Institut fur Nutzpflanzenwissenschaften und Ressoucenschutz

Pollination of Medicinal Plants (*Nigella sativa and Coriandrum sativum) and Cucurbita pepo* in Jordan.

Doktor der Agrawissenschaften
(Dr.agr.)
Doktor-Ingenieur

der
Hohen Landwirtschaftlichen Fakultat

der
Rheinischen Friedrich- Wilhelms-Universitat

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von: Khaled Abu- Hammour

aus: Jordan-Al-Salt

Referent: Prof. Dr. Dieter Wittmann.

Korreferent: Prof. Dr. Marc Janssens.

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Dedication

For All Medicinal and Squash Plants Farmers.

Abstract

The aim of this doctorate thesis was to reveal the reproductive system and to identify the main pollinators in Jordan of Black Cumin (*Nigella sativa*) Coriander (*Coriandrumer sativum*) and squash (*Cucurbita pepo*). These plants are wide spread in Jordan and are used for medical treatments and as spices and food. Furthermore two surveys of solitary bees and the mellitophilous flowering plants were conducted in the Jordan Valley at 150 m below and in Northern Jordan at 200m above sea level from January until April.

On these transects 261 bee individuals out of 11 Genera were recorded. The density of bees in the Jordan Valley was higher than in the northern study site. Most bees were active during March. 106 species out of 23 families of melittophilous plants were found. Most species were from the families Caryophyllaceae, Gramineae and Compositae.

The following pollination treatments were conducted.

1. Open pollination (flower is accessible for all visitors);
2. Spontaneous selfing (bagged flower, no pollinator can touch the flower, but selfing can happen);
3. Forced selfing (emasculation and hand pollination, afterwards bagged); and
4. Cross pollination.

Measured and compared were the seed set after these trials. They were repeated in two years at the two study sites in order to see whether different pollinators are present.

Nigella sativa: Black cumin produced most seed after open pollination (86% of the ovaries set seed), when the flowers were forced to self pollination seed set reached at 85%, after cross pollination seed set was 75%, seed set in emasculated flowers (selfing) rendered only 13%. Nigella has a special way to assure spontaneous self pollination. This mechanism was described and compared with a similar mechanism in *Nigella arvensis*. The only pollinators were honey bees.

Coriander sativum: Coriander has hermaphroditic male and female flowers. The hermaphroditic flowers are strictly self-incompatible. However geitonogamie between male and hermaphroditic flowers renders at least 25%. Analyses of fertilized seeds showed that they contain 14,9% Protein while non fertilized ones had a significant lower content of 4.7%. This again shows that pollination does not only produce well shaped fruits but also increases the quality of the content of seeds. Only honeybees pollinated the Coriander flowers.

Cucurbita pepo: Open pollination gave the best results (88%) while hand cross pollination was less effective (86.1%). Geitonogamy reached similar results as Hand cross-pollination with 84%. In these flowers only honeybees were recorded.

Zusammenfassung

Eines der Ziele der vorliegenden Arbeit war, die Bienenfauna und ihre Trachtpflanzen an zwei Standorten im nördlichen Hochland (200m über NN) und im Jordan Tal (150 unter NN) zu erfassen. Des Weiteren wurden die Reproduktionssysteme von Schwarzkümmel *Nigella sativa* L., Koriander, *Corinander sativum* L. und Kürbis, *Cucurbita pepo* L. untersucht. Hierzu war es notwendig, den Beginn und die Dauer der jeweiligen männlichen und weiblichen Phase der Blüten zu ermitteln. Folgende Versuchsansätze wurden durchgeführt: Offene Bestäubung, Fremdbestäubung, Test auf spontane Autogamie, Test auf Selbstkompatibilität und Emaskulation. Auch sollte geklärt werden, welche Funktion die autogenen Bewegungen der Stamina und des Stylus bei *Nigella* und Koriander haben. Die Charakteristika dieser Bewegungen sollten mit bereits bekannten Mechanismen zur Selbstbestäubung bei verwandten Arten verglichen werden.

Die Bienenfauna im Jordan Tal und in Nord-Jordanien wurde vom Januar bis April 2007 untersucht. Bei den Begehungen wurden mittels Handfängen 261 Bienen gesammelt, die zu 11 Gattungen gehören. Die Anzahl der Bienen zur diesen Zeit war in Jordan Valley höher als die in Nord-Jordanien. Die Phase der höchsten Flugaktivität der Bienen war März.

Bei der Erfassung des Artenbestandes der Pflanzen wurden 106 Arten aus 23 Familien nachgewiesen. Die 3 größten Familien waren Caryophyllaceae, Gramineae und Compositae.

Nigella sativa L. zeigte bei der offenen Bestäubung einen hohen relativen Samenansatz von 86%; beim Test auf Selbstkompatibilität lag der Samenansatz bei 85%, bei der Fremdbestäubung bei 75%. Bei der Autogamie ergab sich einen Samenansatz von 46.5%, die emaskulierten Blüten zeigten dagegen den geringsten Samenansatz von 13%. Der einzige Bestäuber war die Honigbiene. 67% der besuchenden Bienen waren Pollensammlerinnen und 33% waren Nektarsammlerinnen. *Nigella* hat einen speziellen Mechanismus zur aktiven Selbstbestäubung. Dieser wurde beobachtet und beschrieben und mit dem ähnlichen Mechanismus bei *Nigella arvensis* verglichen.

Corinander sativum L.: Koriander hat drei Typen von Blüten, zwittrige, männliche und weibliche. Die zwittrigen Blüten sind strickt selbst-inkompatibel. Bei der

Nachbarbestäubung zwischen dem Pollen einer männlichen Blüte mit einer zwittrigen können sich aus ca. 25% der Ovarien Samen bilden. Die offene Bestäubung war die effektivste mit 52% Samenansatz. Auf den Blüten wurden ausschließlich Honigbienen nachgewiesen. Es wurde gezeigt, dass die unbefruchteten Samen einen Proteinanteil von 4.7% haben, während bei den befruchteten Samen der Proteinanteil bei 14,9% lag.

Cucurbita pepo L.: Bei der offenen Bestäubung bildeten 90,7% der Ovarien Samen, bei Fremdbestäubung 85,1% und bei der Nachbarbestäubung (Geitonogamie) lag der Samenansatz bei 86%. Der Bestäuber waren auch hier ausschließlich Honigbienen.

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List of Abbreviations:

cm: centimeter (10^{-2} meter).
End of the daylight: recommended to be 15:30.
Fig.: Figure.
g: gram.
km^2: Kilometer Square.
mg: milligram (10^{-3} gm.).
NCARTT: National Center for Agricultural Research and Technology Transfer.
SPSS: Standard Package for Social Science.
Tab.: Table
µl Microliter (10^{-6} Liter).

1. INTRODUCTION

Although Jordan is a small country with an area of 90,000 km^2, it is one of the richest countries in the number of aromatic and medicinal plant species. The total number of vascular plant species in the world is about 250,000 whereas Jordan's vascular flora is numbering 2500 species which is almost 1/100 of the total world flora. Medicinal plants comprise and about 25% of the total flora in Jordan about 485 species of wild medicinal plants. Medicinal plants were neglected in Jordan as a governmental concern. Many families have an interest in planting medicinal species for their own uses.

1.1. *Nigella sativa*

Black cumin "*Nigella sativa*" (Ranunculaceae) is an annual herbaceous plant. The genus *Nigella* is represented in 20 species of Mediterranean-western Asian origin (DANTUONO *et al*., 2002). Only *N. sativa, N. damascene* and *N. arvensis* are of interest in Jordan, *Nigella sativa* is the only species planted by farmers. There is no accurate data about the planted area. Annual production was 3-5 tons for the year 2005/2006 (personal communication).

Description of the Plant

Nigella sativa is a hermaphroditic species with determinate flowering patterns, starting with the flower terminating the main shoot and ending with the flowers on the lower-most branches. In its natural form, the flowers are delicate, and usually pale blue and white color, with five to ten petals and characterized by the presence of nectaries. The androecium comprises a large number of stamens, which shed their pollen as the filament curves outward during the male phase. The gynoecium consists of up to five completely united follicles, each with a long, indehiscent style and composed of a variable number of multi ovule carpels, developing into a follicle after pollination, with a single fruit partially connected to form a capsule like structure. Seeds are generally small size (1-5mg) dark grey or black (FILIPPO *et al*., 2002). The fruit is large and its inflated capsule contains numerous seeds.

Medicinal Use

Nigella sativa is in traditional medicine extensively used for healing various respiratory disorders from Morocco to Pakistan and in southern Europe (FILIPPO *et al*., 2002). The seeds have been widely added as a spice to a variety of foods such as bread, yoghurt, pickles, sauces, and salads for flavoring. They are also used in Jordanian folk traditional medicines for some respiratory, gastrointestinal, rheumatic and inflammatory disorder (AMIN, 1991; NAFISY, 1989; and ZARGARI, 1990). *Nigella sativa* seeds have been reported to contain essential oil, fixed oil, flavonoids, saponins, alkaloids, and proteins (ZARGARI, 1990; BURITS and BUCAR, 2000; and AL–GHAMDI, 2001). Because the traditional and folkloric uses of *Nigella sativa* seed are supported by a long history of human experience, this plant may be an important source for the isolation of potential drugs. The companies in Jordan nowadays are interested in *Nigella sativa,* especially the oil for medicinal usage. All registered effects make *Nigella sativa* an ideal candidate for use in cancer prevention and cure (NAFISY, 1989; AMIN, 1991; and ZARGARI, 1990). Yield instability is a common problem in *Nigella sativa* grown in Jordan. Little attention has been paid for crop pollination in the area and the *Nigella sativa* plant was never evaluated for pollination requirement under Jordanian weather conditions. Pollination studies of *Nigella sativa* are very limited in the literature. LLOYD (1979) showed that *Nigella sativa* is self pollinated without mention of the mechanism; ZOHARY (1983) showed that *Nigella sativa* is capable of setting seed without being cross pollinated. The flowers of *Nigella sativa* are visited by honeybees (RICCIARDELLI and ODDO, 1981).

1.2. *Coriandrum sativum*

Coriander (*Coriandrum sativum*) is a culinary and medicinal plant from the Umbelliferae. It is an annual herb originally from the Mediterranean area. Nevertheless, several authors have named *Coriandrum sativum* as a wild plant. LINNAEUS (1780) reported that *Coriandrum sativum* also occurs as a weed in cereals. ALEFELD (1866) mentioned that *Coriandrum sativum* was a common weed spread from southeastern Europe to southern Russia. STOLETOVA (1930) also reported on wild *Coriandrum sativum* from Armenia. All parts of the plant have a strong odor, from which the plant takes its name. The cultivation of *Coriandrum sativum* is widespread, but is planted on a small scale only. In

Jordan, it is where found in gardens rather than in large fields, in contrast with Germany where there are in many landraces of *Coriandrum sativum* (DIEDERICHSEN, 1996). It is cultivated as a summer or winter crop.

Description of the Plant

The plant can reach heights of 20 to 80cm. The stem is more or less erect, branched sometimes with several side branches at the basal node. Each branch finishes with an inflorescence. The color of the more or less ribbed stem is green and sometimes turns to red or violet during the flowering period. The leaves alternate. The first ones are often gathered in a rosette. The leaves are of two types, the lower with leaflets and the upper divided into narrow linear segments (DIEDERICHSEN, 1996). The *Coriandrum sativum* flower has five irregular-shaped petals, five stamens, five sepals, and two styles. Flowering starts with the primary umbel. The first umbel bloom has hermaphrodite flowers, with possibly a few staminate ones (DIEDERICHSEN, 1996). The inner flowers of umbellets are staminate. The central flowers are circular, with small inflexed petals.

The color of the petals are pale pink or sometimes white. Their flowering period is shorter (DIEDERICHSEN, 1996). In a single flower, the five filaments of the staminate are located between the five petals. After the flower opens, the white filaments are visible between the petals. Under optimum conditions, many different insect species are pollinators or visitors of *Coriandrum sativum* umbels (DIEDERICHSEN, 1996). The species of insects that pollinate *Coriandrum sativum* depend on the area of cultivation. Flowering and pollination biology of *Coriandrum sativum* is typically of that for umbelliferous plants, according to BELL (1971). Depending on the weather conditions, 2-3 days after opening of the first flower, the pollen sacs open and spread the pollen. MC GREGOR (1976) showed that selfing of the *Coriandrum sativum* is impossible but GLUKHOV (1955) showed that *Coriandrum sativum* is partially self-fertile. He suggested that geitongamy is common and cross is possible. Bees are beneficial to *Coriandrum sativum*, GLUKHOV (1955) reported that when they were excluded only 49.4 percent of the seeds set, but when they were present 68.3 percent of the seeds set. BOGOYAVLENSEII and AKIMENKO (1966) associated seed yields with greater insect visitation.

Use of *Coriandrum sativum*

This plant is of economic importance since it has been used as a flavoring agent in food product, perfumes and cosmetics. Moreover the essential oils of the fruits and various extracts from *Coriandrum sativum* have been shown to possess antibacterial (BURT, 2004; CANTORE *et al.,* 2004; and KUBO *et al.*, 2004), anticancerous and antimutagenic (CHITHRA and LEELAMMA, 2000) activities. *Coriandrum sativum* has been used in medicine for thousands of years. In Jordan the primary product is the fresh green herb of *Coriandrum sativum*, used for its specific flavor, which is completely different from that of the ripe fruits.

In other countries the fruits are used as a spice and vegetable. OLEUM (1993) stated that Russian produces high quality *Coriandrum sativum* oil, with a linalool content of 55%. BAUER (1942) found that *Coriandrum sativum* attains its greatest yield of volatile oil (0.9%). The fatty oil of *Coriandrum sativum* is of interest because of the high level of petroselinic acid. Adipic acidic is used for the manufacture of a wide range polymers including high grade engineering plastic and has a global marketing.

1.3. *Cucurbita pepo*

Squash (*Cucurbita pepo*) belongs to the Cucurbitaceae family. *Cucurbita pepo* has been grown in North America for thousands of years. The word *Cucurbita* comes from the Massachusetts Indian word askuta, meaning “eaten uncooked”.

Description of the Plant

There are many different varieties of *Cucurbita pepo*. They all share the common plant characteristic of broad, dark green leaves. Generally, it grows as seasonal crops, although it can grow, wild out of season, mixed with natural vegetation. All of the Cucurbita species are annuals, most of them are prostrate with trailing branches, reaching a length of 12 to 15m, but some have a short, semi erect stem. The leaves are large and are borne one petioles up to 61cm in length. Leaves vary in size and shape, but typically have 3 to 5 lobes. *Cucurbita pepo* has creeping growth; its growth cycle is short. *Cucurbita pepo* plants are monoecious.

Staminate flowers are at the end of a thin stem, and have three anthers producing relatively large pollen grains. Plants produce male flowers 3 to 4 days before producing female flowers (ROULSTON *et al.,* 1996). Usually three male flowers are formed for each female, but temperature and photoperiod can change this ratio. Female flowers are open for only one day. The male flowers are easily recognizable because they are to be found above the foliage on long stems.

The male flowers posses both pollen and nectar; the female flowers only nectar. Pistillate flowers with single a pistil of three inferior united carpels. Pistillate flowers are on a short peduncle, the style is thick, and the stigma two lobed. The showy corolla of the pistillate flower is attached to the end of the easily recognizable ovary. It was recorded 24 to 34 pistillate blooms per *Cucurbita pepo* plant with 5.5 to 43.7 percent set. Many researches are concerned about improving quality and quantity of food production. But they did not take pollination as a basic subject for study, although it is considered the most important factor of production. Monoecious and dioecious plants generally require cross-pollination (BASHAM and ELLS, 1999).

Because the anthers are in one flower and the stigma is in another, the mechanical transfer of pollen is essential to fruit set. Some researchers stated that the seed number and fruit weight was increased in proportion to the amount of pollen deposited on the stigma. The plants produce pollen that is heavy sticky and not blown easily from flower to flower. Therefore, *Cucurbita pepo* requires means such as insects to transfer pollen.

Cucurbita pepo pollen is large, and not easily moved by insects. Honey bees are the principle means by which pollen is transferred from the male to the female flower. Some attempts observed that honey bees worked the flowers most intensively from 6 a.m. to noon with maximum activity from 8 to 9 a.m. It was stated that most bees visit the *Cucurbita pepo* flowers for nectar only. Nectar is secreted from a ring of tissue surrounding the style and just inside the perianth tube. As the number of bee visits increase, seeds weight and number increase. Furthermore, fruit size and shape get better (WILLS and WEARNING, 1993). Practically all authorities give primary credit to the honey bee in pollinating *Cucurbita pepo* (WOLFENBARGER, 1965).

DURHAM (1928) gave some credit to the cucumber beetle; whereas others to ants; and to the wild bees, *Peponapsis* species and *Zenoglossa* species, with a minor role played by *Diabrotica* species to beetles. Studies stated that "other insects are involved such as cucumber, scarab and meloid beetles, and flies and moths but to a lesser extent than are bees. Even though honey bees are poorly adapted as pollinators of *Cucurbita pepo*, pumpkin, and gourd, because of the small size of the insect and the relatively large pollen grains, still the importance of honey bees as pollinators of these crops should not be minimized. If pollination was inadequate, the introduction of honey bees was the only solution.

Use of *Cucurbita pepo*

Cucurbita pepo has a very long shelf life, which has contributed to their decorative uses. They are also one of the few vegetables that do not lose nutritional quality after picking. Vitamin A levels actually increase during storage. The high levels of Vitamin A found in deep yellow *Cucurbita pepo* such as butternut and acorn have an anti-oxidant effect that may lower the risk of some cancers. Although summer *Cucurbita pepo*, unlike their winter relatives, are low in Vitamin A, both varieties of *Cucurbita pepo* are high in Vitamin C. (BIOBASICS, 2006).

1.4. Standard Questions

The study answers the following questions:

a) What are the main descriptive features that differentiate the research sites?
b) Which type of reproductive system has the three selected species, protondric or protgamy?
c) Which type of pollination results in maximum seed set open, self, cross or Geitonogamy?
d) Do plants set fruit when anthers are removed from the whole flower?
e) Does the competitive ability of a plant produced by self pollination differ from that produced by cross pollination?
f) Which pollinators are performing pollination technique upon the selected plant?
g) What are the protein percentages in fecundated and non fecundated seeds for *Coriandrum sativum*?
h) What are the dominant types of bees in The Jordan Valley and what is their behavior?
i) What are the most supportive plants for bees at the two study sites?

2. MATERIALS AND METHODS

2.1. Research Sites

The research was conducted in Jordan at two different locations. The first one is located 150m below sea level. This area is wet with warm temperatures in winter, and dry and hot in summer. The other is located 200m above sea level. It is characterized by rainy cold winter and dry mild summer (Fig. 2). The site has produced relatively high biodiversity in Flora and biogeographically units. Jordan valley (Fig. 1), which extends down the entire flank of Jordan 50km away from Amman, is the country's most distinctive natural feature. The Jordan valley is located between 22° 40′ 0′′ latitudes, and 35° 30′ 0′′ longitudes. The northern segment of the Jordan valley, known in Arabic as the Ghor, is the nation's most fertile region. It contains the Jordan River and extends from the northern border down to the Dead Sea. Several degrees warmer than the rest of the country, its year-round agricultural climate, fertile soils, high winter rainfall and extensive summer irrigation have made the Ghor the food bowl of Jordan. According to MD (2002), the mean maximum and minimum temperature is 29.9°C and 16.98°C respectively, with rainfall amount around 77-392mm with over 44.84 rainy days per year. Its mean relative humidity is (72.45%) in winter and 48% in summer for the last 30 years. The Jordan Valley is experienced to ground frost nearly 2.5 days per year.

Fig. 1: Jordan Valley Overview of Vegetation Covers.

Location A: 150m (below sea level) **Location B: 200m (above sea level)**

Fig. 2: Research Sites (53, 15, 30, 30, are the Main International Highway)

2.2. Plant Materials

Nigella sativa Plantation

The research represented specific plant species (landraces), which were planted on site at different elevations. *Nigella sativa* was planted 150m below sea level and 200m above sea level at location A and location B. *Nigella sativa* was obtained from botanical gardens in Jordan (NCARTT). The seeds were planted in hills 30cm apart on November 5th 2005. The rows were 20m long, with 1m between rows. Water was supplied daily by drip irrigation and extra fertilizers (N P K) were applied. Black plastic mulch was used. Each plant was represented by three rows per location. *Nigella sativa* prefers light and requires well drained soil. Missing hills were replanted when necessary. The plants were thinned to two plants per hill when they were at two to three leaf stages. The two locations were kept weed free by cultivation and hand weeding.

Coriandrum sativum Plantation

Coriandrum sativum was planted in location A and location B. Seeds were obtained form local markets (landraces). The seeds were planted on November 5th 2006. The rows were 20m long with 1m between rows. Water was supplied daily by drip irrigation, and extra fertilizers (N P K) were applied. Each two locations were kept weed free by cultivation and hand weeding; because *Coriandrum sativum* is a weak competitor for weed, in spite of the black plastic mulch that was used.

Cucurbita pepo plantation

ANITA variety was planted in both locations. Seeds were obtained form local markets. The seeds were planted on November 5th 2006. *Cucurbita pepo* is a warm-season crop planted in well-drained, sandy loams with high organic matter content and a pH of 6.0 to 6.5.

2.3. Survey of Bees

By the start of flowering of the wild plants in the period from November 2006 to April 2007, I collected the flowers that were visited by bees, and I identified the most bee-supportive plants, i.e. the most visited plants by bees. I have sampled the bees visiting the flowers of plant using a hand-net. Then, I monitored the activity of bees for pollination in order to define the pollinator of each and calculate in percent basis of the total bees' number and the time they were active. Hence, I recorded the most dominant bees around the selected plants. I studied their behavior of feeding on nectar or pollen. In addition to this, I collected other bees' samples from the northern parts of Jordan. Voucher specimens of bees were kept in the Institute of Ecology and Resources of Landscape of Bonn University. I classified the specimens up to the genus level.

2.4. Survey of Flora

During the period of November 2005 to March 2006, a field tour was made once a day every week to monitor the wild and cultivated plants grown around *Nigella sativa*, regardless of the elevations of plants areas. Five plants were collected and identified at the flowering stage of each plant and the species reserved in a laboratory. However, by the start of flowering of the wild plants in the next period from November 2006 to April 2007, I collected flowers that were visited by bees.

2.5. Determination of Anthesis and Receptivity Periods

Receptivity

The time of stigmatic receptivity for *Nigella sativa, Coriandrum sativum*, and *Cucurbita pepo* was examined under a dissecting microscope. The direct test of receptivity was an assay that detects the presence of stigmatic peroxides.

To determine receptive, the stigmas were treated with hydrogen peroxide 3%, small air bubbles were formed by maturation of the stigma, which means that the flower is in the female phase (DAFNI and MAUES 1998). To determine receptive periods, 50 flower buds of each plant species were marked, 10 flower buds of the same age were bagged a day before the opening of flowers for 5-days. Finally, 10 flower buds were taken to the laboratory to check the availability of stigma receptivity.

Anthesis

For the three selective plants, timing of anthesis was checked in the field using a hand magnifier. After bending, anther capsules were observed by the naked eye. The mechanism of pollen releasing has been described through direct observations in the field. Any rupturing of the capsule for *Nigella sativa* and *Cucurbita pepo* will cause a pollen release where it is verified by anther dehiscence in the *Coriandrum sativum*. To re-insure the anthesis period due to small dense accumulated flowers of *Coriandrum sativum*' cluster, I observed the anther bending and dehiscent through the dissecting microscope.

2.6. Visitors and Pollinators

The observation periods were evenly distributed between 7:00 a.m. and 16:00 p.m. To quantify pollinator visitation, observations were made from sunrise to sunset, when most visitation occurs. (Collected the pollinators with a hand – held net). Insect visitors were caught by sweep netting; the bees that had visited the flowers in transect. Bees were collected 2-3 times a week on sunny days during the entire flowering period. The collected specimens were kept in a special insect box supplied with naphthalene galls to keep them through long storage without pest damage. Voucher specimens of bees were kept in the Institute of Ecology and Resources of landscape of Bonn University. I recorded if a visitor collected nectar, pollen or both, and counted the number of visiting individuals per visitor species.

Only those visitors were included that were visiting a minimum of three flowers in sequence or stayed more than three seconds in a flower to exclude accidental visitors. During the observations, I also recorded also the first insect that came to the plant, the type of landing on the flowers (sepal, petal, anther, or on stigma), type of departure from the flower (from sepal, anther, stigma or on petal), type of motion in the flower, and specifically the nature of the landing on the *Coriandrum sativum* flower (center of cluster or edge of cluster). The determination of the bee collector types was via the type of bearings and the part where landing on/departure off was observed. For *Nigella sativa,* the collectors that had circular motion on petals were classified as pollen and nectar collectors, while the collectors landing on petals without circular motion were classified as nectar collectors, and the collectors landing on anthers were classified as pollen

collectors. For *Coriandrum sativum*, the collectors with random motion on the cluster' edges were considered as nectar collectors, whereas the collectors with circular motion on the center of the cluster were classified as pollen collectors. For *Cucurbita pepo,* the collectors landing on anthers were classified as pollen collectors. However, flowers visited by pollinators were marked. I compared locations by monitoring which bees exist in Jordan Valley around the study area. I sampled wild bees within crop fields. I counted the spending time for pollinators on flowers of selected plants with a stopwatch. I monitored (10) bees pollinators for all locations in the maximum visit rate period then the average for all replicates. The non-native *Apis mellifera* was excluded in the collector as it is an abundant species in any agricultural locality of Jordan.

2.7. Amount of Nectar

Amount of nectar was determined from covered plants plots to ensure that the nectar was not taken by bees. Ten mature flowers from *Nigella sativa* and *Cucurbita pepo* plants were taken during pollination time. Nectar volume in micro-liter (µl) was measured with a graduated micropipette from a signed flower as described by (NEPI and PACINI, 1993).

2.8. Amount of Pollen

The weight of pollen grains of male flowers from covered plants was estimated using a method described by KEARNS and INOUYE (1993). Ten flowers were covered to ensure that the pollen was not taken by bees. A flower was dispersed by ethanol evaporation. The dish was weighed again to assess the weight of pollen grains per flower (applied on *Coriandrum sativum* and *Cucurbita pepo*) in milligrams. Because of no synchronization maturity of anthers and the long period for anthesis in *Nigella sativa*, it is difficult to estimate the weight of total pollen in one flower. Pollen output per flower was calculated by estimating the weight of pollen grains per anther and multiplying by the average number of anthers. This provided an estimate of total pollen output per flower.

2.9. Visitation Rate

In order to observe the pollinator visitation tour, the number of visits per bee was estimated by counting the number of visits from beginning of pollination to fertilization,

those with anther or stigma contact. The counts were conducted daily in *Nigella sativa* every 15 minutes per hour; while the plant species flowers were open. Whereas for *Coriandrum sativum* and *Cucurbita pepo,* counts were made for a one hour period, (8) hours a day while plant species flowers were open (three replicates were made).

2.10. Pollination

Nigella sativa Pollination

Controlled pollinations were carried out on the selected individual's plant at the time of maximum stigma receptivity and anthesis. Pollinated flowers were observed periodically for fruit set. The reproductive success of the study species was assessed by performing a spontaneous self pollination, manual self and cross pollination treatments. Following the initiation of the first flower bud, flowers were selected randomly and tagged; I assigned 180 flower buds of *Nigella sativa* in each location. 30 flower buds were marked per each pollination treatment. Pollination treatments were performed from February to March 2006 in order to know the best pollination treatment results in both locations. In order to conduct Geitongamy and Xenogamy types of pollination, all of the stamens organs of each flower were removed using special scissors (Emasculation). The flowers were pollinated using pollen from freshly dehisced anthers from the male flowers by using a fine brush (of the same plant) for Geitongamy pollination treatment and from another plant for Xenogamy pollination treatment (Cross pollination).

The flowers were left exposed to any insect as it occurs in nature for open pollination treatment. To prove the bagged self pollination, flower buds were bagged till the end of the pollination stage, and were left untreated and then revealed again in order to avoid any negative impact on their germination.

In order to check forced self pollination on the same hermaphrodite flower, flowers were bagged till the last day of the male stage while the beginning of the female stage. The flowers were pollinated using pollen from freshly dehisced anthers from the male flower to the female flower on the same hermaphrodite flower by using a fine brush. Regarding emasculation, flower buds of nearly the same age were selected in order to remove male

flowers to investigate the differences between the role of pollinator and the role of plant by the number of fruit set.

The anthers were removed with a pair of tweezers and were left to pollinate by a pollinator. If an emasculated flower sets fruit, then it must have received pollen from a pollinator. However, if an emasculated flower fails to set fruit, the pollinator will have no role in fertilization.

Coriandrum sativum Pollination

Controlled pollinations were carried out on selected individual's plant at the time of maximum stigma receptivity and anthesis. Pollinated flowers were observed periodically for fruit set. The reproductive success of the study species was assessed by performing a spontaneous self pollination, manual self and cross pollination treatments. Following the initiation of the first flower bud, clusters were selected randomly and tagged; I assigned 180 cluster of *Coriandrum sativum* for each location. 30 clusters were marked per each pollination treatment for many reasons, including the diversity types of flowers, their sizes, and their numbers in one cluster. Pollination treatments were performed from November 2006 to April 2007 in both locations designed to know the best pollination type.

In order to conduct Geitongamy and Xenogamy types of pollination, all stamens' organs of each flower were removed using special scissors (Emasculation). The flowers were pollinated using pollen from freshly dehisced anthers from male flower by using a fine brush (of the same plant) for Geitongamy pollination treatment and from another plant for Xenogamy pollination treatment (Cross pollination).

For the hand Geitongamy, hand Xenogamy, hand forced pollination and bagged self pollination for one hermaphrodite flower. In the 30 chosen clusters per each treatment, one flower was chosen dependent on pollination treatment whereas the rest, per each cluster, were removed.

For hand forced pollination, the selected hermaphrodite flowers at bud stage were tagged and bagged until the last two hours of the male stage. I cut all stamens from hermaphrodite flower in order to keep the pollen grains until stigmas were receptive. Then pollen grains were used to pollinate the hermaphrodite flowers. I covered the selected hermaphrodite flowers until the end of pollination stage to obtain the conditions of bagged self pollination for one hermaphrodite flower. The chosen flowers were let exposed to avoid any negative effects upon germination.

I covered the selected clusters until the end of pollination stage to obtain the conditions of bagged self pollination for one cluster. The chosen flowers were let exposed, and were revealed to avoid any negative effects upon germination. The selected clusters for open pollination treatment were left exposed to any insect as it occurs in nature.

Cucurbita pepo Pollination

Controlled pollinations were carried out on selected individual's plant at the time of maximum stigma receptivity and anthesis. Pollinated flowers were observed periodically for fruit set. The reproductive success of the study species was assessed by performing a spontaneous self pollination, manual self and cross pollination treatments. The reproductive success of the study species was assessed by performing open pollination and manual pollination treatments. Following the initiation of the first flower bud, I assigned 120 flower buds of *Cucurbita pepo* in each location. 30 flower buds were marked per each pollination treatment. Pollination treatments were performed from February to March 2007 in season one and January to March 2008 in season two, in order to know the best pollination treatment results in both locations.

The flowers were pollinated using pollen from freshly dehisced anthers from male flower by using a fine brush (of the same plant) for Geitongamy pollination treatment and from another plant for Xenogamy pollination treatment (Cross pollination). The flowers were left exposed to any insect as it occurs in nature for open pollination treatment.

To prove the bagged self pollination, flower buds, bagged till the end of pollination stage, were left untreated and revealed again in order to avoid any negative impact on their

germination. To prove the bagged self pollination, 30 female flowers buds were bagged till the end of pollination stage and left untreated in order to check the parthenocarpy, and were revealed again in order to avoid any negative impact on their germination.

2.11. Movement in Stigma and Anther Separation

Nigella sativa

I have documented the changes in the relative positions of anthers releasing pollen and the styles. A total of 30 flower buds were monitored during the study period using a hand magnifier. A single flower from 30 individual flowers was monitored from the morning to the end of the day. Each flower was scored for the number of the anthers on the flower, the number of anther dehisced, the position of the dehisced anthers and the positions of anthers relative to the stigma. The length of anther and style were measured by using a special caliber. Representative photographs were taken of the flowers at each stage.

Coriandrum sativum

To document changes in the relative positions of anthers releasing pollen and the stigma, a single flower from 30 individual flowers were monitored from the morning to the end of the day. Each flower was scored for the number of anthers dehisced, the position of the dehisced anthers and the positions of anthers relative to the stigma. Representative photographs were taken of the flowers at each stage.

Cucurbita pepo

To document changes, a single flower from 30 individual flowers was monitored from the morning to the end of the day. Representative photographs were taken of flowers at each stage.

2.12. Average Number of Ovules

30 marked flower buds were assigned to count the number of ovules in order to know the standard number of ovules in the stigma. I counted the number of ovules per each capsule, then took the average number of ovules in both locations and used the average number as a reference in the calculation.

2.13. Proportion of Hermaphrodite to Staminate and Pistilate Flowers for *Coriandrum sativum*

Measurements were carried out in all research population throughout their flowering period. Plants were marked and studied from the beginning to the end of their flowering (n =30 cluster). When in flower, the marked clusters were collected from each plant and their hermaphrodite, staminate, and pistilate flowers. They were counted using a dissecting microscope.

2.14. Proportion of Male to Female Flowers for *Cucurbita pepo*

Measurements were carried out in all research populations throughout their flowering period. Plants were marked and studied from the beginning to the end of their flowering (n =30 cluster). When in flower, the marked flowers were collected from each plant. Flowers were counted by the naked eye.

2.15. Seed Content

a) Determination of Seed Content

To determine the chemical constituents of *Coriandrum sativum* and *Cucurbita pepo* seeds, I chose an accredited lab, using internationally standard methods for high level and accurate results, which is The Royal Scientific Society (RSS) chemist's lab and the National Center for Agricultural Research and Technology Transfer (NCARTT) chemist's lab. I collected 400g of plant seeds from each location. The preparation requirements of seeds for extraction were applied including: drying of seeds, crushing, and solvent extraction to facilitate the extraction operation (different solvents were used for determining a specific constituent in percent basis, i.e. methyl esters for fatty acid

determination). The following detailed table (Tab. 1) shows the analyzed constituents and the international standards methods used:

Tab.1: Analyzed Constituents for *Coriandrum sativum* and *Cucurbita pepo*

No.			**Standard method used**
1.	Seed constituent	Ash	SOP: 130M01-009
		Moisture	SOP: 130M01-010
		Oil	SOP: 130M01-001
		Proteins	SOP: 130M01-006
		Trace elements: Mg – Mn – Cu Ca – K	 SOP NO :131M02-005 SOP NO :131M02-002
2.	Oil constituent	Fatty acids	COI/T.20/Doc. No. 24 (2001) – AOCS Ch 2-91 (1997)

b) Protein Percentage in Fecundated and Non Fecundated Seeds for *Coriandrum sativum*

The protein content in *Coriandrum sativum* seeds was determined by the National Center for Agricultural Research and Technology Transfer (NCARTT) chemist's lab.

I collected 400g of *Coriandrum sativum*' seeds from treatments which were assigned for open pollination and bagged self for one cluster. The preparation requirements of seeds for extraction were applied, including: drying of seeds, crushing, and solvent extraction to facilitate the extraction operation.

2.16. Data Analysis

Data was analyzed as complete randomized design with three replicates. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities lend (SPSS). For statistical data, standard descriptive statistics were performed for each of the following quantitative parameters: the number of produced fruits, the number of seed for each stigma, the number of ovules, the number of chambers per each capsule, the number of non fecundated seeds and the total number of fecundated seeds. Mean number of buds and stigmas of plants, standard deviation, and differences between pollination treatments in term of seed set per fruit were calculated. The statistical program package SPSS was used. Insect visits were standardized by calculating the number of visits per flower per plant. The data was summarized over the season by taking an average of the observations. Minimum and maximum value was observed and graphical analyses were applied.

3. RESULTS

3.1. Survey of Bees

During the study period, a total of 261 bee individuals were collected from flowers of wild plants. Specimens were identified up to the genus level. Eleven genera were recorded which belong to six families. It is interesting to point out that *Sinapis alba* is the most supportive plant for bees (Tab. 2). From January to March, the total numbers of bees illustrated were more in Jordan Valley than in the Northern parts of Jordan. Moderate temperatures and hence, dense vegetation cover in Jordan Valley aggravated the ultimate flowering activity, in contrast with the Northern parts of Jordan.

Tab. 2: Bees survey

1. Anthophora	**2.Eucera**	**4. Lasioglossum**	**6.Osmia**	**8.Anthidium**
Jordan Valley	**Jordan Valley**	**Jordan Valley**	**Northern**	**Jordan Valley**
sp1 ♀J:	**sp1**♂J: Anchusa	**sp1** ♀ J:	**sp1**♀A:	**sp**1♂ M:
Anchusa strigosa	strigosa	*Senecio vernalic*	*Sinapis alba*	*Phlomis viscose*
Sillene damacena	*Calendula arvensis*	*Sinapis alba*	**sp** ♂M:	**sp2** ♂ F:
Silene aegyptica	*Silene damasene*	**sp2** ♀ J:	*Anchusa strigosa*	*Senecio vernalis*
Sinapis alba	**Northern**	*Sinapis alba*	**sp3**♀M: *Sinapis*	**9. Halictus**
sp2 ♂**F:**	**sp2,3,4**♀F: *Sinapis*	**sp4**♀J:	*alba*	**Jordan Valley**
Anchusa strigosa	*alba*	*Calendula arvensis*	**sp4**♀F:	**sp** ♀ F:
sp3 ♀M:	*Sinapis vulgaris*	**Northern**	*Calundula arvensis*	*Sinapis alba*
Anchusa strigosa	**sp5**♀A :	**sp 3,5** ♀:	**sp5**♀ F:	**10. Bombus**
sp4 ♀J:	*Sinapis alba*	*Silene aegyptica*	*Sinapisalba*	**Northern**
Anchusa strigosa	**sp6**♀M: *Anchusa*	**5. Megachile**	7.**Nomada**	**sp** ♀M: Malva
Malva Sp.	*strigosa*	**Northern**	**Jordan Valley**	**11.Colletes**
Selene damcene	**3.Andrena**	**sp** ♀ M:	**sp1** ♀J:	**Jordan Valley**
Silene aegyptica	**Jordan Valley**	*Sinapis alba*	*Sencio vernalis*	**sp1**♂JF:
Sinapis alba	**sp1,2** ♂ J :	**Northern**	**sp2** ♀ J*:*	*Calendula arvensis*
Northern:	**sp 3,4,5,6,7,10,12** ♀ J:	**sp 8,9** ♀F	*Lupinus sp.*	**Northern**
sp5,6,7 ♀**M:**	*Calendula arvensis*	*Sinapis alba*		**sp2** ♂♀M A :
Anchusa strigosa	*Sinapis alba*	**sp 11** ♀ M		*Sinapis arvensis*
		Sinapis alba		*Sinapis alba*

Legend:

J: January.
F: February.
M: March.
A: April
Sp: Species
♂: Male
♀: Female

3.2. Survey of Plant Species

A total of 106 plant species were recorded (Tab. 3) belonging to 91 genera and 23 families. The best represented families were the Compositae (18 genera and 21 species), Graminaea (11 genera and 13 species, Papilionaceae (10 genera and 15 species), and Caryophllaceae (10 genera and 12 species). On the other hand, other families were represented only one species (Chenopodiaceae, Papeveraceae, Rosaceae, Geraniaceae, Linaceae, Euphorbiaceae, Primulaceae, Plumbaginaceae, and Plantaginaceae).

Tab. 3: The Flora of the Surveyed Area

	1. Arizoaceae
1.1	*Arizoon hispanicum* L.
	2. Boraginaceae
2.1	*Alkanna galilaea* Boiss
2.2	*Alkanna strigosa* Boiss.et Hohen
2.3	*Anchusa strigosa* Bank et Sol
2.4	*Ecchium judaeum* Lacaita
2.5	*Heliotropium europaeum* L.
2.6	*Podonosma orientalis* Feinbr
	3. Charyophyllaceae
3.1	*Arenaria leptoclados* Guss.
3.2	*Dianthus strictus* Banks et Sol.
3.3	*Gypsophila Arabica* Barkouda
3.4	*Herniaria hirsuts* L.
3.5	*Paronychia argentea* Lam.
3.6	*Paronychia sinaica* Fresen.
3.7	*Pteranthus dichotomus* Forssk.
3.8	*Silene conoidea* L.
3.9	*Silene vivianii* Steud.
3.10	*Spergularia diandra* Heldr. Et Sart.
3.11	*Vaccaria pyramidata* Medik.
3.12	*Velezia rigida* L.
	4. Chenopodiaceae

4.1	*Atriplex halimus* L.
	5. Cistaceae
5.1	*Helianthemum aegyptiacum* Mill.
5.2	*Helianthemum salicifolium* Mill.
	6. Compositae
6.1	*Anthemis Palestine* Reut. subsp*. Palestine*
6.2	*Atractylis cancellata* L.
6.3	*Calendula arvensis* L.
6.4	*Carthamus nitidus* Boiss.
6.5	*Centaurea hyalolepis* Boiss. subsp*. Hyalolepis*
6.6	*Centaurea pallescens* Del. supsp*. pallescens.*
6.7	*Cichorium pumilum* Jacq.
6.8	*Crepis aspera* L. var. *aspera*
6.9	*Crepis sacta* Bornm. Subsp. *Sancta*
6.10	*Filago contracta* Chrtek et Holub
6.11	*Filago pyramidata* L.
6.12	*Geropogan hybridus* Sch. Bib.
6.13	*Gundelia tournefortii* L.
6.14	*Gymnarrhena micrantha* Desf.
6.15	*Hedypnois rhagadioloides* F.W. Schmidt subsp. *Tubae formis* Havek
6.16	*Koelpinia linearis* Pall.
6.17	*Pallenis spinosa* Cass.
6.18	*Phagnalon rupestre* Dc.
6.19	*Rhagadiolus stellatus* Gaertn.
6.20	*Scorzonera papposa* DC.
6.21	*Sonchus oleraceae*
	7. Cruciferae
7.1	*Carrichtera annua* DC.
7.2	*Erucaria hispanica* Druce.
7.3	*Eruca sativa* Mill.
7.4	*Matthiola aspera* Bioss.
7.5	*Notoceras bicorne* Caruel.

	8. Dipsacaceae
8.1	*Pterocephalus plumosus* Coult.
8.2	*Scbiosa prolifera* L.
	9. Euphorbiaceae
9.1	*Euphorbia chamaepeplus* Boiss. et Gaill.
	10. Geraniaceae
10.1	*Erodium gruinum* L'He'r.
	11. Gramineae
11.1	*Aristida adscensionis* L.
11.2	*Avena sterilis* L.subsp. sterilis
11.3	*Bromus madritensis* L.subsp. delilei Maire & Weiller
11.4	*Bromus rubens* L.
11.5	*Catapodium rigidum* C.E. Hubbard
11.6	*Crithopsis delileana* Roshev.
11.7	*Lolium subulatum* Vis.emend.Terrell
11.8	*Lophochloa pumila* Bor
11.9	*Phalaris brachystachys* Link
11.10	*Poa sinaica* Steud.
11.11	*Stipa capensis* Thunb.
11.12	*Trticum ovatum* Raspail. Syn. *Aegilops ovata* L.
11.13	*Triticum peregrinum* Hack & Fraser Syn. *Aegilops variabilis* Eig.
	12. Labiatae
12.1	*Ballota undulata* Benth
12.2	*Origanum syriacum* L.
12.3	*Salvia horminum* L.
12.4	*Salvia syriaca* L.
12.5	*Teucrium polium* L.
	13. Liliaceae
13.1	*Allium stamineum* Boiss.
13.2	*Gagea chlorantha* Schult. & Schult. Fil.
13.3	*Ornithogalum narbonense* L. *subsp.narbonense*
13.4	*Tulipa polychrome* Stapf.

13.5	*Urginea maritime* Baker.
	14. Linaceae
14.1	*Linum strictum* L. *var.spicatum Pers.*
	15. Papaveraceae
15.1	*Paperver polytrichum* Boiss.et Ky.
	16. Papilionaceae
16.1	*Astragalus cruciatus* Link
16.2	*Astragalus hamosus* L.
16.3	*Astragalus intercedens* Sam.
16.4	*Hippocrepis multisiliquosa* L.
16.5	*Hippocrepis unisilquosa* L.
16.6	*Hymenocarpos circinnatus* Savi.
16.7	*Medicago polymorpha* L.
16.8	*Medicago radiate* L.
16.9	*Onobrychis crista-galli* Lam.
16.10	*Ononis biflora* Desf.
16.11	*Ononis natrix* L.
16.12	*Physanthyllis tetraphylla* Boiss.
16.13	*Scorpiurus muricatus* L.
16.14	*Trigonella monspeliaca* L.
16.15	*Vicia sativa* L.
	17. Plantaginaceae
17.1	*Plantago coronopus* L. *subsp. Commutata Pilger* *Var. crassipes coss. Et Daveau*
	18. Plumbaginaceae
18.1	*Limonium thouinii* O.Kuntze
	19. Primulaceae
19.1	*Anagallis arvensis* L.*var. caerulea Gouan.*
	20. Rosaceae
20.1	*Sarcopoterium spinosum sp.*
	21. Rubiaceae
21.1	*Callipeltis cucullaria* Stev.

21.2	*Crucianella manrostachya* Boiss.
	22. Ranunculaceae
22.1	*Adonis cupaniana* Guss.
22.2	*Anemone coronaria* L.
	23. Umbelliferae
23.1	*Bupleurum lancifolium* Hornem.
23.2	*Daucus subsessilis* Boiss.
23.3	*Eryngium creticum* Lam.
23.4	*Lagoecia cuminoides* L.
23.5	*Pimpinella anisum* L.
23.6	*Tordylium aegyptiacum* Lam.*var.aegyptiacum*

3.3. *Nigella sativa*

Anthesis and Receptivity

Styles are the first floral organ to emerge and extend, followed by extension of the stamens. When the style has almost straightened, the anthers began to dehisce along about a half an hour, after the dehiscence of the anthers when it is considered to be the first day for pollen shedding, until the fifth day, the male stage activated between 8:30 a.m. to end of the day and then anthers sank down. The male phase was initiated a few days before the stigmas became receptive and the male stage lasted for five days. By the fifth day of the male stage, the female stage started. Stigmatic peroxides tests indicated that receptivity occurred between 8:00-13:00 p.m. and for one day only. Male and female stages synchronized on the last day of the flowering period (Fig. 3).

The weight of pollen for the *Nigella sativa* plant was 0.064 mg/flower, whereas the volume of nectar was 0.13 µl. Affluent floral rewards (both nectar and pollen) are noted during the male phase of the flowers.

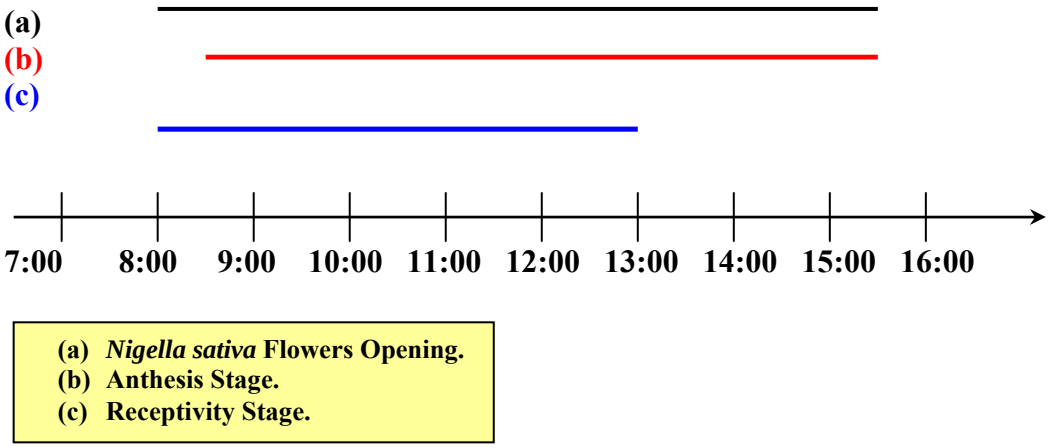


Fig. 3: Blooming Stages of *Nigella sativa*, Anthesis and Receptivity.

Movements of Stigma and Anthers in *Nigella sativa*

The male and female organs at bud stage are presented in (Fig. 4a). At onset of the male stage, all the stamens stand erect (Fig. 4b). They curve outwards one by one, roughly in whorls and strictly reflecting the order of initiation (Fig. 4c). When the anthers reach a horizontal position, the pollen is released (Fig. 4d). Then, the stamens sink down. An anther takes 4-7 hours to empty its contents. The stamen movement is not continuous, but it is divided into three phases. In the first phase (12-14 hours) the lower part of the filament inclines slightly, while the upper part curves more strongly, so that the anther is brought into a horizontal position. After reaching this position, movement comes to a standstill. The second phase, towards the ends of the male stage, the styles of the five carpels usually curve down (Fig. 4e) and twist (Fig. 4f). This ensures that in the female phase the stigmatic crests, whose bends were making an angle of 45°, continue to make a right angle with the ovary to run down nearly the whole length of the style to touch the top of the anther at several points (Fig. 4f). The third stage, in which the stamen sinks down, is much shorter than the previous ones (4-6 hours). Finally, the empty anthers curve up. This is a purely passive movement, apparently without any function. After uptaking the pollen, the stigma was pollinated (Fig. 4g), and then the stigma inclined upwardly erect as the order of initiation and made an angle of 180° with the ovary (Fig. 4h). The maximum style length reached 1.73 cm, whereas the maximum anther length was 1.72cm. This indicates the equal lengths of the style and the anther.

Fig. 4a: Plant at bud stage.

Fig. 4b: The stamens stand erect.

Fig. 4c: First phase of stamens movement: The stamens curve outwardly in whorls

Fig. 4d: Pollens releasing

Fig. 4e: First phase of style movement: the styles of the five carpels curve down.

Fig. 4f: Twisting point of style with anther.

Fig. 4g: The stigma is pollinated.

Fig. 4h: The stigma inclined upwardly erect.

Pollination

Season one

a) Location A

Nigella sativa' flowers produced a non significant number of ovules under all treatment conditions with an average of 96 ±0.5, as shown in (Tab. 4). Generally, all flowers under the different treatments produced seeds (Tab. 4). Open pollinated flowers produced significantly higher seeds as compared with other treatments 74.9±1.4. Hand cross, hand geitongamy and hand forced self ranked secondly in seed set and produced nonsignificant differences between them with a seed set average of 82.9±1.6, 73.5±1.5 and 78.9±1.6 respectively. A non-fecundated seed production is also a common feature of *Nigella sativa'* flowers under the different treatments. In the first location, open pollination occupied the lowest average of non-fecundated seeds (Tab. 4) compared to other treatments. Hand cross, hand geitongamy and hand forced self ranked second in producing a non-significant fecundated seeds with an average of 12 ± 1.7, 21.6 ± 1.6 and 18 ± 1.4 respectively.

There were significant differences ($P \leq 0.05$) in the percentage of seed set between treatments (Tab. 3). Seed set percentage after open pollination (86.8% seed) was significantly higher than all other treatments ($P \leq 0.05$). Non significant differences were found between the average percentage of seed set when hand cross, hand geitongamy and hand forced self was used on flowers (79.8%, 75.4 % and 81 % respectively).

***Nigella sativa* Location A season one:**

Tab. 4: Seeds set after different pollination treatments in *Nigella sativa*, location A.

Treatment of Pollination	No. of Ovules/Capsule	No. of Fecundated Seed/Capsule	No. of Non Fecundated Seed/Capsule	Percentage of Seed Set/Capsule
Open	93.3±0.5 a	74.9±1.4 a	19.0±1.5 a	86.8±1.2 a
Hand Cross	95.6±0.7 b	82.9±1.6 b	12.0±1.7 b	79.8±1.3 b
Hand Geitongamy	95.1±0.6 b	73.5±1.5 b	21.6±1.6 b	75.4±1.2 b
Hand Forced Self	96.0±0.5 b	78.9±1.6 b	18.0±1.4 b	81.0±1.4 b

a and b; are symbols related to difference in comparison.

b) Location B

Nigella sativa' flowers produced a non significant number of ovules under all treatment conditions with an average of 91.1±0.5, as shown in (Tab. 5). Generally, all flowers under the different treatments produced seeds (Tab. 5). Open pollinated flowers produced significantly higher seeds as compared with other treatments 82.9±1.5. Hand cross, hand geitongamy and hand forced self ranked second in seed set and produced nonsignificant differences between them with a seed set average of 72.4±1.4, 70±1.3, and 77.5±1.1 respectively for the first location. Characteristics of producing fecundated seeds in the second location were fairly constant in value and regulated mainly by treatments conditions (Tab. 5).

A non-fecundated seed production is also a common feature of *Nigella sativa'* flowers under the different treatments. In the first location, open pollination occupied the lowest average of non-fecundated seeds (Tab. 5) compared to other treatments. Hand crossed, hand geitongamy and hand forced self ranked second in producing a non-significant fecundated seeds with an average of 18.7 ± 1.3, 25 ± 1.5 and 18.1 ± 1, respectively.

There were significant differences ($P \leq 0.05$) in the percentage of seed set between treatments (Fig. 5). Seed set percentage after open pollination (87 % seed) was

significantly higher than all other treatments ($P \leq 0.05$). Non significant differences were found between the average percentage of seed set when crossed, hand geitonogamy and hand forced self was used on flowers (79 %, 73 % and 80 % respectively).

Nigella sativa Location B, Season One

Tab. 5: Seeds set after different pollination treatment in *Nigella sativa* location B.

Treatment of Pollination	No. of Ovules/Capsule	No. of Fecundated Seed/Capsule	No. of Non Fecundated Seed/Capsule	Percentage of Seed Set/Capsule
Open	95.6±0.48 a	82.9±1.5 a	12.8±1.5 a	87±1.3 a
Hand Cross	91.1±0.5 b	72.4±1.3 a	18.7±1.3 b	79±1.2 b
Hand Geitongamy	95±0.5 b	70±1.3 b	25±1.5 b	73.6±1 b
Hand Forced Self	96±0.6 b	77.5±1.1 b	18.1±1 b	80±0.99 b

a and b; are symbols related to difference in comparison.

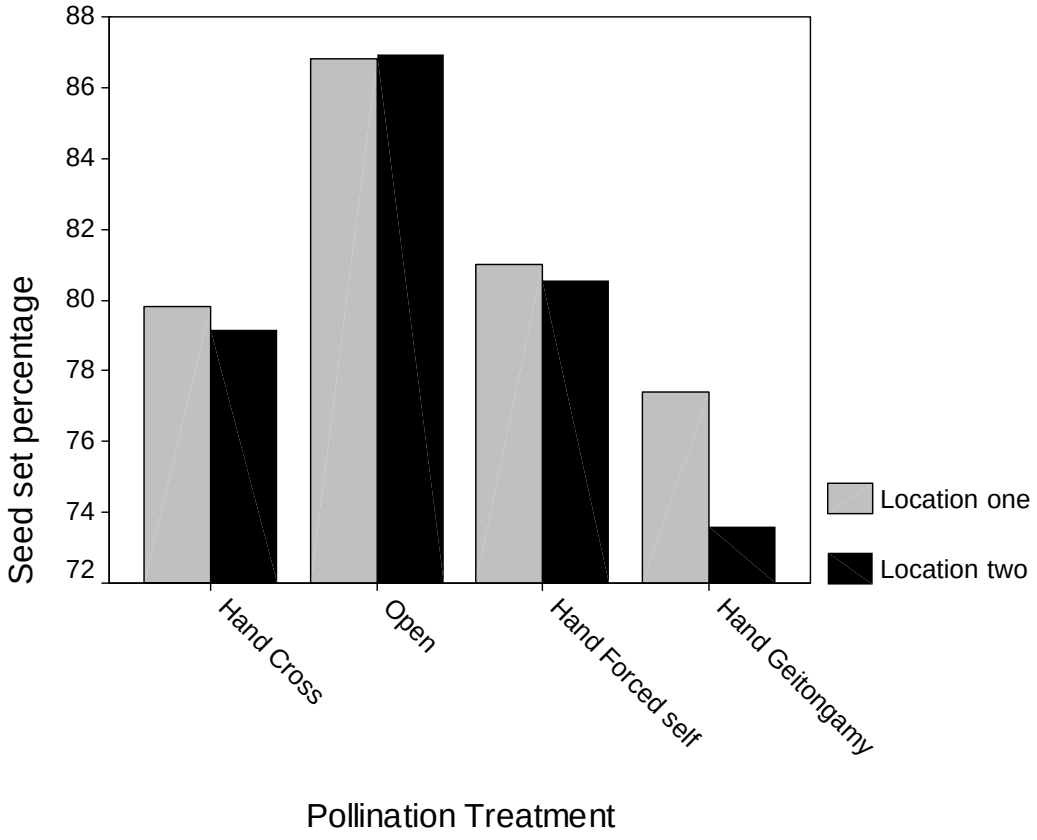


Fig. 5: *Nigella sativa* Seed Set Percentage upon Pollination Treatments in Location A and B season one.

Season Two

a) Location A

Nigella sativa' flowers produced a non significant number of ovules under all treatments conditions with an average of 92.3 ± 1.42, as shown in (Tab. 6). Generally, all flowers under the different treatments produced seeds (Tab. 6). Open pollinated flowers produced significantly higher seeds as compared with other treatments in both locations 83.4 ± 0.67. Hand cross, hand geitongamy and hand forced self ranked second in seed set and produced a non-significant differences between them with a seed set average of 74.6 ± 0.68, 73.6 ± 0.67, and 79.7 ± 0.32 respectively for the first location.

Bagged self pollinated flowers ranked third and produced 44.1 ± 0.75 seeds. The lowest seed set was recorded in the case of emasculated flowers with an average seed production of 12.4 ± 0.33. Hand cross, hand geitongamy and hand forced self ranked second. A non-fecundated seed production is also a common feature of *Nigella sativa'* flowers under the different treatments. In the first location, open pollination produced non-significant fecundated seeds with an average of 12 ± 1.7 , 21.6 ± 1.6 and 18 ± 1.4, respectively.

There were significant differences ($P \leq 0.05$) in the percentage of seed set between treatments (Fig. 6). Seed set percentage after open pollination (87% seed) was significantly higher than all other treatments ($P \leq 0.05$). Non significant differences were found between the average percentage of seed set when hand crossed, hand geitongamy and hand forced self was used on flowers (79%, 78% and 83%, respectively). Nearly half of the produced set seed in bagged flowers was with an average of 47%. Emasculated flowers (13%) recorded the lowest seed set from other treatments with significant differences.

Nigella sativa Location A, Season Two

Tab. 6: Seeds set after different pollination treatment in *Nigella sativa* location A.

Treatment of Pollination	No. of Ovules/Capsule	No. of Fecundated Seed/Capsule	No. of Non Fecundated Seed/Capsule	Percentage of Seed Set/Capsule
Open	96.8±2.19 a	83.4±0.67 a	13.4±1.93 d	87.0±1.67 a
Hand Cross	96.0±2.31 a	74.6±0.68 b	21.4±2.39 c	79.0± 1.98 b
Hand Geitonogamy	93.0±2.81 a	73.6±0.67 b	21.1±1.81 c	78.0±1.46 b
Hand Forced Self	97.2±1.67 a	79.7±0.32 b	17.6±1.77 c	83.0±1.54 b
Bagged Self	94.2±1.50 a	44.1±0.75 c	51.9±0.73 b	74.0±1.10 c
Emasculation	97.6±1.67 a	12.2±0.33 d	85.4±0.33 a	13.0±0.46 d

a, b, c, and d; are symbols related to difference in comparison.

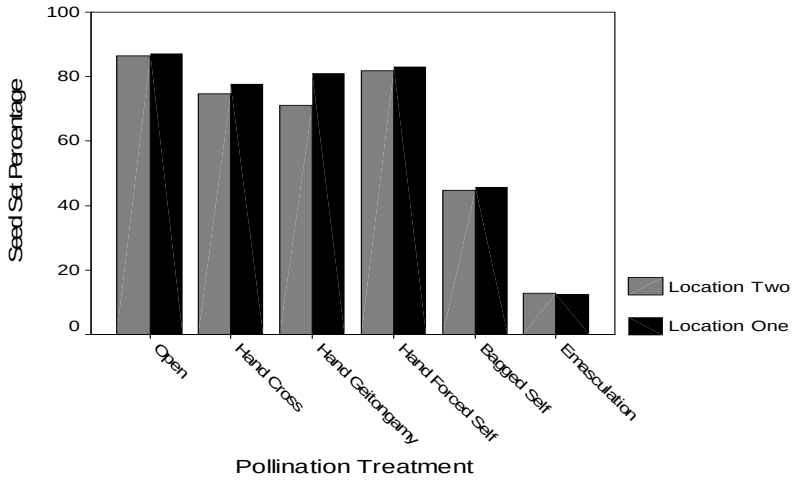


Fig. 6: *Nigella sativa* Seed Set Percentage upon Pollination Treatments in both Locations, Season Two.

b) Location B

Nigella sativa' flowers produced a non significant number of ovules under all treatment conditions with an average of 97.2 ± 1.67, as shown in (Tab. 7). Generally, all flowers under the different treatments produced seeds (Tab. 7). Open pollinated flowers produced significantly higher seeds as compared with other treatments in both locations 82.4 ± 0.57. Hand cross, hand geitongamy and hand forced self ranked second in seed set and produced non-significant differences between them with a seed set average of 71.8 ± 0.57, 67.9 ± 0.62, and 78.6 ± 0.5, respectively. Bagged self pollinated flowers ranked third and produced 43 ± 0.74 seeds. The lowest seed set was recorded in the case of emasculated flowers with an average seed production of 12.4 ± 0.5.

Open pollination occupied the lowest average of non-fecundated seeds 13.7 ± 1.76 (Tab. 7) compared to other treatments. Hand cross, hand geitongamy and hand forced self ranked second, a non fecundated seed production is also a common feature of *Nigella sativa'* flowers under the different treatments in producing a non-significant fecundated seeds with an average of 20.2± 1.57, 25.1 ± 1.62 and 14.5 ± 1.6, respectively.

There were significant differences ($P \leq 0.05$) in the percentage of seed set between treatments (Fig. 7). Seed set percentage after open pollination (87% seed) was significantly higher than all other treatments ($P \leq 0.05$). Non significant differences were found between the average percentage of seed set when hand crossed, hand geitongamy and hand forced self was used on flowers (78%, 73% and 85% respectively). Nearly half of the produced set seed in bagged flowers was with an average of 46%. Emasculated flowers (13%) recorded the lowest seed set from other treatments with significant differences.

Nigella sativa Location B, Season Two

Tab.7: Seeds set after different pollination treatments in *Nigella sativa* location B.

Treatment of Pollination	No. of Ovules/Capsule	No. of Fecundated Seed/Capsule	No. of Non Fecundated Seed/Capsule	Percentage of Seed Set/Capsule
Open	96.1±1.76 a	82.4±0.57 a	13.7±1.76 d	87±1.56 a
Hand Cross	92.3±1.42 a	71.8±0.57 b	20.2±1.57 c	78±1.28 b
Hand Geitonogamy	93.1±1.86 a	67.9±0.62 b	25.1±1.62 c	73±1.22 b
Hand Forced Self	93.1±1.53 a	78.6±0.50 b	14.5±1.60 c	85±1.39 b
Bagged Self	93.6±1.50 a	43.0±0.47 c	50.6±1.71 b	46±1.10 c
Emasculation	94.9±1.90 a	12.4±0.50 d	82.5±2.01 a	13±0.62 d

a, b, c, and d; are symbols related to difference in comparison.

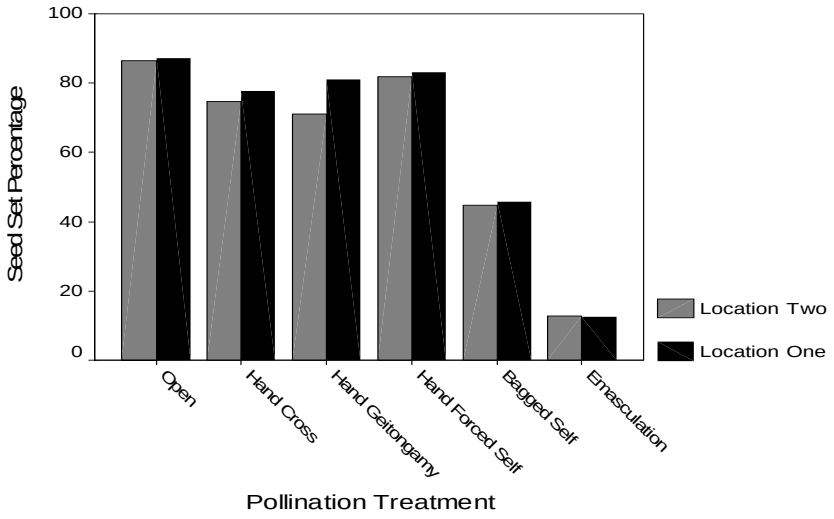


Fig. 7: *Nigella sativa* Seed Set Percentage upon Pollination Treatments in both Locations season two.

Behavior of Honey Bee Visitors

During our observation, honey bees were the only visitor and pollinator that visited *Nigella sativa* in the morning around 7:00 a.m. Every flower had at least one bee. Each bee spent a different amount of time with an average of 12.5sec for nectar collecting, 8sec for pollen collectors. The only diurnal visitors and pollinators were honey bees. Honey bees were frequent visitors to *Nigella sativa* in The Jordan Valley. The honey bee showed the same behavior in the two locations. In the evening, no pollinators were found in the flowers at both sites. The major pollinator was the honey bee. *Nigella sativa'* flowers' mean visit rates for the three replicates in both locations were 14.9 and 14.6 daily visiting tours, respectively. The ultimate activity during the three replicates was approximately from 9:30 a.m. to 12:30 p.m. in both locations. 33% of the total bees observed were pollen collectors, while the remaining 67% were nectar collectors (Tab. 8).

Tab. 8: Behavior of honey bees and their bearings for *Nigella sativa*.

Behaviors of Bees	Average Spending Time/Flower (Second)	Landing on			Departure of			Percent of Bees According to Their Bearings
		Petals	Anthers	Twisting	Petals	Anthers	Twisting	
Nectar Collector were observed	12.5	•			•			67%
Pollen collector were observed	8			•			•	33%

Honey bees visiting tours were conducted in two stages during five days. Anthesis period took place in the first four days, and anthesis and receptivity periods were in the fifth day. Visiting tours were for functional nectar collecting. On the first day, honey bees landed on petals and then collected nectar during circular stepping upon petals, without getting directly exposed to anthers.

On the second till the fourth day, the same behavior occurred. The pollen grains fell down upon bees back from the horizontal anthers during circular motion.

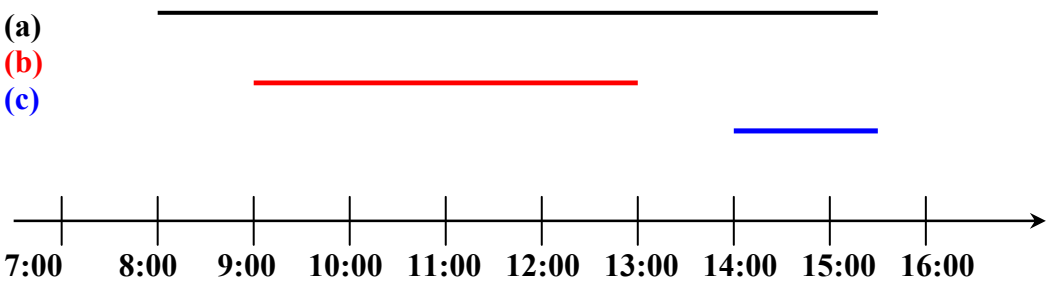
On the fifth day, receptivity period began, in which the styles inclined towards anthers, and then the styles twisted themselves around the anthers. Honey bees were landing directly on this synapse (not on petals). After that, they left and flew to another flower.

3.4. *Coriandrum sativum*

Anthesis and Receptivity

The flowers of the *Coriandrum sativum* are protandrous. Young stamens and petals are incurved at the beginning of anthesis. Stamens stand vertically over the stigma, when the anthers freshly dehisce and become fully expanded after shedding their pollen. The male phase starts in the morning, while the female phase activates after noon with a non overlapping period between them. The male stage starts at 9:00 a.m. and ceases by the afternoon, at about 13:00. After a while, the female stage initiates until the evening of the same day (Fig. 8). The anther stage did not develop reassembly or at the same time. Furthermore, this maturation process of the anthers did not lead to self pollination because stigmas are receptive much later.

The weight of the pollen for the *Coriandrum sativum* plant was 0.006 mg, whereas the amount of nectar in *Coriandrum sativum* was immeasurable.



(a) *Coriandrum sativum* Flowers Opening.
(b) Anthesis Stage.
(c) Receptivity Stage.

Fig. 8: Blooming Stages of *Coriandrum sativum*, Anthesis and Receptivity.

Movements of stigma and anthers in *Coriandrum sativum*

a) Description for One Cluster

Coriandrum sativum's umbel contains (5-9) clusters, each cluster has (19-21) flowers (Fig. 9a), Three types of flowers, the hermaphrodite flower (Fig. 9b), the staminate flower (Fig. 9c) and the pistilate (Fig. 9d) are found in each cluster, but the number of male flowers are more than the female and hermaphrodite. The first blooming flower is a hermaphrodite and the later flower has only a staminate flower (Fig. 10). The later staminate flower developed while the receptivity of the stigma initiated. The anther stands vertically (Fig. 11a) then partially bends toward the surface of the stigma out of their flower (Fig. 11b) that lies beneath (Fig. 11c). The pollen of anther in a staminate flower is thrown (Fig. 11d) and falls to stigmas of neighboring flowers where it's potentially pollinated. The inner flowers of umbellets are staminate. The central flowers are circular. The umbels of higher order usually contain more staminate flowers and the flowering period is short.

Fig. 9a: *Coriandrum sativum*' s cluster contains (9-21) flowers.

Fig. 9b: Hermaphrodite flower.

Fig. 9c: Female Flower.

Fig. 9d: Male Flower.

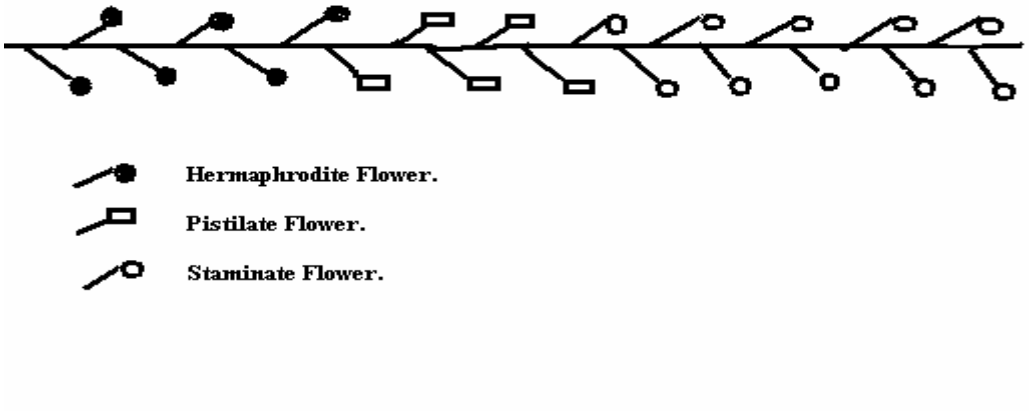


Fig. 10: Order of the Opening of *Coriandrum sativum* Flowers (Left to Right).

Fig. 11a: The anther stands vertically.

Fig. 11.b. Partially bending toward the surface of stigma.

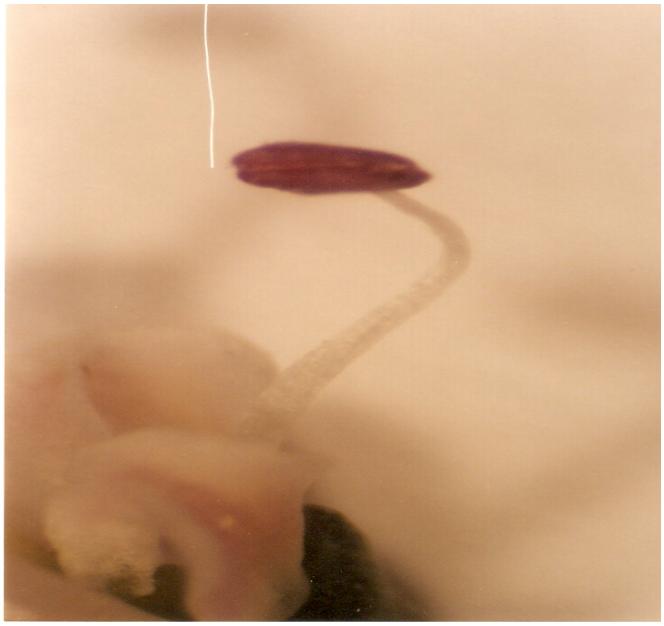


Fig. 11c: Anther lies beneath.

Fig. 11d: The pollen is thrown.

b) Description for One Hermaphrodite Flower

A flower has five petals, five stamens and two styles. In a hermaphrodite flower, the male developed earlier than females during inflorescence stage, and so the male stage began before the female stage. The anthers dehisce and release the pollen so the pollen is gone while the stigma is unreceptive.

The Ratio of Staminate Flowers to Pistilate and Hermaphrodite Flowers in One Cluster

Coriandrum sativum flowers had more male flowers than female and hermaphrodite flowers (Fig. 12). Male, female and hermaphrodite flowers occupy average numbers of 9.8, 4.8 and 5.4 respectively.

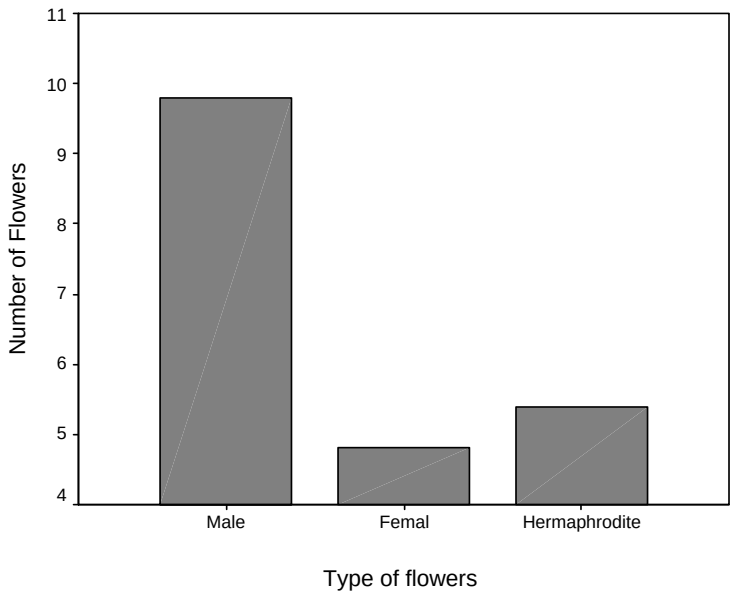


Fig. 12: *Coriandrum sativum*' s flower types/Cluster

Pollination

Season one

a) Location A

In general, each cluster is composed of 19.7 ± 0.12 flowers. Not all flowers in all treatments from the first site went on to set seeds. There were no significant differences in pollinated flowers per cluster between open pollinated flowers 10.5 ± 0.2, hand crossed pollinated 9.6 ± 0.14 and hand geitongamy 9.5 ± 0.15. There was no significant difference emphasized in pollinated flowers between all pollination treatments. There was also no significant difference in the average number of fecundated seeds of the open pollinated flowers 21 ± 0.1, hand crossed 19.3 ± 0.29 and hand geitongamy 19.7 ± 0.31.

Open pollination scored a low average of non-fecundated seeds 0 ± 0 (Tab. 9). No significant difference were noted between the open pollination and all other treatments. Open pollination occupies the highest seed set percentage compared to other pollination treatments 52.2 ± 0.5 (Fig. 13). There were no significant differences in percentage of seed set in open pollination; hand crossed 47.9 ± 0.7 and hand geitongamy 49.7 ± 0.7. There was a significant difference in percentage of seed set between all treatments.

***Coriandrum sativum* Location A, Season One**

Tab. 9: Seeds set after different pollination treatment in *Coriandrum sativum* location A.

Treatments of Pollination	No. of Flower/Cluster	No. of Pollinated Flower/Cluster	No.of Fecundated Seed/Cluster	No. of Non Fecundated Seed/Cluster	Percentage of Seed Set/Cluster
Open	20.1±0.13 a	10.5±0.2 a	21.0±0.1 a	0.00±0.00 a	52.2±0.5 a
Hand Cross	20.0±0.14 a	9.6±0.14 a	19.3±0.29 a	0.13±0.07 a	47.9±0.7 a
Hand Geitonogamy	19.7±0.12 a	9.8±0.15 a	19.7±0.31 a	0.26±0.09 a	49.7±0.7 a

a, b, c, and d; are symbols related to difference in comparison.

b) Location B

In general, each cluster is composed of 20 ± 0.12 flowers. Not all flowers in all treatments for site B went on to set seeds. There was no significant difference in pollinated flowers per cluster between open pollinated flowers 10.4 ± 0.12 , hand crossed pollinated 9.9 ± 0.15 and hand geitongamy 9.8 ± 0.15 in location A. There was no significant difference emphasized in pollinated flowers between all pollination treatments. There was also no significant difference in the average number of fecundated seeds of the open pollinated flowers 20.8 ± 0.2, hand cross 19.7 ± 0.31 and hand geitongamy 19.6 ± 0.31 in location A.

Open pollination scored a low average of non-fecundated seeds 0.1 ± 0.05 in location A (Tab. 10). There was no significant difference between the open pollination and all treatments. Open pollination occupied the highest seed set percentage compared to other pollination treatments 51 ± 1.1 in location A (Fig. 14). There was no significant difference in percentage of seed set in open pollination, hand cross 49.9 ± 1.1 and hand geitongamy 49.1 ± 0.72. There was a significant difference in percentage of seed set between all treatments.

***Coriandrum sativum* Location B, Season One**

Tab. 10: Seeds set after different pollination treatment in *Coriandrum sativum* location B.

Treatments of Pollination	No. of Flower/Cluster	No. of Pollinated Flower/Cluster	No.of Fecundated Seed/Cluster	No. of Non Fecundated Seed/Cluster	Percentage of Seed Set/Cluster
Open	20.0±0.12 a	10.4±0.12 a	20.8±0.2 a	0.1±0.05 a	51.0± 1.1a
Hand Cross	19.9±0.13 a	9.9±0.15 a	19.7±0.31 a	0.26±0.1 a	49.9±1.1 a
Hand Geitonogamy	19.9±0.13 a	9.8±0.15 a	19.6±0.31 a	0.26±0.15 a	49.1±0.72 a

a, b, c, and d; are symbols related to difference in comparison.

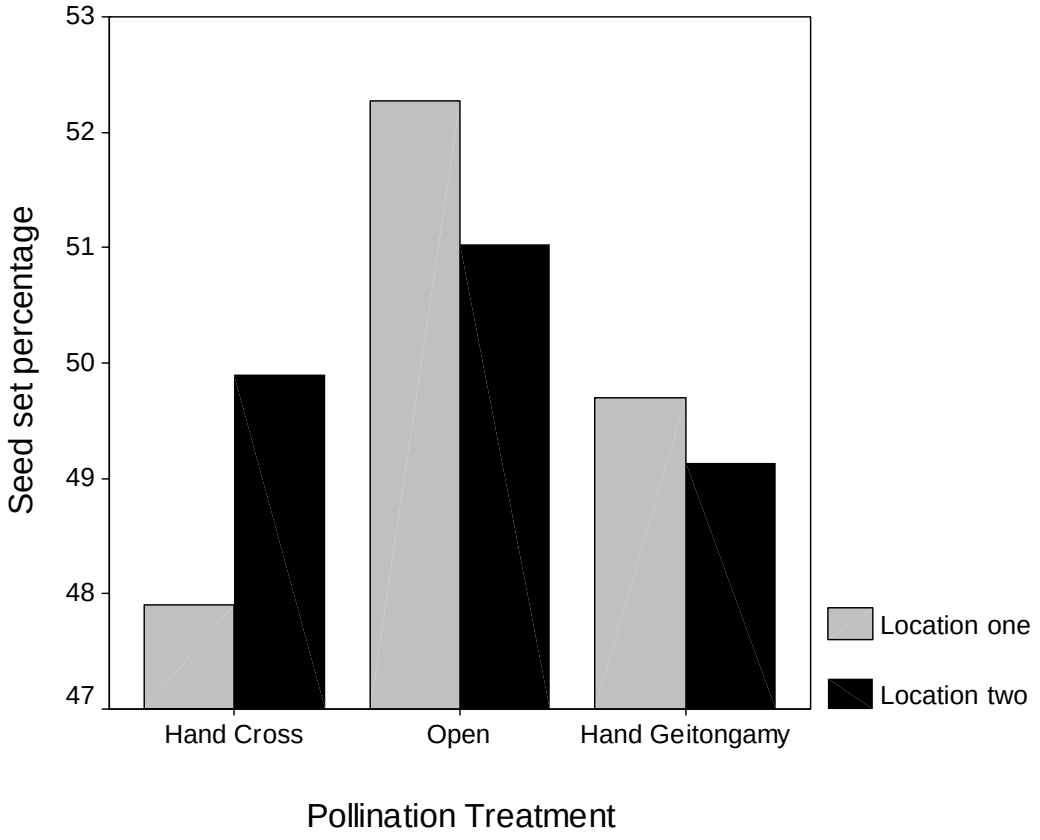


Fig. 13: *Coriandrum sativum* Seed Set Percentage upon Pollination Treats in both Locations season one.

Season Two

a) Location A

Not all flowers in all treatments for both sites went on to set seeds. There was no significant difference in pollinated flowers per cluster between open pollinated flowers 10.6 ± 0.10, hand crossed pollinated 9.2 ± 0.16 and hand geitongamy 9.6 ± 0.16. But, there was a significant difference emphasized in pollinated flowers between all pollination treatments and bagged/untreated flowers at cluster level, which was 4.6 ± 0.47. the average number of fecundated seeds of the open pollinated flowers 21.2 ± 0.21, hand crossed 18.6 ± 0.33 and hand geitongamy 18.9 ± 0.29 were not significantly different in location A. However, there was significant difference in fecundated seeds between all treatments and flowers that were bagged and untreated at cluster level, which was 7.5 ± 0.68 at location A.

Open pollination scored a low average of non-fecundated seeds 0 ± 0.56 in location A (Tab. 11). There was no significant difference between the open pollination and all treatments except the bagged self for one cluster 1.74 ± 0.38. Open pollination occupies the highest seed set percentage compared to other pollination treatments 52.2 ± 0.52 (Fig. 14). There was no significant difference in percentage of seed set in open pollination, hand cross 49 ± 0.82 and hand geitongamy 49 ± 0.75. But, there was a significant difference in the percentage of seed set between all treatments and flowers that were bagged and untreated at the cluster level (Bagged self for one cluster) 23 ± 2.27. Seed set in *Coriandrum sativum* under bagged self pollination conditions in single hermaphrodite flower and hand forced self pollination was almost zero (Tab. 10).

Coriandrum sativum Location A, Season Two

Tab. 11: Seeds set after different pollination treatment in *Coriandrum sativum* location A.

Treatments of Pollination	No. of Flower/Cluster	No. of Pollinated Flower/Cluster	No.of Fecundated Seed/Cluster	No. of Non Fecundated Seed/Cluster	Percentage of Seed Set/Cluster
Open	20.1±0.13 a	10.6±0.10 a	21.2±0.21 a	0.00±0.0 b	53.0±0.52 a
Hand Cross	20.0±0.13 a	9.20±0.16 a	18.6±0.33 a	0.20±0.09 b	47.0±0.74 a
Hand Geitonogamy	19.6±0.13 a	9.6±0.16 a	18.6±0.33 a	0.20±0.09 b	49.0±0.75 a
Bagged Self for One Cluster	19.9±0.15 a	4.6±0.47 b	7.50±0.68 b	1.74±0.38 a	23.0±2.27 b
Hand Forced Self	19.8±0.15 a	0	0	0	0
Bagged Self for One Flower	19.5±0.15 a	0	0	0	0

a, b, c, and d; are symbols related to difference in comparison.

b) Location B

In general, each cluster is composed of 20.1 ± 0.13 flowers. Not all flowers in all treatments for the second site went on to set seeds. There was no significant difference in pollinated flowers per cluster between open pollinated flowers 10.2 ± 0.16, hand crossed pollinated 8.67 ± 0.18 and hand geitongamy 9.8 ± 0.15. But, there was a significant difference emphasized in pollinated flowers between all pollination treatments and bagged/untreated flowers at cluster level, which was 5.4 ± 0.55. The average number of fecundated seeds of the open pollinated flowers 20.3 ± 0.31, hand crossed 17.3 ± 0.33 and hand geitongamy 19.4 ± 0.25 were not significantly different in location A.

But, there was a significant difference in fecundated seeds between all treatments and flowers that were bagged and untreated at cluster level, which was 8.7 ± 0.91.

Open pollination scored the lowest average of non-fecundated seeds 0.1 ± 0.06 (Tab. 12). There was no significant difference between the open pollination and all treatments except the bagged self for one cluster 1.96 ± 0.38.

Open pollination occupies the highest seed set percentage compared to other pollination treatments 51 ± 0.65 (Fig. 14). There was no significant difference in percentage of seed set in open pollination, hand crossed 49 ± 0.82 and hand geitongamy 50 ± 0.66. But, there was a significant difference in percentage of seed set between all treatments and flowers that were bagged and untreated at cluster level (Bagged self for one cluster) 27 ± 2.79. Seed set in *Coriandrum sativum* under bagged self pollination conditions in single hermaphrodite flower and hand forced self pollination was almost zero (Tab. 11).

Coriandrum sativum Location B, Season Two

Tab. 12: Seeds set after different pollination treatment in *Coriandrum sativum* location B.

Treatments of Pollination	No. of Flower/Cluster	No. of Pollinated Flower/Cluster	No.of Fecundated Seed/Cluster	No. of Non Fecundated Seed/Cluster	Percentage of Seed Set/Cluster
Open	19.9±0.14 a	10.2±0.16 a	20.3±0.31 a	0.10±0.06 b	51.0±0.65 a
Hand Cross	19.8±0.13 a	8.67±0.18 a	17.3±0.33 a	0.24±0.10 b	49.0±0.82 a
Hand Geitonogamy	19.8±0.14 a	9.80±0.15 a	19.4±0.25 a	0.30±0.12 b	50.0±0.66 a
Bagged Self for One Cluster	20.0±0.15 a	5.40±0.55 b	8.7±0.91 b	1.96±0.38 a	27.0±2.79 b
Hand Forced Self	19.7±0.15 a	0	0	0	0
Bagged Self for One Flower	19.6±0.15 a	0	0	0	0

a, b, c, and d; are symbols related to difference in comparison.

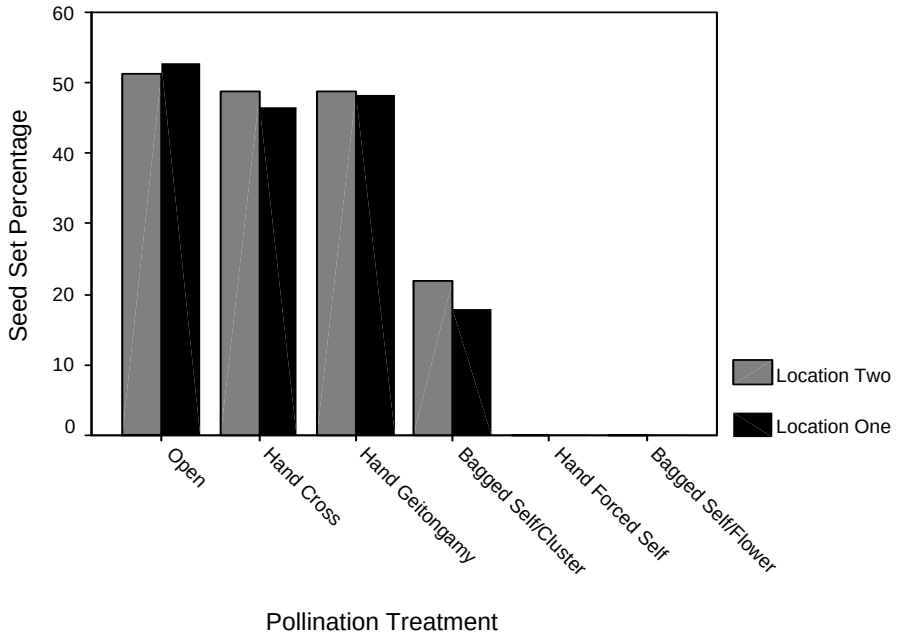


Fig. 14: *Coriandrum sativum* Seed Set Percentage upon Pollination Treats in both Locations season two.

Pollinators and Visitors

The visitors and pollinators that visited *Coriandrum sativum* in both locations were honey bees. Bees visited after the flowers were open and reached a maximum between 9:30-11:30 a.m. on sunny days. Bees spent at least 12sec for pollen collection and 14sec for nectar collection. Pollen collector bees occupy 70% of the total bees visiting the cluster, and the remaining 30% total bees are occupied by nectar collector bees (Tab. 13). What is worth mentioning is that the honey bees do not visit the flowers during periods of cold or wet weather. No other insect visitors were noticed in both locations.

Honey bees landed on *Coriandrum sativum*'s cluster in order to collect nectar. The mechanisms were being observed through random motion of honey bees over *Coriandrum sativum*'s cluster looking for nectar. Some honey bees were bearing pollen on their legs while visiting the cluster. Then, the honey bees were flying to another cluster behaving the same. The same behavior was observed in the second location.

Tab. 13: Behavior of honey bees and their bearings for *Coriandrum sativum*.

Behaviors of Bees	Average Spending Time/Cluster	Landing on Cluster		Behavioral Motion			Percent of Bees According to Their Bearings and Landing on
		Center	Edge	Random	Zigzag	Circular	
Nectar Collector were observed	14		●	●			30%
Pollen collector were observed	12	●				●	70%

Mean Visit Rate for Honey Bees

Coriandrum sativum had mean visit rates for the three replicates in both locations were 14.14 and 11.67, respectively. The ultimate activity during the three replicates was approximately from 8:30 a.m. to 11:30 a.m. in both locations.

Seed Content

The seed contains the following essential oils, based on the analysis applied (Tab. 14a, 14b, and 14c). Seeds tested were chosen from open treatment of pollination for fatty acids composition, essential oils and mineral contents. Moisture content was 8.30, protein content was 15.78%. Oil content was low in all seeds and was 7.40% and ash was 6.31%.

Oleic acid was significantly the most concentrated (80.10%). All minerals containing Manganese and Copper were the most prevalent minerals. The linalool acid was the common essential oil in *Coriandrum sativum* and was 66.7%. The fatty oil of *Coriandrum sativum* contained high level of petroselinic acid.

Tab. 14a: *Coriandrum sativum* Essential Oils.

No.	Main Component	% Total Essential Oil
1.	Linalool	66.7
2.	Alpha-pinene	9.8
3.	Gamma-terpinene	8.3
4.	Geranylacetate	3.3
5.	Camphor	3.0
6.	Geraniol	1.9

Tab. 14b: *Coriandrum sativum* Seed Constituents

Content	Result	Unit
Moisture	8.30	%
Ash	6.31	%
Protein	15.78	%
Oil	7.40	%
Magnesium	0.34	%
Calcium	0.40	%
Potassium	1.46	%
Manganese	18.8	mg/kg
Cupper	11.6	mg/kg

Tab. 14c: *Coriandrum sativum* Fatty Acids Constituents.

Fatty acid	Trivial name	Systematic name	%
C 14:0	Myristic acid	Tetradecanoic acid	0.10
C 16:0	Palmitic acid	Hexadecanoic acid	3.33
C 16:1	Palmitoleic acid	*cis*-9-Hexadecenoic acid	0.42
C 17:0		Hexadecanoic acid	0.03
C 17:1		Desaturation of *cis*-9-Hexadecenoic acid	0.04
C 18:0	Stearic acid	Octadecanoic acid	0.88
C 18:1, n-7	Vaccenic acid	*cis*-11-Octadecenoic acid	80.10
C 18:2	Linoleic acid	*cis*-9, 12 -Octadecadienoic acid	14.63
C 18:3	α –Linolenic acid	*cis*-9, 12, 15 -Octadecatrienoic acid	0.29
C 20:0	Arachidic acid	Eicosanoic acid	0.09
C 22:0	Behenic acid	Docosanoic acid	0.03
C 20:1	Gadoleic acid	*cis*-9- Eicosenoic acid	0.04
C 24:0	Lignoceric acid	Tetracosanoic acid	0.02

3.5. *Cucurbita pepo*

Receptivity and Anthesis

Both male and female *Cucurbita pepo* flowers opened during the early morning (6:30 a.m.) and were generally closed by mid afternoon (3:30 p.m.). At complete blooming stage for *Cucurbita pepo*, the male stage started at 7:00 a.m. and stopped at 12:00 p.m. The female stage started early in the morning around 8:30 a.m. and stopped around 3:30 p.m. (Fig. 15). The pollen weight for the *Cucurbita pepo* plant on average was 0.17 mg, whereas the volume of nectar was 0.24 µl in the *Cucurbita pepo* flower.

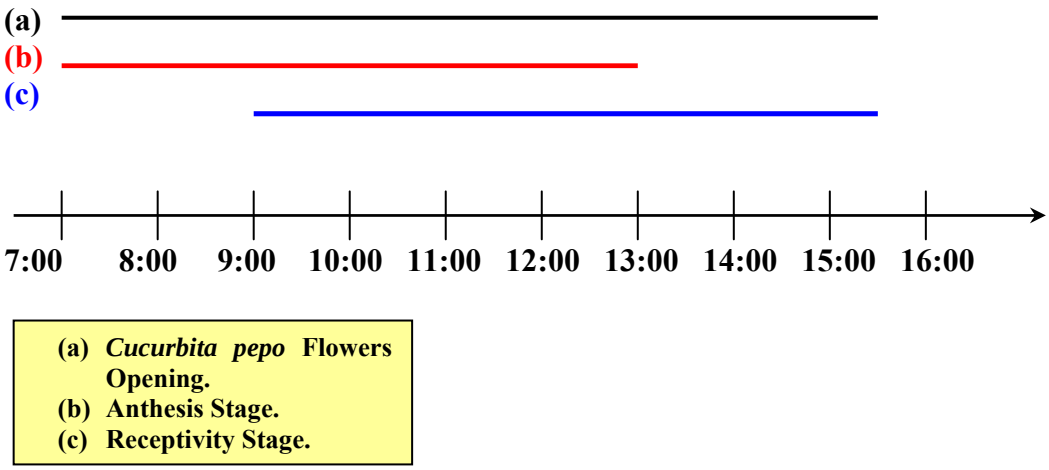


Fig. 15: Blooming Stages of *Cucurbita pepo*, Anthesis and Receptivity.

Changes in Stigma and Anther Separation during Development

The male flowers are easily recognizable because they are to be found above the foliage on long stems. The female flowers are also easily recognizable because the future fruit is to be found at their base and the ovary already has a well defined shape. (Fig. 16a and 16b).

Fig. 16a: Male Flower

Fig. 16b: Female Flower

The Ratio of Male Flowers to Female Flowers

Male flowers occupy a ratio to female flower 3:1.

Season One

Pollination

a) Location A

Flowering began December 15 and paused on February 5. In the pollination of *Cucurbita pepo*, many seeds must be formed or many pollen grains deposited on the stigma for the fruit to be regular in shape and of commercial value. Fruit weight presented was much higher in open pollination with little loss when crossing and selfing. There was no significant difference between treatments (1.6 ± 0.09, 1.5 ± 0.05 and 1.4 ± 0.04, respectively). There was no significant difference in seed weight between open pollinated treatment 14.8 ± 1.1, hand crossed pollinated treatment 14.7 ± 0.9 and hand geitongamy treatment 13.8 ± 0.7 in location A. Open treatment, hand crossed and hand geitongamy produced non-significant differences in a number of seeds with an average

of 190.5 ± 7.2, 190 ± 8.5 and 180.4 ± 6.9, respectively for the first location. A non fecundated seed production is also a common feature of *Cucurbita pepo* flowers under the different treatments. Open pollination occupied the lowest average among non-fecundated seeds (Tab. 15) compared to other treatments. There was no significant difference between all treatments. Hand crossed, hand geitongamy ranked secondly in producing a non-significant fecundated seeds with an average of 31.7 ± 1.4, 49.2 ± 2.1, respectively.

Cucurbita pepo flowers produced a non significant number of ovules under all treatment conditions with an average of open treatment at 209.5 ± 7.1, hand crossed at 221.8 ± 9.4 and hand geitongamy at 229.6 ± 8.5. Non significant differences were found between the average percentages of seed set when flowers underwent open treatment, hand crossed and hand geitongamy (90.7%, 85.1 % and 78.5 %, respectively). Fruit abortion can reach 100% in flowers bagged to exclude insect visitors.

Tab. 15: Seeds set after different pollination treatment in *Cucurbita pepo* location A.

Treatments of Pollination	Total Fruit Weight (kg)	Seed Weight (g)	No. of Seeds	No. of Non Fecundated Seed	No. of Ovules	Percentage of Seed Set
Open	1.6±0.09 a	14.8±1.1 a	190±7.2 a	19±1.3 a	209.5±7 a	90.7±0.68 a
Hand Cross	1.5±0.05 a	14.7±0.9 a	190±8.5 a	31.7±1.4 a	221.8±9 a	85.1±0.7 a
Hand Geitonogamy	1.5±0.04 a	13.8±0.7 a	180.4±6.9 a	49.2±2.1 a	229.6±8.5 a	78.5±0.6 a
Bagged Self	0	0	0	0	0	0

(a) is symbol related to difference in comparison.

b) Location B

Flowering began January 25 and paused on March 15. In the pollination of *Cucurbita pepo*, many seeds must be formed or many pollen grains deposited on the stigma for the fruit to be regular in shape and have commercial value. Fruit weight appeared much higher in open pollination in location A and there was little loss when crossing and

selfing. There was no significant difference between treatments a (1.73 ± 0.06, 1.58 ± 0.05 and 1.58 ± 0.05, respectively). There was no significant difference in seed weight between open pollinated treatment 16.4 ± 0.4, hand crossed pollinated treatment 14.9 ± 0.7 and hand geitongamy treatment 13.8 ± 0.4 in location A. Open treatment, hand crossed and hand geitongamy produced non-significant differences in the number of seeds between them , which produced an average of 212 ± 10.7, 179.2 ± 6.9 and 171± 5.2, respectively. A non fecundated seed production is also a common feature of *Cucurbita pepo* flowers under the different treatments. Open pollination occupied the lowest average among non-fecundated seeds (Tab. 16) compared to other treatments and there was no significant difference between all treatments.

Hand crossed and hand geitongamy ranked second in producing non-significant fecundated seeds with an average of 26.7 ± 1.9, 31.7 ± 2, respectively. *Cucurbita pepo* flowers produced a non significant number of ovules under all treatment conditions with an open treatment average of 228 ± 11.9; hand crossed 206 ± 8.1 and hand geitongamy 202 ± 6.5. Non significant differences were found between the average percentages of seed set (Fig. 17) when flowers underwent open treatment, hand crossed and hand geitongamy (92.9 %, 87.2 % and 84.5 %, respectively). Fruit abortion can reach 100% in flowers bagged to exclude insect visitors.

Tab. 16. Seeds set after different pollination treatment in *Cucurbita pepo* location B

Treatments of Pollination	Total Fruit Weight (kg)	Seed Weight (g)	No. of Seeds	No. of Non Fecundated Seed	No. of Ovules	Percentage of Seed Set
Open	1.73±0.06 a	16.4±0.4 a	212±10.7 a	16.6±1.3 a	228±11 a	92.9±0.3 a
Hand Cross	1.68±0.05 a	14.9±0.7a	179.2±6.9 a	26.7±1.9 a	206±8.1 a	87.2±0.8 a
Hand Geitonogamy	1.68±0.05 a	13.8±0.4 a	171±5.2 a	31.7±2.0 a	202±6.5 a	84.5±0.7 a
Bagged Self	0	0	0	0	0	0

a, b, c, and d; are symbols related to difference in comparison.

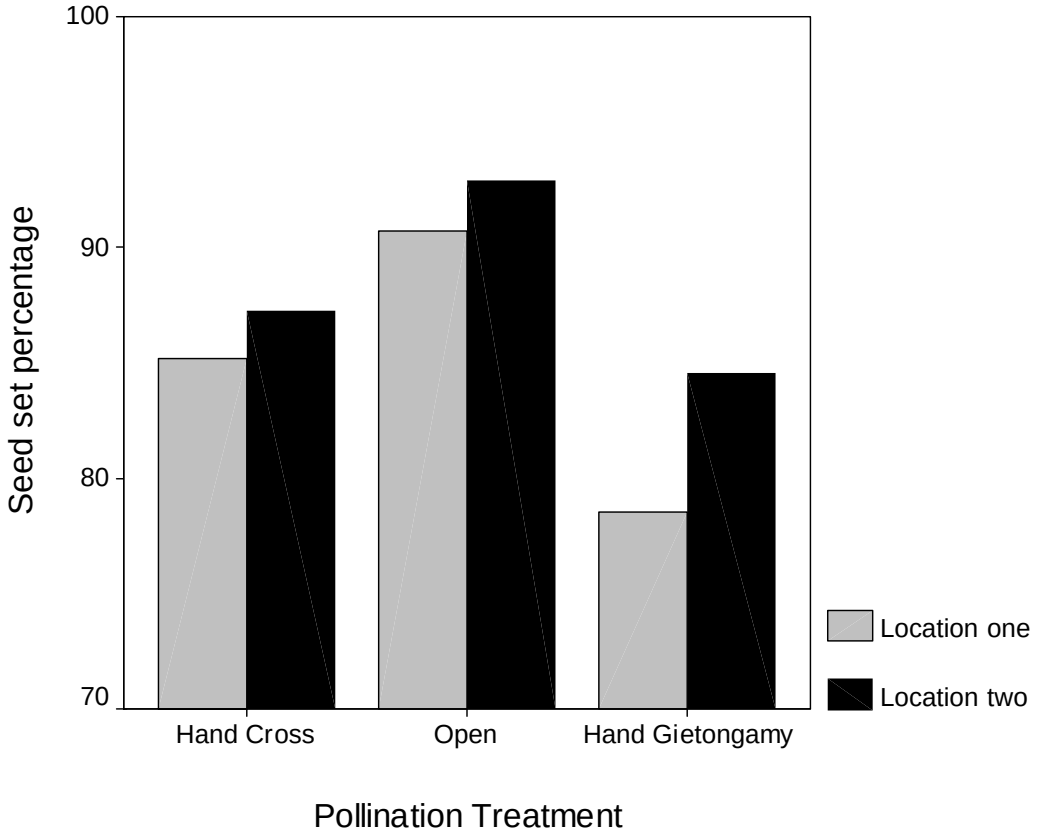


Fig. 17: *Cucurbita pepo* at season one in both locations

Pollinator and Visitor

Pollination is almost exclusively performed by honey bees. Bee visits after the flowers are open reached a maximum between 8:30a.m.and 10:30a.m. on sunny days. Male flowers were visited more than female flowers. In male flowers, the honey bee lands near the edge of the petals and moves towards on the anther, through which it takes the pollen. After that it flies on to the next flower. Sometimes honey bees land near the edge of a petal and move towards the pore, through which it takes the nectar. While in the female flower, the insect usually lands on the edge of a petal, sometimes even on the tip of the stigma, loads the pollen and then flies away (Tab. 17a).

The bee loses pollen on the way in, especially if it comes from a male flower and is already loaded with pollen and on the way out because exit is more difficult than entry. In spite of honey bees continued visits in location B, visitation numbers for *Cucurbita pepo* seemed to be in huge number during the first two weeks. After that, the number of bees decreased with the progression of the plant's age and the appearance of wild plants. Flowering of wild plants started on the first of February then increased until the end of March when the flowers began to dry..

Tab. 17a: Landing on and departure of honey bees:

Part / **Behavior**		**Anther**	**Stigma**	**Sepal**	**Petal**
Male	**Landing on**				=
	Departure	=			
Female	**Landing on**				=
	Departure		=		

No visitors appeared in location A, however, in the beginning of flowering in location B, a new visitor (Black Fly) played an essential role in the feeding of nectar. The visitor visited the flowers after are they opened and reached its maximum (between 9:30a.m.-10:30a.m.). The number of visitors increased as the flower's main visitor until February 15 then decreased at the end of February. In the male flower, the black Fly usually lands on the petal, goes toward the base of the anther, and takes the nectar through the pores; while in a vertical position, the Black Fly returns to its landing position then flies away. In the female flower, the Black Fly lands on the petal, goes towards the base of stigma, then takes the nectar through the pores, and once in a vertical position, returns to its landing position then flies away (Tab. 17b). The visitor showed to spread its collection to the nearby *Cucurbita pepo* farm in high intensity. In contrast with honey bees, the availability period for the Black Fly visitor is longer. The average time spent on a flower was 18 seconds and 26 seconds, respectively in both locations. I noticed the visitor spent more time on the flowers than honey bees.

Tab. 17b: Landing on and departure of Black Fly.

Part / Behavior		Base of Anther	Base of Stigma	Sepal	Petal
Male	**Landing on**				=
	Departure				=
	Moving	=			
Female	**Landing on**				=
	Departure				=
	Moving		=		

Amount of Pollen and Nectar

The amount of nectar found in the female and the perfect flower was 24 µg, while the nectar found in the male was 8 µg. The amount of pollen grains of the male flower was 17µg.

The Productivity

The productivity of the total marked fruit was 13.25 for location A and 15.25 for location B, while it ranged between 1.15 to 1.7 kg for one fruit.

Mean Visit Rate for Honey Bees

Cucurbita pepo had mean visit rates for the male three replicates in both locations at 20.92 and 14.70 respectively. While three female replicates in both locations were 14.76 and 12.84, respectively (Tab. 18).The maximum activity during the three replicates was approximately from 8:30a.m. to 10:30a.m. in both locations. The visit rate of bees in location A was more than location B.

Tab. 18: Mean Visit Rate and Mean Spending Time for season one:

Visit Rate	Sex	Mean Visit Rate/ 15 Min.	Mean Spending Time / Sec.
Honey bee \ Location A	**Male**	20.92	19.3
	Female	14.76	20
Honey bee \ Location B	**Male**	14.70	16.85
	Female	12.84	20.85
Black Fly \ Location B	**Male**	21.2	18
	Female	22.9	26

Season Two

Pollination

a) Location A

Flowering began February 5 and paused at the beginning of March. In the pollination of *Cucurbita pepo*, many seeds must be formed or many pollen grains deposited on the stigma for the fruit to be regular in shape and of commercial value. The fruit weight appeared much higher in open pollination and there was little loss when cross and selfed. There is no significant difference between any of the treatments (1.58 ± 0.05, 1.56 ± 0.05 and 1.65 ± 0.0, respectively).

There was no significant difference in seed weight between open pollinated treatments (15.3 ± 0.7), hand crossed pollinated treatment (14.3 ± 0.9) and hand geitongamy treatment (16.6 ± 0.4). Open treatment, hand crossed and hand geitongamy produced non-significant differences in the number of seeds. They produced an average of 186.4 ± 7.5, 191.7 ± 7.7 and 191.9± 6.6, respectively. A non fecundated seed production is also a common feature of *Cucurbita pepo* flowers under the different treatments.

Open pollination occupied the lowest average among non-fecundated seeds (Tab. 19) when compared to other treatments. In addition, there was no significant difference between all treatments. Hand cross, hand geitongamy ranked second in producing a non-significant fecundated seeds with an average of 35.3 ± 1.3, 27.9 ± 1.1, respectively.

Cucurbita pepo flowers produced a non significant number of ovules under all treatment conditions with an average of open treatment (220 ± 8.3), hand crossed (227 ± 8.7) and hand geitongamy (219 ± 7.2). Non significant differences were found between the average percentages of seed set when flowers underwent open treatment, hand crossed and hand geitongamy (84.2%, 84.3 % and 87.1 % respectively). Fruit abortion can reach 100% in flowers bagged to exclude insect visitors.

Tab. 19: Seeds set after different pollination treatment in *Cucurbita pepo* location A season two

Treatments of Pollination	Total Fruit Weight (kg)	Seed Weight (g)	No. of Seeds	No. of Non Fecundated Seed	No. of Ovules	Percentage of Seed Set
Open	1.58±0.05 a	1.53±0.7 a	186±7.5 a	33.9±1.2 a	220±8.3 a	84.2±0.5 a
Hand Cross	1.56±0.05 a	14.3±0.9 a	191±7.7 a	35.3±1.3 a	227±8.7 a	84.3±0.4 a
Hand Geitonogamy	1.65± 0.0 a	16.6±0.4 a	191±6.6 a	27.9±1.1 a	219±7.2 a	87.1±0.4 a
Bagged Self	0	0	0	0	0	0

a is symbol related to difference in comparison.

b) Location B

Flowering began January 13 and paused at the beginning of March. In the pollination of *Cucurbita pepo*, many seeds must be formed or many pollen grains deposited on the stigma for the fruit to be regular in shape and commercial value. Fruit weight appeared much higher in open pollination in location A and there was little loss by crossing and selfing. There was no significant difference between all treatments (1.69 ± 0.03, 1.69 ± 0.04 and 1.67 ± 0.03, respectively).

There was no significant difference in seed weight between open pollinated treatment (17.2 ± 0.4), hand crossed pollinated treatment (17.2 ± 0.5)and hand geitongamy treatment (17.3 ± 0.4) in location A. Open treatment, hand cross and hand geitongamy produced non-significant differences in the number of seeds between them. They produced an average of 179.3 ± 5.7, 192.6 ± 6.2 and 185.4± 5.8, respectively. A non

fecundated seed production is also a common feature of *Cucurbita pepo* flowers under the different treatments. Open pollination occupied the lowest average among non-fecundated seeds (Tab. 20) compared to the other treatments. In addition, no significant difference was found between treatments. Hand crossed and hand geitongamy ranked second in producing non-significant fecundated seeds, with an average of 30.8 ±0.9 and 28 ± 1.3, respectively. *Cucurbita pepo* flowers produced a non significant number of ovules under all treatment conditions, with an average of open treatment at 210 ±6.5, handcrossed at 223± 6.8 and geitongamy at 213 ±6.8. Non significant differences were found between the average percentages of seed set (Fig. 18) when flowers underwent open treatment, hand cross and hand geitongamy (85%,86% and 86% respectively). Fruit abortion can reach 100% in flowers bagged to exclude insect visitors. *Cucurbita pepo* flowers had more male flowers than female. Male flowers occupied a ratio to female flower of 3:1.The male flowers did not produce fruit, but they did supply the pollen that fertilizes female flowers

Tab. 20: Seeds set after different pollination treatment in *Cucurbita pepo* location B

Treatments of Pollination	Total Fruit Weight (kg)	Seed Weight (g)	No. of Seeds	No. of Non Fecundated Seed	No. of Ovules	Percentage of Seed Set
Open	1.69±0.03 a	17.2±0.4 a	179±5.7 a	31.5±1.1 a	210±6.5 a	85±0.4 a
Hand Cross	1.69±0.04 a	17.2±0.5 a	192±6.2 a	30.8±0.9 a	223±6.8 a	86±0.4 a
Hand Geitonogamy	1.67±0.03 a	17.3±0.4 a	185±5.8 a	28.0±1.3 a	213±6.8 a	86±0.3 a
Bagged Self	0	0	0	0	0	0

a, b, c, and d are symbols related to difference in comparison.

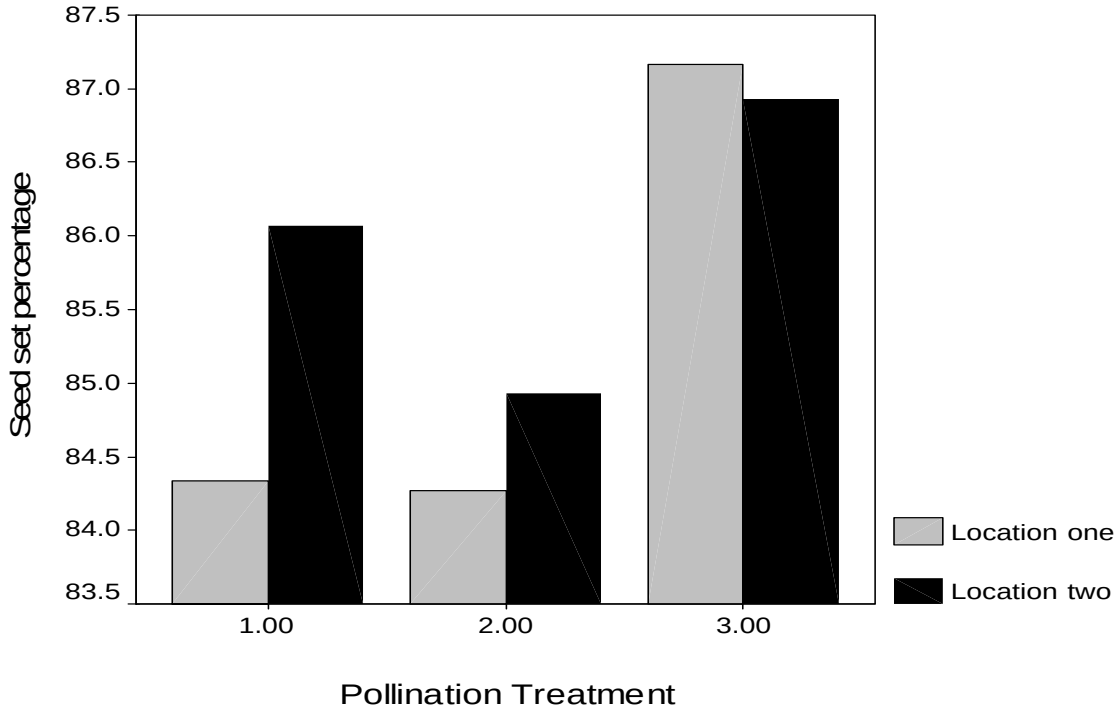


Fig. 18: *Cucurbita pepo* pollination treatment in both locations season two.

Pollinator and Visitor

Pollination is almost exclusively performed by honey bees. Bee visits after the flowers are opened and have reached a maximum (between 8:30a.m. and 10:30a.m. on sunny days). Male flowers are visited more than female flowers. In the male flowers, a honey bee lands near the edge of petal and goes towards the anther, through which it takes the pollen, and then flies on to the next flower. Sometimes, a honey bee lands near the edge of petal and goes towards the pore, through which it takes the nectar. While in the female, the honey bee usually lands on the edge of the petal, sometimes even on the tip of the stigma, loads the pollen then flies away(Tab. 21). The bee loses pollen when entering the flower, especially if it comes from a male flower and the honey bee is already loaded with pollen. The honey bee loses pollen on the way out because exit is more difficult than entry. In spite of honey bees continued visits in location B. Visits to the *Cucurbita pepo* seemed to be in huge numbers during the first two weeks. After that, the number of bees decreased with the progression of the plant's age and the appearance of wild plants. Flowering of wild plants started on the first of February

then increased around the end of March when the flowers began to dry. The Black Fly did not appear during the second season.

Tab. 21: Landing on and departure of honey bees.

Part / Behavior		Anther	Stigma	Sepal	Petal
Male	**Landing on**				=
	Departure	=			
Female	**Landing on**				=
	Departure		=		

Amount of Pollen and Nectar

The amount of nectar found in the female and perfect flowers was 24 µg, while nectar found in the male flower was 8 µg, and the amount of pollen grains of male flower was 17µg.

The Productivity

The productivity of the total marked fruit was 14.25 kg for location A and 15.4 kg for location B. The range of one fruit was between 1.2 to 1.7 kg.

Mean Visit Rate for Honey Bees.

Cucurbita pepo mean visit rates to the three male replicates in both locations were 19.9 and 20.4, respectively, while mean visit rates for the three female replicates in both locations were 15.1 and 16.8, respectively (Tab. 22).The greatest activity during the three replicates was approximately from 8:30a.m. to 10:30a.m. in both locations. The visit rate of bees in location B was more than location A.

Tab. 22: Mean Visit Rate and Mean Spending Time for season Two.

Species / Visit Rate	Sex	Mean Spending Time / Minute	Mean Visit Rate / 15 Minute
Location A	**Male**	19.7	19.9
	Female	20.9	15.1
Location B	**Male**	18.8	20.4
	Female	20.6	16.8

Seed Content

Seeds from the open treatment of seeds *Cucurbita pepo* were evaluated for their proximate analysis, fatty acid composition and mineral content (Tab. 23a, and 23b). Moisture content was 7.05 % and protein value was 26.62. Oil content was high at 37.27%. The ash content was 4.40 %. Furthermore, Magnesium was 1.07%, Calcium was 0.04%, Potassium was 0.76%, Manganese was 34.20% and Copper was10.40 %. Fatty acids composition contains linoleum acid, which was the most concentrated at 56.44% followed by oleic acid at 25.55 and palmitic acid at 11.96%.

Tab. 23a: *Cucurbita pepo* Seed Constituents.

Content	Result	Unit
Moisture	7.05	%
Ash	4.40	%
Protein	26.62	%
Oil	37.27	%
Magnesium	1.07	%
Calcium	0.04	%
Potassium	0.76	%
Manganese	34.20	mg/kg
Cupper	10.40	mg/kg

Tab. 23b: *Cucurbita pepo* Fatty Acids.

Fatty acid	Trivial name	Systematic name	%
C 14:0	Myristic acid	Tetradecanoic acid	0.90
C 16:0	Palmitic acid	Hexadecanoic acid	11.96
C 16:1	Palmitoleic acid	*cis*-9-Hexadecenoic acid	0.11
C 17:0		Hexadecanoic acid	0.07
C 17:1		Desaturation of *cis*-9-Hexadecenoic acid	0.02
C 18:0	Stearic acid	Octadecanoic acid	5.02
C 18:1, n-9	Oleic acid	*cis*-9-Octadecenoic acid	25.55
C 18:2	Linoleic acid	*cis*-9, 12 -Octadecadienoic acid	56.44
C 18:3	A –Linolenic acid	*cis*-9, 12, 15 -Octadecatrienoic acid	0.25
C 20:0	Arachidic acid	Eicosanoic acid	0.3
C 22:0	Behenic acid	Docosanoic acid	0.07
C 20:1	Gadoleic acid	*cis*-9-Eicosenoic acid	0.09
C 24:0	Lignoceric acid	Tetracosanoic acid	0.03

4. DISCUSSION

4.1. Survey of Bees

The number of bees genus were limited

Mediterranean agricultural landscapes change dramatically which requires distinct and appropriate management to prevent further impoverishment of the flora and fauna. This is of special importance for solitary bees and other pollinating insects that provide essential ecosystem services. Through our field surveys, I investigated eleven genera of bees. Water scarcity, land degradation, urban expansion, and/or other human/climate change induced factors could affect the diversity of such biosystems.

4.2. Flora Survey

The best representative families in Jordan were the Compositae, Graminaea, Papilionaceae, and Caryophllaceae

I recorded 106 species belonging to 91 genera and 23 families. During the 2005/2006 season, I monitored the wild and cultivated plants grown around *Nigella sativa* weekly. Five plants were collected at the flowering stage of each plant and the species was reserved and identified in the laboratory. Because of its importance, Jordan's rangeland and forestry was studied by international experts, mainly through UN organizations, in particular, FAOs who submitted reports to the Hashemite Kingdom of Jordan (HTSL, 1956; and LONG, 1957). A number of medicinal plants can be utilized in the drug industry, especially for the treatment of cancer and other diseases. Wild species, with their massive genetic variations, contributed to the development of agriculture and medicine. Many species constitute the foundation for community welfare, especially in rural areas. They provide food, feed, and fuel for large communities in several parts of the world; Jordan is no exception. The flora in the Jordanian mountains has a large and socioeconomic impact on the local people and their welfare. According to classifications developed in Montana, U.S.A., HTSL (1956) classified Jordan as grassland, brush, bare rock, forest and bare ground.

The vegetation in Jordan is in accordance with the association of species that was classified by LONG (1957). He found that vegetation is divided into twenty – nine subdivisions and forest types are divided into nine subdivisions. The presence of unusual ecological conditions in Jordan greatly affects its plant life and biological diversity in terms of species diversity, density and distribution (AL–EISAWI *et al*., 1996). Jordan has been divided into different bioclimatic or biogeographical regions. LONG (1957) classified Jordan into eight bioclimatic regions by gathering the information of 24 stations distributed around Jordan.

4.3. *Nigella sativa*

a) The male phase is initiated a few days before the stigmas become receptive, where the anthesis duration remains for five days.

Full flowering started with the appearance of bright blue petals. The male stage began as the anthers started to shed their pollen, from the first day to the fifth day. The male stage activated between 8:30 a.m. to the end of day. The viability of one anther remained during one day then started to sink down. It is interesting to point out that anthers remain active for five days, which coordinates with the receptivity period on the fifth day. Because the flowering period for *Nigella sativa* coincides with pleasant April temperatures in Jordan, this may lead to an increase in the interval of anthesis since pollen responds to temperature. It is surprising for pollen of *Nigella sativa* to continue for five days. Another reason for this long period of anthesis is the large number of anthers in staminate. Climatic factors affect the anthesis intervals in *Nigella sativa*. There is evidence that high temperatures had a direct effect on pollen performance since pollen responds to temperature. However, at the same time high temperatures are advantageous for pollen as well by hastening its tube growth rate. On the other hand, low temperatures may act against pollen by reducing its germination and growth rate, which could limit the fertilization success (THOMPSON and LIU 1973; JAKOBSEN and MARTENS 1994)

b) The duration of stigmatic receptivity in *Nigella sativa* was approximately hours.

In angiosperms, the stigma is the first female structure the pollen grains and pollen tubes have to face on their way to the female gametophyte. The stigma provides an adequate environment for pollen grain germination (HELSOP-HARRISON and SHIVANNA

1997; KNOX 1984). One of the most important features of stigmas is stigmatic receptivity, defined as the ability of the stigma to support pollen germination. This is a decisive stage in fertilization success and has a large variability among plant species (HELSOP-HARRISON 2000). After the end of the fifth day of the male stage, the female stage began to be active from 8:00 a.m. to 13:00 p.m. and then stopped. It is interesting to point out that the stigma of *Nigella sativa* is receptive throughout anthesis in spite of the flowering period in April when we don't have high temperature that may hurt the plant. The stigma was receptive only for several hours. The explanation is that the stigma has direct exposure to the sun which may increase the exposed area. In addition, the receptivity of the stigma occurred after the stigma lost most of the anthers that surrounded it so that the entire stigma is exposed to the sun which may also increase the exposed area to sun. That means high temperatures affect stigma receptivity and reduces receptivity intervals. There is evidence that ensures the stigma responds to high temperatures. High temperatures are detrimental for the female part by reducing the length of stigmatic receptivity and accelerating ovule degeneration (POSTWEILER *et al*. 1985). It is well documented that the reproductive phase, especially from pollination to fertilization, is highly vulnerable to the prevailing environmental conditions including temperature (HALL 1992; STEPHENSON *et al.,* 1992). The duration of stigmatic receptivity is variable depending on the species, and it is also variable within genera. There is evidence that indicates duration of stigmatic receptivity is variable, that the duration of stigmatic receptivity is variable depending on the species and is usually greater in wind-pollinated than in insect-pollinated species (KHADARI *et al*. 1995).

Thus, the stigma can be receptive for not much more than an hour or so, as in *Avena* or *Dactylis*, to as long as several days, as in other grass species (*Pennisetum* or *Zea*) or *Eucalyptus* in which it can remain receptive for more than a week, particularly in hostile environments (HELSOP-HARRISON 2000). From an agricultural perspective, stigmatic receptivity also has clear practical implication as it limits floral receptivity, the effective pollination period (GUERRERO-PRIETO *et al.* 1985) and hence fruit set (reviewed in SANZOL and HERRERO 2001). Moreover, in an ecologist context, by altering stigmatic receptivity, flowering plants may influence the likelihood of fertilization by indirectly controlling the number and the quality of mating through the control of the number of pollen grains deposited and the time of germination (CRUDEN *et al*., 1984; PRIMACK 1985; GALEN, *et al*., 1986).

c) Autonomous Pollination in *Nigella sativa*

First of all, I would like to define ***Autonomous*** for the reader to understand. As LLOYD, 1992 defines it: Prior self pollination within-flower: self-pollination that occurs before the opportunity for outcross-pollen receipt for that flower has occurred, competing self pollination within-flower; self-pollination that occurs during the opportunity for outcross-pollen receipt for that flower has occurred, and delayed selfing pollination within-flower; self-pollination that occurs after the opportunity for outcross-pollen receipt for that flower has occurred. One of these three types of self pollination occurred in our research in Nigella sativa, which is delayed selfing pollination. Autonomous delayed selfing late in *Nigella sativa* flower's life is favored when honey bees service and thus outcross-pollen receipt is unpredictable. *Nigella sativa* flowers attract honey bees but they can also autonomously perform delayed self pollination, which provides reproductive assurance if pollinators fail to visit. The delayed self pollination occurred in our research because the synchronization between male and female occurred at the end of flowering period.

I agreed with DARWIN, 1877; MULLER, 1883; BAKER, 1955, 1965; LLOYD, 1979, 1992 that pollinator absence or low pollinator abundance during some periods within or among flowering seasons favor shifts from out crossing to autonomous self-fertilization because self-pollinated seeds provide reproductive assurance. Some authors support the research result that absence of pollinators can shift to delayed self pollination. The extinction of pollinators or range expansion in a plant lineage can favor shifts to biotic modes of pollination, including wind pollination and autonomous self fertilization (BAKER, 1955; STEBBINS, 1957; REGAL, 1982; COX, 1991; WELLER *et al*., 1998). The results agreed with BARRETT and HARDER (1996); and RAMSEY and VAUGHTON (1996) that pollinator scarcity and reduced pollinator services may result in high selfing rates. Cross pollination and bagged self pollinations occur; approved by seed set achieved by all treatments applied on the research where bagged selfing and out crossing boosted seed production means of 45 % and 77% respectively. The results agreed with ZOHARY (1983) as he found that *Nigella sativa* are capable of setting seed without being cross-pollinated, but he didn't mention the mechanism for such a result. The results also agreed with FAEGRI and VAN DER PIJL (1971) who reported: There are a few flowers that can self-pollinate on their own, but this limits them to in-breeding. The results agreed with GOODWILLIE (1999) in believing the ability of self pollination

to provide some insurance against pollination failure. In addition to the reproductive assurance benefits, prior selfing could be favored since it reduces the costs associated with the longer floral maintenance time required for out crossing, and sets the stage for the evolution of reduced investment in cues for pollinators and the amount of pollen per flower. In contrast with early selfing, later-selfing species will retain floral traits and costs associated with out crossing (i.e., cues to attract pollinators, pollinator rewards, and prolonging floral maintenance relative to prior selfing species). At one extreme, selfing early in a flower's life (prior) is favored when a population requires pollinators to be chronically absent (LLOYD, 1992), or when a population size is so low as to be undetectable by pollinators (LLOYD, 1992; FAUSTO *et al*., 2001; GOODWILLIE, 2001), or when a population experiences high levels of inter-specific pollen flow (FISHMAN and WYATT 1999).

Many authors are interested in common types of pollination such as cross, open and self pollination, but through our research I have devoted a concerted amount of time to point out some facets of traditional pollination efforts such as delayed self pollination. Delayed selfing may be achieved by either a partial overlap in timing of the male phase with the female phase or changes in the relative position of the anther and stigma during development. For example, delayed selfing in the *Hibiscus laevis* (KLIPS and SNOW, 1979) and the Campanula species (FAEGRI and VANDER PIJL, 1979) is characterized by a progressive downward curling of the stigmatic area towards the style where anthers or pollen are located. Conversely, in the protogynous *Aquilegia Canadensis* (ECKHERT and SCHAEFFER 1998) the stamens progressively elongate towards the exerted stigma. The *Kalmia latifolia* (LYON, 1992) anthers collapse into the stigma on the final day of floral development thereby achieving self pollination. Others have found self pollination late in floral life without changes in morphology. The breakdown of self incompatibility as the flower ages in both Lilium and Longifolium (ASCHER and PELOQUIN, 1966) is attributed to degradation of the proteins that control self incompatibility and can be viewed as another form of delayed selfing. FAEGRI and VAV DER PIJL (1971) used the term "self pollination" or "autogamy" when pollination takes place within one flower (idiogamy), and "allogamy" or "cross pollination" when pollen from one flower is carried out to the stigma of another. Allogamy may further be divided into "geitonogamy" if the flowers are on the same plant and "xenogamy" if they are from different plants. However, it is that geitonogamy that has the ecological properties of cross – fertilizer but the

genetic properties of self fertilization. Thus, geitonogamy appears to be equivalent to autogamy (LLOYD & SCHOEN 1992).

d) Style movement acts towards promoting self-pollination and leads *Nigella sativa* to delayed self-pollination

WEBER (1994) has produced a presentation film showing the pollination mechanism for *Nigella arvensis.* He concisely presented the mechanism in written steps. The mechanism demonstrated style movement in the *Negella arvensis* which exactly resembled our observations on style movement of the *Nigella sativa*; through pictures shown above. I used his written description as a quotation. I measured the style and anther length and style twisting angle. Hence, the equal length of the anther and the stamen demonstrate style twisting, whereas WEBER 1994 did not mention the length. WEBER (1994) mentioned that insects bear pollen on their thorax after touching the horizontal anthers. Our observations showed that honey bees land on the horizontal anthers and twisting point of the style and anther in order to bear pollen grains on their legs. So, how could insects carry pollen to another flower if the pollen is on their thorax? The beginning of receptivity caused a strong twist for stamens and styles that lead to self pollination. This was observed at the end of the male stage and at the beginning of the receptivity stage. Style movement acted towards promoting self-pollination as in *Nigella sativa*. In another plant, style movement lead to avoiding self-pollination and promoting cross-pollination as VERMA and MAGOTRA (2004) reported for *Eremurus himalaicus.* They observed the mechanism of the stigma movement away from the dehiscing anthers, hence, it avoided receiving any left over pollen, and so self pollination was impossible. It is interesting to point out that the *Nigella sativa* plant relied solely on animal vectors to move pollen among individuals. If pollinators are absent or in low numbers at certain times or years, individuals of *Nigella sativ,* that can self pollinate if not previously out crossed, will be at a selective advantage. This reproductive assurance process has been termed “delayed selfing”.

e) ***Nigella sativa*** **mixed mating is a better strategy than selfing alone**

Mixed mating is a better strategy that means an open pollination system is better to seed setting than other pollination treatments. This open system leaves the plant exposed to biotic and abiotic factors.

The plant will be without any restrictions which may cause any reduction in seed setting. The open system includes the role of honey bees and role of plant to pollinate itself by delayed self pollinated flowers. The manual pollination, which included hand crossed, hand geitongamy and hand forced self, ranked second after open pollination. This significant difference is attributed to human performance which is not like natural performance. Excluding the biotic and abiotic factor from the plant by bagged self, means the plant will be restricted without honey bees, and the plant depends on itself to develop its style to reach the maximum length to catch the anthers in order to twist. In spite of the style movement towards the anthers, it gained half of the seed setting from open pollination, and this attributed to the fact that the style's movement occurred once the stigma was receptive and at the final stage of anthesis when there is a small number of anthers, and then sink down. This may not be enough to get a high percent of seed setting as the quantity of pollen is not enough.

f) Honey bees are pollinator to *Nigella sativa* which is considered unattractive to wild bees

The only diurnal visitor and pollinator were honey bees. Honey bees frequently visited *Nigella sativa* in the Jordan Valley. The honey bee had similar behavior in the two locations. In the evening, no pollinators were found in the flowers at both sites and seasons. Flower visitors can only be considered pollinators if four pollination conditions have been met: pollen transfer to the vector observed; pollen transport by the vector observed, pollen transfer from vector to stigma observed; and pollen deposited by the vector shown to result in fertilization of the value (COX and KNOX 1988). The flowers of *Nigella sativa* were unattractive to wild bees' visitors. An important aspect used in many pollination studies is the number of visits made by a pollinator (PROCTOR *et al*., 1996). *Apis mellifera* engaged in pollen and nectar collection as a pollinator of *Nigella sativa* flowers with low frequency. The unattractiveness of *the Nigella sativa* flowers to wilds bees may be attributed to several factors such as the presence of other floral resources.

During our research, *Nigella sativa* flowering coincided with that of other species such as *Centurea syriaca* and *S. arevensis* which are important for apiculture in Jordan due to

their abundant nectar and the large floral patches throughout the area. The attractiveness of any species is a function such as flavor, color, nectar volume, and sugar concentration (FRISCH, 1967), and the bees fly to plant species that yield the greatest nectar and pollen (GARY, 1979).

g) The Role of Honey bees in the pollination of *Nigella sativa* is too small

Honey bees' role as pollinator in fertilizing *Nigella sativa* flower buds was very small compared to the role of plant itself and the role of open natural conditions in pollination. The emasculated buds were left exposed to the pollinators in order to fulfill the pollination where it sets up 12% of seed formation, while the natural conditions and self pollination conditions gave 87% and 45%, respectively. It is necessary to ask whether the removal of stamens affected subsequent flower development, e.g. the growth of the perianths, a factor that would make it difficult to distinguish between the costs of stamens or pistils and the costs of structures associated with display and reward (ANDERSSON, 2003). Such effects seem likely considering the work of ANDERSSON (2000), who detected a cost of producing and maintaining sepals and petals in a related species (N. *degenii*), and PLACK (1957) who found a negative effect of emasculation on corolla size in hermaphroditic plants of the gynodioecious *Glechoma hederacea* (Lamiaceae). In the present study of *Nigella sativa*, stamen removal caused significant reduction in the mean of seed set. The results agreed with ANDERSSON's study (2003) where he observed the stamen removal produced reduction in total seed number. Furthermore *Nigella sativa,* ANDERSSON (2003) carried out the removal of styles from *Nigella sativa'* flowers and he found that; style-less plants initiated almost three times more flowers and invested 57% more biomass in stamens, than plants whose flowers were permitted to set fruit. He found also stamen-less plants produced significantly heavier seeds after hand-pollination.

These observations indicate that stamens draw upon the same pool of resources as the other floral organs and that the removal of immature stamens therefore influences patterns of resource allocation. Furthermore, ANDERSSON and JORGENSEN (2005) carried out the removal of the perianth from *Nigella sativa* flowers and found that; perianth removal produced 12.5% heavier seeds and allocated 15.8% more biomass to seed production than plants in which all perianths were left intact Whereas differences in flower production and total seed number were not significant. Perianth removal did not

significantly affect the proportion of seeds that germinated, but caused a shift towards earlier germination dates.

h) The ultimate Visitation Rates for *Nigella sativa*' flower in both Locations was diurnal visitation type especially at early morning

The maximum visitation rates for *Nigella sativa'* flowers in both locations were between 9:30 a.m. and 12:30 p.m. This was due to the bees activity being limited by environmental factors such as the radiation rate and daily temperatures. Visitation rates were estimated by counting the number of visiting tours, and those with anther or stigma contact. Counts were made for one hour periods during an (8) hour day, while plant the species flowers were open. Pollinators may accidentally take place without any relationship existing between blossom and agent. Even with the concept of a definite relationship in mind, it is not always easy to draw a line between pollinators and accidental visitors. The quantity of pollen transferred from anthers to stigmas, visit frequency to flower, pollinator forage pattern during anthesis, and floral rewards availability are parameters that can adequately explain the pollination efficiency of floral visitors (PRIMACK and SILANDER 1975, HERRERA 1987, 1989). It is generally thought the more visits made, the more efficient is the pollinator, though this also depends on the per visit pollen contribution to the pistillate flower part (PRIMACK and SILANDER 1975, HERRERA 1989).

4.4. *Coriandrum sativum*

a) The length of stigmatic receptivity for *Coriandrum sativum* was approximately hours

In this study, stigma length has been characterized in the *Coriandrum sativum* flower from anthesis to degeneration. The female stage initiated from 14:00. to the evening of the same day. It is interesting to point out that the stigma has short receptivity, which leads to limited fruit set when the pollinator visitation is low. It is interesting to point out that the stigma of *Coriandrum sativum* is unreceptive at anthesis, which leads to impossible self pollination at the single flower level, hence reducing the opportunity to

seed set in case of the absence of a pollinator. GALEN *et al.* 1987 supports our investigation that reported stigma receptivity is a crucial stage in the maturation of a flower, which may greatly influence the rate of self pollination and pollination success at different stages in the flower's life cycle, the relative importance of various pollinators, the interference between male and female functions and the rate of competition via improper pollen transfer. Many plant species are unreceptive at anthesis, like *Chamelaucium uncinatum* (OBRIEN, 1996), and stigma receptive throughout anthesis as CLIFFORD and OWENS (1998) reported that orchid's species showed the stigma is receptive throughout anthesis. Stigma receptivity is a very important factor influencing effective pollination (SANZOL and HERRERO, 2001). When it is restricted to a short period, it may limit fruit set as in the apricot (EGEA *et al*., 1991) or kiwi fruit (GONZALEZ *et al*., 1995) when he stated that stigma receptivity refers to the ability of the stigma to support germination of viable and compatible pollen. HEDHLY *et al*. (2003) found that high temperatures reduced stigmatic receptivity and low temperatures enlarged it. The stigma lost the capacity to offer support first for pollen germination and, finally, to pollen adhesion. The effect of temperature was more pronounced in pollen germination and penetration than in pollen adhesion. In the research of DIEDERICHSEN (1996) in Germany, he found the receptivity period for *Coriandrum sativum* lasted 5 days, whereas in our study in Jordan, it lasted for hours.

This reflects the effect of temperature variance in stigmas receptivity elongation between humid countries in contrast with arid ones. Although the umbelliferea species have a long receptivity period (KOUL *et al*., 1989), *Coriandrum sativum* has few hours receptivity period, according to our research. The carrot, also in the same family as *the Coriandrum sativum*, has 4-5 days receptivity period as noticed by PEREZ-BANON's *et al.* (2006) research in Spain. In angiosperm species, pistil receptivity can last from one to several days. A long duration of pistil receptivity helps high pollination success (TANGMITCHAROEN and OWENS, 1997; SORNSATHAPORNKUL and OWENS, 1998; ALEEMULLAH *et al*., 2000). STPICZYNSKA (2003) reported the stigma of the orchids may retain receptivity for a long time (up to 60 days), while in another plant the pistil receptivity of *Lemus chinesis* lasted only about three hours. This approved the effect of temperature variances in stigmas' receptivity intervals. Optimal receptivity is variable and can be from a few hours after flower opening as in teak

(TANGMITCHAROEN and OWENS, 1997), to a few days after anthesis as in oak (KALINGANIRE *et al*., 2000) and *Salina alba* (YOUNG and GRAVITZ, 2002).

b) The male phase is initiated a day, before the stigmas become receptive, where the anthesis duration remains for a day

The male stage started at 9:00 and remained active until 13:00. The male developed earlier than females during inflorescence stage. The male exhibited strong protandrous, so the male stage began before the female stage. It is interesting to point out that anthers remain active for a day, which leads to unsynchronization of the receptivity period. The successful establishment of angiosperms on land is in part determined by their floral design. Because plants cannot move to find an ideal mate, they have developed a great variety of flowers to provide different mechanisms of pollen release, pollen transfer and deposition of the pollen from the male to the female sexual organs. It is well documented that the reproductive phase, especially from pollination to fertilization, is highly vulnerable to the prevailing environmental conditions including temperatures (HALL 1992 and STEPHENSON *et al*., 1992).

Thus, planet warming may have significant consequences on the reproductive phase with serious implications in agricultural crops in species such as *Coriandrum sativum* in Mediterranean regions. Pollen tube growth is clearly affected by temperature (LEWIS, 1942) and there is good evidence of genetic variability in pollen performance that depend on temperatures among species (ZAMIR *et al.,* 1981; WEINBAUM *et al.,* 1984) or even among genotypes of the same species (WEINBAUM *et al.,* 1984; POLITO *et al*., 1988). Climatic factors affect the anthesis intervals in *Coriandrum sativum*. High temperatures had a direct effect on pollen performance since the pollen responds to temperature. The anthesis in eggplants are mainly influenced by the daylight, temperature and humidity, usually anthesis starts from 7:30 a.m. and continues to 11:00 a.m.. HEDHLY *et al.* (2003) found that high temperatures reduced the germination capacity of the pollen as early as the first day after anthesis. *Coriandrum sativum* has a day anthesis interval period in our research, while carrot, which is in the same family as *Coriandrum sativum*, has 12 days anthesis interval as noticed by PEREZ-BANON's *et al.* (2006) research in Spain. This approved the effect of temperature variance in anthesis interval.

c) *Coriandrum sativum* is complete self incompatible

The Stigma of *Coraindrum sativum* rejected self pollen grains from the same flower for large samples in both locations and seasons. Seed set in *Coriandrum sativum* under hand forced self pollination was almost zero at both sites and seasons. Self pollen rejection leads to self incompatibility. The best mechanisms of self-incompatibility occur by inhibiting the germination of pollen on stigmas, or the elongation of the pollen tube in the styles. These mechanisms are based on protein-protein interactions, each mechanism being controlled by a single locus, which has many different alleles in the species population. Despite their similar morphological and genetic manifestations, these mechanisms have evolved independently, and are based on different cellular components. The single locus contains two basic self-incompatibility; one expressed in the pistil, and the other in the anther. Because of their physical proximity, these genes are genetically linked, and are regarded as a single allele.

The translation products of the two genes from the same allele are two proteins which, by interacting with one another, lead to the arrest of pollen germination and/or pollen tube elongation, and thereby generate a self-incompatibility response, preventing fertilization. I hadn't found any research involving the approval of capability of *Coriandrum sativum* to self-incompatibility but ROMANENKO and SVECNIKOVA research (1988) found male sterility in *Coriandrum sativum*. FISHER (1941) was probably the first to point out that a gene which allowed self fertilization in a self incompatible plant would rapidly reach fixation, unless it was under a severe disadvantage through diminished viability in its carriers. Pollination requires consideration of the plant. Some plants are self-fertile or self-compatible and can pollinate themselves. Other plants have chemical or physical barriers to self – pollination and need to be cross pollinated, with these self – infertile plants, not only pollinators must be considered, but the plant as well. There is no evidence if *Coriandrum sativum* is self compatible because anthesis and receptivity periods were unsynchronized, so the stigma was not useful from pollen grains, hence no benefit for pollination, but it is important for breeding programs. From here it is important to indicate that not only pollinators must be considered but plants as well. In our investigation, it is not important to devote a lot of effort in finding solutions in order to break self incompatibility for pollination requirements.

d) Honey bees are pollinator and visitor to *Coriandrum sativum* which is considered unattractive to bees

The visitors and pollinators were honey bees. Honey bees frequently visited the *Coriandrum sativum* in the Jordan Valley. The honey bee had similar behaviors in the two locations. In the evening no pollinators were found in the flowers at both sites. It is interesting to point out that honey bees' pollen collector acted through random motion over *Coriandrum sativum*'s cluster and flew to another cluster behaving the same. From here, it is important to indicate that honey bees are not only pollen collectors but main pollinators for *Coriandrum sativum*.

In addition, honey bees are not only considered pollinators, but nectar thieves as well which accounted for 30 % of the visits. When a honey bee visits a flower in order to drink nectar there may be restrictions in their way may shift their visiting fate. The restrictions which may face the way of honey bees during their visits are: the biology of *Coriandrum sativum*; the diversity of types of flower, the variation of number of flowers, the small size of flowers and the density of flowers at cluster level. In Jordan, agriculture has come to depend greatly upon honey bees to fulfill its pollination needs. This insect has several valuable qualifications for this role. The honey bee is adapted to many climates and can successfully revert to its original wild state in most parts of the world, quickly becoming part of the natural reservoir of pollinators. Our results agreed with MC GREGOR (1976) in that honey bees are the ideal pollinators for *Coriandrum sativum*. Our results also agreed with GLUKHOV (1955) who reported that bees are beneficial to the *Coriandrum sativum* and when they were excluded, only 49.4 % of the seeds set, but when they were present, 68.3 % of seeds set. BOGOYAVLENSEII and AKIMENKO (1966) support our results when they associated seed yields with increased bee visitation.

DIEDERICHSEN (1996) reported that under optimum conditions, many different insect species are pollinators or visitors of *Coriandrum sativum* umbels, the species of insects that pollinate *Coriandrum sativum* depend on the area of cultivation. In India, studies have been done by KOUL *et al*. (1989) concluded that honey bees are the main pollinators for *Coriandrum sativum.* But in Egypt, EL-BERRY *et al*. (1974) observed that other insects pollinated *Coriandrum sativum.* In contrast, ABDEL-AAL and HUSSEIN (1982) reported that honey bees were frequent visitors of *Coriandrum sativum* in Egypt. 10 different species of insects, some of which are endangered, visit *Coriandrum*

sativum flowers in Germany. The variation in pollinators which visit *Coriandrum sativum* in Jordan, Egypt and Germany is attributed to climatic factors and the practices of farmers which may expose wild bees to danger.

The flowers of *Coriandrum sativum* were unattractive to wild bees as visitors. An important aspect used in many pollination studies is the number of visits made by a pollinator (PROCTOR *et al*., 1996). It is generally thought that the more visits made, the more efficient is the pollinator (PRIMACK and SILANDER 1975, HERRERA, 1989). *Apis mellifera* engaged in pollen and nectar collection as a pollinator of *Coriandrum sativum* flowers with low frequency. The flower is mainly attractive to bees for both pollen and nectar because of their odor and visual nectar guides (FREE, 1993). This attraction is important to ensure arrival of pollen to the stigma of pistillate flowers by bees during their collecting of nectar. Bees are generalized to their nectar sources, capable of visiting many kinds of plants for nectar, but many if not most bees are pollen specialists (WCISLO and CANE, 1996). We must explain all wrong practices to farmers in order to keep pollinators for sustainability, since the first flowering plants were insect – pollinated (THIEN *et al*., 2000), but many plant species utilize other animal taxa or even wind and water to carry pollen between flowers (COX, 1991). Wind pollination, the most common form of a biotic cross pollination, release dioecious and self – incompatible plants from relaxing on animals for pollen transfer (REGAL 1982; BERRY and CALVO 1989; GOODWILLIE 1999; CULLEY *et al*., 2002. Abiotic pollination may also increase phonological and developmental flexibility, which may be particularly beneficial in climates with short growing seasons (COX, 1991).

e) Self-pollination for *Coriandrum sativum* is impossible for single hermaphrodite flower

Self-pollination for a single hermaphrodite flower is impossible. Seed set in *Coriandrum sativum* under bagged self pollination conditions in hermaphrodite flower pollination was almost zero at both sites. The males developed earlier than the females during inflorescence stage, in that the male exhibited strong protandrous resulting in the male stage beginning before the female stage. The anthers dehisce and release the pollen and the pollen is gone while the stigma is unreceptive, so the probability of self pollination at a single flower level is zero.

The strong protandry and strong separation between male and female phases at a single flower level lead to the probability that anthesis and receptivity coinciding is absent. The results agreed with MC GREGOR (1976) who noted the impossibility of self-pollination in the *Coriandrum sativum*, if the pollination is specified in a single hermaphrodite flower. DIEDERICHSEN', in his book (1996), reported that the possibility of self pollination for *Coriandrum sativum* occurred but unfortunately he did not specify the type of self pollination. In our research, I have pointed out those two types of self pollination in *Coriandrum sativum*: self pollination at a single hermaphrodite flower level and self pollination at a cluster level which has (19-20) flowers. This is a problem for the reader to know which type of self pollination he means, did he mean self pollination at one flower level or at all cluster levels? In our investigation, DIEDERICHSEN' considered the whole one cluster as one flower. I agree with DIEDERICHSEN (1996) that out crossing will take place if the pollen from another *Coriandrum sativum* flower reaches the stigma. I agree with DIEDERICHSEN (1996) because through our research I have pointed out that geitongamy took place and there was no significant difference between geitongamy and open pollination. *Coriandrum sativum* was not emasculated but pollinated with the pollen of other flowers while still having a degree of selfing of 25% percent.

f) *Coriandrum sativum* is partially selfing in one cluster

The cluster has (9-21) flowers. The flowers are characterized by its small size, diversity of type, large number of male flowers in contrast to female flowers, hermaphrodite flowers, and its density within flowers. The first blooming flower is a hermaphrodite and the later flower is a staminate flower. The later staminate flowers developed while the receptivity of the stigma had initiated. The anther stands vertically then partially bends toward the surface of the stigma that lies beneath. The pollen is thrown and falls on to stigmas. In this way, some of the stigmas may be pollinated. From here, it is interesting to point out that *the Coriandrum sativum* plant is partially self fertile at the cluster level.

g) The first opening flower being a hermaphrodite flower

The research results seem to suggest that the first opening flowers are hermaphrodite, while the later opening ones are staminate. SINGH and RAMANUJAM (1972) suggested there is a fairly sharp transition, where the central flower is one of the earlier opening

flowers, while the later opening ones are staminate, though a few staminate flowers may have opened before. Environmental and developmental factors might, perhaps, be involved in the switch over from perfect to staminate. I disagree with JOSM's (1965) observation where he reported that there does not appear to be any such clear zonation in the *Coriandrum sativum* cluster. Since the *Coriandrum sativum* cluster consists highly of condensed racemes, our attempt was to decondense it, taking advantage, as it were, of the racemose nature of the inflorescence. I had recorded three types of flowers in *Coriandrum sativum* which are; hermaphrodite flower, staminate flower and pistillate flower. Another aspect of the inflorescence structure is the pattern of distribution of the two types of flowers. According to BRAAK and KHO (1958) there is a well defined pattern of distribution of the two types of flowers in carrot, in the form of zones of one kind of male or female flowers The biology of a flower may help in the occurrence of the partially self pollination. I wrote partially, and with that I mean some of stigmas, may pollinate if these stigmas are at the receptive period and have enough pollen for these stigmas. I agree with GLOKHOVE 1955 who showed *Coriandrum sativum* is partially self pollinated. This self pollination provides reproductive assurance with poor or absent pollinators, so we can get seed setting if honey bees are absent. I agree with the following researchers that self-fertilization can also provide reproductive assurance in lineage with poor pollinator service (STEBBINS 1957; LEVIN 1972; SOLBREG & ROLLINS, 1977; LLOYD, 1979; UYENOYAMA *et al.,* 1993; HOLSINGER, 1996).

h) *Coriandrum sativum.* Sativum mixed mating is a better strategy than partially selfing alone

Mixed mating is a better strategy that means an open pollination system is better to seed setting than other pollination treatments. This open system leaves the plant exposed to biotic and abiotic factors. The plant will be without any restriction which may cause a reduction in seed setting. An open system includes the role of honey bees and the role of plants to pollinate it self by partially self pollinating flowers.

i) Low percentage of protein in *Coriandrum sativum*' seeds

The protein percent in treated fecundated-seeds for *Coriandrum sativum* was 14.9% of the total dry mass, while the protein percent in an empty embryo was 4.7% of the total dry seed mass. Protein contains more fecundated seeds than non-fecundated seeds, which

is attributed to the enzymatic activity of protein synthesis in complete fertilized ovules (embryos) versus the empty ovary (gamete) and the fact that the cell division in fecundated seeds needs more protein to complete their division in contrast with the empty ovary (gamete). Many studies pointed out the seed content in *Coriandrum sativum*, but did not include the percent of protein in fecundated and non-fecundated.

j) High ratio of staminate flowers compared to pistillate and hermaphrodite flowers

The number of male flowers is more than the number of female and hermaphrodite flowers. With an average of 9.8 % of male flowers per cluster, *Coriandrum sativum* has a much higher percentage of male flowers than female and hermaphrodite flowers which range between 4.8 % and 5.4%. In our investigation, the high ratio of male flowers at cluster level was considered a main problem that causes a reduction in seed setting because the male flower does not develop seeds but as a source of pollen grains. Also in our investigation, the problem of sex-expression in *Coriandrum sativum* is attributed *to* auxin level of the flower produced.

HESLOP-HARRISON (1957) suggested that the auxin level at the differentiating apex of *Coriandrum sativum* determines the sex-expression of the flower produced. This level of auxin concentration may further be influenced by a number of environmental factors like temperature, auxin economy, mineral nutrition and photoperiod. There have been few attempts to analyze the problem of sex-expression in *Coriandrum sativum* at the biochemical or physiological level. GALUN (1961) has pointed out the possible relationship between auxins and sex-expression. There have been several reports on the sex-expression being altered by external application of growth hormones, substances with anti-auxin effects and seasonal variations, etc. (CHAUDHARY and PATHAK, 1959, 1960; PIKE and PETERSON, 1969.) I disagree with researchers who try to alter the ratio of staminate, pistillate, and hermaphrodite flowers by using auxins or other chemicals in order to reduce staminates' flower number to increase the seed yields. In our investigation, the researchers have to leave *Coriandrum sativum* in nature without interference, because in view of the protandrous nature of *the Coriandrum sativum,* the presence of male flowers can lead to a considerably greater degree of self pollination than when male flowers were not present. Hence, any changes in the number of male flowers at cluster level may lead to cancel the role of self pollination at cluster level because self pollination depends mainly on male flowers.

k) **The ultimate visitation rates for *Coriandrum sativum*' flower in both locations was diurnal visitation type especially in the early morning**

The ultimate visitation rates for *Coriandrum sativum* flowers at both locations were during 8:30 a.m. to 11:30 p.m.. This was notedbecause the bees' activity was limited by environmental factors; the radiation rate and the daily temperature. An important aspect used in many pollination studies is the visits made by a pollinator. Low visitation rates of females could present a problem for successful seed set when pollinators or pollen is limited. Nevertheless, *Coriandrum sativum* has a diversity of flowers, the number of male flowers is more than the hermaphrodite and the female flower, hence low visitation of honey bees and a low number of female flowers caused a big problem for pollination.

From here, it is interesting to point out that the low visitation rate not only affects pollination, but also the biology of a plant. Therefore, researchers should have taken this into consideration. Although the number of pollen grains removed from a flower and deposited on stigmas increases with an increase in the frequency of flower visits and/or increased residence time of pollinators on a flower, the proportion of deposited pollen to removed pollen declines as the amount of pollen removed increases (HARDER & THOMSON 1989; GALEN & STANTON 1989; HARDER and CRUZAN, 1990). However, restrictions on pollen removed are likely to lower the foraging efficiency of pollinators (HARDER & THOMSON 1989). The amount of pollen available in a flower may influence the frequency of the pollinator's visits. Similarly, efficient removal and deposition of pollen during single flower visits occur in some plant species whose flowers are exploded by single flower visits of pollinators, (FAEGRI and VAN DER PIJL 1971). In addition to visitation rate, individual bees may visit up to 500 individual flowers on a single trip from the hive with 93% - 98% of all visits to the same flower species (GRANT 1950; FREE 1963). The species visited varies between individuals, between hives, and overtime in the same individuals, in response to changing nectar availability in different flower species (BARTH, 1985). Bees rapidly learn to associate nectar availability with a particular flower species, and can use color, scent and shape to identify the correct species. Bees can also learn to distinguish between flowers of the same species according to their age, and to favor the age group which provides the highest reward (GIURFA and NUNEZ, 1993).

l) Seed content

The fatty oil of *Coriandrum sativum* is of high interest because of the high level of petroselinic acid, which has potential non-food applications in oleo chemistry. This oleic acid, like isomer, opens up another potential approach to the manufacture of medium-chain acids, since it can be split into lauric (C 12:0) and adipic (C 6) acids by the oxidative cleavage. However, oil percentages in the range of 30-40% are needed for an economically viable crop.

Adipic acids are used for the manufacture of a wide range of polymers including high grade engineering plastics. At present, adipic acid is derived from mineral oil by a process which releases gasses such as N2O that damage the ozone layer and contribute to global warming. Petroselinic acid is an isomer of oleic acid and is used as a plastics lubricant, in the manufacture of nylons and cosmetics. Oleic acid (C 18:0) is used in many industrial processes, and in the food industry. It is a major constituent of salad cream and mayonnaise. Other more recent uses include the use as a green vegetable by some ethic groups and to flavor dishes and foods such as pickles and sauces. Both the quantity and composition of the oil can change drastically as the plant matures. In the oil from *Coriandrum sativum*, the content of aliphatic aldehydes drops and that of the monoterpens alcohols increases tenfold from the stage of full flowering to green fruit. The composition and quality of oil is affected significantly by the method of isolation and subsequent processing steps. Enzymatic action (fermentation) in the crude plant material prior to distillation or extraction can bring about hydrolysis of glycosides and release oil components. The composition of oil may depend greatly on the isolation method. Steam distillation is the most common process, but sensitive compounds can undergo rearrangement or oxidation on heating. Oils from flower blossoms are extracted by pressing the petals with a purified fat or hydrocarbon solvent and the essential oil is then extracted with alcohol. Petroselinic acid is a characteristic component of the fatty oil of umbellifferous plants, and the fruits of several medicinal and aromatic plants have been screened for this fatty acid.

4.5. *Cucurbita pepo*

a) The male phase is initiated an hour before the stigmas become receptive, where the anthesis duration remains for hours

Both male and female *Cucurbita pepo* flowers are open during early morning (around 6:30 a.m.)_ and generally close by mid afternoon (around 3:30 p.m.) At complete blooming stage for *Cucurbita pepo*, the male stage started at 7:00 a.m. and stopped at 12:00 a.m.

Also, the female stage started early in the morning around 8:00 a.m. and stopped at 14:00 p.m. Not surprisingly, the stigma is most receptive to pollen in the morning. Each stigma should receive several hundred grains of pollen for best fruit-set and quality. In west Tennessee, each *Cucurbita pepo* flower opens for only one day, usually between 5:15 and 11:00 a.m. (SKINNER and LOVETT, 1992). However, if it's hot, flowers close early. Consequently, as with other cucurbits, good bee visitation in the morning is important. The *Cucurbita pepo* flowers open early in the morning and close around noon of the same day, never to reopen (FREE, 1992; NEPI and PACINI, 1993). The variation between the anthesis and receptivity intervals is attributed to climatic factors affecting the country. At our research sites, the temperature from day to day changed so it had a direct effect on anthesis and stigma periods since the pollen responded to the varying temperatures. However, at the same time, high temperatures are advantageous for the pollen by hastening its tube growth rate, which could signify an adaptation of the male part and leads to a short female receptivity. *Cucurbita pepo* has a flowering habit which is unique among the vegetable crops, the biology of *Cucurbita pepo* flowers and the large flowers may lead to expose the male and female flowers to direct sun, hence increasing the exposed surface of the flower. There are evidences that indicate anther and stigma respond to temperature. High temperatures have a direct effect on pollen performance since the pollen responds to temperatures. On the other hand, low temperatures may act against the pollen by reducing its germination and growth rate, which could limit the fertilization success (THOMPSON and LIU, 1973; JAKOBSEN and MARTENS, 1994). In lower temperatures, the pistil seems to compensate the effect on pollen by enlarging stigmatic receptivity and delaying style and ovule degeneration (STOSSER and ANVARI, 1982).

From here it is interesting to point out that *Cucurbita pepo* flowers are usually open and attractive to bees for only one day or less. The opening of the flower, release of pollen, and commencement of nectar secretion normally precedes bee activity.

b) Allogamy in *Cucurbita pepo*

Cucurbita pepo is one of the most important vegetable crops in Jordan. This commodity is produced predominately for the fresh market. Temperature conditions in Jordan are favorable for this crop. There is no significant difference between open, hand geitongamy and hand cross in both seasons and locations in terms of the total fruit weight, seed weight, number of seeds, number of non fecundated seeds, number of ovules and percentage of seed set. Although it was noted that the open pollination gained the highest number, however, there were no significant differences between them. Fruit abortion can reach 100% in flowers bagged to exclude insect visitors, that indicates this varieties of *Cucurbita pepo* is non parthenocarpic.

It is interesting to point out that pollination must take place on a day when the flowers open, since pollen viability, stigmatic receptivity, and attractiveness to bees last only one day. From this point, *Cucurbita pepo* pollination is most effective in the early morning. Our result indicates that hand pollination and open pollination had no significant difference. Subsequently, we can point out that when the bee population in a field is too low for good fruit set, one can substitute the bees by pollinating by hand. Hand pollination is a tedious chore, but if one really likes *Cucurbita pepo* and there are no bees in the field, it's the only way that someone will be able to harvest a crop. Introduction of hives into cultivated fields is a common agriculture practice to get successful pollination in Jordan. However, the current pandemic among honey bees caused by plagues has not only increased the cost of honey bees' hive introduction and maintenance in agriculture zone, but also highlighted the need to find alternative species as managed crop pollinators. Beside honey bees being considered as amain pollinator, we can devote efforts to find alternative pollinators. We can train laborers or agronomists to be a good hand pollinator's. The non significant differences between hand treatment and open treatment is attributed to one reason; *Cucurbita pepo* pollen is large and sticky, this helps specialists to transfer the pollen grain in the right way and carry a sufficient amount in order to get a successful pollination.

The pollen is yellow in color and produced on the structure in the center of the male flower. *Cucurbita pepo* is not a rich source of pollen or nectar, but bees readily visit the plants if there are no more attractive plants nearby. Bees collect pollen on *Cucurbita pepo* mostly in the early morning and switch to nectar later in the morning. In Jordan, pollen foraging is at its highest before 10:00 a.m. and decreases dramatically in the afternoon.

During the main growing season, the ratio of male to female flowers is usually 3:1. This helps ensure pollination of the female flowers which must be pollinated in order to set fruit. The female flower is distinguished by the presence of an ovary at the base. Female flowers are born on very short stems, while male flowers are born on long stems. Farmers often become concerned when many flowers appear early, but fruits fail to set. The reason for this is that all of the early flowers are males. Female flowers develop somewhat later and can be identified by the miniature fruit at the flower base.

c) Honey bees are pollinators to *Cucurbita pepo*

Honey bee hives were rented and placed on *Cucurbita pepo* field edges to promote adequate pollination. Recommendations and advice were given to growers to utilize one to two hives per acre so as to pollinate *Cucurbita pepo* crops. Without good pollination, yields are drastically decreased. The absence in the native bee population has forced growers to rent hives in order to produce *Cucurbita pepo* and other cucurbit crops in Jordan. Honey bees are excellent pollinators of *Cucurbita pepo*; they make more contact with reproductive parts of a flower, work faster, and work earlier in the morning. Although the main pollinator for *Cucurbita pepo* is honey bees in our research in Jordan, there is evidence that honey bees may not always be the most efficient pollinator. SKINNER and LOVETT (1992) found that bumble bees were more efficient than honey bees in promoting a satisfactory fruit-set. Six of eight (75%) single bee flower visits by bumble bees resulted in whole fruit, whereas five of sixteen (31 %) single bee visits by honey bees resulted in whole fruit.

TEPEDINO (1981) reported that bees of genus *Peponapis* are no more efficient than honey bees at setting fruit of *Cucurbita pepo*. Honey bees play an essential role in the pollination of *Cucurbita pepo*. Our result showed that honey bees played an efficient role in the pollination of *Cucurbita pepo* as long as they pollinated it, as they were frequent visitors. Observations included collecting pollen, transporting pollen and contacting the

stigma. The bees take longer to visit a female flower because of the position of the nectary and the greater quantity of nectar. The bees suck the nectar as they circle the nectar receptacle. In the male flower, they suck the nectar through a nectary pore and never move on to the adjacent one. The decrease or absence of visits of honey bees for *Cucurbita pepo* after mid of January may be ascribed to climatic factors and hives being moved by farmers. In addition, a small population of honey bees went to the herbaceous plant beside the cultivated area on sunny days and many of the population didn't fly because the temperature was under 15°C. Honey bees with large pollen loads are often seen visiting male flowers However, honey bees are generalists and they readily move to any competing bloom that offers richer rewards than *Cucurbita*.

This is a problem in the Jordan where wild plants, a rich source of nectar, blooms in competition with early *Cucurbita pepo*. With temperature decreasing to 10°C. The Black Fly appeared in the male and female flowers of *Cucurbita pepo* in high intensity at the second site of the research. The Black Fly is more efficient than honey bees in visiting. I have checked this visitor at other *Cucurbita pepo* farms in The Jordan Valley. I have found the same visitor during the same period. The main purpose of the Black Fly's visitation is nectar theft since it lands on the petals and departs from the base of the male and female flowers. The availability of the Black Fly is due to the substantial water bonds. The Black Fly spent more time in female flowers than males because female flowers have richer nectar than the males. Nevertheless, I have applied the experiment in the second season, however the Black fly never appeared on *Cucurbita pepo*.

d) Seed content

Oil content of the *Cucurbita pepo* seeds varies from 40-50% depending on the genotype. The oil is dark green and contains free fatty acids. Vitamin E content, especially gammatocopherol, which is very high. The naked oil seed *of the Cucurbita pepo* are often eaten as a snack food Oil extracted from the seed is sold as a medicine to relieve prostate problems. The seed is high in unsaturated fats and protein as well as Vitamin E and A. Oil from the seed may be used for deep frying and as a base for mayonnaise, French dressing and margarine. The oil is dark due to the carotenoids and chlorophyll and when extracted in solvent, is high in the linoleic, oleic and palmitic fatty acids. The seed oil is used for salad dressings but also has uses in pharmacology and alternative medicine

(WAGNER, 2000), especially when produced organically. Formation of oil in the plant, and consequently the yield and composition of the oil produced, depends on many factors. Genetic differences in plants of the same species that are otherwise indistinguishable can result in widely different oil content.

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Phone: + 962 5 3559927
Mobile: + 962 777195363
+ 962 799302744
Email: abu_hammour77@yahoo.com
Nationality: Jordanian
Gender: Male
Place & D.O.B: Jordan, 4-1-1978

Khaled Adel Saleh Abu-Hammour

Relevant Experiences

Researcher **2005 – Until Present**

National Center for Agricultural Research and Extension / Ministry of Agriculture

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Agricultural Engineering, Nematode Laboratory. **2003-2005**

National Center for Agricultural Research and Extension / Ministry of Agriculture

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Plant Production Engineering **2001-2003**

Microbiology Laboratory (Sewage Water Treatment)

National Center for Agricultural Research and Extension / Ministry of Agriculture

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Education

- **Philosophy Doctorate of Pollination of Medicinal Plants** **2005-2008**

  University of Bonn, Germany

- **Master Science of Horticulture Science** **2002-2004**

  Jordan University of Science and Technology
  Faculty of Agriculture.

- **Bachelor Science of Agriculture Engineering** **1996-2001**

  University of Jordan
  Faculty of Agriculture.
  Department Of Plant Protection.

- **High School: Secondary Al Salt School.** **1995-1996**

Ability and Interest

- Language skills: English (Fluent Speaking & Writing), Arabic (Fluent Speaking & Writing) and Germany Basics.
- Computer Applications: Microsoft Office and Windows Operating System.
- Computers (Software and Hardware).
