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Analysis of essential oil of *Capparis spinosa* L. leaves and interaction between *Pieris brassicae* L. (Lepidopteran) which attack caper and natural enemy *Cotesia glomerata* (L.)

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Abstract : The investigation was carried out during 2015 - 2016 at the laboratories of Plant Protection Dep., Faculty of Agriculture, Damascus University. the constituents of essential oil extracted from leaves of *Capparis spinosa* L. Capparidaceae family, growing wild in Damascus, Syria,. The essential oil were prepared from dried and powdered leaves with steam distillation .The essential oil were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). A total of 12 components were identified. The major constituents of the *C. spinosa* leaves oil was Thymol, Octanoic acid, methyl isothiocyanate and 2-hexenal. This is the first report of *Pieris brassicae* L. feed on *Capparis spinosa* plants growing wild In Syria. addition, the results indicated that the natural enemy *Cotesia glomerata* (L.) was able to suppressed the *P. brassicae*, where, the parasitism rate varied from 84.25% to 87.54%. Finally, the results suggest that *Pieris brassicae* L. not harmful to *C. spinosa* plants growing wild in Damascus, Syria.
Key words: *Capparis spinosa* L., GC/MS, *P. brassicae*, *C. glomerata*.

1. Introduction

Capparis spinosa L. (caper) belonging to the family Capparidaceae is a xerophytic plant growing in a broad range of climatic conditions, varying from dry deserts to cooler altitudes of mountains[1]. The plant had been a part of the Mediterranean diet for over 5,000 years and several archaeologists has unearthed caper seeds, along with grape, pistachio, almond and olive seeds in Mediterranean basin, which belonged to the middle Bronze age. The famous traveller, Evliya Celebi mentioned caper plants 400 years ago in Osmancik town of Corum city in Anatolia region[2]. Although, the flora of the Mediterranean region has considerable endemism, it is uncertain whether the caper bush is indigenous to these regions. However, it could have originated in the tropics and later naturalized to the Mediterranean basin[1]. Currently, capers naturally grown in Spain, France, Morocco, Monaco, Italy, Malta, former Yugoslavia, Greece, Tunisia, Algeria, Africa, Southeast Asia, Himalayas, Pacific islands and some parts of Australia[3]. Investigated the genetic diversity and relationships among Capparis species growing in Syria using IRAP and ISSR techniques. The groups included genotypes identified as *C. spinosa*, *C. sicula* Duh., and *C. aegyptia* Lam. Based on the morphological description, molecular studies and statistical analyses of this study[4]. The caper is used essentially for flower buds. Also, flower buds, root bark, and fruits are used in folk medicine due to their analgesic, wound healing, cell regeneration, tonic, and diuretic effects[5]. In Baluchistan popular medicine, an herbal tea made of caper root and young shoots is considered to be beneficial against rheumatism, and powder of leaves are use for skin diseases. Extracts of different parts of *C. spinosa* have been shown to possess biological activity against a large number of pathogens: antifungal, antibacterial, anti-amoebic, and anti-worm activities have been demonstrated[6,7]. The leaf oil was composed of isothiocyanates, n-alkanes, terpenoids, a phenyl propanoid, an aldehyde and a fatty

acid. The main components of this oil are thymol (36.4%). Isopropyl isothiocyanate (11%), 2- hexenol (10.2%) and butyl isothiocyanate (6.3%) was reported. The volatile oils of the ripe fruit and the root are composed mainly of the methyl isothiocyanate, isopropyl isothiocyanate and sec-butyl isothiocyanates was reported[8]. Also the leaves of *C. spinosa* have kaempferal, quercetin, isorhamnetin and their O-methyl derivative, thomnocitirin, rhamnetin andrhamnozin[9]. Essential oils of caper (*Capparis ovata* Desf. var. *canescens*) leaves obtained by steam distillation followed by solvent extraction were analyzed by GC and GC/MS. 100 from the caper leaf oil. The major volatile compounds found in caper leaves were methyl isothiocyanate (20.0%), thymol (15.5%), 4-vinyl guaiacol (4.3%), hexyl acetate (3.6%) and trans-theaspirane (2.6%)[10]. Common components of *C. spinosa* L. oils identified were benzyl alcohol, octanoic acid, benzoic acid, α-terpinolene, carvacrol, zingerone and 4-fluoro benzaldehyde, β-Pinene , *P*-Cymene, α-Terpinolene, and Carvone[11].

The literature of entomocenosis caper pests in specific regions, In total, 23 insect species have been reported as major and minor pests. Caper foliage can be attacked by a large phytophagous entomocenosis which includes both sap sucking (heteroptera and homoptera) and defoliator (Lepidoptera and coleopteran) pests[12]. Lepidopteran species which attack caper are generally polyphagous, they occur on caper in the dry season, when other potential host plants are not available[13]. Only pirid species are reported on caper, i.e. *Colotis evagore* Lucas in Spain, *Anaphaeis aurota* F., *Colotis fausta* Olivier, and *Colotis liagore* Klug. in Saudi Arabia[14]. The most severe attack can be caused by *Pieris brassicae* L. commonly called large white butterfly, and *pieris rapae* L., the small white butterfly. *P. brassicae* is a polyphagous insect with a worldwide distribution[15]. Insects regroup the largest number of species at the Earth scale and are present in all landscape. They are also considered as being a major component of communities and ecosystem involved in many multi-species interactions, whether as prey, predator, parasite, pollinator or herbivore[16]. [35-44]Therefore, they are included in many consumer-resource interactions either as the resource or the consumer. Insect herbivores are attacked by a wide range of natural enemies[17,18]. Parasitoids could constitute 20-25% of all insect species, with the most important genera belonging to Hymenoptera wasps and Diptera flies[19]. *Pieris brassicae* L. (Lepidoptera: Pieridae) is a serious pest of cruciferous crops[20], particularly cabbage and related varieties throughout India and North-West Europe [21].

The *Cotesia* genus

The current usage of the generic name *Cotesia* (Hymenoptera: Braconidae: Microgastrinae) is relatively recent, and the previous literature pertaining to *Cotesia* species used the traditional name *Apanteles* Foerster (which now has a more restricted application:)[22]. *Cotesia* species are all koinobiont endoparasitoids, which can develop successive broods on a single host generation[23]. Many *Cotesia* species are important natural enemies of agricultural and forestry pests, and a few have been manipulated as biocontrol agents. One, *Cotesia glomerata* (Linnaeus), is a common parasitoid of the Eurasian cabbage white butterflies (species of *Pieris* Schrank) and has been studied in considerable detail both in the laboratory and in the field, with the generation of a vast associated literature[21]. The *Cotesia* genus lays its eggs in the first/second instar of its host, and the parasitoid larvae egress from the host halfway through its fifth instar[24]. Host larvae parasitized by koinobiont species continue to grow and develop, but larval endoparasitoids can alter the growth and development of their hosts to meet their own nutritional requirements[25].

The objective of this study

The aim of the present study was to evaluate the chemical composition of the essential oil extracts from leaves of *C. spinosa* growing wild in Syria. Also this investigate the first recorded to *Pieris brassicae* (Lepidoptera: pieridae) on *C. spinosa* In Syria and calculate the natural rate of parasitization of caterpillar with the endoparasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) under naturally conditions.

Materials and Methods

This work was conducted in Department of Plant Protection, Faculty of Agriculture Damascus University during 2015-2016.

Plant Material

leaves of *Capparis spinosa* L. plants were collected manually, at, 2015. growing wild in Qudssaya Suburb, Damascus (Near Damascus, 36.19'N, 33.53'E). Governorate, Syria. The plants were identified and deposited in the Department of Renewable Natural Resources and Ecology- Faculty of Agricultural, Damascus University, Syria. The leaves samples were air-dried at room temperature (25°C) and shad, for 10 days.

Essential oil isolation:

The dried samples of *C. spinosa* were ground in a coffee mixer, and subjected to hydrodistillation in Clevenger,s apparatus, (100 g of each sample in 500 ml of distilled water), for 3 hour for the extraction of the essential oil and to three replications. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and calculated average of essential oil yield, for three replication. The essential oils were stored at 4°C until analysis by GC-MS.

Gas chromatography–mass spectrometry analysis

Analysis of oils were carried out by GC-MS chromatography (GC-agilent 7986, indictor: inert-MS) in Atomic Energy Commission(AECS)- Damascus, Syria. Components was ascertained based on the spectra and compared with library and literature data. Also, the identification of each compound was confirmed by comparison of its retention index with those of authentic compounds.

Field collected parasitized larval of *P. brassicae*L:

A total of 800 *Pieris brassicae* caterpillars (from first to fifth instars) collected randomly from *C. spinosa* plant growing wild in Qudssaya Suburb nature reserve during the first May to end June 2015 (100 caterpillars /weekly), were brought to the Laboratory. Caterpillars were purely unknown from how many number of times they were parasitized. Caterpillars were reared separately (each 100 Caterpillars) on leaves of *C. spinosa*, in plastic boxes in 16 h. light/ day at 25^{o}C and 60 - 65% relative humidity. The Caterpillars were reared until they pupate (when they are not parasitized) or the parasitoid larvae egress from the caterpillar. Every two days, frass and unused plant material were removed and fresh plants added to ensure caterpillars were fed. All plant material was removed from the plastic box at the beginning of caterpillar pupation. Caterpillars that died prematurely were dissected to assess whether they were parasitized or not; for some of them the reasons of death remained anyway unknown. The egression of parasitoid's cocoon (from parasitized larvae) or the formation of host pupa (of unparasitized larvae) to determine the parasitism rate. All parasitoid yellow cocoons were placed in cages in a laboratory room permitting their development (temperature: 25°C, photoperiod L:D 16h:8h). Adults of *C. glomerata* emerged 10 days after their pupation. Wasps were collected from the emergence cages for training and deposited in above conditions to prevent premature mortality events due to an excess of activity. All wasps which emerged the same day from one cage were placed together to allow for mating. They were supplied with a 20% honey-water solution.

The taxonomy of larvae and adults of ***P.*** *brassicae* and *Cotesia glomerata* were confirmed with the help of Biological Control Studies and Research Center, and department of plant protection in Faculty of Agricultural, Damascus University, Syria.

Means were separated at the 5% significance level by least significant difference (L.S.D) test, using SPSS.20 software.

Results and Discussion

Chemical composition of the essential oil in leaves of *C. spinosa* .

This study was undertaken to investigate the chemical composition of essential oils obtained from leaves of *C. spinosa* . The essential oils isolated by water distillation were obtained in yield 0.052 % v/w base on dry weight of sample for *C. spinosa*. The results obtained by GC-MS analysis of the essential oils in leaves of *C. spinosa* are presented in Table 1. twelve compounds were identified in the essential oils of *C. spinosa* leaves, representing 67.51% of the total, The major constituents of the *C. spinosa* leaves oils were Thymol (17),

Octanoic acid (16), methyl isothiocyanate (12) and 2-hexenal (8.23%). Also, saturated organic acid: palmitic acid (2.45%), and unsaturated organic acid : olic acid (1.17) and linoleic acid (0.56%). The above identified constituents in the present study were reported by earlier workers, the yield of *C. spinosa* oil (0.04 % pale yellowish oil) was dominated by isopropyl isothiocyanate (28.92 %), methyl isothiocyanate (25.60 %), butyl isothiocyanate (16.65 %), 3-p-menthene (3.08 %), 2-butenyl isothiocyanate (2.24 %) and 3-methylthio-1-hexanol (2.03 %) as major constituents[26,8]. Another researchers revealed that the leaf oil composes of N-alkanes, phenyl propanoid, thymol (26.4%), isopropyl isothiocyanates (11%), 2-hexenal (10.2%), butyl isothiocyanate (6.3%), chlorophyll, proline (amino acid) and starch contents were reported[27]. Also, isolated y -sitosterylglucoside-6'- octadecanoate, 3-methyl-2-butenyl- y –glucoside from *Capparis spinosa* of Jordanian origin [28]. The fatty acid composition of *Capparis spinosa* seeds oils included, palmitic: 10.23%, stearic: 2.61%,oleic: 38.45%, linoleic 23.75% and linolenic 1.17%[29].

Table 1. Main components of essential oil obtained from leaves of *C. spinosa* by GC/MAS chromatography.

Compounds	%
β-Pinene	0.5
P-Cymene	0.1
α-Terpinolene	2.35
Octanoic acid	16
Carvone	0.6
thymol	17
methyl isothiocyanate	12
butyl isothiocyanate	6.55
2-hexenal	8.23
Palmitic acid	2.45
Oleic acid	1.17
Linoleic acid	0.56
Total	67.51

The first report investigation on attach of *Pieris brassicae* insect *to C. spinosa* wild growing In Syria.

In this study the first report about attach of *Pieris brassicae* L. insect to C. spinosa **wild growing** In Syria (Figure1.). These result agreement with many studies*, P. brassicae* attacks several plant species belonging to Brassicacae[30]. On caper, this pest has been found in Southern Spain[13] , and in Italy, on the island of Pantelleria and Salina[31,32].

Figure1. *Pieris brassicae* L. Caterpillars before parasitoid egression on *Capparis spinosa* L.

All larval parasitoid have emerged and begin to spin yellow cocoon

Emergence from yellow coccon of adult wasp

Died of *Pieris brassicae* caterpillar Adult of *Cotesia glomerata*

Figure 2. Successive stages of parasitoid egression from a *Pieris brassicae* (L.) caterpillar.

Field collected parasitized larval of *P. brassicae* by *C. glomerata* development*:*

A total of 800 (in 8 weeks) *P. brassicae* caterpillars were collected and reared, in 2015. Where the mortality of caterpillars due to parasitoids is recorded in the table,2 and Figure, 2. Parasitism rate varied from 84.25% to 87.54% , was observed during the study with no significant between the time collected samples. Also, the number larval parasitoid ranged from 15.50 to 18.59. The emergence of adult wasp ranged from 12 to 15 with no significant between the weeks. This results supported by the other studies, *Cotesia* species are all koinobiont endoparasitoids, which can develop successive broods on a single host generation[23]. Many *Cotesia* species are important natural enemies of agricultural and forestry pests, and a few have been manipulated as biocontrol agents. One, *C. glomerata*, is a common parasitoid of the Eurasian cabbage white butterflies (species of *Pieris* Schrank) and has been studied in considerable detail both in the laboratory and in the field, with the generation of a vast associated literature. Also, The results show that superparasitism of *P. brassicae* by the parasitoid *C. glomerata* reduced survivorship but increased food consumption and weight growth in *P. brassicae* larvae[30]. Some studies have indicated that parasitism by *C. glomerata* can induce physiological alterations in the host[33].In fact, larvae parasitized by *C. glomerata* consume significantly more food during their development than unparasitized ones[34].

The results showed that the percentage of the naturally mortality the caterpillars of *P. brassicae* ranged from 4.25% to 5.53% were observed during the study, with no significant between the weeks (Table, 2 and Figure, 2).

Table 2: Number of caterpillars collected in Qudsia *P. brassicae* population together with their parasitism status, and estimate of parasitism rate

Weeks of collected of larva	Parasitism rate	Naturally mortality rate	No. larval parasitoid	No. emergence of adult wasp
1	87.54	5.35	15.50	14
2	88.46	4.25	17.25	15
3	84.25	4.45	16.75	14
4	86.28	5.53	15.25	13
5	87.67	5.48	16.25	12
6	85.45	5.25	17.75	15
7	86.47	4.89	18.59	15
8	88.45	4.25	16.56	13
Mean	86.82	4.56	16.74	13.83
L.S.D(5%)	3.25	1.56	3.39	2.88

Conclusion:

The results showed that yield of essential oil of *Capparis species* leaves from Damascus (Syria) province were 0.052 % v/w. Also, These results suggest that Caterpillars *of Pieris brassicae* (L.) which attack caper it's not harmful for *Capparis species* wild growing in Damascus, because suffer suppressed from the parasitoid, *Cotesia glomerata*.

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