturity was not affected by different row spacing and planting densities in 2008. However, it was influenced significantly by planting densities in 2009. Maximum plant height of 67 cm was observed in 37.5 cm row spacing as compared to minimum plant height of 61 cm in 15 cm row spacing in 2009 (table 5.19). Plant density of 294 plants m^{-2} led to significant taller plants (65 cm), whereas significant lower plant height (62 cm) was recorded under planting density of 707 plants m^{-2} (table 5.19). Averaged of the year's anise plants were taller in 2009 which might be due to better environmental conditions during vegetative period. Number of primary branches per plant (PBP) and umbel number per plant (UNP) are important characters for fruit yield in anise cultivation. The effect of planting densities on number of primary branches

(PBP) and number of umbels per plant (UNP) were significant in both seasons. In both years, these yield components showed no remarkable variation by different row spacing treatments. The number of primary branches per plant (PBP) varied with each year where higher level was found in 2008 (4.2), ranging from 2.3 to 4.2, than in 2009 (3.8) which it ranged between 2.1 to 3.8 according to the planting densities (table 5.19).

Table 5.19: Effect of row spacing (RS) and planting densities (PD) on plant height (PH) (cm), primary branches per plant (PBP), secondary branches per plant (SBP), and umbels number per plant (UNP) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau 2008-09

RS	PD	Gross-Gerau 2008					Gross-Gerau 2009				
		PL.m^{-2}	PH	PBP	SBP	UNP	PL.m^{-2}	PH	PBP	SBP	UNP
			cm	no	no	no		cm	no	no	no
1		415	44a	2.8a	0.18a	3.9a	562	61c	2.8a	0.22a	4.1a
2		342	45a	3.3a	0.25a	4.6a	520	64b	2.9a	0.43a	4.3a
3		284	46a	3.4a	0.38a	4.9a	456	67a	3.0a	0.72a	4.7a
	1	209	46a	4.2a	0.45a	5.8a	294	65a	3.8a	0.95a	5.7a
	2	333	44a	2.9b	0.21a	4.2b	537	65a	2.8b	0.26b	4.1b
	3	499	43a	2.3b	0.16a	3.5b	707	62b	2.1c	0.16b	3.2c
LSD (5%)											
RS			ns	ns	ns	ns		2.2	ns	ns	ns
PD			ns	0.8	ns	1		2.2	0.4	0.4	0.8
CV X PD			ns	ns	ns	ns		ns	ns	ns	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm, PL.m^{-2}, plants m^{-2}

Plant densities of 209 plants m^{-2} and 294 plants m^{-2} induced significant higher number of PBP (4.2), UNP (5.8) and PBP (3.8), UNP (5.7) respectively in 2008 and 2009, whereas significant lower PBP (2.3), UNP (3.5) and PBP (2.1), UNP (3.2) were noticed under planting density of 499 plants m^{-2} and 707 plants m^{-2} respectively in 2008 and 2009 (table 5.19). Higher number of PBP, SBP and UNP were produced under wider row spacing in both seasons. The highest numbers of primary branches per plant (PBP), secondary branches per plant (SBP), umbel number per plant (UNP) were recorded from 37.5 cm row distances, while least number of these components was attained from the narrow row distances of 15 cm in both seasons. Secondary branches per plant (SBP) were not affected by used treatments in 2008, however significant difference was observed in 2009 regarding planting densities. No interaction was found regarding these anise plant features in both trail years.

Fruit number per plant (FNP) and fruit weight per plant (FWP) were affected by row spacing (RS) as well as by used planting densities (PD) in both years (table 5.20). Plant density of 209 plants m^{-2} and 294 plants m^{-2} induced significant higher FNP (116 and 127 fruits/plant) respectively, whereas significant lower FNP (50 and 41 fruits/plant) was observed from plant density of 209 and 294 plants m^{-2} respectively in 2008 and 2009 (table 5.20). Fruit weight per plant (FWP) ranged from 0.14 to 0.33 g in 2008, where as it varied from 0.09 to 0.38 g in 2009 (table 5. 20). Similar trend of higher fruit weight was observed in lower planting densities in both seasons. In various row spacing, 37.5 cm row spacing induced maximum FNP of 99 fruits per plant and maximum FWP of 0.27 g per plant while minimum FNP (55 fruits) and FWP (0.16 g) were recorded in 15 cm row spacing. Higher number of fruits and higher fruit weight per plant were recorded from lower planting densities and wider row spacing.

In 2008, the effects of planting densities (PD) were not statistically significant for 1000-fruit weight (TFW); however, it showed significant difference in 2009. Averaged over the years, it ranged from 2.41 to 2.44 g in 2008, whereas it ranged from 1.88 to 2.25 g in 2009 (table 5.20). In 2009, planting densities of 294 plants m^{-2} recorded significant higher 1000-fruit weight of 2.25 g, while significantly lower value was observed with 1.88 g at narrow planting densities of 707 plants m^{-2}.

Table 5.20: Effect of row spacing (RS) and planting densities (PD) on fruits number per plant (FNP), fruit weight per plant (FWP), 1000-fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau 2008-09

RS	PD	Gross-Gerau 2008					Gross-Gerau 2009				
		PL.m^{-2}	FNP	FWP	TFW	FY	PL.m^{-2}	FNP	FWP	TFW	FY
			no	g	g	dt/ha		no	g	g	dt/ha
1		415	55b	0.16b	2.44a	4.7a	562	51b	0.14b	2.16a	9.6a
2		342	76ab	0.21ab	2.43a	5.1a	520	82ab	0.21ab	2.08a	9.5a
3		284	99a	0.27a	2.42a	5.0a	456	97a	0.28a	1.90a	9.4a
	1	209	116a	0.33a	2.44a	5.5a	294	127a	0.38a	2.25a	11.3a
	2	333	64b	0.17b	2.44a	4.8ab	537	61b	0.17b	2.00ab	9.8b
	3	499	50b	0.14b	2.41a	4.3b	707	41b	0.09b	1.88b	7.5c
LSD (5%)											
RS			31	0.1	ns	ns		36	0.1	ns	ns
PD			31	0.1	ns	0.8		36	0.1	0.3	0.9
CV X PD			ns	ns	ns	ns		ns	ns	ns	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm, PL.m^{-2}, plants m^{-2}

As seen in table 5. 20, fruit yield (FY) of anise was affected significantly by different planting densities. On the other hand non significant affect was noticed regarding row spacing. The fruit yield of the year 2009 was superior to those of the year 2008. Averaged over both years, the highest fruit yield of 11.3 dt/ha was obtained from the planting density of 294 plants m^{-2}, while the lowest fruit yield of 4.3 dt/ha was recorded by 499 plants m^{-2}. However a trend of lower fruit yield was found in narrow planting densities and wider row spacing. There was no interaction regarding these study parameters.

5.2.1.3 Content, yield and composition of essential oil

Quality parameters of anise fruits including essential oil concentration (EO), essential oil yields (EOY) were not affected by used treatments in 2008. Averaged over the years, within all treatments essential oil concentration reached a maximum level of 3% (2.84 to 3.05%) (table 5.21). In 2009, a tendency of higher essential oil concentration was observed in lower planting densities and close row spaces. A decreasing trend of essential oil accumulation was observed as row spacing distances increased. Interaction effect was noticed regarding essential oil accumulation in 2009 (Fig. 5.17).

Table 5.21: Effect of different 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 Gross-Gerau 2008-09

RS	PD	Gross-Gerau 2008			Gross-Gerau 2009		
		Plants m^{-2}	EO	EOY	Plants m^{-2}	EO	EOY
			%	Kg/ha		%	Kg/ha
1		415	2.89a	13.5a	562	3.05a	29.3a
2		342	2.85a	14.3a	520	2.99a	28.0a
3		284	2.93a	14.6a	456	2.84a	27.0a
	1	209	2.84a	15.7a	294	3.04a	34.1a
	2	333	2.94a	14.2a	537	2.93a	28.5b
	3	499	2.90a	12.5a	707	2.90a	21.8c
LSD (5%)							
RS			ns	ns		ns	ns
PD			ns	ns		ns	2.6
RS X PD			ns	ns		0.3	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm

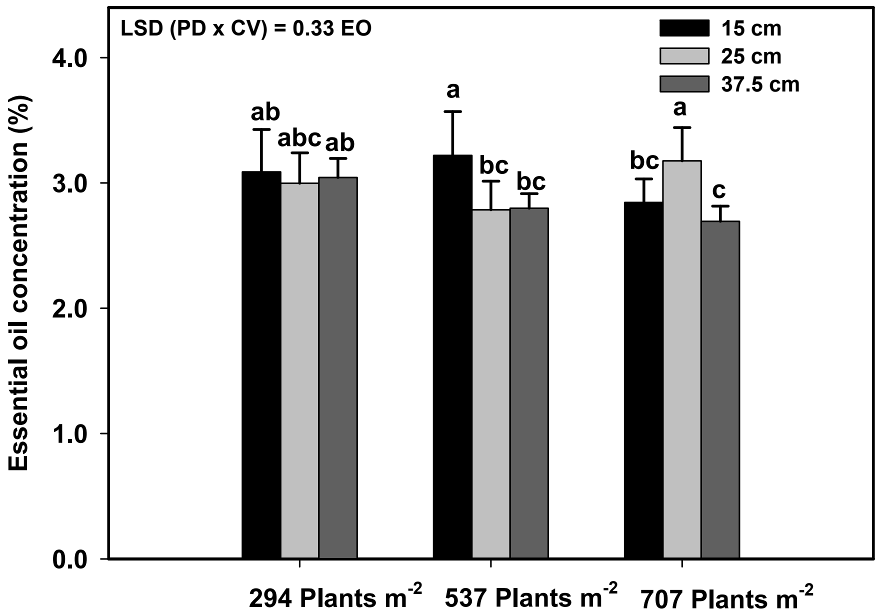


Fig. 5.17: Effect of row spacing (RS) and planting densities (PD) on essential oil concentration (%) of anise at experimental station Gross-Gerau 2009

Higher concentration of essential oil was measured in 2009. Essential oil yield is a trait that depends on the fruit yield and essential oil content. During 2009, essential oil yield was affect significantly by different planting densities. However averaged over years, it ranged from 12.5 to 34.1 kg/ha. Plant density of 294 plants m^{-2} achieved significant higher essential oil yield of 34.1 kg/ha as compared to 537 and 707 plants m^{-2} (table 5.21).

Quantitative study showed that plant density and row spacing had no effect on the percentage of *trans*-anethole, γ-himachalene and estragol in anise essential oil in 2008 (table 5. 22).

Table 5.22: 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 Gross-Gerau 2008-09

RS	PD	Gross-Gerau 2008				Gross-Gerau 2009			
		Plants m^{-2}	ES	GH	TA	Plants m^{-2}	ES	GH	TA
			%	%	%		%	%	%
1		415	0.38a	5.4	91.9a	562	0.51a	5.5	91.4 a
2		342	0.39a	5.5	91.8a	520	0.51a	5.6	91.4a
3		284	0.41a	5.6	91.5a	456	0.51a	5.8	91.1b
	1	209	0.40a	5.6	91.5a	294	0.49b	5.6	91.3ab
	2	333	0.39a	5.5	91.9a	537	0.50b	5.5	91.4a
	3	499	0.39a	5.5	91.8a	707	0.54a	5.7	91.2b
LSD (5%)									
RS			ns	ns	ns		ns	0.1	0.2
PD			ns	ns	ns		0.01	0.1	0.2
RS X PD			ns	ns	ns		ns	ns	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm

The compound estragol which can be characterized as the chemical compound methyl chavicol considered as a relevant quality component was influenced by plant density treatments in 2009 (table 5. 22). 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 of those in 2009. Increasing trend of estragol concentration was observed as planting density increased in 2009. Significant higher concentration of estragol (0.54%) was found with planting density of 707 plants m^{-2} whereas significant lower value (0.49%) was observed in planting density of 294 plants m^{-2} in 2009 (table 5.22). Comparable concentrations of γ-himachalene were detected in both seasons. In both years concentration of γ-himachalene increased as row spacing narrowed. Increasing trend of γ-himachalene was noticed as plant density increase in 2009. Significant higher concentration of γ-himachalene 5.7 and 5.7% was caused with planting density of 707 plants m^{-2} and with 37.5 cm row spacing. Significant variation was evident for *trans*-anethole among treatments in 2009. The *trans*-anethole content in the essential oil of anise varied from 91.1 to 91.9% in both years. In 2009, narrow row spacing of (15 cm) had significant higher concentration of *trans*-anethole (91.4%) whereas significant lower concentration (91.1%) was recorded in wider row spacing of (37.5 cm) (table 5.22). Planting densities of 294 and 537 plants m^{-2} were produced significant higher concentrations of *trans*-anethole 91.3 and 91% respectively in comparison with plant density of 707 plants m^{-2} which produced 91.2%.

5.2.2 Field experiments Giessen 2008-2009

5.2.2.1 Disease and lodging assessment

Data regarding *Cercospora* leaf blight presented in Fig. 5.18. Lower disease rate was observed in 2008 compared with 2009. In 2008 disease rating varied from 2.2 to 2.9 from used treatments. Lower level of disease (2.2) was observed plants grown in wider row spacing of 37.5 cm compared with other row spacing treatments in 2008.

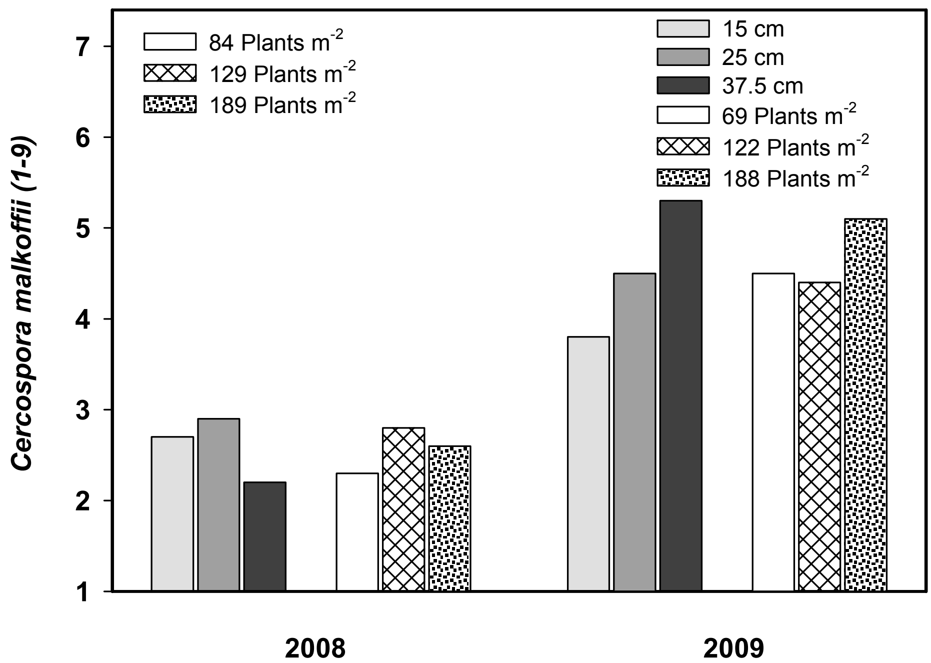


Fig. 5.18: Effect of row spacing and plant densities on *Cercospora malkoffii* (1-9) on anise at experimental station Giessen 2008-09

It was noticed from data that disease level increased as plant densities increased. Higher disease level of *Cercospora malkoffii* was observed from plant densities of 129 and 189 plants m^{-2} compared with 84 plants m^{-2} in 2008 (Fig. 5.18). Higher plant density may increase relative humidity within the canopy and increase the duration of leaf wetness by reducing air movement and sun light penetration which increased disease level.

Opposite results were noticed in 2009 compared with 2008 regarding row spacing treatments. Disease rating on anise plants varied from 3.8 to 5.3 and 4.4 to 5.1 concerning row spacing and plant densities respectively (Fig. 5.18). In 2009, higher disease infection was recorded from plants grown under wider row spacing compared with closed spaced row. It might be a reason that higher lodging was estimated in wider row spacing treatments. Highest disease level of 5.1 was recorded from planting densities of 188 plants m^{-2}. In 2009, lodging was also estimated which ranged from 2.8 to 7.7 in row spacing and plant densities treatments (Fig. 5.19). Increasing trend of disease was observed as distance between the row increases. Highest lodging was recorded from plant grown under wider row spacing of 37.5 cm compared with closed spaced row of 15 cm treatments. Higher level of lodging was observed in narrow plant densities as compared with lower plant densities.

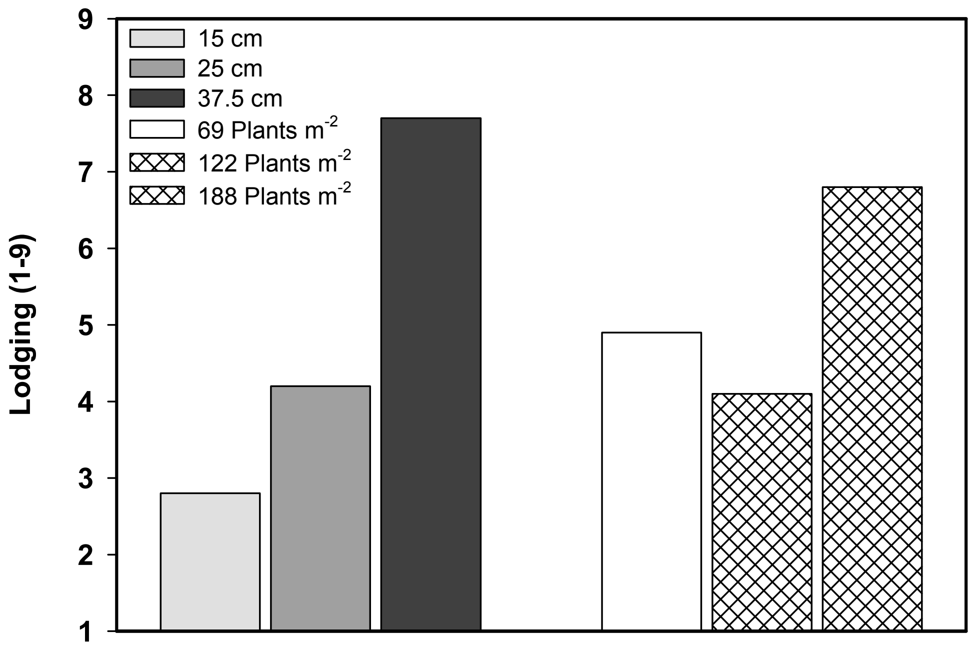


Fig. 5.19: Effect of row spacing and plant densities on lodging (1-9) in anise at experimental station Giessen 2009

5.2.2.2 Growth and fruit yield parameters

Data presented in table 5.23 show that different sowing rates led to differ levels of plant densities determined after germination with 84, 129, 189 and 69, 122, 188 plants m^{-2} respectively in the years 2008 and 2009 (table 5.23). Similar trend of plant densities was observed in both years. However the soil conditions in Giessen induced strong reduction of plant density compared with Gross-Gerau. In both station there was a contrary effect of sowing rate on fruit yield of anise. The results presented in table 5.23 revealed that plant height of anise at maturity was not affected by different row spacing treatments in both seasons.

Averaged over the years anise plant height ranged from 45 to 66 cm. Independent of that plant height decreased in wider row spacing. In 2009, plant height was affected by different planting densities. Maximum plant height of anise 66 cm was recorded by plant density of 69 plants m^{-2} whereas minimum plant height of 61 cm was attained with plant density of 188 plants m^{-2} (table 5.23). A decreasing trend of plant height was noticed with increasing plant densities. Averaged of the year's anise plants were taller in 2009. 1000-fruit weight (TFW) of anise was affected by planting densities as well as by used row spacing in 2008. 1000-fruit weight of anise varied from 2.57 to 3.23 g in both seasons (table 5.23). Significant higher 1000-fruit weight of 2.78 and 2.76 g was induced by 37.5 cm row spacing and plant density of 189 plants m^{-2} respectively in 2008. 1000 fruit weight was not affected by plant densities and used row spacing in 2009. Overall higher TFW was observed in 2009. Different row spacing had significant effect on the fruit yield anise in 2008; opposite to that fruit yield was not affected by row spacing as well as used plant densities in 2009.

Table 5.23: Effect of row spacing (RS) and planting densities (PD) on plant height (PH) (cm), 1000-fruit weight (TFW) (g) and fruit yield (FY) (dt/ha) of anise (*Pimpinella anisum* L.) at experimental station Giessen 2008-09

RS	PD	Giessen 2008				Giessen 2009			
		Plants m^{-2}	PH	TFW	FY	Plants m^{-2}	PH	TFW	FY
			cm	g	dt/ha		cm	g	dt/ha
1		210	47a	2.63b	8.3a	146	62a	3.20a	6.8a
2		111	47a	2.62b	7.2b	112	63a	3.00a	5.9a
3		81	46a	2.78a	3.0c	120	63a	3.07a	5.5a
	1	84	48a	2.70a	6.3a	69	66a	3.03a	5.9a
	2	129	46a	2.57b	5.9a	122	62b	3.23a	6.5a
	3	189	45a	2.76a	6.3a	188	61b	3.00a	5.8a
LSD 5%									
RS			ns	0.1	0.9		ns	ns	ns
PD			ns	0.1	ns		2.2	ns	ns
CV X PD			ns	ns	ns		ns	ns	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm

Fruit yield of anise ranged from 3.0 to 8.3 dt/ha in both seasons (table 5.23). Maximum fruit yield of 8.3 dt/ha was recorded in 15 cm row spacing followed by 7.2 dt/ha in 25 cm while minimum fruit yield of 3.0 dt/ha was in 37.5 cm row spacing (table 5.23). In both years higher fruit yield was induced by close row spacing of 15 cm as compared to other row spacing.

5.2.2.3 Content, yield and composition of essential oil

As seen in table 5.24, the effect of row spacing was statistically significant for essential oil concentration of aniseed in 2008. Planting densities did not showed pronounced effect regarding essential oil accumulation in 2008. Contrary to that essential oil content was affected by plant densities as well as used row spacing. Averaged over years, it ranged from 2.77 to 3.73% (table 5.24). In 2008, 25 cm row spacing led to significant higher concentration of essential oil as compared to other row spacing. On the contrary, 37.5 cm row spacing was synthesized significant higher concentration whereas minimum essential oil of 3.24% was accumulated by 15 cm row spacing in 2009. Increasing trend of essential oil was observed as row spacing increased in 2009 (table 5.24). Higher concentration of essential was synthesized in 2009 as compared to 2008. Essential oil accumulation decreased as planting densities increased. Lower essential oil contents of 2.77 and 3.27% were observed under the planting densities of 189 and 188 plants m^{-2} respectively in 2008 and 2009 (table 5.24). 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.

Table 5.24: 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

RS	PD	Giessen 2008			Giessen 2009		
		Plants m^{-2}	EO	EOY	Plants m^{-2}	EO	EOY
			%	Kg/ha		%	Kg/ha
1		210	2.83ab	23.6a	146	3.24b	22.3a
2		111	3.00a	21.4a	112	3.43b	20.3a
3		81	2.80b	8.6b	120	3.73a	20.7a
	1	84	3.07a	19.2a	69	3.53a	20.8a
	2	129	2.80a	16.7a	122	3.59a	23.5a
	3	189	2.77a	17.6a	188	3.27b	19.0
LSD (5%)							
RS			0.2	3.2		0.2	ns
PD			ns	ns		0.2	ns
CV X PD			ns	ns		ns	ns

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm

As seen in table 5.24 essential oil yields of anise varied between 8.6 and 23.6 kg/ha from different used treatments (table 5.24). Significant higher essential oil yield of 23.6 kg/ha was recorded with 15 cm row spacing whereas significant lower yield of 8.6 kg/ha was noticed under 37.5 cm row spacing (table 5.24). In fact, essential oil yield associated directly with fruit yield.

Data presented in table 5.25 show that quality parameters of anise fruits including estragol, γ-himachalene and *trans*-anethole were significantly affected by different row spacing treatments in 2008. Averaged over the years, the concentration of estragol in the essential of anise varied from 0.51 to 0.61%. Similar level of estragol concentration was observed in both years. Plant grown in 37.5 cm row spacing led to significant higher concentrations of estragol and *trans*-anethole 0.61 and 91.3% respectively in 2008. Averaged over years γ-himachalene concentration ranged from 5.6 to 7.4%. Significant higher concentration of γ-himachalene 6.3% was induced by 15 cm row spacing whereas lower concentration 5.6% was found with wider row spacing 37.5 cm in 2008 (table 5.25). Similar results regarding γ-himachalene were observed in 2009. Overall higher concentration of γ-himachalene recorded in 2009. Estragol concentration was influenced by both study factors row spacing as well as plant densities in 2009.

Table 5.25: 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

RS	PD	Giessen 2008				Giessen 2009			
		Plants m^{-2}	ES	GH	TA	Plants m^{-2}	ES	GH	TA
			%	%	%		%	%	%
1		210	0.57a	6.3a	90.5b	146	0.55ab	7.3ab	88.8ab
2		111	0.52b	6.1a	90.6ab	112	0.57a	7.4a	88.6b
3		81	0.61a	5.6b	91.3a	120	0.52b	6.9b	89.4a
	1	84	0.57a	5.9a	90.9a	69	0.59a	7.3a	88.4b
	2	129	0.57a	6.0a	90.6a	122	0.54b	7.2a	88.9ab
	3	189	0.56a	6.0a	90.9a	188	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

RS1: 15 cm, RS2: 25 cm, RS3: 37.5 cm

Significant higher concentration of estragol 0.59% was noticed with planting density of 69 plants m^{-2} whereas significant lower value 0.51% was observed in planting density of 294 plants m^{-2} in 2009 (table 5.25). In various row spacing, 37.5 cm row spacing led to produced significant higher 89.4% concentration of *trans*-anethole as compared to other row spacing. Plant density of 188 plants m^{-2} led to significant higher concentrations of *trans*-anethole (89.4%) in comparison with plant density of 69 plants m^{-2} which produced 88.4% (table 5.25). Higher concentration of *trans*-anethole was observed in 2008 as compared to 2009. There was no interaction regarding these study parameters. Table 5.26 depicts that total 17 components were identified in the essential of cultivar Enza Zaden. 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 5.26: 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

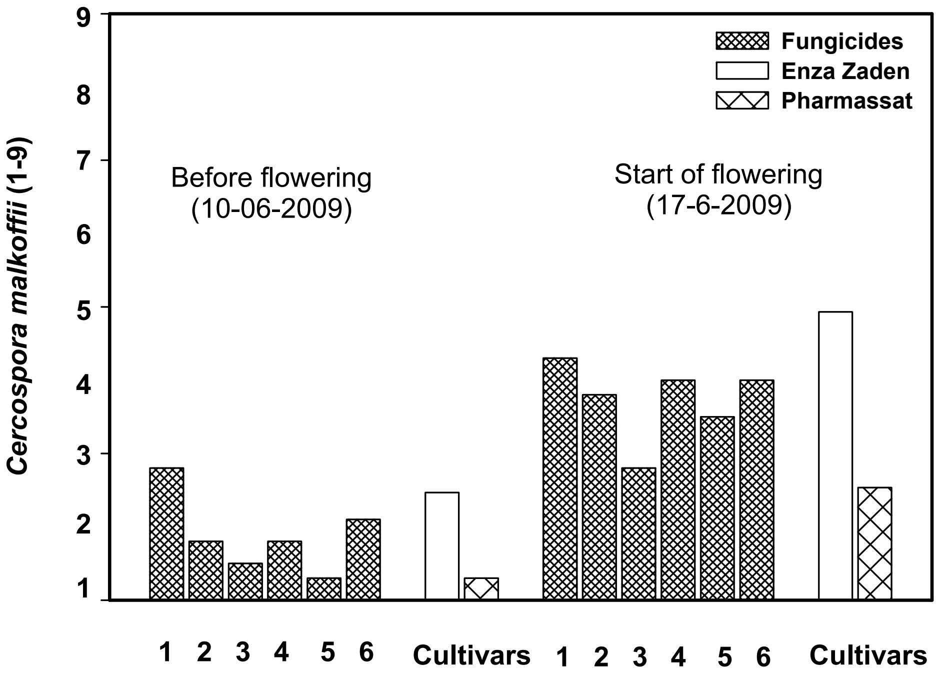
*KI: Kovat´ s retention index

5.3 Effect of fungicide application

5.3.1 Field experiment Gross-Gerau 2009

5.3.1.1 Disease and lodging assessment

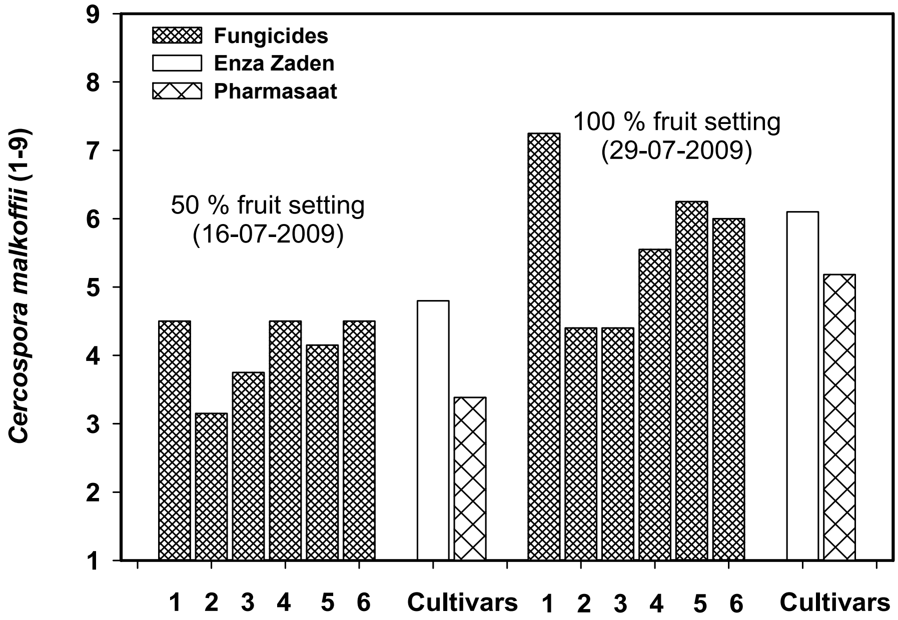
Visual fungal disease incidence of *Cercospora malkoffii* was assessed at different stages of anise plant development. There was no disease incidence (1: without infection) during early stages of anise plant development till 20-05-2009. However first infection with *Cercospora* was observed during the last week of May. Fungicides applications were sprayed on anise plants before start of flowering. First application was done on 1st week of June and second two weeks later. *Cercospora malkoffii* fungus was recorded after application of fungicides before flowering stage which reached a level of 1-2. Higher disease infection was observed where no fungicide was applied as compared to other treatments. Before the flowering stage treatments no. 2 and 4 had similar infection level. When weather conditions become favorable for fungus then disease spread rapidly. As clear from below graph before flowering disease infection was reached a level of 1.2 to 1.9 within all plots (Fig. 5.20). Fosetyl application led to decline disease infection as compared to other treatments. At the start of flowering stage infection level varied from 1.9 to 2.7. After application of fungicide treatment with Azoxystrobin + Difenoconazol (no. 3) disease infection was reduced as compared to other treatments.



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

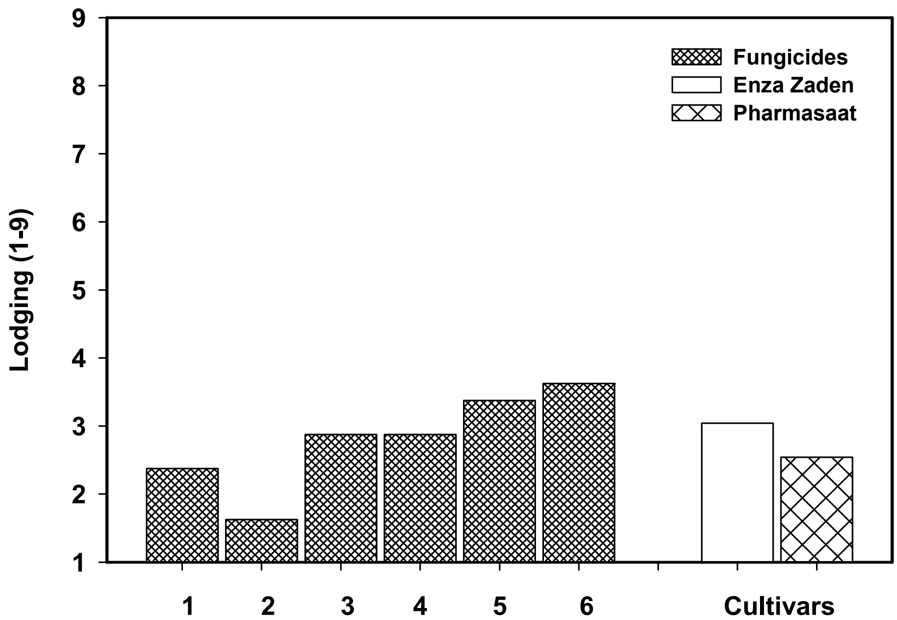
Fig. 5.20: Effect of different fungicides on *Cercospora malkoffii* (1-9) at two stages of anise Gross-Gerau 2009

At the start of flowering stage cv. Enza Zaden was more susceptible with disease level of (3) as compared to cv. Pharmasaat (1.8). At the 50% fruiting stage anise plants were infected by the fungal pathogen in a range from 3.1 to 4.5 (level) in 2009 (Fig. 5.21). During the 50% fruit setting stage, minimum fungal infections (3.2 level) and (3.8 levels) were noticed with application of Mancozeb + Metalaxyl-M (no. 2) and Azoxystrobin + Difenoconazol (no. 3) respectively as compared to other treatments (Fig. 5.21). At the 50% fruit setting stage treatments no. 1, 4 and 6 had similar level of infection within all plots. During the last assessment which was carried out at 100% fruit setting stage higher fungal infection (7.3) was recorded from untreated anise plant as compared to treated plants (Fig. 5.21). Application of treatments Mancozeb + Metalaxyl-M (no. 2) and Azoxystrobin + Difenoconazol (no. 3) were minimizing the disease infection and enhance the photosynthetic area of anise plants and improve anise plant development. Overall in 2009 cv. Enza Zaden was more susceptible as compared to cv. Pharmasaat.



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Fig. 5.21: Effect of different fungicides on *Cercospora malkoffii* (1-9) at two stages of anise Gross-Gerau 2009



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Fig. 5.22: Effect of different fungicides on lodging (1-9) of two anise cultivars at experimental station Gross Gerau 2009

Lodging rate was estimated before the harvesting, and its value ranged from 1.6 to 3.6 during this experiment (Fig. 5.22). Maximum lodging of (3.6) was noted after application of Fosetyl by other treatments which were followed by Propamocarp with lodging rate of (3.4). Anise plants treated with Azoxystrobin + Difenoconazol and Mancozeb + Diemethomorph showed similar results of lodging. Anise plants treated with fungicidal application of Mancozeb + Metalaxyl-M reduced the rate of lodging over other fungicidal treatments. Cv. Enza Zaden was more susceptible to lodging than cv. Pharmasaat (Fig. 5.22).

5.3.1.2 Growth and fruit yield parameters

In 2009 anise plants reached a plant height of around 70 cm (68 to 72 cm) (table 5.27). The application of fungicides did not cause any significant differences between the treated and untreated plants regarding plant height. The used cultivars had no significant differences in plant height. In field experiment 2009 number of primary branches per plant (PBP) ranged between 2.8 and 4.0 (table 5.27). However highest number of PBP (4.0) was found in treatment 3 which was applied with Azoxystrobin + Difenoconazol followed by no. 4 (Mancozeb + Diemethomorph). Minimum number of 2.8 primary branches per plant was recorded where no fungicide was applied (table 5.27).

Table 5.27: Effect of different fungicides (Fu) and cultivars (Cv) on plant height (PH) (cm), primary branches per plant (PBP), secondary branches per plant (SBP) and umbels number per plant (UNP) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau during growing season 2009

Fu.	PH			PBP			SBP			UNP		
	cm			no			no			no		
	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean
1	68	67	68a	2.8	3.5	3.2a	0.3	0.6	0.5a	4.1	5.1	4.6a
2	69	72	70a	3.0	3.4	3.2a	0.3	0.6	0.5a	4.3	5.0	4.7a
3	72	70	71a	3.4	4.0	3.7a	0.5	1.2	0.8a	4.9	6.2	5.5a
4	70	69	69a	3.7	3.6	3.6a	0.8	0.3	0.5a	5.4	4.9	5.2a
5	70	71	71a	3.4	3.7	3.5a	0.6	0.9	0.7a	4.9	5.7	5.3a
6	69	72	71a	3.3	3.6	3.4a	0.5	0.4	0.4a	4.7	5.0	4.9a
Mean	70a	70a		3.3b	3.6a		0.5a	0.7a		4.7b	5.3a	
LSD 5%	ns			CV: 0.5			ns			CV: 0.5		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Despite that it must be stated that fungicidal treatments did not show significant differences in primary branches per plant of anise. Contrary to that a significant difference was found between both cultivars which were used in this trial. The cultivar

Pharmasaat had highest number of primary branches per plant averaging 3.6 whereas cv. Enza Zaden was characterized by lower number of primary branches per plant of 3.3 branches (table 5.27). Secondary branches per plant (SBP) which varied from minimal 0.3 to maximal 1.2 showed no significant differences regarding applied fungicides and used cultivars in 2009. Only small and non significant differences could be observed between the fungicides and cultivars. Umbels number per plant (UNP) of anise ranged from 4.1 to 6.2 (table 5.27). There was a tendency of lower umbel number per plant in treatments without fungicide application but no clear effect of applied fungicides was found. In contrary to that there were statistically significant differences between the cultivars. Cv. Pharmasaat recorded higher number of umbels per plant (5.3), whereas significant lower UNP of 4.7 umbels was produced by cv. Enza Zaden.

As shown in table 5.28 the cultivars differed significantly with respect to fruit number per plant (FNP) and fruit weight per plant (FWP) in 2009. In comparison with cv. Enza Zaden, cv. Pharmasaat was characterized by much higher number of fruits (76) and higher fruit weight (0.23 g) which led to higher fruit yield of this cultivar (5.28). The number of fruits per plant and the fruit weight per plant ranged from 41 to 109 fruits and from 0.10 to 0.37 g respectively but this variation were not significantly induced by applied fungicides (table 5.28).

Table 5.28: Effect of different fungicides (**Fu**) and cultivars (**Cv**) on fruits number per plant (**FNP**), and fruit weight per plant (**FWP**), 1000-fruit weight (**TFW**) and fruit yield (**FY**) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau during growing season 2009

Fu.	**FNP**			**FWP**			**TFW**			**FY**		
	no			g			g			dt/ha at 91%		
	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean
1	41	79	60a	0.11	0.23	0.17a	2.35	2.28	2.31a	6.3	7.5	6.9b
2	60	81	71a	0.17	0.24	0.20a	2.38	2.52	2.45a	9.3	10.9	10.1a
3	62	109	86a	0.18	0.37	0.27a	2.04	1.83	1.94c	9.8	9.3	9.5a
4	76	62	69a	0.21	0.17	0.19a	1.98	2.05	2.02bc	7.5	8.2	7.9b
5	41	59	50a	0.1	0.17	0.14a	2.27	2.18	2.22ab	7.1	7.3	7.2b
6	54	62	58a	0.14	0.18	0.16a	1.99	2.04	2.02bc	7.2	7.2	7.2b
Mean	56b	76a		0.15b	0.23a		2.17a	2.15a		7.9a	8.4a	
LSD 5%	CV: 17			CV: 0.06 g			FU: 0.28 g			FU: 1.1 dt/ha		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Results show that some of the applied fungicides had positive effect on the thousand fruit weight (TFW); whereas no effect was observed regarding used cultivars (table

5.28). TFW of anise plants ranged from 1.83 to 2.52 g. The application of Mancozeb + Metalaxyl-M (no. 2) led to maximum TFW of 2.45 g, whereas significant lower TFW of 1.94 g was noticed by application of Azoxystrobin + Difenoconazol in treatment no. 3 (table 5.28). Fungicidal application with Mancozeb + Metalaxyl-M (no. 2) induced higher fruit yield (10.1 dt/ha) while significant lower fruit yield of 6.9 dt/ha was obtained by control as compared with the other treatments in 2009 (table 5.28). The tested cultivars had not pronounced affect regarding fruit yield.

5.3.1.3 Content, yield and composition of essential oil

Table 5.29 shows that applied fungicides and cultivars caused non significant differences regarding essential oil accumulation (EO) in 2009. Essential oil synthesized by anise plants within all treatments reached a level of 2.46 to 2.97% (table 5.29). Mancozeb + Metalaxyl-M (no. 2) application induced relatively higher concentration of essential oil as compared to other treatments. In present study cultivars exhibited similar results regarding essential oil accumulation. Contrary to essential oil concentration the yield of essential oil (EOY) was significantly influenced by fungicidal treatments in 2009 (table 5.29). Essential oil yield is a trait which directly depended on both essential oil concentration as well as fruit yield. Maximum EOY of 27.4 kg/ha (p= 0.00) was attained by application of Mancozeb + Metalaxyl-M (no. 2) which was significant higher in comparison with control (18.5 kg/ha) but similar to treatment no. 3 (24,3 kg/ha) (table 5.29). Fungicide treatments of no. 4, 5 and 6 had similar EOY to each other and in comparison with the control but lower performance in comparison with the maximal EOY of treatment no. 2.

Table 5.29: Effect of different fungicides (Fu) and cultivars (Cv) on essential oil (EO) and essential oil yield (EOY) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau during growing season 2009

Fu.	EO			EOY		
	%			%		
	Cv1	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**
1	2.6	2.74	2.67a	16.5	20.5	18.5c
2	2.97	2.50	2.74a	27.5	27.3	27.4a
3	2.59	2.46	2.53a	25.6	23.0	24.3ab
4	2.62	2.60	2.61a	19.7	21.5	20.6bc
5	2.62	2.57	2.60a	18.7	18.5	18.6c
6	2.62	2.67	2.65a	19.0	19.2	19.1c
Mean	2.67a	2.59a		21.2a	21.7a	
LSD 5%	ns			FU: 3.6		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Table 5.30 shows that none of the fungicides which are applied had any significant impact on the quality parameters including estragol, γ-himachalene and *trans*-anethole in 2009. However estragol concentration showed significant variation between used cultivars in 2009.

Table 5.30: Effect of different fungicides (Fu) and cultivars (Cv) on estragol (ES) (%), gamma-himachalene (GH) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau in 2009

Fu.	ES			GH			TA		
	%			%			%		
	Cv1	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**
1	0.53	0.58	0.56a	5.8	6.0	5.9a	90.9	90.7	90.8a
2	0.51	0.55	0.53a	6.0	5.9	5.9a	90.7	90.9	90.8a
3	0.53	0.56	0.55a	5.7	5.8	5.8a	90.9	90.9	90.9a
4	0.54	0.56	0.55a	5.8	6.0	5.9a	90.9	90.7	90.8a
5	0.53	0.55	0.54a	6.0	6.0	6.0a	90.6	91.0	90.8a
6	0.54	0.56	0.55a	5.4	5.9	5.7a	91.5	90.8	91.2a
Mean	0.53b	0.56a		5.8a	5.9a		90.9a	90.8a	
LSD $5_{\%}$	CV: 0.02			ns			ns		

Cv 1: Enza Zaden, Cv 2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

The concentration of estragol in the essential varied from 0.51 to 0.58% and was lower in fruit samples of cv. Enza Zaden (0.53%) in comparison with cv. Pharmasaat (0.56%) (table 5.30). Quantitative study showed that applied fungicides had no effect on the percentage of *trans*-anethole, which varied from 90.7 to 91.5% (table 5.30). Averaged over the cultivars maximal concentration of *trans*-anethol (91.2%) was found in treatment no. 6 but without significant differences to the other treatments (table 5.30).

Fig. 5.23: Symptoms of disease infection on basal leaves of anise plants, Gross Gerau 2009

Fig. 5.24: Necrotic spots on underside of anise leave

Fig. 5.25: Disease symptom on inflorescence of anise plants, Gross-Gerau 2009

Fig. 5.26: Infected anise plants with reduced growth

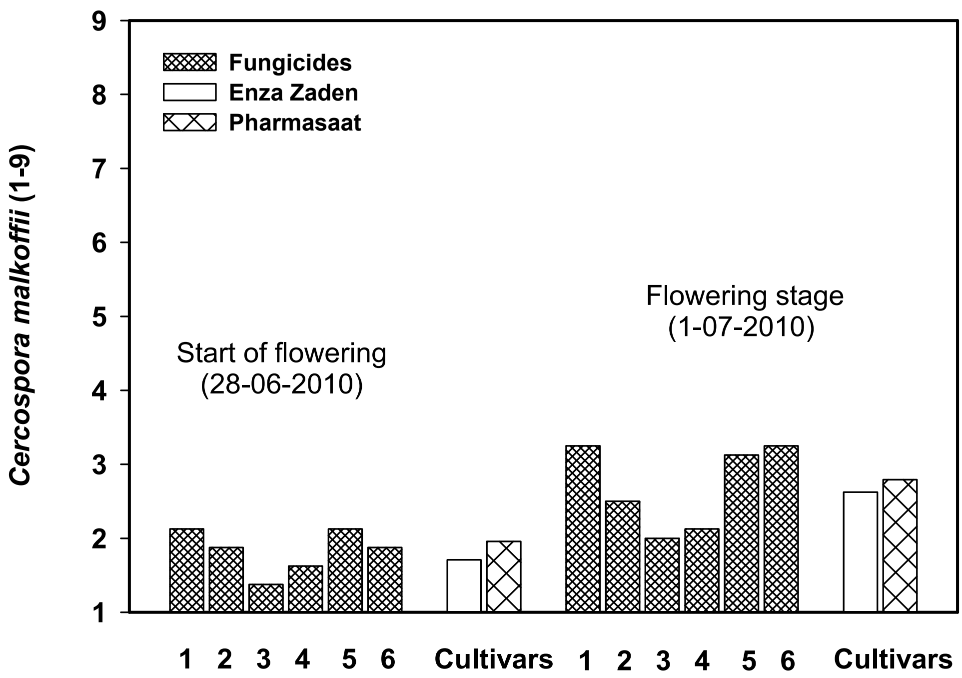
Fig. 5.27: Lash green field of anise at experimental station Gross-Gerau 2009

Fig. 5.28: Anise plants under stress conditions caused by nitrogen deficiency at Gross-Gerau 2010

5.3.2 Fungicide experiment Gross Gerau 2010

5.3.2.1 Disease and lodging assessment

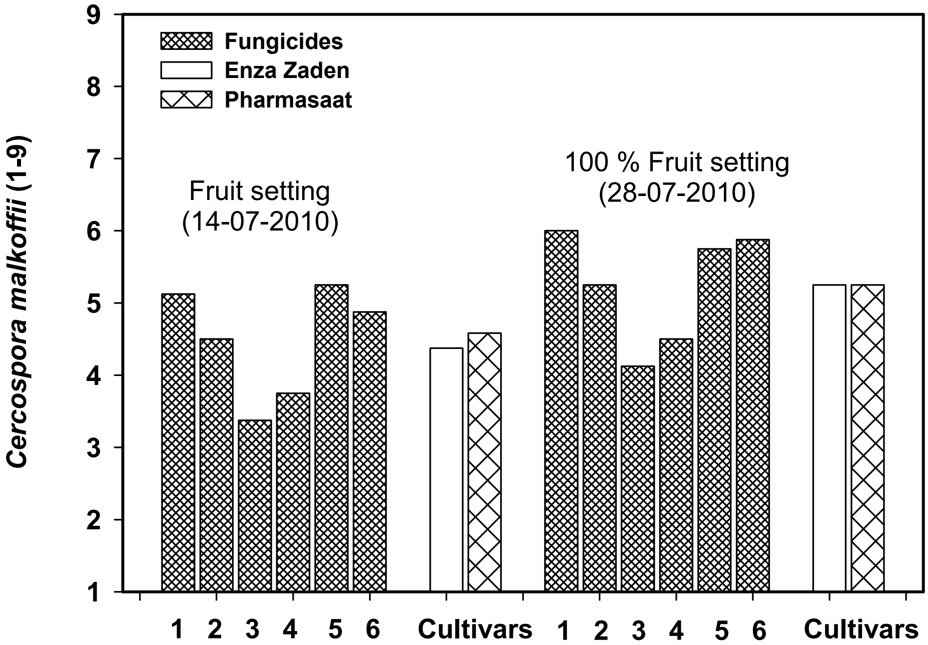
In 2010, first disease evaluation was performed on 7-06-2010 after fungicide application before the flowering period. During 2010, first time fungicides were applied on 1-06-2010 and second application was performed after 14 days. At the start of flowering infection level varied from 1.0 to 2.3 within all plots (Fig. 5.29). Application of Azoxystrobin + Difenoconazol (no. 3) and Mancozeb + Diemethomorph (no. 4) led to reduced infection before flowering stage level as compared to other treatments. Higher anise leaf blight was observed where no fungicides were applied. During early stages of anise cultivars cv. Pharmasaat was more susceptible as compared to cv. Enza Zaden. During the flowering stage fungicidal treatments no. 3, 4 and 2 led to decline infection level as compared to other treatments. At the flowering stage disease level reached a maximum level (3.3) of 2.0 to 3.3 (Fig. 5.29). Higher disease severity was found where no fungicides were applied on anise plants (Fig. 5.29). Next *Cercospora* disease assessment was done on fruit setting stage (14-7-2010) of anise and disease prevalence was increased in treatments no. 5, 6 and in control plots as compared to other treatments. Application of fungicidal treatments Azoxystrobin + Difenoconazol (no. 3) and Mancozeb + Diemethomorph (no. 4) were maximizing the photosynthetic area by reducing infection level.



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

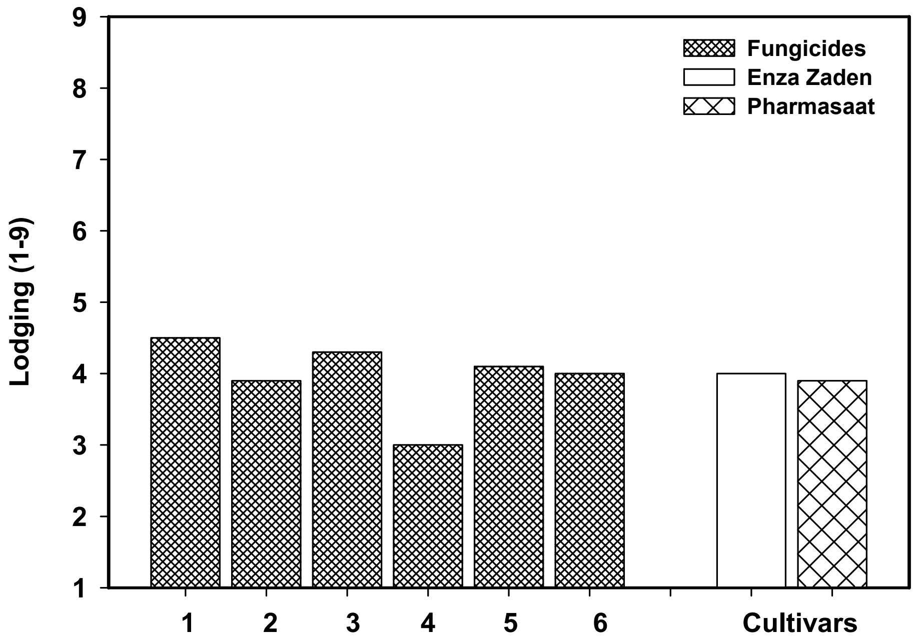
Fig. 5.29: Effect of different fungicides on *Cercospora malkoffii* (1-9) at two stages of anise Gross-Gerau 2010

Application of fungicidal treatment of Azoxystrobin + Difenoconazol (no. 3) gave better results in both seasons (Fig. 5.30).



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Fig. 5.30: Effect of different fungicides on *Cercospora malkoffii* (1-9) at two stages of anise Gross-Gerau 2010



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Fig. 5.31: Effect of different fungicides on lodging (1-9) of two anise cultivars at experimental station Gross Gerau 2010

At the 100% fruit setting anise plants were infected by the fungal pathogen in a range from 4.1 to 6.0 (level) in 2010 (Fig. 5.30). Lowest infection rates 4.1 and 4.3 were recorded after application of treatments Azoxystrobin + Difenoconazol (no. 3), and Mancozeb + Diemethomorph (no. 4) respectively in 2010. Treatments no. 5, 6 and 1 (control) had similar level of infection within all plots. Anise plants were infected by the fungal pathogen *Cercospora malkoffii* in a range from 1.2 (before flowering) to 7.3 (100% fruit setting) and 1.4 (start of flowering) to 6.0 (100% fruit setting) (level) respectively in 2009 and 2010, which led to brown colored leaf spots. Overall higher fungal infection was assessed in 2009 as compared to 2010.

In 2010, fungicidal treatments reduced lodging clearly compared with untreated control (Fig. 5.31). However lodging rate varied from 3.0 to 4.5. Minimum lodging (3.0) was recorded from those plots which were treated with Mancozeb + Diemethomorph followed by Mancozeb + Metalaxyl-M compared with other fungicidal treatments. Anise plants treated with Propamocarp and Fosetyl had similar level of lodging rate. Relatively higher lodging was observed in cv. Enza Zaden compared with cv. Pharmasaat in 2010 (Fig. 5.31).

5.3.2.2 Growth and fruit yield parameters

In 2010 anise plants reached a plant height of around 53 cm (51 to 56 cm) (table 5.31). Neither the applied fungicides nor the cultivars caused significant differences regarding height of plant stand occurred in 2010. Overall taller plants were observed in 2009 in comparison with 2010. Significant differences ($p= 0.00$) among fungicide treatments for primary branches per plant occurred in 2010 (table 5.31). Primary branches per plant recorded by anise ranged from 3.0 to 4.2 in 2010. Azoxystrobin + Difenoconazol (no. 3) and Mancozeb + Diemethomorph (no.4) treated plants induced significant higher number of primary branches per plant (4.1and 3.7) respectively as compared to other treatments. After application of fungicidal treatments no. 2, 5, 6 and control had similar level of branches per plant.

Tested cultivars show similar pattern of primary branches per plant. Secondary branches per plant (SBP) of anise are characterized by lower number per plant but there are no significant differences regarding applied fungicides and used cultivars. Secondary branches per plant of anise ranged from 0.8 to 2.1 (table 5.31). Beside of these results Azoxystrobin + Difenoconazol (no.3) and Mancozeb + Diemethomorph (no.4) sprayed plants were induced higher number of secondary branches per plant in comparison with other treatments. Umbels number per plant (UNP) is important parameter for final fruit yield contribution for anise cultivation which varied in the executed experiment from 4.9 to 7.5 in 2010 (table 5.31). Azoxystrobin + Difenoconazol (no. 3) application led to maximum umbels number per plant (7.2) followed by Mancozeb + Diemethomorph (6.4); whereas significant lower number of umbels per plant (5.4) was observed where no fungicide was applied. Umbels number per plant was not affected by used cultivars. No interaction effect between both study factors was observed regarding these plant features.

Table 5.31: Effect of different fungicides (Fu) and cultivars (Cv) on plant height (PH) (cm), primary branches per plant (PBP), secondary branches per plant (SBP) and umbels number per plant (UNP) at experimental station Gross-Gerau in 2010

Fu.	PH			PBP			SBP			UNP		
	cm			no			no			no		
	Cv1	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**
1	55	52	55a	3.1	3.4	3.3bc	0.8	1.5	1.2a	4.9	5.8	5.4b
2	53	56	53a	3.3	3.1	3.2c	1.5	1.3	1.4a	5.9	5.4	5.6b
3	55	51	55a	4.1	4.2	4.1a	1.9	2.3	2.1a	7.0	7.5	7.2a
4	51	55	51a	3.9	3.6	3.7ab	1.9	1.4	1.7a	6.8	6.0	6.4ab
5	55	52	55a	3.5	3.0	3.3bc	1.4	1.0	1.2a	5.9	5.1	5.5b
6	51	56	51a	3.4	3.4	3.4bc	1.5	1.1	1.3a	5.9	5.5	5.7b
Mean	53a	54a		3.6a	3.4a		1.5a	1.4a		6.1a	5.9a	
LSD 5%	ns			FU: 0.5			ns			FU: 1.1		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2 kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3 kg/ha)

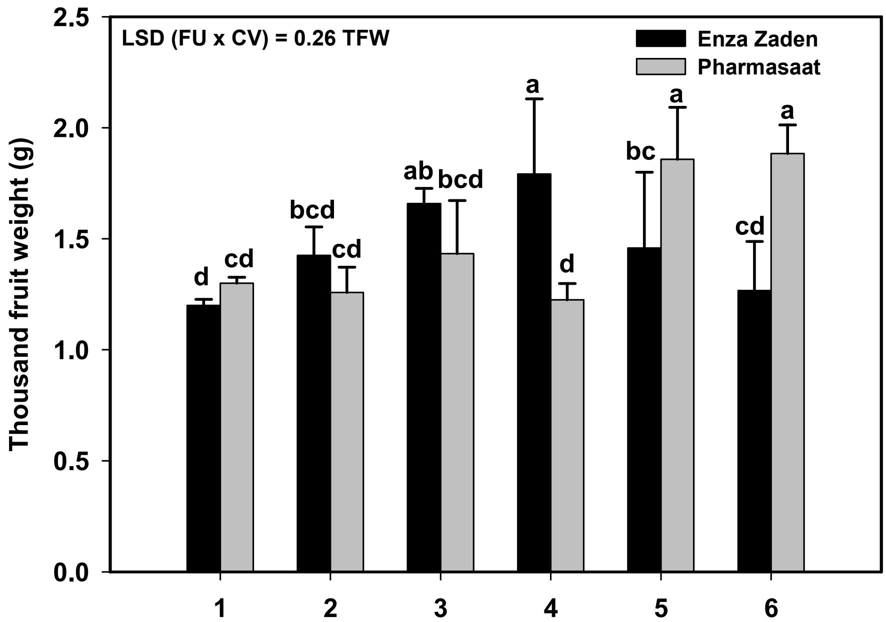
Data presented in table 5.32 show that applied fungicides had pronounced effect on the fruit number per plant (FNP) and on fruit weight per plant (FWP) of anise in 2010. After application of fungicidal treatments fruit number per plant and fruit weight per plant were ranged from minimal 29 to maximal 145 and from minimal 0.05 g to maximal 0.25 g respectively. These fruit yield parameters were not affected by used cultivars. Maximum fruit number per plant (145) and fruit weight per plant (0.25 g) were obtained by application of Azoxystrobin + Difenoconazol (no. 3) followed by Mancozeb + Diemethomorph (no. 4) with 82 fruits/plant and 0.14 g fruit weight whereas treatments no 1, 2 and 6 had similar fruits number and fruit weight per plant. Propamocarp (no. 5) application had vice versa effect on these parameters (29 fruit/plant, 0.05 g fruit weight) (table 5.32).

Data in table 5.32 indicate that there were significant differences among fungicidal treatments regarding thousand fruit weight (TFW) which varied from 1.20 to 1.88 g. Highest TFW of 1.66 g was noticed after application of Propamocarp (no. 5), whereas significant lower value of 1.20 g and 1.34 g were observed in case of control and with application of Mancozeb + Metalaxyl-M. (table 5.32). Treatments no. 3, 4, 5 and 6 led to significant higher but similar level of TFW as compared to other treatments. There was an interaction between fungicides and used cultivars regarding TFW in 2010 which is presented in (Fig. 5.32). Fruit yield of anise was affected by various applied fungicides in 2010. Fungicidal treatment Azoxystrobin + Difenoconazol (no. 3) induced higher fruit yield of 6.3 dt/ha followed by Mancozeb + Diemethomorph (no.4) which attained fruit yield of 4.6 dt/ha while lower fruit yield of 3.5 dt/ha was obtained by Propamocarp (no. 5) application as compared with the other treatments (table 5.32). The tested cultivars had no pronounced effect regarding fruit yield. Plants of the fungicidal treatments no. 1, 2, 5 and 6 had statistically similar pattern of fruit yield.

Table 5.32: Effect of different fungicides (Fu) and cultivars (Cv) on fruits number per plant (FNP), and fruit weight per plant (FWP), thousand fruit weight (TFW) and fruit yield (FY) at experimental station Gross-Gerau in 2010

Fu.	FNP			FWP			TFW			FY		
	no			g			g			dt/ha at 91%		
	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean	Cv1	Cv2	Mean
1	51	77	64bc	0.07	0.12	0.10bc	1.20	1.30	1.25c	3.8	3.5	3.6c
2	77	43	60bc	0.12	0.06	0.09bc	1.43	1.26	1.34bc	3.9	3.8	3.9bc
3	102	145	124a	0.19	0.25	0.22a	1.66	1.43	1.55a	6.4	6.1	6.3a
4	97	67	82b	0.17	0.11	0.14b	1.79	1.23	1.51ab	4.5	4.7	4.6b
5	39	29	34c	0.07	0.05	0.06c	1.46	1.86	1.66a	3.8	3.1	3.5c
6	73	47	60bc	0.12	0.07	0.09bc	1.27	1.88	1.58a	4.0	4.2	4.1bc
Mean	73a	68a		0.12a	0.11a		1.47a	1.49a		4.4a	4.2a	
LSD 5%	FU: 31			FU: 0.06			FU: 0.2, FU X CV: 0.3			FU: 0.8		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl- M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb+ Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)



1: Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb + Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Fig. 5.32: Effect of different fungicides on 1000-fruit weight (g) of two anise cultivars at experimental station Gross-Gerau 2010

5.3.2.3 Content, yield and composition of essential oil

In 2010 fungicide application had significant impact regarding essential oil concentration among used treatments (table 5.33). However maximum essential oil concentration of anise synthesized within all treatments reached a level of 3.01% (2.37 to 3.01%) (table 5.33). CV. Pharmasaat synthesized significant higher concentration of essential oil (2.75%), whereas significant lower concentration of essential oil (2.61%) was accumulated by cv. Enza Zaden (table 5.33). All fungicide applications caused considerable changes in essential oil accumulation. Propamocarp (no. 5) application led to minimal concentration of essential oil (2.50%) of anise seeds and Azoxystrobin + Difenoconazol (no. 3) application enhanced essential oil accumulation (2.92%) it to maximal as compared to other treatments (table 5.33). Plants treated with fungicidal treatments no. 2, 4, 5 and control synthesized similar level of essential oil in anise fruits.

Essential oil yield is traits which directly depend on fruit yield and essential oil concentration. Maximum essential oil yield of 18.3 kg/ha (p= 0.00) was attained by application of Azoxystrobin + Difenoconazol (no. 3) followed by Mancozeb + Diemethomorph (11.8 kg/ha) and Fosetyl (11.7 kg/ha) which were significant higher in comparison with Propamocarp (no. 5) which produced 8.6 kg/ha (table 5.33).

Table 5.33: Effect of different fungicides (Fu) and cultivars (Cv) on essential oil (EO) and essential oil yield (EOY) (kg/ha) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau during growing season 2010

Fu.	**EO**			**EOY**		
	%			%		
	Cv1	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**
1	2.62	2.56	2.59b	10.0	9.0	9.5bcd
2	2.82	2.54	2.68b	11.0	9.8	10.4bcd
3	3.01	2.83	2.92a	19.4	17.1	18.3a
4	2.56	2.51	2.53b	11.6	11.9	11.8b
5	2.63	2.37	2.50b	9.8	7.4	8.6d
6	2.83	2.88	2.86a	11.3	12.2	11.7bc
Mean	2.75a	2.61b		12.2a	11.2a	
LSD $5_{\%}$	FU: 0.20, CV: 0.11			FU: 2.5		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb+ Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Table 5.34 shows that none of the fungicides had any significant impact on γ-himachalene concentration of anise in 2010. γ-himachalene concentration ranged from 3.1 to 3.8%. Cultivars showed non significant affect with respect to gamma-himachalene in 2010. The compound estragol which can be characterized as the chemical methyl chavicol considered as a relevant quality component was not found in the essential oil samples of anise in 2010. Overall higher concentration of γ-himachalene was noticed in 2009 as compared to 2010. It is obvious from results that the fungicides had pronounced effect on the quality parameter *trans*-anethole in 2010 (table 5.34). The concentration of *trans*-anethole in the essential oil varied from 93.5 to 95.0% in 2010. Application of Fosetyl (no. 6) led to significant higher concentration of *trans*-anethole (95.0%), whereas significant lower concentration (94.0%) was observed in control (no. 1) (table 5.34). Higher concentration of *trans*-anethole was observed in 2010 as compared to 2009. However *trans*-anethole concentration showed significant variation between used cultivars in 2010. *Trans*-anethole percentage was lower in fruit samples of cv. Pharmasaat (94.3%) in comparison with cv. Enza Zaden (94.6%) (table 5.34).

Table 5.34: Effect of different fungicides (Fu) and cultivars (Cv) on gamma-himachalene (GH) (%) and *trans*-anethole (TA) (%) of anise (*Pimpinella anisum* L.) at experimental station Gross-Gerau during growing season 2010

Fu.	**GH**			**TA**		
	%			%		
	Cv1	**Cv2**	**Mean**	**Cv1**	**Cv2**	**Mean**
1	3.5	3.8	3.6a	94.4	93.5	94.0b
2	3.3	3.4	3.3a	94.2	94.1	94.2b
3	3.3	3.3	3.3a	94.5	94.6	94.6ab
4	3.2	3.3	3.3a	94.8	94.5	94.7ab
5	3.4	3.5	3.4a	94.5	94.1	94.3ab
6	3.1	3.3	3.2a	95.2	94.8	95.0a
Mean	3.3a	3.4a		94.6a	94.3b	
LSD $5_{\%}$	ns			Fu: 0.8, CV: 0.01		

Cv1: Enza Zaden, Cv2: Pharmasaat, **1:** Control, **2:** Mancozeb + Metalaxyl-M (2kg/ha), **3:** Azoxystrobin + Difenoconazol (2x1.0 L/ha), **4:** Mancozeb+ Diemethomorph (2kg/ha), **5:** Propamocarp (3 L/ha), **6:** Fosetyl (3kg/ha)

Data presented in table 5.35 show the composition of used cultivars in current trials. Totally 16 and 15 components were identified in cultivars Enza Zaden and Pharmasaat respectively. In both cultivars the main constituent was *trans*-anethole which was more than 80% followed by γ-Himachalene around 6.0%. The minor constituents in the current experiments were estragol, *cis* anethole, cyclosativene, beta elemene, α-himachalene, γ-himachalene, α-amorphane, methylisoeugenol, α-zingiberene, β-himachalene, α-muurolene, β-bisabolene, β -sesquiphellandrene and spathulenol (table 5.35).

Table 5.35: Chemical composition (%) in essential oil of two anise cultivars Enza Zaden and Pharmasaat analyzed by GC-MS

Compound	*KI	Enza Zaden	Pharmasaat
		%	%
Estragol	1197	0.4	0.3
Cis anethole	1252	0.2	0.2
trans-anethole	1287	83.1	85.3
Elemene (delta)	1333	0.3	0.3
Cyclosativene	1367	0.1	0.0
Beta elemene	1388	0.1	0.1
α-himachalene	1449	0.6	0.6
γ-himachalene	1478	6.3	5.8
α-amorphane	1482	0.2	0.1
(E)-Methylisoeugenol	1489	0.2	0.2
α-zingiberene	1493	0.6	0.5
β-himachalene	1499	0.4	0.3
α-muurolene	1502	0.1	0.1
β-bisabolene	1506	0.3	0.2
β -sesquiphellandrene	1522	0.1	0.0
Spathulenol	1580	0.1	0.0
Unknown	1629	0.1	0.0
Unknown	1831	5.6	5.3
Unknown	1886	0.7	0.6
Unknown	2109	0.7	0.0
Mean		100	100
No. of identified compound		16	15

*KI: Kovat´ s retention index

6. Discussion

6.1 Effect of cultivar, plant density and sowing time

6.1.1 Fruit yield and yield components

In current study there was a strong variation of fruit yields of anise ranged from minimal 2.4 dt/ha (in early sowing GG 2008) until maximal 12.0 dt/ha (in early sowing at Giessen), which was caused by the treatments as well as by fungus infection (*Cercospora malkoffii*). Highest variation was observed in Giessen 2009 where fruit yields decreased by three weeks delayed sowing from 9.0 - 12.0 dt/ha to 4.2 to 4.8 dt/ha. This effect can be explained by shortening the juvenile phase (germination to flowering) of anise of 13 days which not only reduced the cumulative temperature but also caused an earlier beginning of reproductive phase of the plants. The reduction in development period due to delayed sowing may have reduced the vegetative growth of plants which results in smaller plants with lower branches and umbels per plant and lead to lower fruit number. Current results confirms the observations of Zehtab-Salmasi et al. (2001) who reported that lower anise fruit yield due to late sowing which was related to reduction in vegetative growth of the plants. It can be concluded that delayed sowing resulted in insufficient vegetative growth of anise and plants immediately responded to photoperiod which led to reduced plant length, lower number of umbels per plant and reduced fruit yield (Zolleh et al. 2009, Mirshekari, 2010).

Contrary to Giessen (Gie) 2009, nearly the same level of fruit yield in both sowing times was found in Gross Gerau (GG) 2008 as well as in GG 2009. In GG 2008, relatively higher fruit yield was obtained in delayed sowing time compared with early sown anise plants. The small differences in fruit yields between both sowing times can be explained by the disease infection with *Cercospora malkoffii*. The lower fruit yield of anise in early sowing time was related to higher infection rate of *Cercospora malkoffii* (level 6.2) compared with delayed sowing (infection level 4.6). Overall higher fungus infection was found at experimental station GG which might be explained by higher relative air humidity and higher air temperature during cultivation period of anise. Anyway in present study, a tendency of higher fungal infection was observed in narrow planting densities compared to lower plant densities. Higher plant density may increase relative humidity within the canopy and increase the duration of leaf wetness by reducing air movement and sun light penetration. Therefore it can be supposed that plant density could have significant impact on plant disease incidence in anise (Burdona and Chilvers, 1982, Copes and Scherm 2005).

For both experimental years, an increase in planting density reduced the fruit yield of anise. Current findings are different from the results of Tuncturk and Yildirm (2006), who evaluated different seed rates of (5, 10, 15 and 20 kg/ha) in field experiments with anise and showed a close positive relationship between seed rate and anise seed yield with an optimal seed rate of 15 kg/ha. The authors further reported that with application of high sowing rate of 20 kg/ha led to clear reduction of anise fruit yields. But current results agree with the previous work carried out in Germany which showed that narrow planting densities had negative impact on seed yield of anise (Yan et al. 2011). The plant densities of 189 and 374 plants m^{-2} produced highest fruit yields in 2008 and 2009, respectively at experimental station Gross-Gerau. Contrary to those, 39 plants m^{-2} achieved highest fruit yield at experimental station Giessen. These differences among the studies were probably related to lower plant density which induced higher number of yield contributing components which enhance fruit yield.

Lowest fruit yields of 0.7 (1^{st} sowing) and 2.4 dt/ha (1^{st} sowing) were recorded by cv. Hild Samen in Gie and GG respectively in 2008. This effect could be caused by higher susceptibility of cv. Hild Samen to *Cercospora malkoffii* compared with other used cultivars. In present study, fruit yield and plant height were positively and significantly correlated (r = 0.68). Photosynthetic surface in case of all cultivars resulting in better source of photosynthates available for grain filling phase (Cosge et al. 2009).

The number of branches of anise plant was different in each field experiment, ranging from 2.4 to 4.6 and 1.5 to 3.6 in GG 2008 and 2009, respectively and from 4.2 to 6.4 and 5.6 to 8.5 in 2008 and 2009 respectively at Giessen. This affect can be explained by lower germination rate of anise which was found in Giessen 2009 and by different soil properties especially water holding capacity of the soil of both stations. In current results number of primary branches, umbels, fruits and fruit weight per plant were decreased as plant densities increased. It can be concluded that at lower plant density, plants have efficient use of available resources such as water, light, and nutrients while at higher planting densities, competition among plants will be more. These findings are in line with the previous work that yield components are decreased by increasing the planting densities (Tunctürk et al. 2005, Tuncturk and Yildirim 2006, Yan et al. 2011).

In executed trials, delayed sowing significantly decreased the yield contributing components such as branches, umbels, fruits and fruit weight per plant in both experimental years in GG. These findings are also in conformity with previous work that showed clear decline in number of branches, umbels and fruits per plant due to delayed sowing (Tuncturk and Yildirim 2006, Yan et al. 2011).

Cv. Hild Samen was characterized by higher number of yield contributing parameters (branches/plant, umbels/plant, fruits/plant, fruit weight/plant) as compared to other cultivars in GG 2008. It can be suggested that lower plant density of this cultivar reduced the competition for available resources (water, nutrients, light) and increased these yield parameters because more space available for plant spreading. In executed trials, the number of branches per plant was significantly associated with the number of umbels per plant.

Thousand fruit weight (TFW) of anise ranged from 1.81 to 3.43 g among all treatments at both experimental stations. Highest variation was observed in Giessen 2009 which was caused by sowing time as well as by plant density and cultivar. Anise plants in delayed sowing plots were characterized by long ripening phase of fruits with unfavorable weather conditions. High fungus infection rate during this phase contributed to smaller fruit formation. Beside that a significant effect of plant density on TFW was observed, which was caused by higher competition between anise plants. In 2008, significant higher TFW was produced by cv. Hild Samen compared with other cultivars in delayed sowing times at both stations which might be a result of lower germination rate with this cultivar which increase individual fruit size.

Plant height of anise varied from 40 to 44 cm and 47 to 55 cm in GG 2008 and GG 2009 respectively, whereas it ranged from 40 to 47 cm and 62 to 70 cm in Gie 2008 and Gie 2009 respectively. This different level of plant length was caused mainly by different soil properties and environmental conditions. It can be suggested that higher water holding capacity of the clay soil in Giessen improved plant growth of anise. Additionally lower germination rate and plant density at this station could be contributed to higher plant length of anise in this experiment. In both stations in 2009 taller anise plants were observed. It can be supposed that the shortening of the anise stems is related to the precipitation during the vegetative plant stage which ranged from 184 mm (2008) to 236 mm in GG (2009), whereas it varied from 153 mm to 232 mm in 2008 and 2009 respectively. There was a positively correlation between plant height and number of umbels/plant (r = 0.65) which emphasizes the importance of the stem length for formation of morphological parameters and fruit yield. In 2008, cv. Hild Samen produced smaller plant height compared with other cultivars at Giessen station which might be caused by genetic variation of used cultivars. In current trials only in Giessen 2009 plant height was significantly affected by plant densities. The reduction in plant height induced by higher plant densities in both sowing times is due to higher competition within plant stand which made worse the growing conditions of anise.

Delayed sowing time resulted in smaller plant heights in all experiments. This effect could be due to shorter period of vegetative growth which is determined by sum of air temperature as well as by photoperiodic conditions (day length). The plant height findings obtained from the study are contrary to Tuncturk and Yildirim (2006) who reported that anise plant height increased as planting densities narrowed these contradictory results might have been due to climatic variation and genetic traits of the crop plants.

6.1.2 Content and yield of essential oil

According to European Pharmacopoeia, anise fruit must have an essential oil concentration higher than 2% (European Pharmacopoeia, 2000). In current field experiments essential oil content of anise varied from 2.30 to 3.67%. Therefore it can be concluded that anise which is cultivated in Germany (Hessen) compiles the demand of the European Pharmacopeia. The level of essential oil in anise fruits was nearly the same like in field experiments of subtropical regions. Cultivar determined effects on essential contents were observed in 2008 at both stations (both sowing times). This affect which was caused by lower essential oil content of cv. Hild Samen can be due to higher fungus infection of this cultivar which reduced the capacity of oil accumulation. In present study delayed sowing increase essential oil accumulation in both years at experimental station Giessen. This effect can be explain that higher sum of air higher temperature during flowering-maturity stage in delayed sowing which ranged from 983 (1^{st} sowing) to 1221 (2^{nd} sowing) °C and 986 (1^{st} sowing) to 1375 (2^{nd} sowing) °C respectively in 2008 and 2009.

In executed trials only delayed sowing led to increased essential oil concentration at experimental station GG 2009. Higher essential oil concentration in delayed sowing can be explained by higher temperature of (20.2 °C) and lower precipitation (40 mm) was occurred during fruit formation stage (August) of anise which enhanced essential synthesis compared with 2008 where lower temperature (18.4 °C) and higher precipitation (72 mm) was occurred during fruiting stage. Zehtab-Salmasi et al. (2001) carried out a study to investigate the effect of water supply and sowing dates on performance and essential oil production of anise in ecological conditions of Iran. The authors showed that essential oil percentage was decreased significantly with delayed sowing, but water limitation resulted in an increase in essential oil contents. Current findings confirm the conclusions that the amount of essential oil produced under drought conditions was either maintained or enhanced, depending on the cultivars and extent of stress (Zehtab-Salmasi et al. 2001, Azizi et al. 2009). Anise essential oil percentage depends not only on genetic resources but also on the development of anise fruits. A significant change in essential oil accumulation was reported during the development of anise fruits with higher values of 5.5% at waxy stage than 3.4% at ripening stage (Omidbaigi et al. 2003, Özel, 2009). In present study, essential oil contents were lower than waxy stage but similar to that harvested at ripening stage.

Yields of essential oil from anise have been reported in the range of 10 to 24 kg/ha (Zehtab-Salmasi et al. 2001, Tuncturk and Yildirim 2006). Relative to the anise oil yields reported in the literature, essential oil yield in our study ranged from 1.6 to 41 kg/ha. Highest essential oil yields were found in early sowing at both experimental stations and at lower plant densities. Current results are in contrast with other findings that showed an increase in essential oil yields by increasing the seed rates

(Tuncturk and Yildirim 2006). In fact, essential oil yield was associated directly with fruit yield and essential oil concentration. Difference in these components directly affect essential oil yield (Tunctürk et al. 2005, Tuncturk and Yildirim 2006). Significant and positive correlation was found between fruit yield ($r = 0.96$) and essential oil yield (Tuncturk and Yildirim 2006).

6.1.3 Chemical composition of essential oil

More than 15 different components have been reported which were found in anise essential oil (Lawrence 1984, Askari et al. 1998, Özcan and Chalchat, 2006, Orav et al. 2008, Yan et al. 2011). In current studies, *trans*-anethole was the dominant constituent varied from 89.5 to 96.9% of the essential oil which is similar with previous studies of anise (Maheshwari et al. 1989, Askari et al. 1998, Orav et al. 2008, Yan et al. 2011). The minor constituents in anise essential oil from present experiments were similar to that found in previous studies including, γ-himachalene (2-8%), α-himachalene (0.13-0.86%), estragol (0.32-0.76%), *cis* anethole (0.10-0.18%), elemene delta (0.08-0.54%), α-amorphane (0.03-0.17%), α-zingiberene (0.45-0.96%), β-himachalene (0.15-0.53%), α-muurolene (0.03-0.20%) and β-bisabolene (0.21-0.50%) (Arslan et al. 2004, Orav et al. 2008, Yan et al. 2011).

In current field trials a tendency of higher concentration of *trans*-anethole was observed in early sowing times at both experimental stations. Current findings are in agreement with that of Maheshwari et al. (1989), who reported higher *trans*-anethole concentrations in early-sown anise plants compared with delayed-sown plants under ecological conditions of India. This is different from the results of others who showed a marked change of the percentage of *trans*-anethole essential oil from 90% to 80% as the anise fruit developed from waxy to full mature stage Omidbaigi et al. (2003). In current trials *trans*-anethole concentrations were similar in anise fruits harvested at waxy stage in tropical and subtropical regions but higher than fruits harvested at full ripening stage. It can be suggested from current and previous studies that to achieve higher concentration of *trans*-anethole, anise fruits harvested at ripening stage in temperate conditions whereas waxy stage was recommended to meet higher concentration of *trans*-anethole due to higher temperature for tropical and subtropical regions.

Estragol, the flavoring agent, is considered to have negative effects on animal and human health and was deleted from the list of flavors in food stuffs (Burt 2004). In present study estragol was found in all essential oil samples which varied from 0.37 to 1.41% at both locations. The European Pharmacopoeia limit of estragol in essential oil of anise (0.5-6.0%) was not exceeded in investigated samples. Cv. Hild Samen was characterized by a higher concentration of estragol and *trans*-anethole from both stations in 2008. These contradictory might be caused by genetic variations of used cultivars. Orav et al. (2008) analyzed anise fruits from various European countries, samples from Germany named as Germany1 and Germany2 contained (2.0% estragol and 92.2% *trans*-anethole) and (2.3% estragol and 92.7% *trans*-anethole) respectively. In Giessen 2009, cv. Pharmasaat induced higher concentration of estragol in early and delayed sowing time. These contradictory results caused by genetic variation of used cultivars. In comparison to previous studies, the values of the present results were not higher than the values reported by Askari et al. (1998) (1.04%), Rodrigues et al. (2003) (0.9-1.5%), Özcan and Chalchat, (2006) (2.4%) and Orav et al. (2008) (0.5-2.3%). Significant but negative correlation was noticed between estragol and *trans*-anethole (r= -0.63).

In current study γ-himachalene concentration affected by used cultivars in 2008 at both stations which ranged from 2.1 to 7.4 and 2.2 to 5.5 respectively in Gie and GG. Cv. Hild Samen characterized by higher concentration of γ-himachalene compared with other cultivars which might be caused by genetic variation of used cultivars.

An increasing trend of *trans*-anethole was observed as plant density increased in early sowing at experimental station Giessen 2009. This effect may be caused by higher *Cercospora malkoffii* infection of (4.9 levels) in narrow plant density compared with lower plant density (2.6 levels). Sander and Heitefuss (1998) conducted experiments with wheat and reported that in response to infection with a compatible race of powdery mildew (*Erysiphe graminis* f.sp. *tritici*), levels of phenolic acids slightly increased in leaves of the cv. Syros grown with low and medium nitrogen supply. It can be explained that activity of PAL enzyme which is responsible for the synthesis for phenolic compounds and phenylpropanoids increased under low nutrient level, light (through its effect on phytochrome) and by fungal infection. Fungal invasion triggers the transcription of messenger RNA that codes for PAL that enhances the amount of PAL in plant, which then stimulates the synthesis of phenolic compounds (Logemann et al. 1995). It can be concluded from current trials and previous studies that secondary metabolites increased when plants face stress conditions.

It can be concluded that anise is sensitive to plant density and sowing date. Under ecological conditions of the middle Hessen in Germany, an early sowing date at the beginning of April with planting densities of 50-200 plants/m² concerning experimental sites proofs to be a decisive cropping practice in order to increase fruit yield. Delayed sowing causes a yield decrease. Higher sowing rate results in narrow planting densities, but reduces fruit yield components, like number of branches, umbels and fruits per plant which leads to lower fruit yield. From all tested cultivars cv. Hild Samen characterized by lowest fruit yield as well as lowest essential oil concentration. The cultivars tested in our study showed high essential oil concentrations with more than 90% *t*rans-anethole and less than 1% estragol and is good chemotype of anise cultivar.

6.2 Effect of row spacing and plant density

6.2.1 Fruit yield and yield components

In 2008 at experimental station Gross-Gerau much lower fruit yields were observed as compared to 2009. The reason for these differences are due to heavily disease infection of anise plants caused by the fungus *Cercospora 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 as well which lemmatized photosynthetic surface area of anise plants. Contrary to that in Giessen 2009 anise plants were characterized by lower infection level of 3.8 to 5.5 caused by *Cercospora malkoffii*. Overall higher fungus infection was found at experimental station GG which might be explained by higher relative air humidity and higher air temperature during cultivation period of anise.

A second factor which influenced the fruit yields of anise was lodging of anise plants. At the Giessen experimental station anise plants had lodging at the level of 2.8 to 7.7 which reduced fruit yield in comparison with 2008. It seems that higher level of lodging (7.7) and higher infection rate (6.8) are supported in wider row spacing and narrow plant densities. Severe lodging in plants during fruit initiation may prevent the transport of water, nutrients and assimilates through the xylem and phloem, resulting in a reduction in assimilates for grain filling and premature fruit formation occur. Higher moisture levels in a lodged plant population may be favourable for fungal growth and for the development of diseases, which have detrimental effects on plant growth and fruit quality of anise plants.

In current trials fruit yield of anise was not affected by different row spacing whereas plant density had pronounced effect at Gross Gerau in both seasons where fruit yields varied between 4.3 and 11.3 dt/ha. Plant density of 294 plants m^{-2} produced 127 fruits and fruit weight 0.38 g per plant which was reduced to 41 fruits and 0.09 g fruit weight per plant under maximal plant density of 707 plants m^{-2} in GG 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. It can be concluded from current study that narrow plant densities obtained limited nutrition, water uptake as well as light 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 number of branches, number of umbels as well as fruit number and fruit weight per plant significantly.

Contrary to that, in Giessen 2008 fruit yield of anise was only affected by row spacing treatments. Plants grown under 15 cm row distances produced significant higher fruit yield of 8.3 dt/ha compared with 3.0 dt/ha received with 37.5 cm row spacing. The plants which were grown in wider row spacing increase competition among individual plants for available resources (water and nutrients) and reduced fruit yield. Maheshwari et al. (1989) carried out field experiments with anise reported that higher seed yield was noticed by sowing seed broadcast or in 15 cm close spaced row in comparison with wider row spacing of 45 cm. In observation of (Kizil et al. 2008) who reported that lower row distances produced higher seed yield compared to wider row distances in cumin (*Cuminum cyminum* L.).

In executed trials plant height, number of primary branches and number of umbels per plant were significantly correlated with fruit yield. Similar results were also reported by Sanker and Khader (1991) who showed significant relationship between seed yield and number of branches in coriander (*Coriandrum sativum*) which is belonging to the same family (*Apiaceae*) like anise.

The germination rate was decreased as distance between the rows increased in both seasons which can be explained by excessive competition of the plants for nutrients and water results in embryo death which led to lower germination rate. Overall higher germination rate was observed in 2009 which can be attributed to higher air temperature 15 °C and lower precipitation (36 mm) during germination period (sowing-emergence) of anise. In 2008, lower germination rate might be a reason of low temperatures 8.8 °C and 5.5 °C in GG and Gie respectively. Peter (2001) reported that optimum soil temperature for anise seed germination is at the level from 18 to 21 °C. Lower soil temperature of 6 °C induces depressed anise plant growth Weiss (2002).

In executed trials plant height of anise varied from 44 to 46 cm and 61 to 67 cm in Gross Gerau 2008 and 2009 respectively, whereas it ranged from 45 to 48 cm and 61 to 66 cm in Giessen 2008 and 2009 respectively. Average over the year taller plants were observed in 2009 at both stations because anise plants received higher precipitation during vegetative stage in 2009 (GG 236 mm, Gie 232 mm May to July) compared with 2008 (GG 184 mm, Gie 153 mm) at both stations. In current study row spacing effect regarding plant height of anise was only observed in GG 2009. The highest plant height of 67 cm was achieved under wider row spacing of 37.5 cm whereas significant smaller plant height of 60 cm recorded in row spacing of 15 cm. The plants grown with wider row spacing had lower germination rate which provide more area of land around them to draw the nutrition, water and had more solar radiation to absorb for better photosynthetic process and hence performed better as individual plants. Current results are different from the findings of Kizil et al. (2008) and Ahmad et al. (2004) who carried out field trials with cumin (*Cuminum cyminum*) and fennel (*Foeniculum vulgare* L.) respectively which are belonging to *Apiaceae* family. The researchers reported plant height decreased as row distance increased under field conditions. Current investigation showed that plant height was markedly affected by different plant densities in 2009 at both stations. In current trials highest plant height was recorded under lower plant densities. These findings are contrary to previous work of Tuncturk and Yildirim (2006) who reported that highest plant height was obtained from the application of 20 kg/ha seed rates and the lowest value from 5 kg/ha seed rate in anise plants. These contradictory results might be due to climatic variation and genetic traits of the crop plants. However it can be explained that under lower level of plant density plants have more nutrition, water and light, and therefore have better growth and finally produced taller plants.

In current study number of branches and number of umbels per plant were affected by plant density in both seasons which ranged from 2.1 to 4.2 and 3.2 to 5.8 respectively in Gross Gerau 2008 and 2009. Number of primary branches, and umbels per plant were higher in lower plant densities compared with narrow densities. It can be supposed that aniseed plant stand is characterized by plasticity of yield components formation within a range of 200 to 300 plants m^{-2}. These results are in agreement with the findings of Tuncturk and Yildirim (2006) and Yan et al. (2011) who reported that higher seed rates resulted in higher plant density but reduced number of branches and umbels per plant. It can be explained that under lower plant density, anise plants show efficient use of available water, light and nutrient while under high plant density, there is competition among plants which led to lower yield contributing components.

Number of primary branches, secondary branches and umbels per plant were not affected by various row spacing in both seasons at experimental station GG. Independent of that higher number of these yield components were recorded in wider row spacing of 37.5 cm. Current results are in line with findings of Kizil et al. (2008) who reported that wider row distance produced maximum number of branches and umbels per plant, while the least number of these yield components were obtained from narrow row distance. It can be concluded from current study that under wider row spacing better growth of anise plants occur which attributed to the better use of water, nutrients and UV radiation and resulted higher photosynthesis which led ultimately higher yield components. There was a positive relationship (r = 0.97) between number of branches and umbels per plant in current trials. The results are in line with the findings of Cosge et al. (2009) received with fennel (*Foeniculum vulgare* L.) who reported that branches are positively and significantly correlated with number of umbels per plant.

In executed trials, it was found that significant higher number of fruits and fruit weight per plant were recorded in lower plant densities and wider row spacing in both seasons at experimental station GG. Present results are accordance with the findings of Yan et al. (2011) who reported that with increasing plant density the fruit number per plant decreased significantly. In present trials fruit weight per plant increased with increasing distance between the rows in both years. These observations agree with Kizil et al. (2008) who reported fruit weight per plant decrease in closed spaced rows of 20 cm 0.91 g whereas increased by increasing row distance of 60 cm 1.34 g in cumin (*Cuminum cyminum*) plants. In lower plant density, anise plants have more nutrition, water and light and therefore have better growth which produced higher branches, umbels per plant and finally produce higher number of fruits and fruit weight per plant. In current study higher seed rate application resulted higher plant densities but reduced yield components of anise. It can be supposed that aniseed plant stand is characterized by plasticity of fruit yield formation within range of 200 to 300 plants m^{-2} caused by the effect of fruit yield components. Wider space availability between the wider rows might have increased the root spread which eventually utilized the resources such as water, nutrients, and light very effectively which increased yield components. Further it can be explain that at wider row spacings there is better source sink relationship due to which yield attributing characters get increased. The results of this study showed significant correlation between fruits number per plant and fruit weight per plant (r = 0.99 in 2008 and r = 0.90 in 2009).

In current study, TFW weight of anise varied from minimal 1.88 to maximal 3.23 g. This TFW range is in accordance with the findings of Tuncturk and Yildirim (2006) and Zehtab-Salmasi et al. (2001). In current trials different row distances had no pronounced affect regarding thousand fruit weight (TFW) in GG whereas it was affected by row spacing in Giessen 2008. Higher TFW was caused by lower germination rate compared with other row spacing treatments. In all executed trials TFW was affected by various plant density treatments in Gie 2008 as well as in GG 2009. The plant densities of 294 and 707 plants m^{-2} led to TFW values of 2.25 and 1.88 g respectively at GG. It can be explained that fruit set depends on availability of sufficient nutrients while shift from vegetative to reproductive stage, increased plant densities resulted in limited availability of nutrients, light and water so the size of individual fruit decrease which led to lower TFW of the anise. Overall higher 1000-fruit weight was noticed at experimental station Gie which might be a result of lower plant density compared with GG and better soil properties (especially water holding capacity) which reduced competition among plants for available resources and increase individual fruit weight.

6.2.2 Content and yield of essential oil

In executed trials essential oil content of anise ranged from 2.77 to 3.73% in both experimental stations. Essential oil synthesis was not influenced by row spacing as well as by plant density at Gross Gerau in both seasons. However an interaction of RS x PD was noticed with respect to essential oil concentration at GG 2009. Opposite to that essential oil accumulation was influenced by various row spacing treatments as well as by plant density treatments. Wider row spacing induced markedly higher essential oil compared with plants grown in closed spaced rows. It might be a reason that under wider row spacing there was a 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 current findings higher essential oil contents were accumulated in lower plant densities compared with narrow plant densities. Khorshidi et al. (2009) reported that essential oil percentage was affected significantly by different densities of planting, as the maximum essential oil percentage of (3.53%) was obtained with the minimum plant density whereas the minimum essential oil percentage (3.1%) was recorded with the maximum plant density. In own trials overall more essential oil was synthesized in 2009 compared with 2008 at experimental station Giessen which can be attributed to better weather conditions especially higher air temperature (18.5 °C) and low precipitation (40 mm) during fruit formation stage. It can be supposed that under stress conditions the amount of essential oil increased because in case of stress, higher numbers of ducts/cavities are produced per fruit in which essential oil accumulated and more metabolites are produced in plants and substances prevent from oxidation in the cells.

In current trials essential yield of anise varied from 12.5 to 43.1 kg/ha at GG whereas it ranged from 8.6 to 23.6 kg/ha at experimental station Giessen. 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 (Zehtab-Salmasi et al. 2001, Tuncturk and Yildirim 2006). Present results are differed with other findings that showed an increase in essential oil yields by increasing the seed rates (Tuncturk and Yildirim 2006). In current study significant correlation was found between fruit yield ($r = 0.98$) and essential oil yield.

6.2.3 Chemical composition of essential oil

Essential oil of anise fruits contains mainly phenylpropanoids and sesquiterpenoid hydrocarbons. It is of interest to note the presence of *trans*-anethole more than 90% 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 (Askari et al. 1998, Özcan and Chalchat, 2006, Orav et al, 2008, Yan et al. 2011).

In present study *trans*-anethole, γ-himachalene and estragol were affected by various row spacing treatments in both seasons at Giessen. Higher concentration of *trans*-anethole was observed under wider row spacing and narrow planting densities. It can be assumed that under wider row spacing anise plants were heavily logged due to competition between plants which improve 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 and Paiva 1995).

Sander and Heitefuss (1998) conducted experiments with wheat and reported that in response to infection with a compatible race of powdery mildew (*Erysiphe graminis* f.sp *tritici*), levels of phenolic acids slightly increased in leaves of the cv. Syros grown with low and medium nitrogen supply. It can be concluded from current results that phenylpropanoids are also synthesized in similar way as phenolic compounds and can be increased under stress conditions. 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 accordance with previous work (Orav et al. 2008, Yan et al. 2011). Further it was observed from current investigations that with increase in level of *trans*-anethole, 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.

Contrary to that quantitative study showed that plant density and row spacing had no effect on the percentage of *trans*-anethole, γ-himachalene and estragol in Gross Gerau 2008 whereas these compounds were affected in Gross Gerau 2009. It can be supposed that 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 and Zeiger, 2002). It can be assumed that phenylpropanoids and phenolic compound concentrations increased when plants face stress conditions.

It can be concluded that anise plants with lower plant density produced higher level of yield contributing parameters including number of primary branches, umbels, fruits and fruit weight per plant. 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 200 to 300 plants m^{-2} gave highest fruit yield due to reduced competition among plants. The tested cultivar (Enza Zaden) in current study showed high essential oil percentage with more than 90% *t*rans-anethole and less than 1% estragol and is good chemotype of anise cultivar.

6.3 Fungicides and cultivars

6.3.1 Impact of fungicides on plant growth and fruit yield

Anise blight caused by *Passalora malkoffii* (Bubak, 1906) U. Braun was reported for the first time in 1906 from plant samples collected at Sadovo (Bulgaria), called as *Cercospora malkoffii* (Braun and Melnik 1997). *Cercospora* is a genus of ascomycete fungi belonging to the family of *Mycosphaerellaceae*. *C. malkoffii* is one of the species which overwinters as mycelium in dead leaves, infected seeds or in plant residues. The spores are wind-blown or rain splashed to new anise tissue where infection occurs. Anise leaf blight symptoms starts from shooting stage at the edges of leaflets as a sectorial necrosis and whole plant will be infected on later plant developments stages. Abundant conidia and conidiophores were observed, especially on the lower surfaces of the leaves, as a velvety cover (Erzurum et al. 2005). In current trials it was observed that the infection with *Cercospora malkoffii* started also at shooting stage of anise plants. For that reason it can be suggested that for effective control of *Cercospora* an early application of fungicides during at the beginning of shooting stage is necessary. In current trials fungicides were applied in 1st week of June in which the anise plants reached the shooting stage (before starting flowering). It can be concluded that early application of fungicide in June could be the right time for successful control of *Cercospora malkoffii* in anise plants under temperate conditions in Germany.

In current trials higher infection level with *Cercospora malkoffii* was recorded in 2009 in comparison with 2010 which might be caused by different climate conditions between both years. In June of the second year 2010 higher precipitation sum and air temperature was observed which could be contributed to higher disease infection. Suitable environmental conditions for the development of *Cercospora* leaf blight are day temperatures of 15 to 30 °C, night temperatures of around 16 °C and prolonged periods of relative humidity of more than 90% or free moisture on leaves (Forsyth et al. 1963, Erzurum et al. 2005). For that reason it can be suggested that more spreading of *Cercospora* leaf blight can be found under wet and warm weather conditions which caused higher infection level in own trials in 2009.

In current trials the application of fungicides had significant effects on fruit yield in both years. In 2009 the application of Mancozeb + Metalaxyl-M led to maximal fruit yield followed by Azoxystrobin + Difenoconazol whereas in 2010 only the treatment with Azoxystrobin + Difenoconazol increased fruit yields by reducing disease level. It seems that the combination of the two compounds Azoxystrobin and Difenoconazol which are belonging to the groups of strobilurins and triazoles respectively getting better fungicide effect. That could be explained by the physiological effects of these fungicides. Azoxystrobin possesses a new mechanism of action consisting of inhibition of mitochondrial respiration by binding at the Qo (quinine oxidizing) site of cytochrome b (Gisi et al. 2002). Inhibition of mitochondrial respiration is achieved by blocking the electron transport between cytochrome *b* and cytochrome *c*1 which, in consequence, leads to a disruption of the energy cycle (Gisi et al. 2002). Difenoconazol is taken by the plant and acts on the fungal pathogen during penetration and haustoria formation by interfering with the biosynthesis of sterols in cell membrane (Nithyameenakashi et al. 2006).

Anise blight caused by *Passalora malkoffii* has been recognized as a major disease of anise in Turkey, causing serious economic losses. Control of the disease in Turkey has been achieved mainly by the fungicides likes azoxystrobin, chlorothalonil + carbendazim with seed treatment as well as with foliar application (Erzurum et al. 2005). Positive fungicide effect was also found in the spice crop caraway (*Carum carvi*) which is belonging to *Apiacea*e family just as anise. In investigations carried out by Odstrcilova (2007) were observed that application of Dimoxystrobin (strobilurin) and Prothioconazole (triazole) which are belonging to different groups of fungicides strongly inhibited mycoflora on caraway and increased seed yield in comparison with other treatments. Besides controlling diseases strobilurin fungicides can modify physiological processes in the crop which could be an additional reason for their yield increasing effect. So it could be found that strobilurin fungicides inhibit ethylene biosynthesis via a reduction in endogenous 1-aminocyclopropane-1-carboxylic acid (ACC) syntheses which delayed leaf senescence and consequently prolonged photosynthetic activity of green tissue (Venancio et al. 2003). These physiological relations till now are not investigated in spice crops like anise. But it can be suggested that this effect does not depend on plant species but is established in crops belonging to different families.

Besides their influence on fruit yield the applied fungicides also modified morphological and plant stand parameters. Therefore in current experiments it could be found that the fungicide treatments with Mancozeb + Metalaxyl-M (in 2009) and with Mancozeb + Diemethomorph (in 2010) reduced lodging of anise plants. This effect can be explained by lower disease infection of the plants caused by *Cercospora malkoffii* which was found on leaves as well as on stems. *Cercospora* infection especially on the basal part of the main stem could be a reason of reduced standing ability of the plants. For that reason a successful control of disease infection might be contribute to reduced lodging of anise plants.

Fungicide application had significant impact on the yield contributing parameters only in 2010 and not in 2009. This effect might be caused by different levels of disease infection as well as different lodging of the plants in both years. In 2010, fruit yield contributing features reached higher level as compared with 2009. The differences between the years probably reflect the influence of ecological conditions. In 2010, anise plants received higher precipitation of 496 mm compared with 2009 in which total 405 mm were measured. Further it was observed that anise plants were more infected by *Cercospora malkoffii* in 2009 (level of 7.0 in untreated plots) in comparison with 2010 (level of 6.0 in untreated plots). Higher fungal infection in 2009 could be additionally reduced the formation of side branches and inflorescence of anise plants in that year.

In current study fungicides had significant effects on thousand fruit weight (TFW) in both years which ranged from 1.94 to 2.52 g in 2009 whereas it varied from 1.25 to 1.66 in 2010. Among the yield contributing parameters only TFW was affected in both seasons. Application of Mancozeb + Metalaxyl-M led to maximal TFW of anise compared with other fungicide treatments in 2009. Contrary to that in 2010, anise plants treated with Propamocarp produced higher TFW compared with other treatments. It might be that these fungicides reduced disease level as well as lodging which increased photosynthetic surface area and established good source and sink relationship for fruit formation. These results are in accordance with the finding of Odstrcilova (2007) who reported that fungicide application of Azoxystrobin and Dimoxystrobin + Boscalid against mycoflora on caraway (*Carum carvi* L.) enhanced TFW in both seasons as compared with other treatments.

6.3.2 Essential oil synthesis and yield

In field experiments carried out in this study essential oil content of anise fruits reached a maximum level of 3.01% (2.50 to 3.01%) in both seasons. Similar level of essential oil contents from anise could be found in experiments from Iran with a level from 2.21 to 2.75% (Zehtab-Salmasi et al. 2001) and in investigations carried out in Turkey with a level from 2.66 to 2.74% essential oil (Tuncturk and Yildrim 2006). Contrary to that higher level of 3.5 to 5.5% was observed in another experiment carried out in Iran (Omidbaigi et al. 2003). Omidbaigi et al. (2003) obtained their results from anise fruits harvested at earlier waxy stage of the crop. Contrary to that essential oil content of the fruits which were harvested at later ripening stage was lower (3.4%). Reducing of essential oil content in ripening stage of spice plants under tropical climate conditions is due to higher losses of oil caused by evaporation. It can be concluded from current and previous studies that to achieve higher essential oil content anise plants should be harvested at waxy stage in tropical and sub tropical regions while ripening stage is recommended for temperate regions.

In the field experiment carried out in 2010 was found that anise plants which are treated with Azoxystrobin + Difenoconazol significantly enhanced essential oil accumulation until 2.92% in comparison with other treatments. The reason for this effect can be explained by improved growth conditions for anise plants caused by fungicide application. Azoxystrobin and Difenoconazol might have physiological effect which delayed leaf senescence and extended the duration of photosynthetic activity of green tissue. It can be suggested that this effect enhanced the oil synthesis of the glandular cells in anise fruits as compared with other treatments. Current results are in line with the finding of Odstrcilova (2007) who carried out field experiments with caraway (*Carum carvi* L.) to control mycoflora on the seeds of that plant. They reported that fungicide application with Prothioconazole had a positive effect on essential oil content compared with other treatments.

In addition to fungicide effect in own experiments could be found that used cultivars had also a significant influence on essential oil content in 2010. After application of different fungicides cv. Enza Zaden accumulated higher concentration of essential oil as compared with cv. Pharmasaat. These contradictory results might have been due to genetic variation of the crop plants. It can be suggested that cv. Enza Zaden has higher potential for essential oil accumulation which might be caused by larger size or higher density of glandular cells in the two seeds which are located in the anise fruits.

6.3.3 Essential oil composition

More than 15 components were identified in essential oil of anise fruits out of which *trans*-anethole was the major compound varied from 90.6 to 95.0% followed by γ-himachalene which ranged from 3.1 to 6.0%. Only small concentrations had the minor compounds like, α-himachalene, estragol, *cis* anethole, delta-elemene, α-amorphane, α-zingiberene, β-himachalene, α-muurolene and β-bisabolene. The composition of essential oil of anise fruits received from current field experiments was similar to those reported in scientific literature (Askari et al. 1998, Özcan and Chalchat, 2006, Orav et al. 2008).

The application with Fosetyl induced higher percentage of *trans*-anethole while lower concentration of this compound was obtained in untreated plots and in other treatments which were found in both years. Recent studies suggest that application with Fosetyl fungicide can also affect the secondary metabolism of plants to a relatively important extent (Guest and Bompeix 1990). The possible effect of Fosetyl on the composition of essential oil can be explained as follows: One of the consequences of phosphonate (Fosetyl-AL) treatment is that it induces a strong and rapid defence response in the challenged host plant. That was found in tobacco, capsicum and cowpea plants were it fully limited the pathogen. Researchers examined the effects of phosphonates on ethylene biosynthesis, phenylalanine ammonia lyase activity, lignin biosynthesis and phytoalexins accumulation (Guest, 1984a, Guest and Bompeix 1990). In each case it was found, that the defense response was more rapid in treated plants. Fosetyl application has complex mode of action ranging from direct toxicity against invading pathogens to indirect effects such as activation of host defense mechanism by break down of phosphorous acid in the plant (Guest and Bompeix 1990). It might be that Fosetyl application activates the host defense mechanism and increases the activity of PAL (phenylalanine ammonia-lyase) which is responsible for the synthesis of phenylpropanoids like *trans*-anethole.

Further it can be suggested that PAL activity which is increased by higher fungal infection triggers the transcription of messenger RNA that codes for PAL. That could enhance the amount of PAL in the plant, which stimulates the synthesis of phenolic compounds as well as metabolic stress caused by Fosetyl application by modifying the defense mechanism of host plants (Logemann et al., 1995). It can be suggested that fungicides applied to the plant can modify the stress conditions influencing the plant metabolism. The abiotic stress influences the formation and the composition of secondary metabolites, especially the production of phytoalexins (Reilly and Klarman 1972, Cartwright et al. 1980). Zaky et al. (2006) which are used as different chemical activators (IAA, ethephon, kinetin, salicylic acid, humic acid and nicotinic acid) and fungicides (Eminent, Tilt, Plantvax and plant Gaurd) against rust disease (*Puccinia pimpinellae*) on anise plants in Egypt. The authors reported that spraying anise plants with each one of the treatments tested increased activities of chitinase and peroxidase enzymes as well as phenolic compounds accumulation in plant leaves in comparison with the control.

Phytoalexin production appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants. For example, in leguminous plants such as alfalfa and soybeans, isoflavonoids are common phytoalexins, where as in solanaceous plants such as, potato, tobacco and tomato various sesquiterpenes are produced as phytoalexin (Taiz and Zeiger 2002). Tobacco transformed with a gene catalyzing the biosynthesis of phenylpropanoid phytoalexin resveratrol becomes much more resistance to a fungal pathogen than non transformed control plants (Hain et al. 1993).

Generally it can be concluded that a fungicide management with Azoxystrobin + Difenoconazol can be an option to reduce the fungal infection with *Cercospora malkoffii* and to improve the fruit yield of aniseed under field conditions in Germany (Hessen).

7. Summary

From 2008 to 2010 field experiments with the medicinal and spice plant *Pimpinella anisum* L. were carried out to clarify the effect of different agronomic factors on fruit yield and its components as well as on essential oil content and its composition.

Three field experiments were carried out at two experimental stations in Giessen and Gross-Gerau. First experiment included three factors: sowing rates, sowing times and cultivars. Second experiment included different row spacing (15, 25 and 35.5 cm) and sowing rates (6, 12 and 24g/10 m^2). Third field experiment was carried out to evaluate the efficacy of various fungicides against the fungal infection caused by *Cercospora malkoffii.* The field trials regarding sowing time and fungicide were designed in RCBD factorial plot arrangements whereas row spacing as under split plot arrangement with four replications. Anise fruits were hydro-distillated using a distillatory apparatus of Neo-Clevenger. The essential oil was analyzed by GC-FID and GC-MS. All data were statistically analyzed by using PIAF software.

In the first experiment it was observed that delayed sowing induced strong effect on plant height as well as on yield contributing parameters like primary branches/plant and umbels/plant which were reduced in both stations. In 2008, fruit yield was significantly modified by the used cultivars and by the established plant densities in both stations. Contrary to that in 2009, fruit yield was not significantly affected by different plant densities as well as by used cultivars in delayed sowing times at both stations. However highest fruit yield was related to lower plant density of 39 plants m^{-2} in Giessen 2009 and 189 and 374 plants m^{-2} in Gross-Gerau 2008 and 2009 respectively. In all executed experiments anise plants grown under higher plant density reduced branches/plant, umbel/plant, fruits/plant, fruit weight/plant and led to lower fruit yield in both stations. The essential oil concentration of anise ranged from 2.30 to 3.67% and was affected by used cultivars in 2008 at both experimental stations. From all tested cultivars cv. Hild Samen was characterized by lowest fruit yield as well as by lowest essential oil concentration. In current trials higher concentration of essential oil was found in delayed sowing times in experimental station Giessen in both years as compared to earlier sown anise plants. This effect can be explained by higher sum of air temperature during the phase from flowering to maturity in delayed sowing compared with early sown anise plants. The variation of plant density had no effect on essential oil concentration of anise. Differences in the composition of the essential oil were found between the investigated cultivars. Cv. Hild Samen synthesized significant higher concentration of the compounds estragol and *trans*-anethole compared with other cultivars.

In the second experiment it was found that the parameters like plant height, number of primary branches/plant, secondary branches/plant, umbels/plant, thousand fruit weight and fruit yield were not affected significantly by various row spacing at experimental station Gross-Gerau in both seasons. However plants grown under wider row spacing recorded significant higher fruit number and fruit weight per plant. Plants grown with lower plant densities produced higher level of yield contributing parameters including number of primary branches, umbels, fruits and fruit weight per plant compared with narrow plant densities. Two consecutive years study showed that lower planting densities from 200 to 300 plants m^{-2} led to highest fruit yield due to reduced competition among the anise plants. The essential oil accumulation was not affected by different row spacing as well as plant densities in Gross-Gerau where as it was affected in Giessen. An interaction of row spacing x plant density was found regarding essential oil accumulation in Gross-Gerau 2009. The interaction is characterized by differ response of plants grown in various row spacing and different level of plant densities. Within row spacing of 15 and 37.5 cm there was a reducing effect in essential oil concentration observed from minimal to maximal plant density. It can be supposed that interspecific competition of high plant densities reduced the size and number of glandular cavities.

In the fungicide field experiments it was found that two of the tested fungicides Mancozeb + Metalaxyl-M and Azoxystrobin + Difenoconazol reduced *Cercospora malkoffii* severity to a great extent in both years. This was consistent over two seasons with different levels of disease in each. It was observed that applied fungicides had no significant impact regarding yield contributing parameters including primary branches, umbels, fruits and fruit weight per plant in 2009 whereas significantly effects were observed in 2010. Cultivar Pharmasaat was characterized by higher level of fruit yield parameters compared with cv. Enza Zaden in 2009. It was found that anise plants sprayed with Azoxystrobin + Difenoconazol induced higher fruit yield parameters compared with other fungicides. Further it was found that essential oil concentration of anise fruits was affected by fungicides as well by used cultivars in 2010. Plants treated with Azoxystrobin + Difenoconazol and Fosetyl accumulated significant more essential oil compared with other treatments. Contrary to that essential oil concentration of anise showed no remarkable variation regarding fungicides application in 2009. The compound *trans*-anethole reached maximal concentration in anise fruits of the plots which were applied with Fosetyl in both seasons. It can be concluded that Fosetyl improved host defense mechanism which could be a reason of higher level of trans-anethole in anis fruits.

8. Zusammenfassung

In den Jahren 2008 bis 2010 wurden Feldversuche mit der Arznei- und Gewürzpflanze *Pimpinella anisum* L. durchgeführt, um den Einfluss unterschiedlicher agronomischer Faktoren auf den Fruchtertrag und die Ertragskomponenten sowie auf den Gehalt und die Zusammensetzung des ätherischen Öls dieser Pflanzen zu untersuchen.

Es wurden drei Feldversuche in den Versuchsstationen Gießen (schluffiger Lehmboden) und Groß-Gerau (Sandboden) durchgeführt. Im ersten Versuch wurde die Wirkung der Prüffaktoren Saatmenge, Saatzeit und Sorte untersucht. Im zweiten Feldversuch wurden unterschiedliche Reihenentfernungen (15, 25 und 35 cm) mit drei Aussaatmengen (6, 12 und 24g/10 m^2) kombiniert. Der dritte Feldversuch wurde durchgeführt, um die Wirksamkeit unterschiedlicher Fungizide auf die Infektion mit *Cercospora malkoffii* zu klären.

Die Feldversuche wurden als randomisierte Blockanlagen mit vier Wiederholungen durchgeführt. Das ätherische Öl der Anisfrüchte wurde durch Wasserdampfdestillation mit einer Neo-Clevenger-Apparatur gravimetrisch gemessen. Die Zusammensetzung des gewonnenen ätherischen Öls wurde anschließend mit GC-FID und GC-MS analysiert. Alle Versuchsergebnisse wurden mit Hilfe des Programms PIAF statistisch evaluiert.

Insgesamt wurden folgende Ergebnisse erzielt: Die verzögerte Saatzeit führte zu einer signifikanten Verminderung der Pflanzenlänge, der Anzahl an Primärtrieben pro Pflanze und Dolden pro Pflanze. Im Jahr 2008 wurde eine signifikante Beeinflussung des Fruchtertrages durch die Prüffaktoren Sorte und Pflanzendichte festgestellt. Im Gegensatz dazu war im Jahr 2009 kein gesicherter Ertragseffekt zu beobachten. Die höchsten Fruchterträge wurden durch die geringsten Pflanzendichten von 39 Pfl./m^2 (Gießen 2009) bzw. 189 Pfl./m^2 (Gross-Gerau 2008) und 374 Pfl./m^2 (Gross Gerau 2009) erreicht. Die Zunahme der Pflanzendichte bewirkte in allen Versuchen eine Verminderung der Triebzahl, der Doldenzahl, der Fruchtzahl und der Fruchtmasse pro Pflanze, was zu einer Verminderung der Fruchterträge führte. Die Gehalte an ätherischem Öl in den Anisfrüchten variierten von 2,30 bis 3,67% und wurden durch die verwendete Sorte determiniert. Von allen geprüften Sorten war die Sorte Hild Samen durch den geringsten Fruchtertrag sowie durch den geringsten Gehalt an ätherischem Öl charakterisiert. Darüber hinaus wurde in der Versuchsstation Gießen auch ein positiver Effekt der Saatzeitverzögerung auf den Gehalt an ätherischem Öl beobachtet. Dieser Effekt wird mit der höheren Temperatursumme erklärt, die während der generativen Entwicklung der Anispflanzen vorhanden war. Die Variation der Pflanzendichte hatte keine Änderung der Gehalte an ätherischem Öl zur Folge.

Die Komponenten des ätherischen Öls, trans-Anethol und Estragol, wiesen bei der Sorte Hild Samen höhere Anteile auf als bei den Vergleichssorten.

Im zweiten Experiment wurden unterschiedliche Reihenweiten geprüft. Diese hatten am Standort Groß-Gerau keinen gesicherten Einfluss auf die Pflanzenlänge, die Verzweigungen/Pflanze, die Doldenzahl/Pflanze, die Tausendfruchtmasse und den Fruchtertrag. Große Reihenweiten hatten jedoch eine größere Anzahl an Früchten pro Pflanze und eine höhere Fruchtmasse pro Pflanze zur Folge. Die Verminderung der Pflanzendichte bewirkte eine signifikante Zunahme der wichtigsten Ertragsparameter wie Verzweigungszahl, Doldenzahl, Fruchtzahl und Fruchtmasse pro Pflanze. Geringe Pflanzendichten von 200 bis 300 Pfl./m^2 führten auf Grund der verminderten interspezifischen Konkurrenz zu den höchsten Fruchterträgen. Darüber hinaus wurde ein positiver Effekt geringer Reihenweiten auf den Fruchtertrag von Anis festgestellt.
Die Gehalte an ätherischem Öl unterlagen keinem gesicherten Einfluss durch die Veränderung der Reihenweiten und Pflanzendichten. Im Jahr 2009 wurde jedoch in Groß-Gerau bezüglich der Ölgehalte eine Interaktion zwischen Reihenweite und Pflanzendichte beobachtet. Insgesamt war festzustellen, dass die Prüffaktoren Reihenweite und Pflanzendichte nur eine geringe Variation der Gehalte an ätherischem Öl in den Anisfrüchten verursachten.

Die durchgeführten Fungizidversuche brachten zum Ausdruck, dass die Wirkstoff-Mischungen „Mancozeb + Metalaxyl-M“ sowie „Azoxystrobin + Difenoconazol“ in beiden Versuchsjahren eine wirksame Verminderung der Infektion mit *Cercospora malkoffii* bewirkten. Im Jahr 2009 bewirkte die Applikation der Fungizide keine Veränderung der Ertragsparameter der Anispflanzen. Demgegenüber wurde im Jahr 2010 eine signifikante und positive Beeinflussung der Ertragsparameter durch die Wirkstoffe Azoxystrobin + Difenoconazol beobachtet. Darüber hinaus wurden im Jahr 2010 durch die Fungizide sowie durch die Sorten auch die Gehalte an ätherischem Öl beeinflusst. Die Wirkstoffe Azoxystrobin + Difenoconazol sowie Fosetyl bewirkten eine signifikante Zunahme der Ölakkumulation in den Anisfrüchten. Der Wirkstoff Fosetyl induzierte außerdem eine höhere Konzentration an trans-Anethol im ätherischen Öl, was mit der Stimulierung der Abwehrreaktion der Pflanze durch Abbau von Phosphorsäure begründet wird.

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10. Appendices

Table A1: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of fruit yield and yield components in early and delayed sowing times Gross-Gerau 2008

Parameters	Gross-Gerau 2008 1st sowing time			Gross-Gerau 2008 2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Plant height (cm)	0.2368	0.1393	0.8671	0.2277	0.8261	0.7660
Primary branches per plant	0.0013	0.0038	0.6946	0.0052	0.0151	0.0248
Secondary branches per plant	0.0142	0.3360	0.2966	0.0530	0.5064	0.7562
Umbels per plant	0.0003	0.0448	0.7253	0.0056	0.0910	0.1637
Fruits per pant	0.0243	0.9175	0.5158	0.0487	0.0154	0.0189
Fruit weight per plant	0.0154	0.9060	0.7228	0.0374	0.0133	0.0711
1000-fruit weight (g)	0.1184	0.0860	0.8600	0.0020	0.4681	0.9872
Fruit yield (dt/ha)	0.0001	0.0131	0.9390	0.0001	0.0524	0.5324
Essential oil yield (kg/ha)	0.0001	0.0002	0.6980	0.0001	0.0696	0.3321

Table A2: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of quality parameters in early and delayed sowing times Gross-Gerau 2008

Parameters	Gross-Gerau 2008 1st sowing time			Gross-Gerau 2008 2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Essential oil content (%)	0.0125	0.0001	0.0139	0.0001	0.3617	0.5244
Estragol (%)	0.0001	0.7857	0.1816	0.0001	0.3213	0.2437
γ-himachalene (%)	0.0001	0.0651	0.4087	0.0001	0.0009	0.0556
Trans-anethole	0.0001	0.2403	0.6027	0.0001	0.1298	0.0925

Table A3: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of fruit yield and yield components in early and delayed sowing times Gross-Gerau 2009

Parameters	Gross-Gerau 2009 1st sowing time			Gross-Gerau 2009 2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Plant height (cm)	0.8920	0.0550	0.9089	0.8605	0.7061	0.7209
Primary branches (per plant)	0.1837	0.0001	0.5459	0.7015	0.0084	0.6310
Secondary branches per plant	0.7614	0.0041	0.8913	0.4104	0.1821	0.0685
Umbels per plant	0.2402	0.0001	0.4552	0.7449	0.0093	0.5270
Fruits per pant	0.0764	0.0001	0.1669	0.8740	0.0726	0.1366
Fruit weight per plant	0.0350	0.0001	0.0665	0.8441	0.0581	0.2738
1000-fruit weight (g)	0.4157	0.0001	0.0598	0.0776	0.3738	0.6674
Fruit yield (dt/ha)	0.0905	0.0001	0.8314	0.0748	0.0503	0.1809
Essential oil yield (kg/ha)	0.1578	0.0001	0.8184	0.0522	0.1272	0.2363

Table A4: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of quality parameters in early and delayed sowing times Gross-Gerau 2009

Parameters	Gross-Gerau 2009 1st sowing time			Gross-Gerau 2009 2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Essential oil content (%)	0.7055	0.3263	0.2108	0.3399	0.9577	0.1947
Estragol (%)	0.0010	0.0731	0.3898	0.0001	0.1216	0.8753
γ-himachalene (%)	0.0001	0.1776	0.0450	0.0011	0.7780	0.7100
Trans-anethole	0.0006	0.1150	0.0188	0.0027	0.3979	0.5664

Table A5: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of fruit yield and yield components in early and delayed sowing times Giessen 2008

Parameters	Giessen 2008 1st sowing time			Giessen 2008 2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Plant height (cm)	0.1057	0.5622	0.9979	0.0213	0.6129	0.9794
Primary branches (per plant)	0.0001	0.0001	0.3921	0.0001	0.0001	0.0173
Umbels per plant	0.0001	0.0001	0.0993	0.0001	0.0001	0.0015
1000-fruit weight (g)	0.2993	0.0156	0.0489	0.0001	0.1475	0.7558
Fruit yield (dt/ha)	0.0001	0.0088	0.2782	0.0001	0.0134	0.3042
Essential oil yield (kg/ha)	0.0001	0.0375	0.3991	0.0001	0.0145	0.4989

Table A6: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of quality parameters in early and delayed sowing times Giessen 2008

Parameters	Giessen 2008			Giessen 2008		
	1st sowing time			2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Essential oil content (%)	0.0002	0.0203	0.8714	0.0001	0.0741	0.3319
Estragol (%)	0.0029	0.1534	0.3960	0.0001	0.6290	0.4454
γ-himachalene (%)	0.0001	0.8147	0.9472	0.0001	0.0910	0.1629
Trans-anethole	0.0001	0.3126	0.3773	0.0001	0.0396	0.0755

Table A7: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of fruit yield and yield components in early and delayed sowing times Giessen 2009

Parameters	Giessen 2009			Giessen 2009		
	1st sowing time			2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Plant height (cm)	0.1284	0.0001	0.0120	0.8662	0.0767	0.0580
Primary branches (per plant)	-	-	-	0.1881	0.0001	0.7429
Secondary branches per plant	-	-	-	0.0358	0.0001	0.0285
Umbels per plant	-	-	-	0.2620	0.0001	0.3367
1000-fruit weight (g)	0.0939	0.6091	0.0611	0.0407	0.0035	0.5432
Fruit yield (dt/ha)	0.9196	0.0013	0.6607	0.5423	0.4573	0.3875
Essential oil yield (kg/ha)	0.7958	0.0001	0.5043	0.9930	0.2668	0.4824

Table A8: Results of ANOVA: p values for main effect and interaction between plant density (PD) and cultivars (CV) of quality parameters in early and delayed sowing times Giessen 2009

Parameters	Giessen 2009			Giessen 2009		
	1st sowing time			2nd sowing time		
	CV	PD	CV X PD	CV	PD	CV X PD
Essential oil content (%)	0.4346	0.0786	0.0794	0.0061	0.2605	0.0167
Estragol (%)	0.0001	0.0002	0.1319	0.0001	0.0008	0.2430
γ-himachalene (%)	0.4606	0.0001	0.9067	0.0087	0.4272	0.3250
Trans-anethole (%)	0.1047	0.0001	0.8607	0.0232	0.3233	0.2667

Table A9: Results of ANOVA: p values for main effect and interaction between plant density (PD) and row spacing (RS) of fruit yield and yield components of anise at experimental station Gross-Gerau 2008-2009

Parameters	Gross-Gerau 2008			Gross-Gerau 2009		
	RS	PD	RS X PD	RS	PD	RS X PD
Plant height (cm)	0.3357	0.0913	0.8804	0.0001	0.0114	0.0956
Primary branches (per plant)	0.189	0.0001	0.8814	0.7421	0.0001	0.1147
Secondary branches per plant	0.4937	0.2519	0.8331	0.0856	0.0016	0.1013
Umbels per plant	0.1722	0.0003	0.9847	0.2435	0.0001	0.0567
Fruits per pant	0.0297	0.0005	0.9167	0.0441	0.0001	0.0686
Fruit weight per plant	0.0156	0.0001	0.944	0.0412	0.0001	0.0583
1000-fruit weight (g)	0.8827	0.8827	0.0702	0.1253	0.0301	0.8083
Fruit yield (dt/ha)	0.6076	0.0246	0.5516	0.9024	0.0001	0.6104
Essential oil yield (kg/ha)	0.6375	0.051	0.6004	0.3262	0.0001	0.1103

Table A10: Results of ANOVA: p values for main effect and interaction between plant density (PD) and row spacing (RS) of quality parameters of anise at experimental station Gross-Gerau 2008-2009

Parameters	Gross-Gerau 2008			Gross-Gerau 2009		
	RS	PD	RS X PD	RS	PD	RS X PD
Essential oil content (%)	0.4965	0.3602	0.7751	0.1524	0.3076	0.0187
Estragol (%)	0.3827	0.3827	0.4269	0.8847	0.0001	0.0887
γ-himachalene (%)	0.4261	0.2890	0.6322	0.0002	0.0437	0.9934
Trans-anethole	0.0996	0.1617	0.4902	0.0005	0.0336	0.7351

Table A11: Results of ANOVA: p values for main effect and interaction between plant density (PD) and row spacing (RS) of fruit yield and yield components of anise at experimental station Giessen 2008-2009

Parameters	Giessen 2008			Giessen 2009		
	RS	PD	RS X PD	RS	PD	RS X PD
Plant height (cm)	0.7968	0.4419	0.9910	0.5574	0.0005	0.3164
1000-fruit weight (g)	0.0335	0.0222	0.9937	0.5285	0.2978	0.1933
Fruit yield (dt/ha)	0.0001	0.5390	0.3110	0.2569	0.5570	0.2761
Essential oil yield (kg/ha)	0.0001	0.2799	0.6502	0.7622	0.1686	0.2789

Table A12: Results of ANOVA: p values for main effect and interaction between plant density (PD) and row spacing (RS) of quality parameters of anise at experimental station Giessen 2008-2009

Parameters	Giessen 2008			Giessen 2009		
	RS	PD	RS X PD	RS	PD	RS X PD
Essential oil content (%)	0.3344	0.0445	0.9883	0.0004	0.0120	0.9657
Estragol (%)	0.0019	0.9134	0.1267	0.0242	0.0006	0.2111
γ-himachalene (%)	0.0003	0.9560	0.9252	0.0385	0.1498	0.8136
Trans-anethole	0.0118	0.5097	0.5645	0.0280	0.0081	0.2049

Table A13: Results of ANOVA: p values for main effect and interaction between fungicides (FU) and cultivars (CV) of fruit yield and yield components of anise at experimental station Gross-Gerau 2009-2010

Parameters	Gross-Gerau 2009			Gross-Gerau 2010		
	CV	FU	CV X FU	CV	FU	CV X FU
Plant height (cm)	0.7664	0.8525	0.9194	0.6912	0.9552	0.0949
Primary branches per plant	0.0215	0.2521	0.6618	0.4098	0.0060	0.7970
Sec. branches per plant	0.1127	0.4849	0.4114	0.6903	0.0594	0.4034
Umbels per plant	0.0328	0.2622	0.3739	0.5691	0.0146	0.4928
Fruits per pant	0.0267	0.2368	0.3778	0.5863	0.0001	0.0856
Fruit weight per plant	0.0191	0.1656	0.3604	0.5945	0.0001	0.1457
1000-fruit weight (g)	0.8786	0.0043	0.8264	0.6131	0.0008	0.0001
Fruit yield (dt/ha)	0.1196	0.0001	0.3944	0.4745	0.0001	0.8524
Essential oil yield (kg/ha)	0.6399	0.0001	0.5806	0.1777	0.0001	0.7239

Table A14: Results of ANOVA: p values for main effect and interaction between fungicides (FU) and cultivars (CV) of quality parameters of anise at experimental station Gross-Gerau 2009-2010

Parameters	Gross-Gerau 2009			Gross-Gerau 2010		
	CV	FU	CV X FU	CV	FU	CV X FU
Essential oil content (%)	0.2082	0.5048	0.1181	0.0286	0.0005	0.5206
Estragol (%)	0.0009	0.5232	0.7324	-	-	-
γ-himachalene (%)	0.1652	0.1867	0.4296	0.1344	0.0632	0.9263
Trans-anethole	0.6045	0.5713	0.1857	0.0186	0.0042	0.5506

Declaration / Erklärung

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the “Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice”.

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ nie dergelegt sind, eingehalten.“

Giessen, May 04, 2012 **(Habib Ullah)**

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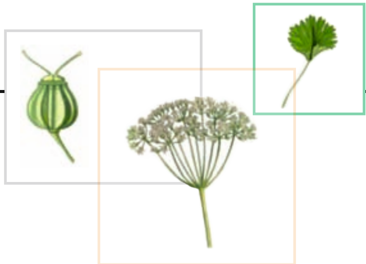

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