UNIVERSITA' DEGLI STUDI DI MILANO

DOCTORAL SCHOOL IN:

MOLECULAR SCIENCE AND PLANT, FOOD

AND ENVIRONMENTAL BIOTECHNOLOGY

PHD IN

Plant Biology and Production

Department of Plant Production

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STUDY OF THE EFFECT OF ABIOTIC AND BIOTIC STRESS ON THE GROWTH DEVELOPMENT AND SECONDARY METABOLISM OF MEDICINAL PLANT SPECIES

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ACADEMIC YRAR 2011/2012

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my leading supervisor, Professor Maurizio Cocucci, the Director of Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano. His wide knowledge and logical way of thinking have been of great value to me. His understanding, encouragement and personal guidance have provided a good basis for the present thesis. I would also like to take this opportunity to express my sincere thanks to Mrs. Cocucci, who made the specially-built equipment for the experiment on mechanical damage.

I am deeply grateful to my supporting supervisor, Dr. Annamaria Giorgi, Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano,for her detailed and constructive comments and for her important support throughout the work. Her encouragement and personal guidance have also provided a good support for the present thesis.

I wish to express my warm and sincere thanks to Dr. Sara Panseri,Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare Laboratorio di Ispezione degli Alimenti di Origine Animale, Università Degli Studi Di Milano,for her guidance, advice and support in solid phase micro extraction techniques, gas chromatography, mass spectroscopy and data analysis.

I owe my most sincere gratitude to Professor Giuseppe Carlo Lozzia, Dipartimento di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversita',Università Degli Studi Di Milano, for the opportunity to work in his Department and for his guidance and advice on aphids' colony maintenance.

I also wish to express my grateful and sincere thanks to Professor Daniela Bassi, Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano, for his supplement of peach seeds and advice on growing and maintaining peach plants in the greenhouse.

I warmly thank Dr. Emanuele Mazzoni, Istituto di Entomologia e Patologia vegetaleUniversità Cattolica del Sacro Cuore- Piacenza,for his supply of aphids and for his valuable advice on growing and maintaining them.

My warm thanks also go to Professor Pilu Roberto , Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano, his subordinate, Dr. Elena Cassani, and a PhD student, Cerino Badone Francesco, who directed my RT- PCR (retro-transcription) expression analysis. Their kind support and guidance have been of great value in this study.

I wish to thank Professor Zocci , Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano and his subordinates for their guidance and support in protein extractions.

My sincere thanks to Professor Fabbio, Dipartimento di Produzione Vegetale, Università Degli Studi Di Milano, for his supplement of jasmonic acid and advice in the use of jasmonic acid.

I also wish to thank Ms. Manuela Sonia Mattara GeSDiMont, Università Degli Studi Di Milano,

for her support in statistical analysis and data checking of the thesis tables.

I am grateful to Dr. Jocelyn Rose, Director, Department of Plant Biology, Institute for Biotechnology and Life Science Technologies, Cornell University, USA, for his advice in novel method development of in-vitro studies.

During this work I have collaborated with many colleagues, for whom I have great regard, and I wish to extend my warmest thanks to all those who have helped me with my work in the Department of Produzione Vegetale, Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversita', Sicurezza Alimentare Laboratorio di Ispezione degli Alimenti di Origine Animale, of Università Degli Studi Di Milano.

I owe my loving thanks to my husband, Mr. Ajantha Rodrigo, and my parents, Mr & Mrs Edmond Nanayakkarawasam. Without their encouragement and understanding it would have been impossible for me to finish this work. Last, but not least, special gratitude goes to my dearest daughter, Aurora Samadi Rodrogo, who, from the age of 4 months, had to become accustomed to my prolonged absences when I was occupied with doctoral work and conferences abroad.

The financial support of the Università Degli Studi Di Milano, is gratefully acknowledged.

Milan, Italy, November 2011

Nanayakkarawasam Masachchige Chandrika Niranjala Nanayakkara

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SUMMARY

Achillea collina Becker ex Rchb., a medicinal plant rich in volatile compounds, was used to study the effects of biotic and abiotic stresses over plant growth and secondary metabolism. Biotic stress was induced by *Myzus persiceae* Sulzer and *Macrosiphoniella millefolii* (De Geer), a generalist and specialist aphid species respectively. Abiotic stress was caused by mechanical damages provoked by a pin and a specially built equipment which apply a controlled and extended pressure to the plants. Plant growth and volatile compounds emissions were evaluated in the different experimental conditions analyzed. The effect of jasmonic acid on the plant volatile fingerprint was also evaluated. The volatile emission patterns obtained in the different conditions were compared in order to have suggestions regarding the metabolic pathways activated in each situation. Furthermore pea (*Pisum sativum* L.) and peach (*Prunus persica* L. Batsch) volatile fingerprints due to *M. persicae* infestation were analyzed and compared to those obtained from *A. collina*. The comparison of the results lead to the identification of volatile compounds induced only by the aphids in all the plant species studied, suggesting the activation of a common metabolic pathway due to infestation. Preliminary molecular approach seems to confirm pytochemical data.

1. INTRODUCTION

1.1.0 Medicinal plants and secondary metabolism

The use of plant extracts, which for centuries have represented the only form of treatment for curing several human diseases, is presently strongly reappraised as an alternative to the use of synthetic products from the pharmaceutical, beauty industries and dietetics but also in organic agricultural activities and crop management, supporting an interesting and increasing market section. Bioactive compounds from plant kingdom often belong to the so called class of the "secondary metabolites" defined as compounds with a restricted occurrence in taxonomic group, that are not necessary for a cell (organism) to live but ensure the survival of the organism in its ecosystem (Veporte & Alferman, 2000). Plant secondary metabolites have a central ecological role and might represent a potentially enormous economic value due to their use as drugs, flavours, fragrance, insecticides and dyes (Smith, 2007; Veporte & Alferman, 2000). Indeed the use of herbs is peculiarly linked to the beneficial effect of secondary metabolites.

Being almost fixed in the same site, plants developed different types of adaptive mechanisms to survive in the changing environment and to face with different types of *stimuli* (Hall, 2006). Some of them regards morphological adaptaments such as leaves with a thick waxy cuticle and stems with thorns or spines used by the plants to overcome environmental and physical stress conditions (Henckel, 1964).

Plant protection can occur also by the use of previously synthesized secondary metabolites or *ex-novo* biosynthesis of secondary metabolites. According to the definition these bioactive compounds are not nutritional and typically occur in small quantities in different plant species, with an high level of specie-specificity. Secondary metabolites can be produced when the plants need to defence against pests and diseases as well as attractant of pollinators and as signal compounds.

Hormone signaling pathways such as jasmonic acid, salicylic acid, abscisic acid, ethylene, are involved in direct and indirect defence (Walling, 2000; Kessler &

Baldwin 2002; França et al., 2001; Grace et al., 2000; Goossens et al., 2003; Mithöfer et al., 2005; Fujita et al., 2006). Some plants contain anti-nutritional constituents such as lectins and amilasy inhibitors, acting directly on insect physiology binding vital molecules or inhibiting digestive enzymes, receptors and chemosensory sensilla (Gatehouse et al., 2002; Murdock et al., 2002).

1.1.1 Classification of secondary metabolites

Secondary metabolites classification can be based on chemical characteristics, plant or biosynthetic origin. The classification based on the biosynthetic pathway from which they derive divide them into three main groups: the terpenoids, the alkaloids and the phenylpropanoids (Croteau et al., 2000). The terpenoids derive from the isoprenoid biosynthetic pathway which uses building blocks of five-carbon compounds. The terpenoids are classified based on the number of the isoprenic unit in hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), steroids / triterpenes (C_{30}) and caratenoids (C_{40}) . Apart these differences in the C skeleton, different types of terpenoids vary on the basis of modifications involving oxidation, reduction, isomerization and conjugation reactions (Croteau et al., 2000). Terpenoids represent the largest and most diverse class of plant volatile metabolites. Low-molecular-weight terpenes such as isoprene, monoterpenes and sesquiterpenes are common constituents of floral and herbivore-induced leaf volatile blends (Dudareva et al., 2006). Volatile terpenes are believed to improve the thermotolerance of photosynthetic tissues since they are likely to intercalate into thylakoid membranes and may stabilize them at high temperatures (Loreto et al., 1998). There is increasing evidence showing that terpene volatiles exhibit antioxidant activities in plant by quenching reactive oxygen species (Loreto & Velikova, 2001; Loreto et al., 2001).

The alkaloids are nitrogen-containing compounds and most of them are derived through the decarboxylation of amino-acid precursors (i.e. ornithine, lysine, tyrosine, tryptophan,and histidine). Alkaloids are end products of different biosynthetic pathways and they are relatively stable compounds which are

bioactive as medicinal or toxic agents. Due to their high bioactivity, alkaloids represent the most potent class of compounds of the chemical defense arsenal used by plants against by herbivore and micro-organism attacks (Schowalter, 2006).

Phenylpropanoids originate from aromatic amino acids, from phenylalanine to cinnamic acid and from tyrosine to *p*-coumaric acid by the action of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), respectively. A series of enzymatic hydroxylations and methylations leads to production of monolignols, *p*-coumaryl, coniferyl and synapil alcohols. Their further hydroxylations and cyclization lead to the phenolic acids, lignans, lignins and flavonoids (Croteau et al., 2000).

The biosynthesis of polyketides appears to be similar to that of long-chain fatty acids (Veporte & Alferman 2000).

1.2.0Abiotic and biotic stress conditions

The plants face different abiotic and biotic stress in their natural environments. Research has shown that biotic stresses are at their most harmful level when they occur together or in combinations of abiotic stress factors (Mittler, 2006). Plants developed different mechanisms to cope up with abiotic and biotic stress conditions. Natural selection has favorite a lot of different defensive mechanisms in plants over millions of years. They have evolved an array of defensive compounds that can make their organs (e.g. leaves, flowers, stems, roots and fruits) distasteful or poisonous to predators. In response, however, the animals that feed on them have evolved over successive generations a range of measures to overcome these compounds and eat the plant safely (Grassmann, et al., 2002).

The plant in turn develops defenses still further which will protect itself until the predator again 'learns' to overcome the same and so on (Pare et al., 1999). The result can be a sort of ongoing chemical "arms race" between plant and predator. Often this increasing specialization means that an herbivore is able to cope with the compounds present in only one species of plant, and so the "war" can be narrowed to one plant species and one insect species (Vollenweider et al., 2005). The phenomenon of plant defences has great relevance for human beings because the substances concerned are often useful as important source medicines and chemicals in agricultutal practice and/or agrochemical industries (Veporte & Alferman, 2000).

1. 2. 1 Abiotic stresses

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment (Mittler, 2006). Abiotic stresses can be due to different environmental variables changing in an unfavorable way for plant surviving, such as climatic changes e.g., high irradiation, salinity, heat, late frost, drought, flooding, heavy metals (Mithöfer et al., 2005; Vollenweider et al., 2005; Mittler, 2006) or to edaphic conditions like nutritional availability, nutrient imbalance, contamination by toxic chemical compounds (Mittler, 2006; Molinier et al., 2006; Fujita et al., 2006).When the environment conditions change and are beyond the normality, abiotic stress conditions can occur and adversely affect the population performance or individual physiology of the living organisms. The influence of mineral nutrition over the primary and secondary metabolism was reported by Rausch et al., (2005) and Amtmann et al., (2008). Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide (Ji-Ping et al 2007).

1.2.2 Biotic stress

Biotic stress occurs as a result of plants interaction with other living organisms, such as arthropods, bacteria, viruses, fungi, parasites, insects, weeds, and cultivated or native plants (Bilgin et al., 2010). Herbivores can be harmful to the plants causing mechanical damages, water stress, wilting and reduction of growth rate meanwhile plant pathogens can cause different type of plant diseases. Once an attack is perceived, plant metabolism must balance the demands for resources to support defense mechanisms against the requirements for cellular maintenance, growth and reproduction (Herms et al., 1992; Zangerl et at., 1997; Berger et al., 2007). Hence defense processes can be costly in terms of plant growth and fitness (Zavala et al., 2004). Plants, herbivores and their natural enemies have coexisted for at least 100 million years, evolving a variety of beneficial and deleterious interactions (Stotz et al., 1999; Karimzadeh et al., 2008).

1.2.3 Plant- insect interactions

In natural ecosystems, plants and insects rapresent an example of organisms coexisting dynamically, interacting continuously in a complex way (Mello et al., 2002; Fernandes et al., 2009; Stotz et al., 1999; Karimzadeh et al., 2008). Plant insect association and interaction have long been a pivotal subject for many biologists and entomologists among others, because of their importance in ecological systems and their economic relevance in agricultural practice. In fact, depending on the intensity of insect attack, insects might be extremely harmful to plants leading them to death (Mello et al., 2002; Fernandes et al., 2009). Plants are able to use different mechanisms to counteract insect attacks (Fernandes et al., 2009). including the activation of specific responses involving different metabolic pathways which can considerably alter their physiology. In addition, as consequence of co-evolution, insects developed several strategies to overcome plants different barriers allowing them to feed, grow and reproduce on their host

plants. As a response to the attack of insects, plants react also releasing volatile compounds that can either warn neighbor plants about the presence of predator or attract parasitoids of the predator, reducing the efficiency of the attack. Indeed, volatiles exert an important role in shaping plants-insect interactions acting as repellents (direct defense) or attractants to other organisms (indirect defense) as well as in plants reproduction (Gershenzon et al., 2007). It is well known that specialized volatiles act as attractants to pollinators and seed dispersers (Fernandes et al., 2009). The identification of these molecules will represent a starting point to find new agrochemicals which can be used in sustainable agricultural practice i.e. as pesticide, herbicides, fungicides or insecticides (Beninger et al., 2004; Prajapati et al, 2005). Moreover, plants secondary metabolites which occur in defence processes lead to crop quantitative and qualitative variations to be carefully studied and evaluated to guarantee proper crops managment (Karimzadeh et al., 2008, Schowalter, 2006).

1.3.0 Plants volatile compounds fingerprint and pathways

Plants normally produce organic volatile compounds (VOCs) playing various and important ecological functions; environmental stimuli can influence significantly the biosynthesis of such molecules, involving hormone signaling pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene. The VOCs which are implicated in plant responses to stress factors play an important role in plant-environment interaction, being involved in very important processes in plant life cycle, such as reproduction, defense, communication, etc. For example, volatiles induced by infestations can attract natural enemies of the herbivores, but they may also induce defense responses in neighboring plants (Pare´ et al., 1999). The chemical identity and composition of the volatile compounds varies with the

plant species as well as in respect with the herbivorous species (Hadacek, et al., 2010).

The volatiles playing important roles in communication between and among species are called semiochemicals (Law and Regnier, 1971). Volatile phytochemicals can serve as airborne semiochemicals promoting or deterring interactions between plants and insect herbivores. As an example, wheat seedlings without herbivore damage attract aphids, while volatile produced by wheat seedlings with a high density of aphids repel other aphid species (Quiroz et al., 1997; Pare´ et al., 1999).

The plants have got the ability to differentiate herbivore damages from a general wound response (Loughrin et al., 1994; Pare´ et al., 1999) as well as the host-seeking insects have the ability to recognize and respond to such chemical cues and differentiate them from background odors (Pare´ et al., 1999). This indications suggest that the presence of elicitors associated with insect feeding are absent from other types of leaf damage. Pare´ et al., (1999) in fact reported that plants respond to insect feeding damage by releasing a variety of volatiles from the damaged site, and the profile of the volatiles emitted is markedly different from those of undamaged or plants.

Some findings indicate that there is a basic structural uniformity in the chemical emissions of different plant species due to insect feeding, suggesting the activation of a common set of biosynthetic pathways shared by a wide range of plant families, and that these products are detectable to a broad spectrum of insect parasitoids and predators (Loughrin et al., 1994; Pare´ et al., 1999).

Plants produce bio active VOCs triggering various secondary metabolic pathways by activating specific gene/s. Secondary metabolites show specific effect on other organisms such as herbivores and pathogens (Dixon et al., 2001) having, normally, low impact on the environment (Foster et al., 2007). Hence the uses of natural

compounds in management of crops and cultivations have been increased due to their user friendly behavior in agricultural and ecological systems.

1.4.0 Methodologies to study plant volatile fingerprint and pathways

The complexity of secondary metabolism translates in different research approaches, including the study of signal transduction, gene expression, protein regulation, molecular regulatory mechanisms and metabolic pathways. Gene expression, proteins and enzymatic modifications should be considered to evaluate the molecular rearrangement and the *ex-novo* biosynthesis of secondary metabolites as plant reactions to environment controversy conditions. Genomics, transcriptomics, proteomics and metabolomic approaches should be useful for profiling targeted groups of secondary metabolites in particular species of plant (Fonville et al., 2010).To deeply investigate the living organisms metabolic aspects, sophisticated, sensitive and sensible analytical approaches are required such as NMR (nuclear magnetic resonance), HPLC-MS (high- performance liquid chromatography-mass spectrometry), GC-MS (gas- chromatography-mass spectrometry), CE-MS (capillary electrophoresis- mass spectrometry), FTIR (Fourier transformation infrared spectroscopy) NIR (Near infrared spectroscopy), (Fonville et al., 2010; Hall et al., 2006).

GC (gas-chromatography) is commonly applied in the analysis of volatiles including many classes of molecules. To identify and quantify compounds present in a chromatographic fingerprint apropriate instrumental and software methods are required (Robards, 2003; Kang et al., 2011).

1.4.1 Solid–Phase Micro Extraction technique (SPME)

Solid–Phase Micro Extraction (SPME) technique, developed by Pawliszyn and co-workers in 1990, was previously used to evaluate the volatile emissions of plants under different stress conditions (Hiroyuki Kataoka et al.2000). SPME is a sample extraction technique using a fused silica fiber that is coated on the outside with an appropriate stationary phase. Analytes in the sample are directly extracted and concentrated to the fiber coating. The method saves preparation time, solvent purchases and disposal costs, and can improve the detection limits. It has been used routinely in combination with GC and GC-MS, and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi volatile organic compounds from environmental, biological and food samples (Kataoka et al., 2000). In addition, HS-SPME technique combined with Gas Chromatography/Mass Spectrometry is capable of producing full scan mass spectra at very low concentration levels.

1.4.2 Real-Time Polymerase Chain Reaction (RT-PCR)

Invention of polymerase chain reaction (PCR) technology by Kary Mullis in 1984 gave birth to real-time (RT)PCR. RT- PCR detection and expression analysis of gene(s) in real-time has revolutionized the 21st century biological science due to its vast applications in quantitative genotyping eg. evaluation of inter and intra organisms genetic, early diagnosis of disease, forensic questions etc (Deepack et al,. 2007). Gene expression profile has been widely used to address the relationship between ecologically influenced or disease phenotypes and the cellular expression patterns. PCR–based detection technologies utilizing species-specific primers are proving indispensable as research tools providing enhanced information on biology of plant/microbe or plant insect interactions specialy regards to the ecological point of view (Pilu et al., 2009).

1.5.0 Aim of the Study

- Determine the volatile fingerprint of yarrow (*Achillea collina* Becker), pea (*Pisum sativum* L.) and peach (*Prunus persica* L. Batsch) in normal, abiotic and biotic stress conditions.
- Compare the volatile patterns obtained in the different conditions in order to identify the specie-specific and common bioactive plant volatiles specially involved in plant-insect interaction phenomena.
- Add knowledge on plant insect interaction topic and, eventually, to identify volatile compounds to be tested as useful molecule/s for the management of insects pests and crops .

1.6.0 Plant species

1.6.1 *Achillea collina* Becker ex Rchb. (yarrow)

Fig. 01: *Achillea collina* (Becker ex Rchb.)

The genus *Achillea* (*Asteraceae*) includes more than 100 species widespread all around the northern hemisphere, mainly distributed in Europe, Asia and North Africa. Among them, *A. collina*, is a tetraploid proazulenes-containing species of the *Achillea millefolium* aggregate cultivated in European alpine areas known as mountain yarrow (Giorgi et al., 2009 a). This type of yarrow is a good source of important bio active compounds and the growth at high altitude may constitute an effective way to enhance its quality for both medicinal and nutritional uses (Giorgi et al., 2010a). The plant is a well-known herb used in traditional medicine for its various effects. The indications include gastric and intestinal disorders, inflammations of skin and mucosa as well as hemorrhages (kastner et al., 1993; Sosa et al., 2001). In particular, the sesquiterpenes fraction was shown to contribute to the antiphlogistic effect and to be responsible for the pharmacological activity of plant extract (Loggia et al.,1992 ; kastner et al,1993; Sosa et al., 2001). *Achillea collina* is widely used in the herbal, liquor and medicinal product industries due to its richness in bioactive compounds (Willuhn, 2002). *A. collina* is infested by lies, beetles, aphids and other insects (Morlacchi, Ph.D. declaration, 2010). Morlacchi, et al., 2011 reported *Myzus persicae (*Sulzer), as the generalist and *Macrosiphoniella millefolii* (De Geer), as the specialist aphid species infesting

A. collina. In addition *A. collina* secondary metabolites associated with infestations has been previously reported (Giorgi et al., 2009a ; Nanayakkarawasam, et al., 2010) and preliminary data regarding the induced volatiles associate with abiotic and mechanical stresses has been recently published (Panseri, et al., 2011; Nanayakkarawasam, et al., 2011b). Moreover, the effect on aphid development of their feeding on *A. collina* has been also reported (Giorgi et al., 2010a ; Morlacchi, Ph.D. dissertation ,2010).

1.6.2 *Pisum sativum* L. (pea)

Fig. 02: *Pisum sativum* (L.)

Pisum sativum, known as pea, is an annual plant. The seeds are eatable and the pod is commonly associated with green, purple or golden yellow in colour (Blixt 1962). The average seed weighs is between 0.1 - 0.36 g. The plant is widely grown as a cool season vegetable crop, may be planted as soon as the soil temperature reaches 10 °C and the best temperature for growing is identified as 13 to 18 °C. The plant matures in about 60 days after planting (Blixt 1962). *Pisum sativum* is a host plant for *Myzus persiceae* (Sulzer) also face the virus infection propagated by the same aphid species. (Blackman et al., 1985). The plant growing season is compatible with the aphids' dispersion seasons therefore our attention was

focussed on *P.sativum* plant as an extended plant for the concerned study of plant insect interactions.

1.6.3 *Prunus persica* (L. Batsch) Peach

Fig. 03: *Prunus persica* (L. Batsch)

Prunus persica, known as peach, is a deciduous tree about 4–10 m tall. It bears an edible juicy fruit called peaches. The peach is a species of *Prunus* native to China, where it was first cultivated. The leaves are lanceolate, 7–16 cm (2.8–6.3 in) long, 2–3 cm (0.79–1.2 in) broad, pinnately veined (Bassi, 2003). The flowers are produced in early spring before the leaves and they are solitary or paired, 2.5–3 cm diameter, pink, with five petals (Bassi & Selli, 1990).

The seeds take about 1- 3 month for the germination in lower temperature (winter) while it takes about three years for the tree to begin producing fruits (Bassi et al., 1988). Several species of aphids can become established on peaches. *Myzus persicae* (Sulzer), uses peach trees and their hybrids (*Prunus* spp.) for the primary overwintering (Blackman & Eastop, 1985). Therefore our attention was focused also on *Prunus persica.*

1.7.0 Aphid species

1.7. 1 *Myzus persiceae* (Sulzer),

Fig. 04: *Myzus persicae* (Sulzer), (green peach aphids)

The green peach aphids *Myzus persicae* (Sulzer), can feed on hundreds of host plants belonging to over 40 plant families including vegetables and ornamental plants grown in greenhouses (Dancewicz et at., 2008). Yarrow was reported as one of the possible host plant for *M. persicae* (Giogi et at., 2010b; Morlacchi et al., 2011).This aphid species is found throughout the world, including all areas of North America and Europe, where it is viewed as a pest principally due to its ability to transmit more than hundred plant viruses (Blackman & Eastop, 1985). This allows high levels of survival in areas with inclement weather, and favors ready transport on plant material (Blackman & Eastop, 1985). However green peach aphids are viviparous (giving birth to living young without eggs) in the summer. The oviparous (egg producing) winter stages are much more restrictive in their diet choice. In temperate latitudes the primary or overwintering hosts are trees of the genus *Prunus* (Blackman & Eastop, 1985) particularly peach and peach hybrids, but also apricot and plum (van Emden et al., 1969). There are several synthetic diets suitable for aphid culture, such as the one described by Blackman & Eastop (1985) reported about the keys to identifiy the green peach aphid, from many other common aphids. Stoetzel et al., (1996) published a key for cotton

aphids that is also useful to distinguish green peach aphid from most other common vegetable-infesting aphids. *M. persicae* is widely used in ecological studies due to the versatility in colony maintaining (Moran et al., 2002).

1.7.2 *Macrosiphoniella millefolii* (De Geer),

Fig. 05: *Macrosiphoniella millefolii* (De Geer),

Macrosiphoniella millefolii (De Geer), is a a holocyclic species of specialist aphid that feeds almost exclusively on the species of *Achillea* and related genera such as *Tanacetum* spp. (Morlacchi et al., 2011). Our group has noted that in alpine fields *M. millefolii* is present while *M. persicae* was absent from yarrow (Morlacchi et al., 2011). This is an important component of the yarrow-aphid-natural enemy system which association represents an example of co-evolution process, widely found in nature in the evolutionary phenomenon of trophic specializations (Morlacchi et al., 2011).

1.7.3 The host damage given by *Myzus persiceae* and *Macrosiphoniella millefolii*

Myzus persiceae and *Macrosiphoniella millefolii* being phloem-feeding insects are highly specialized in their mode of feeding (inserting their stylet into the plant tissues) and present a unique stress on plant fitness by removing large amounts of plant sap (Jones, 2001). The aphids can attain very high densities on young plant tissue, causing water stress, wilting, and reduced growth rate of the plant (Hawkins et al., 1993; Nanayakkarawasam et al, 2011a). The relative decreases in root biomass of infested plants were accompanied by similar reductions in distribution to the above-ground parts of the seedlings (Hawkins et al., 1993). Prolonged aphid infestation can cause appreciable reduction in yield of root crops and foliage crops. Even if the aphids are subsequently removed, the contamination of harvestable plant material with aphid honeydew can damaged the harvest (Miles, 1999). However, green peach aphid does not seem to produce the high volume of honeydew observed with some other aphid's species (Miles, 1999). Blemishes to the plant tissue, usually in the form of yellow spots, may result from aphid feeding. Leaf distortions are not common except on the primary host. Contamination of vegetables by aphids sometimes presents quarantine problems (Miles, 1999).

2. MATERIAL AND METHODS

2.1.0 Plant material

2.1.1 *Achillea collina* Becker ex Rchb. plants growing

Achillea collina Becker ex Rchb. cultivar "SPAK" seeds supplied by the Valplantons Bio Company (Saillons, Switzerland), were sown in the greenhouse in a mixture of sterilized soil (alkaline peat, organic C: 43.7%, total N: 1.13%, organic substance: 75.3%, pH=5.3-6.0) and perlite. After germination, the seedlings were transplanted individually into plastic pots containing the same soil and subjected to daily watering under the following controlled greenhouse conditions: humidity of 70%, photoperiod of 16/8 h light/dark, with 200-250 μmol $m^{-2}s^{-1}$ PAR supplied by OSRAM metal halide Powerstar HQI – BT, day /night temperature cycle stands for 26/22°C.

2.1.2 *Pisum sativum* (L.) plants growing

Pisum sativum (L.) seeds supplied by Magnani Sementi S.P.A company (Milan, Italy), were sown in the greenhouse in a mixture of sterilized soil (alkaline peat,organic C: 43.7%, total N: 1.13%, organic substance: 75.3%, pH=5.3-6.0) and perlite. After germination, the plants were transplanted individually into plastic pots containing the same soil and subjected to daily watering under the controlled greenhouse conditions as described in 2.1.1.

2.1.3 *Prunus persica* (L. Batsch) plants growing

Prunus persica (L. Batsch) 'GF 305' (a common peach seedlings rootstocks) seeds supplied by the department of crop production were stratified in wet paper for 2.5 months at 4^0C in a plastic bag. Seeds were than germinated at room temperature (20° C) and transplanted individually into plastic pots containing sterilized soil (alkaline peat, organic C: 43.7%, total N: 1.13%, organic substance: 75.3%,

pH=5.3-6.0) and subjected to daily watering under controlled greenhouse conditions as described in 2.1.1 (Fig. 06).

Fig. 06: *Prunus persica* (L. Batsch) in the greenhouse

2.2.0 Aphyds culture

2.2.1 *Myzus persiceae* (Sulzer),

The generalist green peach aphids, *Myzus persiceae* (Sulzer), were maintained on *Pisum sativum* plants cultured in pots with perlite and kept in a temperature-controlled glasshouse set at 21°C, humidity of 70%, and photoperiod of 16/8 h light/dark.

2.2.2 *Macrosiphoniella millefolii* (De Geer),

Macrosiphoniella millefolii (De Geer) being a specialist aphid, feeds almost exclusively on the *Achillea* spp. The colonies were collected in July-August from *Achillea spp.* plants in the North of Italy area (Valtellina). *M. millefolii* colonies were then maintained on *A. collina* Becker ex Rchb. cultivar "SPAK", grown in standard soil and kept in the same greenhouse under controlled conditions as described in 2.1.1.

2.3.0 Extraction of Volatile Organic Compound (VOCs)

2.3.1 Headspace–Solid phase micro extraction optimization for *in vivo* experiment

HS-SPME methodologies require optimal conditions such as correct number of *in vivo* plants with adequate volatile amounts, suitable fiber with accurate extraction time in order to obtain a good portion of the volatile compounds between the matrix and the headspace. The optimization of the SPME conditions is based either on the peak areas of some compounds present in the chromatogram or on the sum of the peak areas of all compounds identified in the sample (Guo et al., 2006). However the response based on the sum of the peak areas is the most frequently used parameter to optimize the SPME extraction conditions. (Guo et al., 2006).

Different 4 fibers supplied by Supelco, Bellefonte, PA, USA, were evaluated using the developed HS-SPME method of powder form. Divinylbenzene-carbowax-polydimethylsiloxane (DVB-CAR-PDMS, 50/30 μm) fiber was selected due its better ability to extract volatile organic compounds in the headspace and the matrix (data not shown). The cage Fig. 07 which was built with 70 cm (H) X 50 cm (W) X 70 cm (L) dimensions, covered with fine, gas-permeable, light transmitting mesh on the top and on two sides, and with polyethylene on the remaining sides was elected as the best container. The leakage of volatile compound trough the wall appears to be very low in dependence of the high affinity of the fiber for volatile compounds.

Fig. 07: cage for plant-aphid growth

2.4.0 Biotic stress -Plants infestation

2.4.1 *Achillea collina* Becker ex Rchb. plants infestation with *Myzus persiceae and Macrosiphoniella millefolii*

After 20 days in the greenhouse, 18 plants in 3 pots, were infested with 10 mg of adult *M. persicae,* about 20- 30 of adult aphids, or *M. millefolii,* about 15- 25 of adult aphids. The same pots of plants were kept aphid free as control.

The plants sets, infested and control, were inserted into a cage with the wall of muslin mesh (Fig. 8). Three replicates, a pot in a cage each, were maintained for the control and infested plants. The experiments were carried out in the greenhouse under the same controlled conditions as described in 2.1.1.

Fig. 08: *Achillea collina* (Becker) infested and control plants in the greenhouse

2.4.2 *Pisum sativum* (L.) plants infestation with *Myzus persiceae*

After 20 days in the greenhouse, each of the 18 plants was infested with 10 mg of adult *M. persicae,* about 20- 30 of adult aphids. The same amount of plants were kept aphid free as control. The plants sets, infested and control, were inserted into a special built cage as described in 3.1 (Fig. 9). Three replicates, a pot in a cage each, were maintained for the control and infested plants. The experiments were carried

out in the greenhouse under the same controlled conditions as previously described in 2.1.1.

Fig. 09: *Pisum sativum* (L.) infested and control plants in the greenhouse

2.4.3 *Prunus persica*(L.Batsch) plants infestation with *Myzus persiceae*

After 15 days in the greenhouse, each of the 18 plants, separately grown in pots, was infested with 10 mg of adult *M. persicae*, about 20- 30 of adult aphids. The same amount of plants were kept aphid free as control. The plants sets, infested and control, were inserted into a cage as described in 3.1 (Fig.10). Three replicates, a pot in a cage each, were maintained for the control and infested plants. The experiments were carried out in the greenhouse under the same controlled conditions previously described in 2.1.1.

Fig. 10: *Prunus persica* (L. Batsch) infested and control plants in the greenhouse

2.5.0 Abiotic stress

2.5.1 Mechanical stress

Each leaf of 18 *A. collina* plants which were being 20 days in the greenhouse, was damaged using 5 psi pressure gage by a equipment (Fig. 11), which is made out of wood with 2 surfaces (3 cm x 5cm) covered by a velvet cloth. Leafs are put into the two part and pressure applied for 5 seconds (Fig. 12). Then all 18 plants were included into the cage as described. Separate set of plants were maintained as the control without any kind of damage. The day 7 from the initial damage was considered for solid phase micro extraction SPME analysis.

Fig. 11: specially built equipment

Fig.12: The way making damages with the specially built equipment

2.5.2 Mechanical stress by a needle

Each leaf of 18 *A. collina* plants, after 20 days in the greenhouse, was damaged twice a day (11.00 a.m. and 2.00 p.m.) for 5 days using a needle (Fig. 13), which is made out of steel with a plastic top. Then all the plants were included into the cage as described above (2.4.1). Separate set of plants were maintained as the control without any kind of damage. The day 7 from the initial damage was considered for the SPME extractions as shown in (Fig. 14).

Fig. 13: The way *A.collina* Plants damage by the needle

Fig.14: The volatile extractions from the head space of the stressed plant

2.5.3 Jasmonic acid treatment

Each leaf of 18 *A. collina* plants, after 20 days in the greenhouse, was spread by 0.1 μM solution of jasmonic acid (Fig. 15). Then all the plants were included into the cage as described above (2.4.1). Separate set of plants were maintained as the control. The day 7 from the treatment was considered for the Solid phase Micro Extraction (SPME) analysis as shown in (Fig. 14).

Fig.15: *A. collina* Treatment with jasmonic acid

2.6.0 Gas chromatography-mass spectrometry analysis of VOCs

HS-SPME analysis was performed using a Trace GC Ultra (Thermo-Fisher Scientific; Waltham, MA, USA) Gas Chromatograph coupled to a quadrupole Mass Spectrometer Trace DSQ (Thermo-Fisher Scientific; Waltham, MA, USA) and equipped with an Rtx-Wax column (30 m; 0.25 mm i.d.; 0.25 μm film thickness, Restek, USA). The oven temperature program was: from 35 °C, hold 8 min, to 60 °C at 4 °C/min, then from 60 °C to 160 °C at 6°C/min and finally from 160 to 200 at 20°/min. Carry over and peaks originating from the fibre were regularly assessed by running blank samples. After each analysis fibers were immediately thermally desorbed in the GC injector for 5 min at 250°C to prevent

contamination. The injections were performed in splitless mode (5 min) . The carrier gas was helium at the constant flow of 1 ml $^{-1}$. An *n*-Alkanes mixture (C_8-C_{22}, Sigma R 8769, Saint Louis, MO, USA) was run under the same chromatographic conditions as the samples to calculate the Kovats retention indices (KI) of detected compounds. The transfer line to the mass spectrometer was maintained at 230 °C, and the ion source temperature was set at 250 °C. The mass spectra were obtained by using a mass selective detector with the electronic impact at 70 eV, a multiplier voltage of 1456 V, and by collecting the data at rate of 1 scan s^{-1} over the *m/z* range of 30-350. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions when available, or by comparing the Kovats retention indices with the literature data. The comparison of MS fragmentation patterns with those of pure compounds and mass spectrum database search was performed using the National Institute of Standards and Technology (NIST) MS spectral database.

2.7.0 Cell free extract of *A.collina* plant and its activity on farnesylpyrophosphate

Cell free extracts were prepared starting from control and *M. persicae* infested plants. One g of leaf tissue were harvested from control and 7 days infested plants and frozen in liquid nitrogen, insects were removed from infested plants. Then the tissues were ground and homogenized in 1 volume in a buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 10% (v/v) glycerol, 1 mM ethylene diamine tetraacetic acid (EDTA) and 2% PVP (polyvinylpirrolidone). The homogenate was centrifuged 13,000 g at 0°C for 15 minutes and the supernatant was again centrifuged at 100,000g at 0°C, for 40 minutes. One ml of the soluble fraction containing proteins were put in to a vial of 20 ml volume and 1 ml containing 1μM

of FDPP (farnesylpyrophosphate). The vial were sealed properly and kept at the room temperature for 2 hours. In the free space of the vial was introduced 3 fibers (Divenivinylbenzene/Carboxen^{tm}/polydimethylsiloxane) CAR/PDMS/DVB StableFlex^{tm} fiber (Supelco; Bellefonte, PA The 3 fibers were removed from the headspace after 2hours and was performed with a Trace GC Ultra (Thermo-Fisher Scientific; Waltham, MA, USA) Gas Chromatograph coupled to a quadrupole Mass Spectrometer Trace DSQ (Thermo-Fisher Scientific; Waltham, MA, USA).

2.8.0 RT- PCR expression Analysis

Total RNA was extracted from frozen whole plant leaf from infested (*Myzus persiceae* (Sulzer), and control plants of *A.collina* using the method described by van Tunen et al., (1988). Reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect *Pogostemon cablin patchoulol synthase* gene (accession number DQ355151) transcripts. A set of primers specific for the the higly conserved chloroplast *rbcL* (RuBisCO large subunit) gene was used to standardize the concentration of the different samples (Ortola-Vidal et al., 2007). *rbcL* specific sequences were amplified using the following primers: rbc5, 5'-ATACTAGCTTGGCTCATTATTGC-3' and rbc6, 5'-TCCACCAGACATACGTAACGC-3'. The length of the amplified product was 140 bp. Several cycles of successive cDNA dilutions and *rbcL* amplification were done in order to obtain similar amplification signals in the different samples. *Pogostemon cablin patchoulol synthase* mRNA detection was conducted with specific primers designed on coding sequence: Ac5F+1377 (upstream primer 5'-ACGAGAGCACGTTCGCACT-3') and Ac4R+1626 (downstream primer 5'-GATGATGTTTTGCATTGCAGG-3'). A 250 bp amplificate is obtained after 35 cycles of denaturation at 94 °C for 45 s, annealing at 55° for 1 min, extension at 72° for 1.5 min.

PCR products were loaded on 2% (w/v) agarose gels and visualized by ethidium bromide staining under UV light.

2.9.0 Standards and chemicals

Compounds used as references were purchased from Sigma-Aldrich-Fluka (Milan, Italy) (from the General and Flavors and Fragrances catalog).

2.10.0 Statistical analysis

All the experiments were performed in triplicate and results presented are mean with relative standard deviation.

The SPSS software version 17.0 was used for statistical analyses of data. One way Anova test with significance level of 0.05 and S-N-K (Student-Newman-Keuls) tests for pairwise comparisons were used.

3.RESULTS

3.1.0 DETERMINATION OF THE SAMPLING TIMING

3.1.1 CAR/PDMS/DVB- Fiber saturation

The DVB/CAR/PDMS fiber was selected for the in *vivo* experiments also concerning the results obtained by the HS-SPME-GC/MS powder form analyses (Giogi et al., 2009 b). The total volatile emissions by in *vivo A. collina* plants with respect to 6, 18, 24, 30 and 40 hours are reported in Fig.16.The results indicated 24 hour as the best sampling time in our experimental conditions.

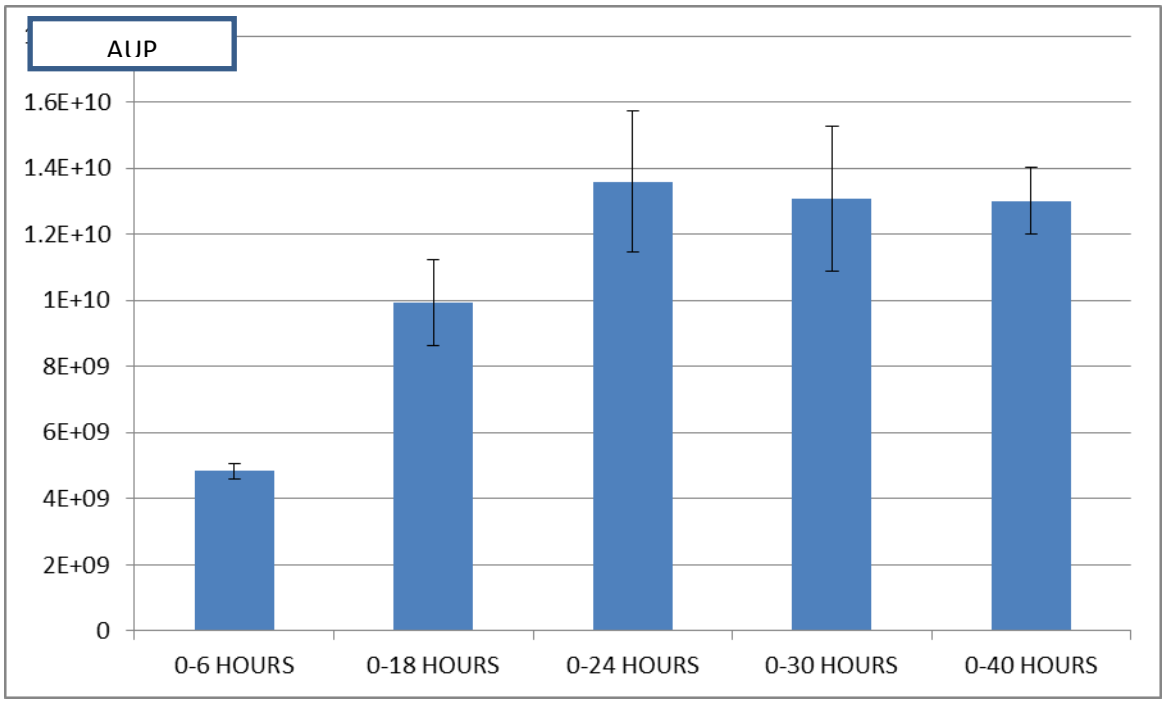

Fig.16: Total volatile amounts, expressed as area under the peak (AUP), detected in *A. collina in vivo* plants in different sampling times, from 6 to 40 hours (HS-SPME analyses; fiber DVB/CAR/PDMS); values are the mean of 3 replicates ± standard deviation.

The Fig.17 showed the variations in of the most abundant volatiles characterizing *A. collina* fingerprint. These results also confirmed that 24h is the best time of sampling in order to have reliable results for the major compounds produced in *in vivo A. collina* plants.

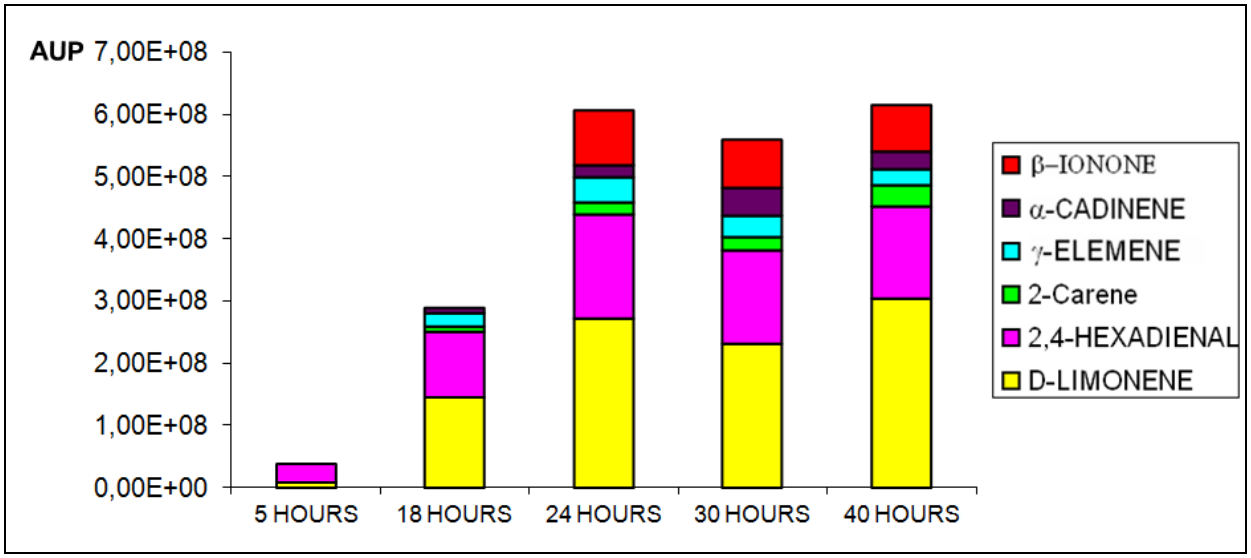

Fig. 17: Trend of the six most abundant volatile compounds in respect to the time of extraction. The amount of each compound from 24 hours on didn't show significant variations ($p < 0,05$). Total areas of compounds are express in: Arbitary unit = Area under the peak.

3.1.2 Time screening on total volatile emissions of *in vivo* control and infested *Achillea collina* plants on week basis

The total volatile emissions of *in vivo A. collina* plants, infested with *M. persicae* and control for 3 weeks were reported in Fig. 18 on each week. The results indicate that the total volatile emissions in the infested plants were significantly ($p<0.05$) higher than in the control plants only in the first week. In fact, from the second week on the total volatile emissions in the control plants were greater than in the infested plants. It is concluded to carry out *in vivo* experiments to evaluate the total volatile emissions of *A. collina* infested and control plants during the first week.

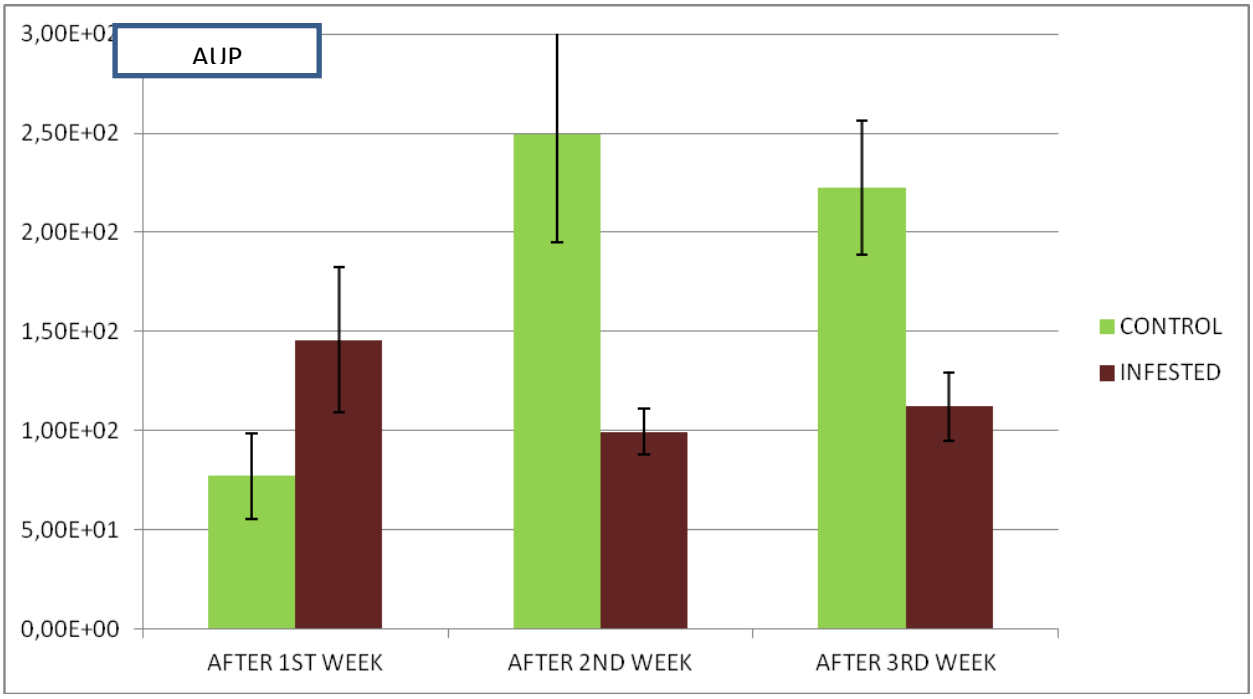

Fig. 18: Total volatile emissions, as area under the peak (AUP), of *in vivo A. collina* plants, infested and control, on week basis for 3 weeks using the developed method for HS-SPME-GC/MS analyses. Each value is the mean ± SD (n = 4).

3.1.3 Time screening on the total volatile emissions of in *vivo Achillea collina* infested by *M. persicae* and control plants on daily basis for 8 days.

The total volatile emissions of, *in vivo A. collina* plants, infested with *M. persicae* and control, on daily basis for 8 days are reported in Fig. 19. The results indicated that the 7^{th} day after the infestation is the best day to evaluate the volatile emissions. In fact from this day on the differences between total volatile emissions of control and infested plants are statistically different ($p<0,05$).

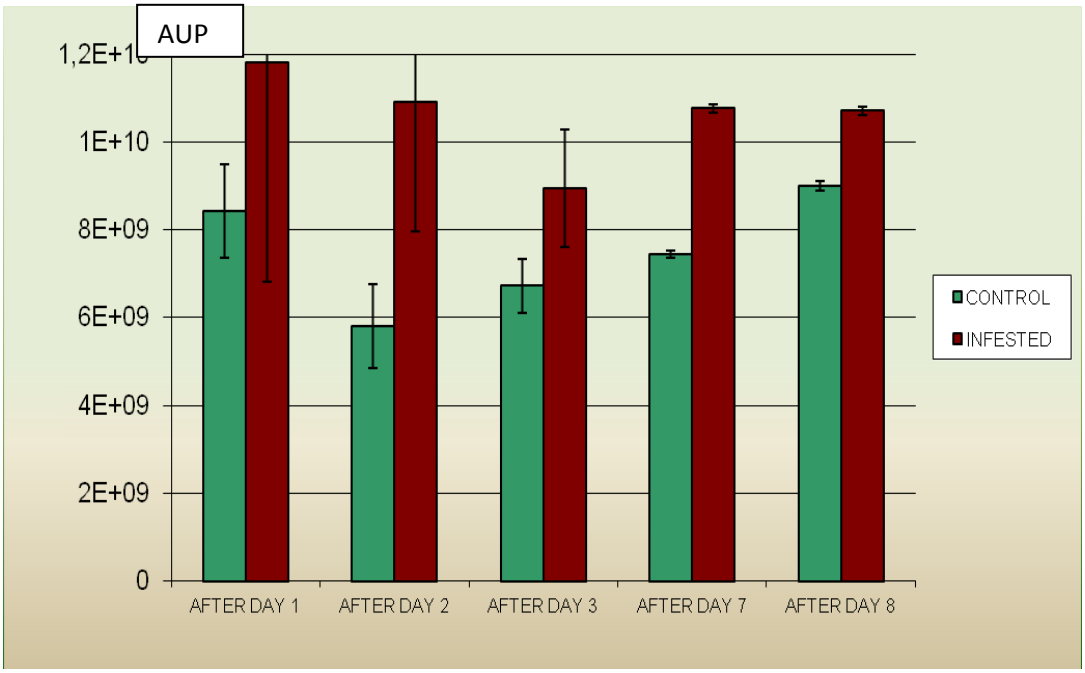

Fig. 19:Total volatile emissions, as area under the peak (AUP), of *in vivo A. collina* plants, infested by *M. persiceae* and control, in day basis for 8 days using the set up method for HS-SPME-GC/MS analyses. Each value is the mean ± SD (n = 4).

3.2.0 Biotic stress on *Achillea collina* by specialist and generalist aphids infestation.

Achillea collina plants were infested by the generalist aphid *M. persiceae* and the specialist aphids *M. millefolii*. The plants were analyzed after one week of infestation. .The compounds in the chromatograms were individually identified using a data base and the results were reported in Table 01. The amounts of each compound in the different experimental conditions were reported as an arbitrary value corresponding to the area under the peak /1000000.

Table 01 - *Achillea collina* volatiles fingerprint in control plants and in the plants infested by the aphids *M. persiceae* and *M. millefolii*

R.T.[a]	Compounds	Control plants n=3		Plants infested by *M. persiceae* n=3		Plants infested by *M. millefolii* n=3	
		mean [bd]	SD[d]	mean	SD	mean	SD
	HYDROCARBONS						
1,37	PENTANE	1.31	0.93	8.75	2.49	5.34	0.16
2,47	1-OCTENE	1.96	0.57	24.52	7.25	22.23	0.76
2,79	n-NONANE	2.93	0.36	2.99	0.37	2.82	0.1
3,16	3-METHYLOCTANE	1.84	0.38	1.4	0.13	3.54	0.04
3,31	BENZENE	2.64	0.88	3.67	0.61	3.6	0.15
3,79	HYDROCARBON	ND	-	1.35	0.15	1.82	0.1
3,95	3-METHYLCYCLOOCTANE	2.48	0.19	3.23	1.11	3.66	0.01
4,01	3,5-DIMETHYLOCTANE	0.7	0.08	0.49	0.02	0.42	0.31
4,32	HYDROCARBON	0.2	0.06	0.28	0.17	0.25	0.16
4,49	α-TRIDECENE	0.6	0.22	0.77	0.08	ND	-
4,64	HYDROCARBON	0.73	0.01	0.34	0.01	3.37	0.2
4,94	HYDROCARBON	5	4.9	3.86	0.2	2.28	0.1
5,53	DECANE	2.61	0.89	2.52	0.32	2.25	0.14
6,45	HYDROCARBON	ND	-	1.85	0.72	1.79	0.09
6,71	TOLUENE	1.35	0.28	45.6	4.05	42.11	1.53
6,87	HEXADECANE	1.18	0.19	1.44	0.05	1.56	0.06
6,97	HYDROCARBON	1.28	0.29	1.84	0.05	1.76	0.12
7,14	HEPTADECANE	0.43	0.04	0.83	0.08	0.85	0.03
8,09	HYDROCARBON	0.65	0.01	0.66	0.31	0.62	0.01
8,36	HYDROCARBON	0.35	0.09	0.49	0.03	0.55	0.16
8,58	HYDROCARBON	6.57	0.13	3.8	0.39	3.48	0.31
9,18	n-TRIDECANE	ND	-	4.44	0.71	4.21	0.15
10,16	o–XYLENE	11.11	0.08	5.91	0.97	4.13	3.06
10,34	m-XYLENE	1.63	0.02	4.02	1.88	3.81	0.09
10,49	p-XYLENE	25.82	1.76	34.24	2.32	13.81	0.09
11,29	HYDROCARBON	0.55	0.08	1.18	0.28	2.31	1.16
11,95	DODECANE	ND	-	0.61	0.14	ND	-
13,82	n-PENTYLCYCLOHEXANE	4.24	0.4	1.25	0.55	1.54	0.4
14,34	n-PENTADECANE	2.38	1.06	2.44	0.37	ND	-
15,03	2-ISOPROPHYL-1-METHOXY-4-METHYLBENZENE	1.48	0.05	2.9	0.69	2.21	1.57
15,82	STYRENE	2.71	0.05	2.96	1.34	2.61	0.49
15,92	o-ETHYLTOLUENE	8.7	0.09	5.11	0.72	2.3	0.37
16,56	1-ETHYL-2,4-DIMETHYLBENZENE	0.32	0	1.15	0.35	1.54	0.4
17,97	HYDROCARBON	2.15	1.76	3.02	0.27	3.24	0.1
18,22	HYDROCARBON	28.09	0.15	18.8	0.45	18.82	0.1
18,92	2-ETHYL-p-XYLENE	2.48	0.04	2	0.12	2.09	0.15
19,09	4-ETHYL-m-XYLENE	4.72	0.12	3.32	0.08	3.38	0.43
19,62	ISODURENE	1.56	0.12	0.21	0.03	0.21	0.08
20,26	4-ETHYL-1,2-DIMETHYLBENZENE	1.16	0.04	0.77	0.21	0.74	0.03

20,51	HYDROCARBON	ND	-	0.73	0.16	0.76	0.03
21,54	1,4-DIMETHYLBENZENE	4.86	0.05	0.62	0.07	0.55	0.07
21,95	HYDROCARBON	ND	-	0.63	0.01	0.14	0.09
26,17	HYDROCARBON	ND	-	0.27	0.12	ND	-
26,88	HYDROCARBON	3.67	0.45	3.42	0.83	3.81	0.09
29,10	HYDROCARBON	1.67	0.02	0.16	0.06	0.22	0.16
30,31	TETRACOSANE	1.4	0.02	0.19	0.08	2.19	0.82
TOT		**145.51**	**16.86**	**211.03**	**31.4**	**178.92**	**14.47**
	ALDEHYDES						
2,07	2-METHYLPROPANAL	16.5	1.21	8.06	2.34	5.11	1.01
3,04	2-METHYLBUTANAL	0.23	0.1	0.45	0.07	0.36	0.02
8,97	HEXANAL	6.02	0.4	0.56	0.1	0.03	0.02
12,26	LAURALDEHYDE	2.57	0.32	1.74	0.72	1.77	0.03
12,95	ALDEHYDE	ND	-	0.74	0.3	ND	-
15,30	trans-2-HEXENAL	11.59	0.05	9.53	0.19	5.29	0.06
18,86	2-HEPTENAL	3.61	0.03	6.56	0.92	10.71	2.81
19,87	2,6-DIMETHYL-HEPT-5,1-AL	ND	-	1.49	0.3	0.52	0.11
20,98	2,4-HEXADIENAL	12.18	0.17	10.26	0.03	11.36	0.53
21,74	2-OCTENAL	4.4	0.27	3.59	0.05	3.17	2.5
21,96	p-MENTH-1-EN-9-AL	1.89	0.05	0.73	0.59	0.74	0.01
23,42	n-DECANAL	1.14	0.01	1.54	0.29	1.72	0.54
23,59	ALDEHYDE	1.8	0.02	ND	-	1.32	0.12
23,84	BENZALDEHYDE	2.26	0.01	2.84	0.06	2.25	0.09
25,35	2,6-NONADIENAL	ND	-	0.74	0.04	4.84	0.73
26,00	β-CYCLOCITRAL	1.27	0.09	0.23	0.1	0.24	0.25
30,54	ALDEHYDE	0.84	0.15	0.49	0.09	0.96	0.02
32,82	TETRADECANAL	ND	-	0.73	0.1	0.78	0.26
TOT		**66.3**	**2.88**	**50.28**	**6.29**	**51.17**	**9.11**
	ALCOHOLS						
7,34	DECANOL	3.67	0.27	4.92	0.15	7.29	0.06
12,06	CYCLOPENTADECANOL	1.76	1.36	0.39	0.01	0.38	0.01
12,78	ETHYLHEXANOL	1.63	0.13	1.83	0.54	2.82	0.1
14,13	9-TETRADECEN-1-OL	1.27	0.27	1.33	0.1	1.36	0.04
20,01	1-HEXANOL	ND	-	6.11	2.8	0.34	0.04
20,19	ALCOHOL	1.91	0.07	1.51	0.03	1.58	0.72
20,76	3-HEXEN-1-OL	1.7	0.07	0.56	0.03	0.67	0.15
21,16	3-OCTANOL	0.68	0.02	0.41	0.06	1.4	0.71
21,36	2-HEXEN-1-OL	ND	-	4.62	0.61	3.43	0.08
22,48	1-OCTENE-3-OL	4.63	0.05	0.3	0.08	1.59	0.22
24,27	cis-9-OCTADECEN-1-OL	0.61	0.08	1.76	0.05	1.71	0.2
26,23	ALCOHOL	ND	-	0.48	0.01	ND	-
31,03	ALCOHOL	4.21	0.13	0.27	0.01	0.68	0.21
36,24	Z-9-HEXADECEN-1-OL	1.52	0.05	ND	-	ND	-
TOT		**23.59**	**2.5**	**24.49**	**4.48**	**23.25**	**2.54**
	KETONES						

2,21	2-PROPANONE	ND	-	0.85	0.13	0.82	0.21
19,22	2,3-OCTANEDIONE	3.8	0.09	2.31	0.06	2.8	0.4
19,43	6-METHYL-5-HEPTENE-2-ONE	5.58	0.1	2.81	0.18	1.32	0.24
26,75	KETONE	0.67	0.25	0.59	0.22	0.58	0.09
29,22	2–PENTANONE	1.63	0.03	2.84	0.1	1.57	0.26
31,49	KETONE	0.74	0.14	0.37	0.04	2.29	0.8
32,72	2H-2,4A-METHANONAPHTHALEN-8(5H)-ONE	1.32	0.04	1.8	0.03	1.51	0.15
33,44	KETONE	1.45	0.3	0.64	0.06	2.56	0.98
33,73	KETONE	1.44	0.06	0.52	0.11	0.64	0.38
TOT		**16.63**	**1.01**	**12.73**	**0.93**	**14.09**	**3.51**
	ESTERS						
2,60	ACETIC ACID-ETHYL ESTER	ND	-	3.55	0.7	3.5	0.03
17,58	HEXYL ACETATE	0.67	0.15	6.97	0.07	5.71	1.16
17,80	2-PENTANOL-4-METHYL-ACETATE	5.38	0.05	0.76	0.02	0.73	0.01
22,32	ISOVALERIC ACID BUTYL ESTER	1.63	0.14	0.96	0.12	3.1	0.8
22,38	BUTANOIC ACID-3-METHYL HEXYL ESTER	ND	-	0.32	0.09	3.2	0.8
24,12	ESTER	1.02	0.09	1.89	0.13	1.61	0.26
25,28	ESTER	ND	-	6.01	1.21	3.56	0.34
28,40	ESTER	4.54	0.2	0.63	0.09	0.64	0.03
30,37	ESTER	ND	-	0.29	0.14	ND	-
31,83	LAURIC ACID,ETHYL ESTER	ND	-	1.8	0.13	ND	-
34,48	ESTER	ND	-	0.32	0.04	ND	-
38,93	ESTER	4.45	0.3	6.91	0.98	6.56	2.08
39,59	ESTER	ND	-	0.57	0.05	ND	-
43,1	PHTHALIC ACID BUTYL 2-ETHYLHEXYL ESTER	0.65	0.01	ND	-	ND	-
TOT		**18.34**	**0.94**	**30.98**	**3.77**	**28.61**	**5.51**
	FURAN						
4,17	2-ETHYLFURAN	ND	-	0.41	0.13	0.11	0.01
4,71	2-METHYLFURAN	4.44	1.32	0.85	0.5	4.49	0.1
TOT		**4.44**	**1.32**	**1.26**	**0.63**	**4.6**	**0.11**
	TERPENES						
5,33	TERPEN	12.59	3.16	8.43	0.57	8.38	0.1
6,01	α-PINENE	74.51	15	96.83	2.74	90.56	0.35
6,33	α-PHELLANDRENE	1.54	0.01	9.51	0.46	8.02	0.87
7,47	α-FENCHENE	ND	-	2.45	0.05	1.45	0.33
7,76	CAMPHENE	2.05	0.78	1.43	0.08	1.14	0.04
7,95	TERPEN	ND	-	0.62	0.21	0.62	0.01
9,53	γ-TERPINEN	6.37	0.01	4.03	1.09	4.21	0.15
9,91	β-PINENE	15.54	1.42	12.95	1.42	8.27	1.1
10,80	β-PHELLANDRENE	1.26	0.15	10.41	2.81	10.95	0.55
10,91	TERPEN	1.35	0.27	2.64	1.84	2.28	0.1
11,66	TERPEN	0.24	0.01	9.49	2	9.27	1.58

12,53	DIMETHYLFULVENE	13.95	2.47	10.33	5.7	10.27	1.58
13,36	TERPINOLEN	15.38	3.24	12.98	0.08	12.27	2.51
13,37	β-MYRCENE	2.9	0.09	3.39	0.54	3.37	0.3
13,66	TERPEN	1.33	0.06	1.03	0.05	1.11	0.01
14,50	D-LIMONENE	13.59	0.48	26.06	4.12	20.99	9.38
14,78	EUCALYPTOL	17.65	0.31	28.13	1.9	24.06	3.99
16,36	TERPEN	6.39	0.52	5.77	0.03	3.38	0.29
16,46	γ–TERPINENE	27.98	4.61	22.61	1.22	14.47	0.35
16,90	γ –PINENE	34.19	0.63	25.55	0.26	22.37	1.89
17,27	o-CYMENE	1.93	0.05	4.43	3.41	3.37	0.31
17,46	TERPEN	11.57	0.07	0.67	0.38	0.63	0.01
17,71	TERPEN	1.27	0.13	1.33	0.11	1.44	0.25
18,30	TERPEN	8.83	0.29	25.76	6.71	26.03	2.2
18,55	p-CYMENE	ND	-	2.03	0.28	ND	ND
20,42	TERPEN	1.74	0.03	1.06	0.07	1.03	0.16
20,73	TERPEN	ND	-	0.51	0.11	0.51	0.43
21,03	TERPEN	ND	-	0.38	0.01	ND	-
22,19	TERPEN	2.38	0.13	0.81	0.33	0.59	0.28
22,59	α-CUBEBENE	6.41	0.07	15.44	3.02	14.4	0.34
22,72	trans-SABINENE	3.79	0.04	5.49	2.23	4.27	0.03
22,97	γ-ELEMENE	2.71	0.12	1.32	0.33	1.16	0.05
23,01	TERPEN	3.09	0.29	2.37	0.41	2.45	0.32
23,13	α-YLANGENE	5.2	4.02	1.9	0.07	1.15	0.03
23,33	α-COPAENE	2.64	0	1.64	0.5	3.45	1.04
23,63	CAMPHOR	2.71	0.05	1.92	0.98	1.41	0.39
23,94	TERPEN	1.61	1.34	1.75	0.16	1.47	0.24
24,04	TERPEN	ND	-	0.15	0.09	0.21	0.1
24,24	VALENCENE	0.85	0.49	0.42	0.05	0.44	0.25
24,40	TERPEN	0.56	0	0.44	0.09	0.71	0.2
24,59	cis-β–TERPINEOL	ND	-	1.75	0.04	1.2	0.07
24,65	LINALOOL	0.44	0.02	0.4	0.05	0.44	0.13
24,79	TERPEN	0.8	0.02	0.19	0.01	0.2	0.14
24,87	PINOCARVONE	ND	-	0.67	0.08	0.82	0.54
25,19	β-CUBEBENE	0.8	0.1	1.25	0.31	1.49	1.03
25,45	SESQUITERPEN	1.35	0.15	0.75	0.16	0.72	0.21
25,60	β-CARYOPHYLLENE	1.36	0.05	0.4	0.02	1.29	0.86
25,69	SESQUITERPENE	2.57	0.36	4.24	0.12	4.63	1.3
25,84	AROMADENDRENE	ND	-	2.2	0.01	1.02	0.75
26,35	ISOLEDENE	0.38	0.1	0.49	0.08	0.43	0.01
26,51	τ-CADINENE	1.21	0.78	0.54	0.2	1.26	0.88
26,79	TERPINENE-4-ACETATE	ND	-	0.25	0.03	ND	-
27,07	α–HUMULENE	2.44	0.16	3.58	0.7	3.07	0.76
27,10	SESQUITERPEN	0.93	0.03	ND	-	ND	-
27,17	TRANS-β-FARNESENE	1.19	0.86	1.04	0.73	1.77	2.4
27,40	TERPEN	1.69	0.03	1.12	0.01	1.18	0.01
27,52	α-AMORPHENE	0.94	0.02	0.51	0.13	2.04	1.12
27,61	α-TERPINEOL	1.12	0.06	0.83	0.45	3.2	2.27
27,91	CAMAZULENE	16.9	2.6	42.33	10.02	48.58	7.1

27,93	SESQUITERPEN	0.82	0.02	0.95	0.09	0.93	0.01
28,06	TERPEN	0.79	0.08	0.95	0.09	0.95	0.03
28,14	ISOCARYOPHILLENE	ND	-	5.92	0.51	1.03	0.87
28,34	α-BERGAMOTENE	2.07	1.6	3.26	0.07	4.28	0.81
28,55	D-GERMACRENE	ND	-	0.36	0.07	0.53	0.21
28,74	α-FARNESENE	1.15	0.17	3.62	0.07	5.26	1.06
28,85	α-CADINENE	1	0.01	3.65	0.09	4.49	2.3
29,04	SESQUIPHELLANDRENE	1.89	0.01	0.14	0.01	0.18	0.04
29,32	TERPEN	1.16	0.02	0.34	0.03	0.35	0.3
29,47	NAPTHALENE	3.99	0.74	1.27	1.06	1.85	0.27
29,52	α-METHYLNAPHTHALENE	ND	-	3.78	0.04	ND	-
29,71	SESQUITERPEN	2.14	0.04	2.1	0.07	2.24	1.55
30,10	SESQUITERPEN	3.49	0.34	1.96	0.09	2	0.46
30,47	TERPEN	ND	-	0.12	0.01	ND	-
30,71	TERPEN	0.42	0.01	0.3	0.1	0.29	0.2
31,06	SESQUITERPEN	1.12	0	0.23	0.01	0.31	0.21
31,30	α–PATCHOULENE	ND	-	0.75	0.47	0.53	0.23
31,79	β-IONONE	0.7	0.02	1.35	0.02	0.29	0.05
32,27	SESQUITERPEN	1.53	0.08	0.83	0.1	0.66	0.06
32,33	CARYOPHYLLENE OXIDE	ND	-	1.3	0.13	0.68	0.2
32,47	PHENOL	0.84	0.04	0.46	0.16	0.46	0.01
32,56	5,6-EPOXIDE-β-IONON	2.63	0.22	2.03	0	2.24	0.12
32,76	SESQUITERPEN	0.46	0.01	1.13	0.11	1.48	0.41
33,00	CUBENOL	0.58	0.1	0.59	0.11	0.32	0.17
33,07	SESQUITERPEN	1.38	0.03	1.82	0.1	1.36	0.15
33,69	SPATHULENOL	ND	-	ND	-	0.51	0.15
33,91	SESQUITERPEN	0.36	0.02	0.27	0.1	0.26	0.03
34,06	6-ALLYL-O-CRESOL	ND	-	1.85	0.01	ND	-
34,26	CARVACROL	1.52	0.02	0.15	0.03	0.52	0.3
34,54	SESQUITERPEN	ND	-	0.47	0.05	ND	-
34,72	SESQUITERPEN	1.03	0.1	0.15	0.04	1.27	0.9
34,89	SESQUITERPEN	ND	-	0.56	0.19	ND	-
35,83	SESQUITERPEN	0.84	0.02	0.32	0.01	0.5	0.22
38,89	SESQUITERPEN	1.19	0.95	3.72	1.51	3.78	0.14
40,16	THYMOL	0.68	0	0.68	0.1	0.21	0.16
44,35	SESQUITERPEN	1.45	0.48	ND	-	ND	-
44,85	SESQUITERPEN	ND	-	0.33	0.02	ND	-
TOT		**375.45**	**50.81**	**474.71**	**65.33**	**435.26**	**63.31**
	ACIDS						
29,79	ACID	1.35	0.03	1.47	0.01	1.44	0.11
30,25	n-HEXANOIC ACID	1.31	0.13	1.41	0.08	1.38	0.22
31,34	ACID	ND	-	0.81	0.08	ND	-
31,94	ACID	4.09	0.03	5.25	1.12	5.86	1.08
32,89	ACID	0.67	0.05	1.34	0.3	1.32	0.11
33,25	ACID	ND	-	0.22	0.13	ND	-
33,50	ACID	1.76	0.05	1.57	0.23	ND	-
34,21	ACID	1.32	0.04	1.46	0.06	1.46	0.01

34,6	DECANOIC ACID	0.48	0.02	0.45	0.08	0.48	0.22
35,27	ACID	0.34	0.04	0.36	0.01	0.58	0.13
37,36	BENZENECARBOXYLIC ACID	0.31	0.05	0.57	0	ND	-
37,61	BENZOIC ACID	1.71	0.07	1.45	0	ND	-
41,71	HEXADECANOIC ACID	0.38	0.01	0.3	0.06	0.32	0.09
43,61	ACID	4.36	0.05	1.62	0.02	1.64	0.08
44,34	ACID	0.28	0.02	4.67	1.18	4.67	1.18
TOT		**18.36**	**0.59**	**22.95**	**3.36**	**19.15**	**3.23**
	OTHERS						
21,00	UNKNOWN	ND	-	13.48	0.61	10.37	0.18

[a]= Retention time

[b]=means value of area under the peak (n=3)

[c]Arbitary unit = Area under the peak /1000000

[d]= Standard deviation

3.2.1 The volatile emissions of *Achillea collina* according to the class of compounds under biotic stress by specialist and generalist aphid infestations.

The amounts of each compound (in the different biotic experimental conditions with *M. persicae* and *M. millefolii* against control of *Achillea collina* plants) as obtained in 3.2.0, were classified according to the class of compounds. The data were statistically analyzed and the different colors indicated the statistical differences in between the experimental conditions.

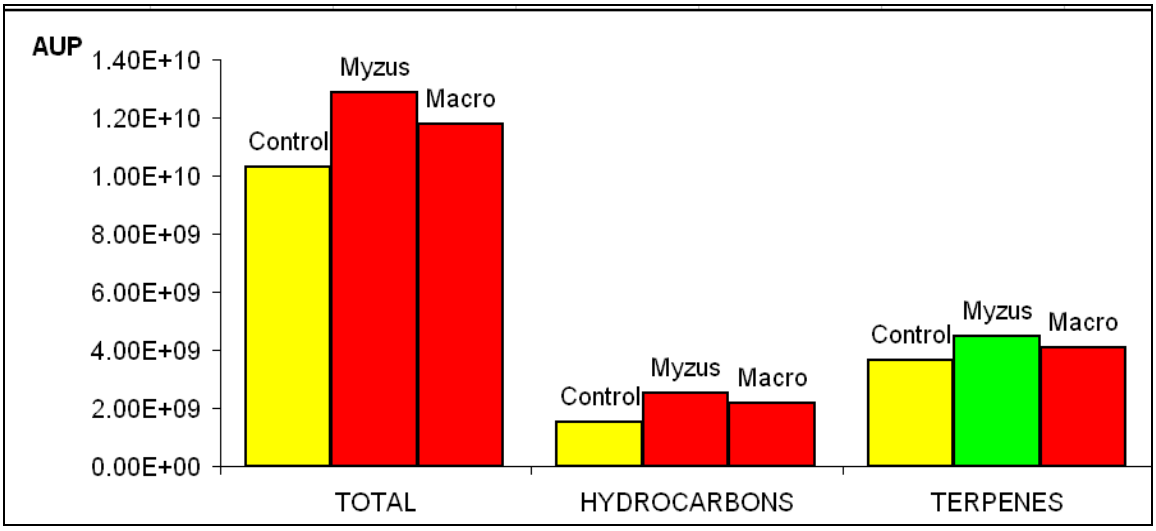

Fig. 20: A : variation of the classes of volatile compounds in the different different biotic stresses conditions (Myzus= *M. persicae* infestation and Macro= *M. millefolii* infestation*)* against the *Achillea collina* Becker ex Rchb. control plants. Data with different colors are statistically significant ($p < 0,05$), differences in each treatment: between the variables.

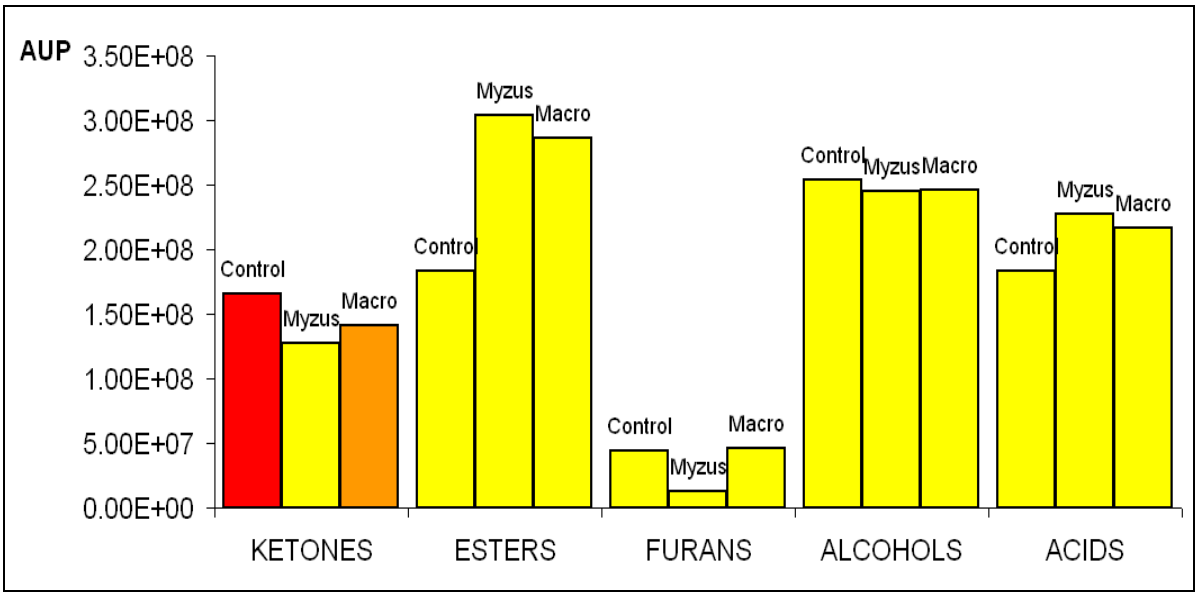

Fig. 20: B : variation of the classes of **v**olatile compounds in the different different biotic stresses conditions (Myzus= *M. persicae* infestation and Macro= *M. millefolii* infestation*)* against the *Achillea collina* Becker ex Rchb. control plants. Data with different colors are statistically significant ($p < 0,05$), differences in each treatment: between the variables.

3.2.2 Newly induced volatiles in *Achillea collina* under biotic stress by specialist and generalist aphid infestations.

Newly induced volatiles in *Achillea collina* plants, when they were infested by the generalist aphid *M. persiceae* and the specialist aphids *M. millefolii* are shown in Fig.21. The volatiles were identified using the developed HS-SPME-GC/MS *in vivo* method as explained in 3.2.0. The compounds which are to be identified, are indicated with the retention time with respect to the class of compounds where it must be included.

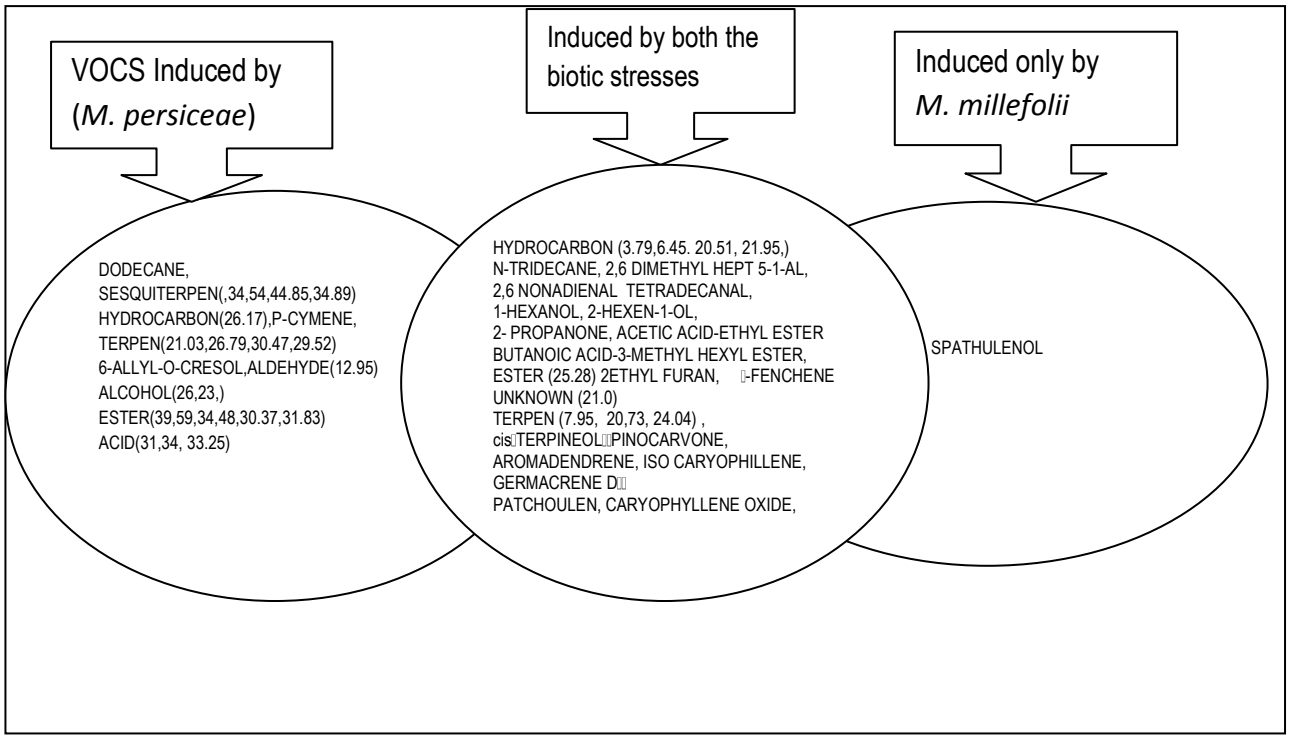

Fig. 21: newly induced volatiles in *Achillea collina* Becker ex Rchb. under the different biotic stresses applied. The numbers represent the retention time of corresponding compound in GC/MS

3.3.0 Abiotic stress on *Achillea collina* by mechanical damages

Achillea collina plants were mechanically damaged in two different manners: by an equipment specially built to apply a constant and measurable pressure to the plants and by a needle injury . The volatile fingerprints of the plants in the different experimental conditions were obtained with the developed *in vivo* method of HS-SPME-GC/MS under optimized parameters. The volatile fingerprint of the chromatograms were individually identified using a data base and the results were reported in Table 02 as an arbitrary value corresponding to the area under the peak /1000000.

Table 02 - volatile emissions of mechanically damaged plants of *Achillea collina* plants (by the specially built equipment and a needle) against control plants.

R.T.[a]	Compounds	Control n=3		Mechanical damage (pressure) n=3		Mechanical damage (Needle) n=3	
		mean [bc]	SD[d]	mean	SD	mean	SD
	HYDROCARBONS						
1,37	PENTANE	1.31	0.93	1.29	0.65	1.16	0.05
2,47	1-OCTENE	1.96	0.57	1.59	1.12	1.6	0.2
2,79	n-NONANE	2.93	0.36	8.27	7.7	3.11	0.05
3,16	3-METHYLOCTANE	1.84	0.38	2	1.42	1.21	0.05
3,31	BENZENE	2.64	0.88	1.5	0.83	2.7	0.25
3,79	HYDROCARBON	ND	-	1.93	0.03	1.81	0.05
3,95	3-METHYLCYCLOOCTANE	2.48	0.19	3.16	0.02	18.97	1
4,01	3,5-DIMETHYLOCTANE	0.7	0.08	0.88	0.02	0.87	0.05
4,32	HYDROCARBON	0.2	0.06	0.35	0.03	0.41	0.19
4,49	α-TRIDECENE	0.6	0.22	0.74	0.11	0.62	0.3
4,64	HYDROCARBON	0.73	0.01	0.42	0.19	0.57	0.25
4,94	HYDROCARBON	5	4.9	8.66	0.73	6.37	4.78
5,53	DECANE	2.61	0.89	1.5	0.16	2.84	0.35
6,45	HYDROCARBON	ND	-	1.92	0.08	4.64	4.08
6,71	TOLUENE	1.35	0.28	2.01	0.07	0.85	0.26
6,87	HEXADECANE	1.18	0.19	1.84	0.15	1.35	0.03
6,97	HYDROCARBON	1.28	0.29	2.31	0.1	1.71	0.13
7,14	HEPTADECANE	0.43	0.04	0.76	0.12	1.47	0.67
8,09	HYDROCARBON	0.65	0.01	0.38	0.17	0.55	0.07
8,36	HYDROCARBON	0.35	0.09	0.3	0.03	0.42	0.07
8,58	HYDROCARBON	6.57	0.13	2.23	0.69	2.91	2.47
9,18	n-TRIDECANE	ND	-	6.01	0.44	4.51	3.92
10,16	o–XYLENE	11.11	0.08	9.23	1.9	13.43	0.72
10,34	m-XYLENE	1.63	0.02	1.35	0	9	7.71
10,49	p-XYLENE	28.82	0.76	22.64	3.69	14.8	13.11
11,29	HYDROCARBON	0.55	0.08	0.66	0.05	0.85	0.11
11,72	HYDROCARBON	ND	-	0.49	0.06	0.69	0.12
11,84	3-ETHYLHEXANE	ND	-	0.4	0.08	0.52	0.07
11,95	DODECANE	ND	-	0.72	0.09	0.67	0.02
13,82	n-PENTAYL-CYCLOHEXANE	4.24	0.4	1.8	0.69	1.65	0.02
14,34	n-PENTADECANE	2.38	1.06	4.97	0.73	3.45	2.57
15,03	2-ISOPROPHYL-1-METHOXY-4-METHYLBENZENE	1.48	0.05	1.8	0.46	11.19	8.60
15,82	STYRENE	2.71	0.05	2.65	0.28	0.98	0.47
15,92	o-ETHYL-TOLUENE	8.7	0.09	2.16	0.67	5.56	3.11
16,56	1-ETHYL-2,4-DIMETHYLBENZENE	0.32	0.02	11.43	10.19	38	0.25

17,97	HYDROCARBON	2.15	1.76	2	1.87	3.87	2.49
18,22	HYDROCARBON	28.09	0.15	54.12	7.68	13.81	7.15
18,92	2-ETHYL-p-XYLENE	2.48	0.04	0.32	0.08	2.76	1.25
19,09	4-ETHYL-m-XYLENE	4.72	0.12	2.98	0.36	4.75	0.64
19,62	ISODURENE	1.56	0.12	3.26	0.01	2.98	2.7
20,26	4-ETHYL-1,2-DIMETHYL-BENZENE	1.16	0.04	0.53	0	1.95	0.87
20,51	HYDROCARBON	ND	-	0.91	0.09	1.53	0.47
21,54	1,4-DIMETHYLBENZENE	4.86	0.05	6.39	1.61	1.75	1.1
21,95	HYDROCARBON	ND	-	0.57	0.27	ND	-
26,88	HYDROCARBON	3.67	0.45	4.54	3.78	2.46	1.99
29,10	HYDROCARBON	1.67	0.02	0.65	0.47	1.87	1.08
30,31	TETRACOSANE	1.4	0.02	1.04	0.3	1.25	0.55
TOT		**148.51**	**15.88**	**187.66**	**50.27**	**200.42**	**76.44**
	ALDEHYDES						
1,58	ACETALDEHYDE	ND	-	0.79	0.08	ND	-
2,07	2-METHYLPROPANAL	16.5	1.21	26.8	26.28	26.78	1.02
3,04	2-METHYLBUTANAL	0.23	0.1	0.68	0.49	0.49	0.01
8,97	HEXANAL	6.02	0.4	12.65	1.17	3.22	2.67
12,26	LAURALDEHYDE	2.57	0.32	1.4	0.04	0.46	0.11
12,95	ALDEHYDE	ND	-	1.14	0.02	1.18	0.11
15,30	trans-2-HEXENAL	11.59	0.05	7.95	0.32	6.58	3.6
18,86	2-HEPTENAL	3.61	0.03	11.22	10.28	1.87	0.86
19,89	ALDEHYDE	4.19	0.64	0.3	0.14	1.04	0.76
20,98	2,4-HEXADIENAL	12.18	0.17	0.4	0.09	1.04	0.22
21,74	2-OCTENAL	4.4	0.27	7.04	0.47	3.05	2.36
21,96	p-MENTH-1-EN-9-AL	1.89	0.05	0.95	0.4	1.29	1.09
23,42	n-DECANAL	1.14	0.01	1.36	0.16	2.29	0.93
23,84	BENZALDEHYDE	2.26	0.01	3.82	0.53	1.87	1.61
25,35	2,6-NONADIENAL	ND	-	0.66	0.16	0.88	0.53
26,00	β-CYCLOCITRAL	1.27	0.09	1.58	0.2	1.82	1.05
30,54	ALDEHYDE	0.84	0.15	0.6	0.07	0.62	0.06
35,57	ALDEHYDE	0.6	0.03	0.52	0.07	0.22	0.01
TOT		**69.29**	**3.53**	**79.86**	**40.97**	**54.7**	**17**
	ALCOHOLS						
7,34	DECANOL	3.67	0.27	5.17	0.05	2.55	1.76
12,06	CYCLOPENTADECANOL	1.76	1.36	0.27	0.02	0.52	0.27
12,78	ETHYLHEXANOL	1.63	0.13	3.87	2.44	6.14	5.06
14,13	9-TETRADECEN-1-OL	1.27	0.27	1.71	0.13	1.32	0.45
20,01	1-HEXANOL	ND	-	76.04	9.39	74.01	16.55
20,19	ALCOHOL	1.91	0.07	3.32	0.37	2.06	0.06
20,76	3-HEXEN-1-OL	1.7	0.07	3.52	2.83	1.57	0.41
21,16	3-OCTANOL	0.68	0.02	0.72	0.13	7.59	6.96
21,36	2-HEXEN-1-OL	ND	-	4.49	0.16	3.87	0.05
22,48	1-OCTENE-3-OL	4.63	0.05	4.05	3.81	0.88	0.67
23,59	ALCOHOL	1.8	0.02	1.32	0.27	1.38	0.17

24,27	cis-9-OCTADECEN-1-OL	0.61	0.08	1.41	0.2	0.83	0.02
26,23	ALCOHOL	ND	-	0.59	0.09	ND	-
36,24	Z-9-HEXADECEN-1-OL	1.52	0.05	0.86	0	0.67	0.05
TOT		**21.18**	**2.39**	**107.34**	**19.89**	**103.39**	**32.48**
	KETONES						
2,21	2-PROPANONE	ND	-	32.03	30.81	ND	-
19,22	2,3-OCTANEDIONE	3.8	0.09	2.73	0.1	4.9	0.98
19,43	6-METHYL-5-HEPTENE-2-ONE	5.58	0.1	3.58	0.04	5	0.58
26,75	KETONE	0.67	0.25	0.7	0.14	0.7	0.2
29,22	2–PENTANONE	1.63	0.03	6.01	2.2	2	1.14
31,49	KETONE	0.74	0.14	0.78	0.1	0.8	0.21
32,72	2H-2,4A-METHANONAPHTHALEN-8(5H)-ONE	1.32	0.04	1.43	0.1	1.57	0.15
33,73	KETONE	1.44	0.06	0.69	0.08	0.55	0.04
TOT		**15.18**	**0.71**	**47.95**	**33.57**	**15.52**	**3.3**
	ESTERS						
2,60	ACETIC ACID-ETHYL ESTER	ND	-	2.54	0.48	3.75	0.25
7,49	ACETIC ACID BUTHYL ESTER	ND	-	1.09	0	3.43	0.34
17,58	HEXYL ACETATE	0.67	0.15	0.15	0	11.83	0.05
17,80	2-PENTANOL-4-METHYL-ACETATE	5.38	0.05	4.88	0	0.94	0.24
22,32	ISOVALERIC ACID BUTYL ESTER	1.63	0.14	1.62	0.12	0.9	0.45
24,12	ESTER	1.02	0.09	0.96	0.03	0.85	0.51
25,28	ESTER	ND	-	2.43	0.2	1.4	0.53
28,96	2-HYDROXYBENZOIC ACID METHYL ESTER	ND	-	0.88	0	0.71	0.12
34,48	ESTER	ND	-	0.5	0	ND	-
40,52	ESTER	0.35	0.05	1.65	0.14	0.44	0.14
TOT		**9.05**	**0.48**	**16.7**	**0.97**	**24.25**	**2.63**
	FURAN						
4,17	2-ETHYLFURAN	ND	-	0.15	0	ND	-
4,71	2-METHYLFURAN	4.44	4.18	0.91	0.41	0.93	0.56
TOT		**4.44**	**4.18**	**1.06**	**0.41**	**0.93**	**0.56**
	TERPENES						
5,33	TERPEN	14.09	11.68	4.84	1.04	7.57	4.58
6,01	α-PINENE	87.94	28.43	20.83	5.62	5.62	1.35
6,33	α-PHELLANDRENE	1.54	0.01	17.33	1.04	9.31	0.69
7,47	α-FENCHENE	ND	-	1.45	0.28	4.41	0.1
7,76	CAMPHENE	2.05	0.78	0.85	0.27	1.84	1.45
7,95	TERPEN	ND	-	0.48	0	0.51	0.02
9,53	γ-TERPINEN	6.37	0.01	8.09	0.57	5.47	3.26

9,91	β-PINENE	18.04	0.08	10.15	1.45	6.83	4.32
10,80	β-PHELLANDRENE	1.26	0.15	4.18	1.32	16.57	12.34
10,91	TERPEN	1.35	0.27	0.59	0.04	2.65	1.78
11,66	TERPEN	0.24	0.01	9.94	0.06	9.83	0.11
12,53	DIMETHYLFULVENE	13.95	2.47	5.16	4.82	5.48	4.71
13,36	TERPINOLEN	16.88	2.74	2.64	0.33	6.38	3.59
13,37	β-MYRCENE	2.9	0.09	7.66	1.54	5.52	4.35
13,66	TERPEN	1.33	0.06	2.93	0.4	2.21	0.78
14,50	D-LIMONENE	13.59	0.48	10.22	2.76	9.26	2.63
14,78	EUCALYPTOL	17.65	0.31	23.82	0.74	9.75	8.04
16,36	TERPEN	6.39	0.52	5.31	0.78	6.84	2.03
16,46	γ-TERPINENE	29.33	24.07	13.4	13.18	4.92	0.02
16,90	γ -PINENE	38.19	0.63	2	1.11	20.31	18.94
17,27	o-CYMENE	1.93	0.05	0.88	0.22	2.17	0.59
17,46	TERPEN	11.57	0.07	8.18	0.45	6.92	3.96
17,71	TERPEN	1.27	0.13	0.61	0.51	0.67	0.01
18,30	TERPEN	8.83	0.29	5.52	0.37	14.88	7.08
18,42	TERPEN	ND	-	1.72	0.2	5.8	2.1
18,55	p-CYMENE	ND	-	18.64	14.72	3.11	0.88
20,42	TERPEN	1.74	0.03	0.82	0.7	1.7	0.21
20,73	TERPEN	ND	-	2.81	2.51	1.52	0.13
21,40	TERPEN	3.76	0.07	4.78	0	2.5	2.18
21,48	TERPEN	24.7	20.26	0.19	0	1.55	1.2
22,19	TERPEN	3.38	0.13	0.73	0.21	0.3	0.11
22,59	α-CUBEBENE	6.41	0.07	6.76	0	5.43	5.18
22,67	TERPEN	ND	-	ND	-	6.01	5.61
22,72	trans-SABINENE	3.79	0.04	7.59	1.56	3.46	3.03
22,85	2-CARENE	ND	-	ND	-	3.55	3.33
22,97	γ-ELEMENE	2.71	0.12	2.69	2.44	2.16	1.9
23,01	TERPEN	3.09	0.29	2.15	0.73	3.85	0.71
23,13	α-YLANGENE	5.2	4.02	1.94	0.17	3.26	0.18
23,33	α-COPAENE	2.64	0	2.42	0.28	3.15	0.03
23,63	CAMPHOR	2.71	0.05	4.11	0.19	2.35	1.08
23,94	TERPEN	1.61	1.34	0.56	0.01	1.31	1.02
24,04	TERPEN	ND	-	0.22	0.02	1.43	1.12
24,24	VALENCENE	0.85	0.49	0.61	0.12	1.59	0.77
24,40	TERPEN	0.56	0	0.86	0.64	0.52	0.37
24,65	LINALOOL	0.44	0.02	1.61	0.27	1.1	0.6
24,79	TERPEN	0.8	0.02	2.2	0.07	0.46	0.03
24,87	PINOCARVONE	ND	-	0.91	0.21	0.7	0.07
25,05	TERPEN	1.42	0.15	2.83	0.76	1.43	0.65
25,19	β-CUBEBENE	0.8	0.1	1.05	0.34	1.76	0.42
25,45	SESQUITERPEN	1.35	0.15	0.5	0.12	0.79	0.01
25,60	β-CARYOPHYLLENE	1.36	0.05	0.97	0.28	1.15	0.35
25,69	SESQUITERPENE	2.57	0.36	5.81	0.62	2.08	0.49
26,35	ISOLEDENE	0.38	0.1	0.96	0.16	2.2	1.42
26,51	τ-CADINENE	1.21	0.78	0.94	0.09	2.1	1.61
26,79	TERPINENE 4-ACETATE	ND	-	1.1	0.22	0.69	0.19

27,07	α–HUMULENE	2.44	0.16	4.34	2.67	3.57	0.97
27,10	SESQUITERPEN	0.93	0.03	0.98	0.32	1.72	1.18
27,17	trans-β-FARNESENE	1.19	0.86	1.31	0.3	0.43	0.13
27,40	TERPEN	1.69	0.03	0.78	0.55	1.09	0.72
27,52	α-AMORPHENE	0.94	0.02	1.81	0.48	1.44	0.47
27,61	α-TERPINEOL	1.12	0.06	1.09	0.43	0.73	0.26
27,91	CAMAZULENE	16.9	2.6	16.92	0.04	8.19	7.69
27,93	SESQUITERPEN	0.82	0.02	0.42	0.02	9.76	9.12
28,06	TERPEN	0.79	0.08	0.59	0.08	0.5	0.17
28,14	ISOCARYOPHILLENE	ND	-	5.48	0.04	0.45	0.09
28,34	α-BERGAMOTENE	2.07	1.6	0.4	0.02	0.43	0.14
28,40	TERPEN	4.54	0.2	5.72	0.45	1.21	0.9
28,55	D-GERMACRENE	ND	-	0.87	0.1	1.39	1.22
28,74	α-FARNESENE	1.15	0.17	1.08	0.36	0.45	0.34
28,85	α-CADINENE	1	0.01	1.78	0.13	0.73	0.16
29,04	SESQUIPHELLANDRENE	1.89	0.01	0.42	0.08	0.71	0.01
29,32	TERPEN	1.16	0	1.65	0.2	4.83	3.87
29,47	NAPTHALENE	3.99	0.74	3.66	0.68	5	3.9
29,71	SESQUITERPEN	2.14	0.04	2.75	0.1	1.82	0.62
29,79	SESQUITERPEN	1.35	0.03	1.03	0.11	2.12	0.53
29,90	SESQUITERPEN	5.92	0.44	6.57	0.21	4.16	2.27
30,10	SESQUITERPEN	3.49	0.34	2.81	0.53	3.38	3.10
30,71	TERPEN	0.42	0.01	0.28	0.21	0.4	0.19
31,03	SESQUITERPEN	4.21	0.13	1.03	0.03	0.43	0.2
31,06	SESQUITERPEN	1.12	0	0.87	0.06	0.57	0.09
31,79	β-IONONE	0.7	0.02	2.85	0.22	1.46	0.35
32,27	SESQUITERPEN	1.53	0.08	3.23	1.05	9.8	7.97
32,47	PHENOL	0.84	0.04	0.67	0.04	1.1	0.8
32,56	5,6–EPOXIDE–β-IONON	2.63	0.22	3.14	0.45	3.43	0.2
32,76	SESQUITERPEN	0.46	0.01	1.22	0.86	0.63	0.03
32,89	SESQUITERPEN	0.67	0.05	1.94	0.13	0.81	0.11
32,94	SESQUITERPEN	ND	-	0.48	0.08	ND	-
33,00	CUBENOL	0.58	0.1	0.92	0.16	0.85	0.11
33,07	SESQUITERPEN	1.38	0.03	1.81	0.08	1.12	0.35
33,18	SESQUITERPEN	ND	-	1.03	0.44	1.68	0.09
33,25	SESQUITERPEN	ND	-	1.16	0.29	5.29	3.4
33,44	SESQUITERPEN	1.45	0.3	4.52	0.19	5.17	3.71
33,69	SPATHULENOL	ND	-	ND	-	0.99	0.45
33,91	SESQUITERPEN	0.36	0.02	2.11	0.24	0.39	0.19
34,06	6-ALLYL-O-CRESOL	ND	-	3.02	0.35	1.24	1.06
34,26	CARVACROL	1.52	0.02	0.18	0.03	0.54	0.09
34,54	SESQUITERPEN	ND	-	2.38	0.11	ND	-
34,72	SESQUITERPEN	1.03	0.1	0.47	0.03	0.57	0.11
34,89	SESQUITERPEN	ND	-	1	0.07	ND	-
35,83	SESQUITERPEN	0.84	0.02	0.53	0.03	0.42	0.19
36,75	SESQUITERPEN	1.84	0.03	1.31	0.55	ND	-
38,89	SESQUITERPEN	1.19	0.95	ND	-	3.99	1.52
40,16	THYMOL	0.68	0	2.02	0	4.87	0.91

42,42	SESQUITERPEN	0.47	0	ND	-	ND	-
44,85	SESQUITERPEN	ND	-	0.62	0	ND	-
TOT		**449.61**	**111.54**	**346.79**	**80.14**	**334.79**	**183.69**
	ACIDS						
21,34	ACID	ND	-	1.08	0.32	2.41	1.77
29,61	ACID	ND	-	7.32	6.07	ND	-
31,17	ACID	ND	-	4.03	1.02	ND	-
33,50	ACID	1.76	0.05	2.3	0.12	1.55	0.21
34,21	ACID	1.32	0.04	0.84	0.07	1.41	1.19
34,60	DECANOIC ACID	0.48	0.02	1.2	0.02	0.54	0.1
34,91	ACID	ND	-	0.93	0	1.81	1.33
35,27	ACID	0.34	0.04	0.34	0.08	1.93	1.71
36,54	BENZENEFORMIC ACID	ND	-	5.51	0.24	ND	-
37,36	ACID	0.31	0.05	1.65	0.91	1.05	0.43
38,93	ACID	4.45	0.3	ND	-	4.23	1.36
41,71	HEXADECANOIC ACID	0.38	0.01	0.72	0.14	0.27	0.04
43,61	ACID	4.36	0.05	1.97	0.18	1.69	0.84
44,34	ACID	0.28	0.02	ND	-	ND	-
TOT		**13.68**	**0.58**	**27.89**	**9.17**	**16.89**	**8.98**
	OTHERS						
1,82	DIMETHYLSULFIDE	1.63	0.77	0.61	0.22	1.19	0.05
5,08	UNKNOWN	ND	-	0.17	0.06	ND	-

[a]= Retention time

[b]=means value of area under the peak (n=3)

[c]Arbitary unit = Area under the peak /1000000

[d]= Standard deviation

3.3.1 Newly induced volatiles in *Achillea collina* under abiotic stresses (mechanical damage created by the specially built equipment and the needle)

Newly induced volatiles in *Achillea collina* plants, when they were mechanically damaged by the specially built equipment and the needle are shown in Fig.22. The volatiles were identified using the developed HS-SPME-GC/MS *in vivo* method as explained in 3.2.0. The compounds which are to be identified are indicated with corresponding retention time in GC/MS with respect to the class of compounds where they must be included.

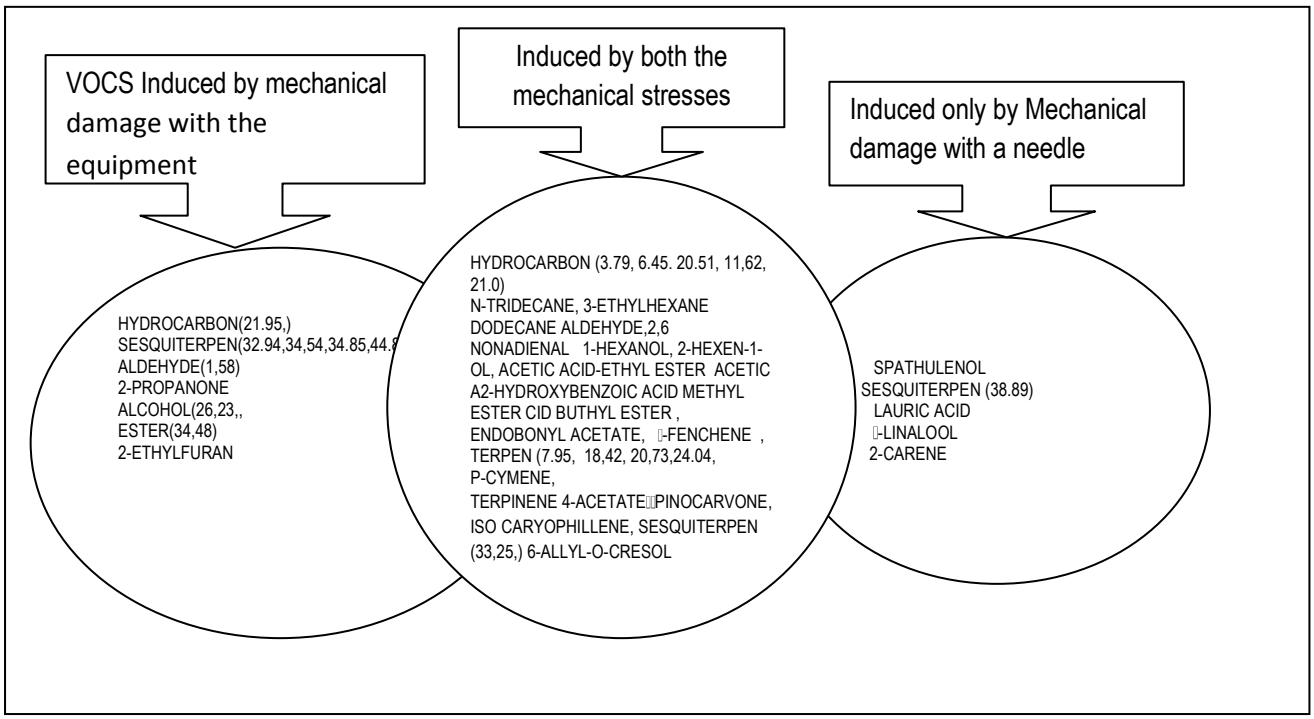

Fig. 22: *A. collina* volatiles newly induced by mechanical damages (needle and the specially built equipment) as compared to control plants. The numbers represent the retention time of corresponding compound in GC/MS.

3.4.0 *Achillea collina* plants volatile emissions after jasmonic acid treatments

Achillea collina plants were treated with jasmonic acid 0.1μM and subjected to volatile compounds analyses with the developed *in vivo* method of HS-SPME-GC/MS under optimized parameters. The volatile fingerprint of the chromatograms were individually identified using a data base and the results were reported in Table 03 as an arbitrary value corresponding to the area under the peak /1000000.

Table 03 - volatile emissions of *Achillea collina* control and treated with jasmonic acid plants.

R.T.[a]	compounds	Control n=3		Jasmonic acid treatment (0.1μM) n=3	
		mean [bc]	SD[d]	mean	SD
	HYDROCARBONS				
1,37	PENTANE	23,57	0,12	36,68	0,11
2,47	1-OCTENE	1,10	0,01	1,69	0,18
2,79	n-NONANE	1,66	0,01	1,99	0,01
3,16	3-METHYLOCTANE	2,14	0,01	2,09	0,01
3,31	BENZENE	0,45	0,01	0,37	0,01
3,79	HYDROCARBON	0,85	0,01	0,79	0,01
3,95	3 METHYLCYCLOOCTANE	0,58	0,01	2,30	0,01
4,01	3,5-DIMETHYLOCTANE	0,65	0,01	ND	-
4,32	HYDROCARBON	0,64	0,01	3,16	0,01
4,49	α-TRIDECENE	2,52	0,01	0,99	0,01
4,64	HYDROCARBON	0,74	0,01	ND	-
4,94	HYDROCARBON	0,14	0,01	0,16	0,01
5,53	DECANE	3,52	0,02	1,80	0,01
6,45	HYDROCARBON	7,85	0,04	ND	-
6,71	TOLUENE	7,85	0,04	15,64	0,04
6,87	HEXADECANE	0,97	0,02	1,42	0,03
6,97	HYDROCARBON	2,85	0,01	2,24	0,01
7,14	HEPTADECANE	0,39	0,01	0,77	0,01
8,09	HYDROCARBON	0,66	0,01	0,66	0,01
8,36	HYDROCARBON	0,45	0,01	0,28	0,01
8,58	HYDROCARBON	6,73	0,03	6,47	0,02
10,16	o-XYLENE	11,08	0,06	11,25	0,03
10,34	m-XYLENE	1,65	0,01	1,67	0,03

10,49	p-XYLENE	29,73	0,15	28,20	0,08
13,82	n-PENTYLCYCLOHEXANE	3,86	0,02	3,61	0,01
14,34	n-PENTADECANE	1,33	0,01	3,45	0,01
15,03	2-ISOPROPHYL-1-METHOXY-4-METHYLBENZENE	0,93	0,01	1,80	0,01
15,82	STYRENE	9,18	0,05	ND	-
15,92	o-ETHYL-TOLUENE,	5,29	0,03	2,87	0,01
16,56	1-ETHYL-2,4-DIMETHYLBENZENE	0,33	0,01	0,34	0,01
17,97	HYDROCARBON	3,93	0,02	0,40	0,01
18,22	HYDROCARBON	28,08	0,14	0,52	0,01
18,92	2-ETHYL- p-XYLENE	2,54	0,01	2,45	0,01
19,09	4-ETHYL- m-XYLENE	4,86	0,02	4,62	0,01
19,62	ISODURENE	6,18	0,03	6,26	0,02
20,26	4-ETHYL-1,2-DIMETHYLBENZENE	4,64	0,02	4,48	0,01
20,51	HYDROCARBON	12,00	0,06	12,00	0,03
21,95	HYDROCARBON	ND	-	27,51	0,08
26,88	HYDROCARBON	3,23	0,02	4,14	0,01
29,90	HYDROCARBON	6,40	0,03	5,50	0,02
30,31	TETRACOSANE	1,39	0,01	1,44	0,01
TOT		**202,94**	**1,13**	**202,01**	**0,92**
	ALDEHYDES				
2,07	2-METHYLPROPANAL	1,85	0,01	15,37	0,04
3,04	2-METHYLBUTANAL	2,95	0,01	2,77	0,01
8,97	HEXANAL	2,16	0,01	1,78	0,06
12,26	LAURALDEHYDE	2,26	0,01	2,91	0,01
12,95	ALDEHYDE	1,34	0,01	2,44	0,01
15,30	trans-2-HEXENAL	3.83	0,02	4,53	0,01
18,86	2-HEPTENAL	3,17	0,02	4,34	0,01
19,87	2,6-DIMETHYL-HEPT-5-1-AL	ND	-	0,25	0,01
19,89	ALDEHYDE	4,85	0,02	3,56	0,01
20,98	2,4-HEXADIENAL	0,13	0,01	1,05	0,03
21,74	2- OCTENAL	19,13	0,10	21.64	0,06
21,96	p-MENTH-1-EN-9-AL	1.95	0,01	ND	-
23,42	N-DECANAL	1,16	0,01	16,43	0,05
23,84	BENZALDEHYDE	6,62	0,03	12,37	0,04
26,00	β-CYCLOCITRAL	85,45	0,43	17,35	2,56
35,57	ALDEHYDE	0,64	0,01	0,58	0,01
TOT		**131,71**	**0,71**	**85,73**	**2,92**
	ALCOHOLS				
7,34	DECANOL	0.79	0,01	0.17	0,01
12,06	CYCLOPENTADECANOL	0,41	0,01	3,14	0,01
12,78	ETHYLHEXANOL	0,93	0,01	1,09	0,05
14,13	9-TETRADECEN-1-OL	1,51	0,01	2,08	0,01
20,01	1-HEXANOL	ND	-	2,83	0,01
20,19	ALCOHOL	1,84	0,01	1,92	0,01
20,76	3-HEXEN-1-OL	21,43	0,11	23,92	0,07

21,16	3-OCTANOL	2,86	0,01	0,17	0,01
21,36	2-HEXEN-1-OL	ND	-	1,80	0,01
21,48	ALCOHOL	4,46	0,02	45,19	0,13
22,48	1-OCTENE-3-OL	6,92	0,03	5,19	0,01
24,27	cis-9-OCTADECEN-1-OL	0,68	0,01	ND	-
36,24	Z-9-HEXADECEN-1-OL	1,58	0,01	1,53	0,03
36,75	ALCOHOL	1,82	0,01	1,87	0,01
TOT		**44,44**	**0,25**	**90,73**	**0,37**
	KETONES				
2,21	2- PROPANONE	ND	-	2,51	0,01
19,22	2,3-OCTANEDIONE	2,18	0,01	4,21	0,01
19,43	6-METHYL-5-HEPTENE-2-ONE	5,52	0,03	1,41	0,03
26,75	KETONE	0,42	0,01	0,93	0,01
29,22	2-PENTANONE	1,67	0,01	10,04	1,18
32,72	2H-2,4A-METHANONAPHTHALEN-8(5H)-ONE	1,36	0,01	1,28	0.05
33,44	KETONE	1,16	0,01	1,75	0,01
33,73	KETONE	41,42	9,21	ND	-
TOT		**53,73**	**9,29**	**22,13**	**1,25**
	ESTERS				
2,60	ACETIC ACID-ETHYL ESTER	22,86	9,89	39,74	6,24
17,58	HEXYL ACETATE	1,02	0,01	0,83	0,01
17,80	2-PENTANOL,4-METHYL-,ACETATE	5,37	0,03	3,83	0,01
22,32	ISOVALERIC ACID BUTYL ESTER	5,01	0,02	0,96	0,01
24,12	ESTER	1,07	0,06	1,17	0,03
28,40	ESTER	4,76	0,02	4,36	0,01
28,96	2-HYDROXYBENZOIC ACID METHYL ESTER	ND	-	23,83	0,07
29,10	ESTER	1,65	0,01	25,25	5,27
31,83	LAURIC ACID,ETHYL ESTER	0.69	0.01	26.39	5,70
34,21	ESTER	1,37	0,01	ND	-
34,54	ESTER	ND	-	7,16	0,02
40,52	ESTER	0,31	0,01	0,41	0,01
43,1	PHTHALIC ACID BUTYL 2-ETHYLHEXYL ESTER	0,65	0,01	0,68	0,01
TOT		**44,07**	**10,07**	**108,22**	**17,39**
4,71	2-METHYLFURAN	0,20	0,01	0,17	0,01
TOT		0,2	0,01	0,17	0,01
	TERPENES				
5,33	TERPEN	25,89	0,13	7,03	0,02
6,01	α-PINENE	7,76	0,04	0,04	0,00
6,33	α-PHELLANDRENE	0,47	0,01	1,59	0,03
7,47	α-FENCHENE	ND	-	0,56	0,01
7,76	CAMPHENE	2,84	0,01	0,43	0,01
7,95	TERPENE	ND	-	0,56	0,01
9,53	γ-TERPINEN	6,39	0,03	6,42	0,02

9,91	β-PINENE	3,90	0,02	4,91	0,01
10,80	β-PHELLANDRENE	5,33	0,03	4,09	0,01
10,91	TERPEN	1,08	0,01	1,65	0,02
11,66	TERPEN	21,81	0,11	ND	-
12,53	DIMETHYLFULVENE	2,90	0,01	2,79	-
13,37	β-MYRCENE	7,29	0,04	7,00	0,02
13,36	TERPINOLEN	1,34	0,01	ND	0,08
13,66	TERPEN	0,31	0,01	1,49	0,03
14,50	D-LIMONENE	3,45	0,02	0,22	0,01
14,78	EUCALYPTOL	2,22	0,01	2,21	0,01
16,36	TERPEN	5,45	0,03	5,38	0,02
16,46	γ-TERPINENE	3,15	0,02	2,49	0,01
16,90	γ -PINENE	0,38	0,01	4,26	0,01
17,27	o-CYMENE	7,31	0,04	9,02	0,03
17,71	TERPEN	7,93	0,04	2,43	0,01
18,30	TERPEN	9,17	0,05	8,58	0,02
18,55	p-CYMENE	3,60	0,02	3,03	0,01
20,42	TERPEN	1,72	0,01	1,79	0,01
20,73	TERPEN	0,18	0,01	0,18	0,01
21,40	TERPEN	3,84	0,02	3,71	0,01
22,19	TERPEN	11,55	0,06	1,27	0,03
22,59	α-CUBEBENE	7,91	0,04	0,09	0,01
22,72	trans-SABINENE	63,17	0,31	0,58	0,01
22,97	γ-ELEMENE	6.62	0.03	7.08	0,02
23,01	TERPEN	2.82	0,01	3.39	0,01
23,13	α-YLANGENE	12.62	0,06	7.08	0,02
23,33	α-COPAENE	9.20	0,05	10.62	0,03
23,63	CAMPHOR	63,17	0,31	58,07	0,17
23,94	TERPEN	2,42	0,01	1,85	0,01
24,04	TERPEN	0,28	0,01	2,43	0,01
24,24	VALENCENE	19,27	0,10	17,62	0,05
24,40	TERPEN	3,69	0,02	3,69	0,01
24,52	TERPEN	1,06	0,01	1,23	0,03
24,65	LINALOOL	58,41	0,29	8,07	0,02
24,79	TERPEN	0,47	0,01	ND	-
24,87	PINOCARVONE	ND	-	4,64	0,01
25,19	β-CUBENE	0,71	0,01	0,91	0,01
25,45	SESQUITERPEN	1,51	0,01	1,25	0,03
25,60	β-CARYOPHYLENE	1,31	0,01	1,46	0,03
25,69	SESQUITERPENE	2,22	0,01	2,94	0,01
25,84	AROMADENDRENE	ND	-	4,15	0,01
26,35	ISOLEDENE	85,45	10,57	69,61	6,15
26,51	τ-CADINENE	1,99	0,01	0,43	0,01
27,07	α-HUMULENE	2,29	0,01	2,61	0,01
27,10	SESQUITERPEN	0,97	0,01	0,91	0,01

27,17	TRANS-β-FARNESENE	11,46	0,06	0,34	0,01
27,40	TERPEN	1,73	0,01	1,72	0,03
27,52	α-AMORPHENE	0,97	0,01	0,93	0,01
27,61	α-TERPINEOL	1,07	0,01	12,27	0,04
27,91	CAMAZULENE	11,46	0,06	69,61	0,20
27,93	SESQUITERPEN	0,85	0,01	0,81	0,01
28,06	TERPEN	0,72	0,01	0,88	0,01
28,14	ISO CARYOPHILLENE	ND	-	8,70	0,02
28,34	α-BERGAMOTENE	3,69	0,02	0,48	0,01
28,74	α-FARNESENE	28,20	0,14	6,19	0,02
28,85	α-CADINENE	1,01	0,01	1,05	0,03
28,96	2-HYDROXYBENZOIC ACID METHYL ESTER	ND	-	23,83	0,07
29,32	TERPEN	1,17	0,01	1,17	0,01
29,47	NAPTHALENE	3,27	0,02	4,75	0,01
29,71	SESQUITERPEN	2,12	0,01	2,19	0,06
29,79	SESQUITERPEN	1,39	0,01	1,38	0,03
30,10	SESQUITERPEN	3,16	0,02	3,85	0,01
30,71	TERPENE	4,96	0,02	9,55	0,03
31,03	SESQUITERPEN	4,36	0,02	4,10	0,01
31,06	SESQUITERPEN	1,12	0,01	1,18	0,03
31,79	β-IONONE	43,84	0,22	26,41	0,08
32,27	SESQUITERPEN	1,62	0,01	1,50	0,03
32,33	CARYOPHYLLENE OXIDE	ND	-	57,86	0,17
32,47	PHENOL	63,18	0,31	ND	-
32,56	5,6-EPOXIDE-β-IONON	2,42	0,01	2,86	0,01
32,76	SESQUITERPEN	0.47	0,01	1,46	0,01
32,94	SESQUITERPEN	ND	-	7,31	0,02
33,00	CUBENOL	5,68	0,03	1,59	0,08
33,07	SESQUITERPEN	1,36	0,01	1,41	0,06
33,18	SESQUITERPEN	ND	-	0,82	0,01
33,91	SESQUITERPEN	0,35	0,01	ND	-
34,26	CARVACROL	1,51	0,01	ND	-
34,72	SESQUITERPEN	1,13	0,01	2,67	0,01
34,89	SESQUITERPEN	ND	-	6,61	0,02
34,91	SESQUITERPEN	ND	-	4,19	0,01
30,04	SESQUITERPEN	ND	-	8,83	0,03
35,56	TERPENE	ND	-	101,96	6,07
35,83	SESQUITERPEN	0,87	0,01	0,83	0,01
38,89	SESQUITERPEN	2,15	0,01	0,26	0,01
40,16	THYMOL	68,75	0,34	91,98	0,26
42,42	SESQUITERPEN	0,48	0,01	0,48	0,01
44,35	SESQUITERPEN	0,98	0,01	1,94	0,01
TOT		**740,59**	**14,14**	**741,62**	**14,7**
	ACID				
30,25	N-HEXANOIC ACID	1,18	0,01	9,12	0,03

32,89	ACID	0,63	0,01	0,73	0,01
35,25	ACID	0,38	0,01	0,31	0,01
37,43	ACID	ND	-	41,45	0,12
37,36	BENZENECARBOXYLIC ACID	41,42	0,21	0,26	0,01
37,61	BENZOIC ACID	1,65	0,01	1,79	0,01
38,93	ACID	4,17	0,02	4,78	0,01
41,71	HEXADECANOIC ACID	0,40	0,01	0,39	0,01
43,61	ACID	4,33	0,02	4,43	0,01
44,34	ACID	0,27	0,01	0,31	0,01
TOT		**54,43**	**0,31**	**63,57**	**0,23**
	OTHERS				
1,82	DIMETHYL SULFIDE	1,02	0,01	0,02	0,00

[a]= Retention time

[b]=means value of area under the peak (n=3)

[c]Arbitary unit = Area under the peak /1000000

[d]= Standard deviation

3.4.1 Newly induced volatiles in *Achillea collina*, under different stresses (biotic, mechanical and jasmonic acid treatments)

Newly induced volatiles in *Achillea collina* plants, when they were stressed with infestations, mechanical damages and jasmonic acid are shown in Fig.23. The volatiles were identified using the developed HS-SPME-GC/MS *in vivo* method as explained in (3.2.0.),(3.3.0) and (3.4.0) respectively. The compounds which are to be identified are indicated with corresponding retention time in GC/MS with respect to the class of compounds where they must be included.

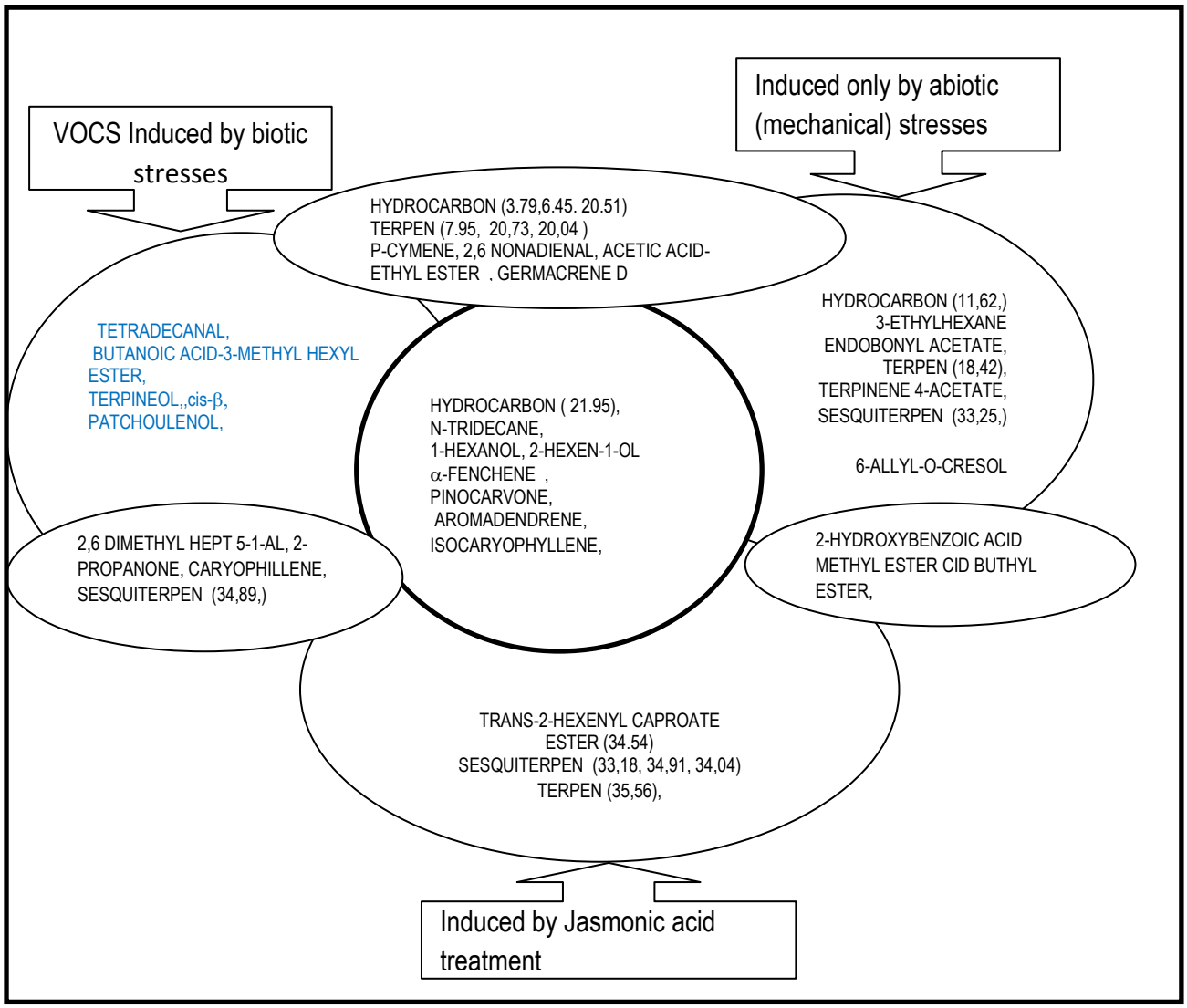

Fig. : 23 Induced volatile emissions of *Achillea collina* Becker ex Rchb. under different stressed conditions (biotic, mechanical and jasmonic acid treatments). The numbers represent the retention time of corresponding compound in GC/MS.

3.5.0 volatile emissions of yarrow *(Achillea collina*), pea(*Pisum sativum*) and peach(*Prunus persica*) after infestation by the generalist aphid *Myzus persicae.*

Yarrow, pea and peach plants were infested by the generalist aphids *Myzus persicae.* The volatile fingerprints of the plants in the different experimental conditions were obtained with the developed *in vivo* method of HS-SPME-GC/MS under optimized parameters. The volatile fingerprint of the chromatograms were individually identified using a data base and the results were reported in Table 04. The amount of each compound in the different experimental conditions were reported as an arbitrary value corresponding to the area under the peak /1000000.

Table 04 - volatile emissions of yarrow, pea and peach in control and infested by the generalist aphids *Myzus persicae*, conditions

R.T.[a]	Compound	Yarrow n=3		Yarrow-infested n=3		Pea n=3		Pea-infested n=3		Peach n=3		Peach-infested n=3	
		mean [bc]	SD[d]	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
	HYDROCARBONS												
1,37	PENTANE	1.3	0.01	8.7	0.06	ND	-	ND	-	21.99	1.56	24.12	0.17
1,54	HEPTANE	ND	-	ND	-	ND	-	0.55	0.01	ND	-	1.48	0.01
1,91	n-OCTANE	ND	-	ND	-	0.22	0.01	ND	-	0.59	0.07	0.65	0.01
2,47	1-OCTENE	1.95	0.01	24.4	3	29.66	1.63	0.84	0.01	48.2	3.9	39.63	0.28
2,79	n-NONANE	2.91	0.02	2.98	0.02	1.13	0.01	ND	-	1.14	0.01	1.6	0.01
3,16	3-METHYLOCTANE	1.83	0.01	1.39	0.01	0.47	0.01	ND	-	0.09	0.02	0.59	0.01
3,31	BENZENE	2.62	0.02	3.66	0.03	1.05	0.01	1.58	0.01	3.29	0.02	3.32	0.02
3,79	HYDROCARBON	ND	-	1.34	0.01	0.59	0.07	0.68	0.02	1.35	0.01	1.29	0.01
3,95	3-METHYLCYCLOOCTANE	2.47	0.02	3.21	0.02	0.72	0.01	0.87	0.01	0.57	0.01	1.29	0.01
4,01	3,5-DIMETHYLOCTANE	0.69	0.01	0.49	0.01	0.29	0.01	1.44	0.01	0.61	0.02	0.46	0.01
4,32	HYDROCARBON	0.2	0.01	0.28	0.02	ND	-	ND	-	ND	-	ND	-
4,49	α-TRIDECENE	0.64	0.07	0.77	0.01	ND	-	ND	-	ND	-	ND	-
4,64	HYDROCARBON	0.72	0.01	0.33	0.01	ND	-	ND	-	ND	-	ND	-
4,94	HYDROCARBON	4.98	0.04	3.84	0.03	3.06	0.02	ND	-	ND	-	ND	-
5,53	DECANE	12.59	0.02	2.5	0.02	ND	-	ND	-	ND	-	ND	-
6,45	HYDROCARBON	ND	-	1.84	0.01	32.63	0.23	ND	-	ND	-	ND	-
6,71	TOLUENE	1.34	0.01	40.39	0.29	0.51	0	0.09	0.02	61.72	1.85	0.81	0.01
6,87	HEXADECANE	1.17	0.01	1.43	0.01	ND	-	0.32	0.02	ND	-	ND	-
6,97	HYDROCARBON	1.28	0.01	1.83	0.01	0.54	0.01	0.32	0.02	1.1	0.01	0.74	0.01
7,14	HEPTADECANE	0.42	0.01	0.82	0.01	ND	-	0.4	0.02	ND	-	ND	-
8,09	HYDROCARBON	0.65	0.02	0.66	0.03	ND	-	ND	-	ND	-	ND	-
8,36	HYDROCARBON	0.35	0.06	0.47	0.04	ND	-	ND	-	ND	-	5.2	0.04

8,58	HYDROCARBON	5.54	0.05	3.78	0.03	ND	-	0.71	0.01	ND	-	ND	-
9,18	n-TRIDECANE	ND	-	4.42	0.03	3.69	0.03	2.36	0.02	ND	-	4.61	0.03
9,78	ETHYLBENZENE	ND	-	ND	-	ND	-	ND	-	ND	-	11.04	0.08
10,16	o–XYLENE	11.05	0.08	5.88	0.04	5.41	0.04	6.31	0.04	ND	-	14.2	0.1
10,34	m-XYLENE	1.62	0.01	4	0.03	ND	-	5.43	0.04	22.77	0.16	28.34	0.2
10,49	p-XYLENE	26.68	2.62	32.33	0.8	1.06	0.01	10.76	0.08	ND	-	5.32	0.04
11,29	HYDROCARBON	0.54	0.01	1.17	0.01	ND	-	ND	-	ND	-	ND	-
11,95	DODECANE	ND	-	0.6	0.01	ND	-	ND	-	ND	-	ND	-
13,82	n-PENTYLCYCLOHEXANE	4.22	0.03	1.25	0.01	0.83	0.01	0.8	0.01	ND	-	ND	-
14,34	n-PENTADECANE	2.37	0.02	2.42	0.02	ND	-	ND	-	ND	-	3.51	0.02
15,03	2-ISOPROPHYL-1-METHOXY-4-METHYLBENZENE	1.47	0.01	2.88	0.02	1.01	0.01	0.06	0.01	ND	-	ND	-
15,82	STYRENE	2.7	0.02	2.95	0.02	ND	-	ND	-	ND	-	ND	-
15,92	o-ETHYL-TOLUENE	8.65	0.06	5.09	0.04	3.64	0.03	3.78	0.03	9	0.21	19.87	0.14
16,56	1-ETHYL-2,4-DIMETHYLBENZENE	0.32	0	1.14	0.01	ND	-	ND	-	16.22	1.3	28.47	1.92
17,97	HYDROCARBON	2.14	0.02	3	0.02	2.57	0.02	1.12	0.01	ND	-	12.71	0.09
18,22	HYDROCARBON	27.95	0.2	18.7	0.13	7.03	0.05	5.67	0.04	60.22	1.84	ND	-
18,92	2-ETHYL-p-XYLENE	2.47	0.02	2.99	0.01	0.85	0.01	0.58	0.02	ND	-	0.29	0.02
19,07	HYDROCARBON	ND	-	ND	-	ND	-	1.26	0.01	ND	-	3.01	0.02
19,09	4-ETHYL-m-XYLENE	4.69	0.03	3.31	0.02	ND	-	ND	-	ND	-	ND	-
19,62	ISODURENE	1.55	0.01	1.21	0.14	ND	-	1.14	0.01	ND	-	ND	-
20,26	4-ETHYL-1,2-DIMETHYL-BENZENE	1.16	0.01	0.77	0.01	ND	-	ND	-	ND	-	ND	-
21,40	HYDROCARBON	3.74	0.03	ND	-	ND	-	ND	-	ND	-	ND	-
21,54	1,4-DIMETHYLBENZENE	4.83	0.03	0.62	0	4.12	0.03	5	0.04	2.74	0.02	10.69	1.49
21,95	HYDROCARBON	ND	-	0.63	0	ND	-	2.91	0.02	ND	-	ND	-
26,88	HYDROCARBON	3.65	0.03	3.4	0.02	3.53	0.03	1.88	0.01	3.94	0.03	ND	-
29,10	HYDROCARBON	1.66	0.01	0.16	0	0.19	0	1	0.01	ND	-	ND	-
30,31	TETRACOSANE	1.4	0.01	0.19	0.02	1.67	0.01	1.08	0.01	0.96	0.01	ND	-
TOT		**158.51**	**3.68**	**204.22**	**5.09**	**106.47**	**2.3**	**58.94**	**0.57**	**256.5**	**11.05**	**223.24**	**4.75**

	ALDEHYDES												
2,07	2 METHYLPROPANAL	16.42	0.12	8.02	0.06	ND	-	ND	-	ND	-	ND	-
3,04	2-METHYLBUTANAL	0.23	0.02	0.47	0.02	ND	-	ND	-	ND	-	ND	-
8,97	HEXANAL	5.99	0.04	0.56	0	ND	-	0.36	0.02	ND	-	ND	-
12,26	LAURALDEHYDE	2.56	0.02	1.73	0.01	ND	-	ND	-	ND	-	ND	-
15,30	trans-2-HEXENAL	11.54	0.08	9.48	0.07	4.23	0.03	4	0.03	ND	-	ND	-
18,86	2-HEPTENAL	3.59	0.03	0.55	0	0.93	0.01	0.56	0.01	1.47	0.01	ND	-
19,87	2,6-DIMETHYL-HEPT-5-1-AL	ND	-	1.49	0.01	5.88	0.04	7.98	0.06	19.06	0.14	4.56	0.03
19,89	ALDEHYDE	4.17	0.03	0.33	0.01	ND	-	ND	-	ND	-	12.17	0.09
20,98	2,4-HEXADIENAL	12.12	0.09	0.26	0.02	ND	-	ND	-	ND	-	ND	-
21,74	2-OCTENAL	4.38	0.03	0.59	0	4.06	0.03	4.96	0.04	2.94	0.02	4.83	0.03
21,81	ALDEHYDE	ND	-	7.75	0.06	ND	-	ND	-	ND	-	ND	-
21,96	p-MENTH-1-EN-9-AL	1.88	0.01	0.72	0.01	4.1	0.03	2.91	0.02	1.55	0.01	2.3	0.02
23,42	n-DECANAL	1.14	0.01	1.53	0.01	1.63	0.01	1.28	0.01	1.17	0.01	1.31	0.01
23,84	BENZALDEHYDE	2.25	0.02	2.83	0.02	1.28	0.01	1.38	0.01	ND	-	ND	-
25,35	2,6 NONADIENAL	ND	-	0.73	0.01	ND	-	1.38	0.01	ND	-	2.84	0.02
26,00	β-CYCLOCITRAL	1.26	0.01	0.23	0.02	2.78	0.02	2.73	0.02	ND	-	ND	-
30,54	ALDEHYDE	0.83	0.01	0.48	0.02	1.14	0.01	0.63	0.03	1.31	0.01	ND	-
32,82	TETRADECANAL	0	0	0.73	0.01	ND	-	0.73	0.01	ND	-	12.66	2.92
32,89	ALDEHYDE	0.67	0.03	1.33	0.01	0.68	0	0.73	0.01	ND	-	ND	-
35,57	ALDEHYDE	0.6	0	1.66	0.01	ND	-	ND	-	ND	-	ND	-
TOT		**69.63**	**0.55**	**41.47**	**0.38**	**26.71**	**0.19**	**29.63**	**0.28**	**27.5**	**0.2**	**40.67**	**3.12**
	ALCOHOLS												
7,34	DECANOL	3.66	0.03	4.9	0.03	1.49	0.01	1.1	0.01	2.33	0.02	2.97	0.02
12,06	CYCLOPENTADECANOL	1.75	0.01	0.39	0	ND	-	0.78	0.01	2.06	0.01	ND	ND
12,78	ETHYLHEXANOL	1.63	0.01	1.82	0.01	ND	-	ND	-	1.93	0.01	ND	ND
14,13	ALCOHOL	1.27	0.01	1.33	0.01	ND	-	ND	-	ND	-	6.05	0.04
20,01	1-HEXANOL	ND	-	4.59	0.03	ND	-	0.42	0.02	ND	-	2.38	0.02
20,19	CYCLOPENTADECANOL	1.9	0.01	1.5	0.01	5.52	0.04	4.2	0.03	7.52	0.05	5.33	0.04

20,76	3-HEXEN-1-OL	1.69	0.01	0.55	0.02	ND	-	ND	-	ND	-	ND	-
21,16	3-OCTANOL	0.67	0.02	0.43	0.03	ND	-	ND	-	ND	-	ND	-
21,36	2-HEXEN-1-OL	ND	-	3.6	0.03	3.9	0.03	9.09	0.06	6.3	0.04	3.3	0.02
22,48	1-OCTENE-3-OL	4.6	0.03	0.31	0.01	2.77	0.02	2.9	0.02	ND	-	ND	-
23,59	ALCOHOL	1.8	0.01	0.32	0.02	ND	-	ND	-	ND	-	ND	-
24,27	cis-9-OCTADECEN-1-OL	0.62	0.01	0.75	0.01	ND	-	ND	-	ND	-	ND	-
31,03	ALCOHOL	4.19	0.03	0.27	0.03	1.14	0.01	0.86	0.01	ND	-	ND	-
36,24	Z-9-HEXADECEN-1-OL	1.51	0.01	ND	-	ND	-	ND	-	ND	-	ND	-
TOT		**25.29**	**0.19**	**20.76**	**0.24**	**14.82**	**0.11**	**19.35**	**0.16**	**20.14**	**0.13**	**20.03**	**0.14**
	KETONES												
2,21	2- PROPANONE	ND	-	0.85	0.01	0.51	0	51.34	0.36	ND	-	1.41	0.01
12,95	OXACYCLODODECANE-2-ONE	ND	-	0.74	0.01	1.12	0.01	0.73	0.01	0.88	0.01	ND	-
19,22	2,3-OCTANEDIONE	3.78	0.03	2.3	0.02	1.21	0.01	1.13	0.01	2.09	0.01	2.35	0.02
19,43	6-METHYL-5-HEPTENE-2-ONE	5.56	0.04	2.8	0.02	2.04	0.01	1.59	0.01	ND	-	ND	-
21,51	KETONE	ND	-	ND	-	ND	-	ND	-	2.74	0.02	ND	-
26,75	KETONE	0.66	0.02	0.59	0.01	ND	-	ND	-	ND	-	ND	-
29,22	2–PENTANONE	1.62	0.01	2.82	0.02	1.9	0.01	1.12	0.01	ND	-	ND	-
31,49	KETONE	0.74	0.01	0.37	0	1.67	0.01	0.57	0.02	ND	-	ND	-
32,72	2H-2,4A-METHANONAPHTHALEN-8(5H)-ONE	1.31	0.01	1.79	0.01	ND	-	ND	-	ND	-	ND	-
33,18	KETONE	ND	-	ND	-	1.42	0.01	0.12	0.02	ND	-	ND	-
33,44	KETONE	1.44	0.01	0.65	0.06	2.28	0.02	0.97	0.01	ND	-	ND	-
33,73	KETONE	1.43	0.01	0.52	0.02	ND	-	ND	-	ND	-	ND	-
TOT		**16.54**	**0.14**	**13.43**	**0.18**	**12.15**	**0.08**	**57.57**	**0.45**	**5.71**	**0.04**	**3.76**	**0.03**
	ESTERS												
2,60	ACETIC ACID-ETHYL ESTER	ND	-	3.54	0.03	ND	-	ND	-	ND	-	ND	-
17,58	HEXYL ACETATE	0.66	0	6.94	0.05	4	4.62	0.23	0.02	ND	-	ND	-
17,80	2-PENTANOL,4-METHYL-ACETATE	5.36	0.04	0.76	0.01	ND	-	ND	-	5.29	0.04	ND	-
18,51	4-HEXENYL ACETATE	ND	-	ND	-	ND	-	ND	-	ND	-	67.59	3.32
20,51	PHENYLETHYLACETATE	ND	-	0.72	0.01	ND	-	ND	-	ND	-	ND	-

22,32	ISOVALERIC ACID, BUTYL ESTER	1.62	0.01	0.96	0.01	1.93	0.01	0.68	0.02	ND	-	ND	-
22,38	BUTANOIC ACID-3-METHYL HEXYL ESTER	ND	-	0.32	0.01	ND	-	0.2	0.02	ND	-	2.49	0.02
24,12	ESTER	1.01	0.01	1.88	0.01	0.79	0.01	0.79	0.01	0.94	0.01	1.33	0.01
25,28	ESTER	ND	-	0.95	0.01	ND	-	ND	-	ND	-	ND	-
28,40	ESTER	4.51	0.03	0.65	0.06	7.39	0.05	0.26	0.01	ND	-	ND	-
30,37	ESTER	ND	-	0.29	0.02	ND	-	ND	-	ND	-	ND	-
31,83	LAURIC ACID ETHYL ESTER	ND	-	0.8	0.01	ND	ND	ND	ND	3.81	0.03	ND	-
34,48	ESTER	ND	-	0.32	0.02	ND	-	ND	-	ND	-	ND	-
43,1	PHTHALIC ACID,BUTYL 2-ETHYLHEXYL ESTER	0.65	0.03	ND	-	ND	-	ND	-	ND	-	ND	-
TOT		**13.81**	**0.12**	**18.13**	**0.25**	**14.11**	**4.69**	**2.16**	**0.08**	**10.04**	**0.08**	**71.41**	**3.35**
	FURANS												
4,17	2-ETHYLFURAN	ND	-	0.42	0.01	ND	-	ND	-	ND	-	0.82	0.01
4,71	2-METHYLFURAN	4.42	0.03	4.85	0.01	0.55	0.01	2.7	0.02	ND	-	ND	-
TOT		**4.42**	**0.03**	**5.27**	**0.02**	**0.55**	**0.01**	**2.7**	**0.02**	**0**	**0**	**0.82**	**0.01**
	TERPENES												
5,33	TERPEN	14.02	0.1	96.38	18.27	ND	-	ND	-	1.21	0.01	ND	-
6,01	α-PINENE	80.5	2.04	91.68	1.64	ND	-	1.71	0.01	ND	-	ND	-
6,33	α-PHELLANDRENE	1.54	0.01	9.47	0.07	ND	-	0.36	0.02	ND	-	1.82	0.01
7,47	α-FENCHENE	ND	-	2.44	0.02	1.01	0.01	0.9	0.01	2.3	0.02	2.53	0.02
7,76	CAMPHENE	2.04	0.01	1.43	0.14	0.95	0.01	0.94	0.01	ND	-	ND	-
7,95	TERPEN	ND	-	0.62	0.02	ND	-	ND	-	ND	-	ND	-
9,53	γ-TERPINEN	6.34	0.05	4.01	0.03	ND	-	ND	-	ND	-	4.25	0.03
9,91	β-PINENE	15.94	0.13	12.41	0.07	6.1	0.04	1.33	0.01	ND	-	ND	-
10,62	TERPEN	ND	-	ND	-	ND	-	3.35	0.02	ND	-	ND	-
10,80	β-PHELLANDRENE	1.25	0.01	10.35	0.07	ND	-	ND	-	ND	-	ND	-
10,91	TERPEN	1.39	0.08	2.63	0.02	ND	-	ND	-	ND	-	ND	-
11,66	TERPEN	0.24	0.02	9.45	0.07	ND	-	0.75	0.01	ND	-	ND	-
12,53	DIMETHYLFULVENE	13.88	0.1	10.28	0.07	4.66	0.03	1.18	0.01	14.64	1.52	12.1	0.09

13,20	TERPEN	ND	-	ND	-	ND	-	ND	-	10.46	1.49	ND	-
13,32	TERPEN	ND	-	ND	-	ND	-	1.63	0.01	ND	-	7.02	0.05
13,36	TERPINOLEN	16.8	0.12	12.96	0.02	ND	-	ND	-	ND	-	4.22	0.03
13,37	β-MYRCENE	2.88	0.02	3.37	0.02	2.85	0.02	0.88	0.01	ND	-	2.76	0.02
13,66	TERPEN	1.33	0.01	1.02	0.01	ND	-	ND	-	ND	-	ND	-
14,50	D-LIMONENE	13.52	0.1	26.03	2.87	2.87	0.02	2.95	0.02	ND	-	9.18	0.07
14,78	EUCALYPTOL	17.56	0.12	22.52	0.16	ND	-	ND	-	2.16	0.02	22.62	0.16
15,16	CUMENE	ND	-	ND	-	ND	-	ND	-	5.75	0.18	0.97	0.01
16,36	TERPEN	6.35	0.05	5.74	0.04	ND	-	ND	-	ND	-	ND	-
16,46	γ –TERPINENE	29.18	0.21	22.5	0.16	3.68	0.03	2.77	0.02	ND	-	ND	-
16,90	γ–PINENE	34.5	0.98	22.55	2.82	15.66	0.11	11.51	0.08	ND	-	ND	-
17,27	o-CYMENE	1.92	0.01	4.4	0.03	2.88	0.02	1.96	0.01	ND	-	2.79	0.02
17,46	TERPEN	11.51	0.08	0.67	0.02	7.3	0.05	4.93	0.04	ND	-	7.38	0.05
17,71	TERPEN	1.26	0.01	1.33	0.28	ND	-	ND	-	ND	-	ND	-
18,30	TERPEN	8.79	0.06	25.63	3.01	3.31	0.02	2.94	0.02	ND	-	4.84	0.03
18,55	p-CYMENE	ND	-	2.02	0.01	ND	-	1.49	0.01	ND	-	3.04	0.02
20,73	TERPEN	ND	-	0.51	0	2.22	0.02	1.13	0.01	7.91	0.06	0.1	0.01
21,03	TERPEN	ND	-	0.38	0.03	10.32	0.07	9.09	0.06	0.53	0.01	ND	ND
21,48	TERPEN	24.58	0.17	24.58	0.17	ND	-	ND	-	ND	-	ND	-
22,19	TERPEN	3.37	0.02	0.8	0.01	ND	-	ND	-	ND	-	1.79	0.01
22,53	β-COPAENE	ND	-	ND	-	ND	-	ND	-	ND	-	46.37	3.91
22,59	α-CUBEBENE	6.38	0.05	15.37	0.11	ND	-	ND	-	10.06	0.07	0.94	0.01
22,72	trans-SABINENE	3.77	0.03	5.47	0.04	64.97	0.46	9.21	0.07	8.56	0.06	5.19	0.04
22,97	γ-ELEMENE	2.69	0.02	1.31	0.01	5.56	0.04	1.15	0.01	ND	-	ND	-
23,01	TERPEN	3.08	0.02	2.35	0.02	ND	-	1.26	0.01	2.83	0.02	2.47	0.02
23,13	α-YLANGENE	5.17	0.04	1.89	0.01	ND	-	ND	-	0.95	0.01	2.21	0.02
23,33	α-COPAENE	2.62	0.02	1.63	0.01	ND	-	1.85	0.01	3.88	0.03	3.12	0.02
23,63	CAMPHOR	2.69	0.02	1.92	0.01	2.13	0.02	2.88	0.02	ND	-	4.14	0.03
23,94	TERPEN	1.6	0.01	1.74	0.01	ND	-	ND	-	ND	-	ND	-
24,04	TERPEN	ND	-	0.16	0.01	ND	-	ND	-	ND	-	ND	-

24,24	VALENCENE	0.85	0.01	0.42	0.02	ND	-	ND	-	1.97	0.01	1.56	0.01
24,40	TERPEN	0.56	0	0.44	0	0.47	0	5.44	0.04	ND	-	ND	-
24,52	TERPEN	ND	-	ND	-	1.41	0.01	1.06	0.01	ND	-	ND	-
24,59	cis-β–TERPINEOL	ND	-	0.74	0.01	ND	-	0.42	0.02	ND	-	1.3	0.01
24,65	LINALOOL	0.44	0.03	0.4	0.03	ND	-	ND	-	ND	-	ND	-
24,79	TERPENE	0.8	0.01	0.19	0.02	0.85	0.01	0.85	0.01	ND	-	3.07	0.02
24,87	PINOCARVONE	ND	-	0.66	0.04	ND	-	0.28	0.02	ND	-	ND	-
25,19	β-CUBEBENE	0.8	0.01	1.24	0.01	1.23	0.01	0.6	0.01	1.97	0.01	ND	-
25,45	SESQUITERPEN	1.34	0.01	0.74	0.01	1.41	0.01	0.58	0.02	ND	-	6.73	0.05
25,60	β-CARYOPHYLLENE	1.35	0.01	0.4	0.04	0.1	0	0.8	0.01	ND	-	ND	-
25,69	SESQUITERPENE	2.56	0.02	4.22	0.03	3.11	0.02	2.67	0.02	ND	-	ND	-
25,84	AROMADENDRENE	ND	-	0.19	0.01	ND	-	0.33	0.01	3.75	0.03	0.96	0.01
26,35	ISOLEDENE	0.37	0.05	0.49	0.01	ND	-	ND	-	ND	-	ND	-
26,51	τ-CADINENE	1.2	0.01	0.54	0.05	ND	-	ND	-	ND	-	ND	-
26,79	TERPINENE-4-ACETATE	ND	-	0.25	0.03	ND	-	ND	-	ND	-	ND	-
27,07	α–HUMULENE	2.43	0.02	3.56	0.03	1.68	0.01	0.8	0.01	ND	-	ND	-
27,10	SESQUITERPEN	0.93	0.01	ND	-	0.99	0.01	1.09	0.01	ND	-	ND	-
27,17	trans-β-FARNESENE	1.18	0.01	1.03	0.01	0.34	0	0.22	0.02	ND	-	ND	-
27,23	TERPEN	ND	-	ND	-	1.63	0.01	0.81	0.01	1.52	0.01	ND	ND
27,40	TERPEN	1.68	0.01	1.12	0.01	7.34	0.05	5.44	0.04	6.8	0.05	ND	-
27,52	α-AMORPHENE	0.94	0.01	0.51	0.03	0.11	0	0.42	0.01	ND	-	ND	-
27,61	α-TERPINEOL	1.11	0.01	0.83	0.01	ND	-	0.28	0.02	ND	-	ND	-
27,91	CAMAZULENE	26.82	1.53	42.27	1.5	ND	-	ND	-	ND	-	ND	-
27,93	SESQUITERPEN	0.82	0.01	0.94	0.01	ND	-	0.4	0.02	ND	-	ND	-
28,06	TERPEN	0.78	0.01	0.95	0.01	ND	-	ND	-	ND	-	ND	-
28,14	ISOCARYOPHILLENE	ND	-	5.89	0.04	ND	-	0.6	0.01	ND	-	ND	-
28,34	α-BERGAMOTENE	2.06	0.01	2.26	0.28	ND	-	ND	-	ND	-	ND	-
28,55	D-GERMACRENE	ND	-	0.36	0.04	ND	-	ND	-	ND	-	ND	-
28,74	α-FARNESENE	1.15	0.01	0.62	0.06	ND	-	ND	-	ND	-	ND	-
28,85	α-CADINENE	0.99	0.01	0.06	0	2.01	0.01	0.88	0.01	ND	-	ND	-

29,04	SESQUIPHELLANDRENE	1.89	0.01	0.14	0.02	ND	-	ND	-	ND	-	9.69	0.07
29,32	TERPEN	1.16	0.01	0.34	0.03	0.74	0.08	0.53	0.01	ND	-	ND	-
29,47	NAPTHALENE	3.97	0.03	1.27	0.01	ND	-	ND	-	ND	-	ND	-
29,52	α-METHYLNAPHTHALENE	ND	-	3.76	0.03	3.61	0.03	2.7	0.02	ND	-	ND	-
29,71	SESQUITERPEN	2.13	0.02	2.09	0.01	ND	-	ND	-	ND	-	ND	-
29,79	SESQUITERPEN	1.34	0.01	0.47	0.05	ND	-	ND	-	ND	-	ND	-
29,90	SESQUITERPEN	5.89	0.04	1.81	0.01	2.59	0.02	4.26	0.03	5.95	0.04	ND	-
30,10	SESQUITERPEN	3.47	0.02	1.96	0.01	1.8	0.01	1.13	0.01	2.51	0.02	ND	-
30,47	TERPEN	ND	-	0.12	0	ND	-	ND	-	ND	-	ND	-
30,71	TERPEN	0.43	0.03	0.3	0.02	ND	-	ND	-	ND	-	ND	-
31,06	SESQUITERPEN	1.11	0.01	1.23	0.28	ND	-	ND	-	ND	-	ND	-
31,17	SESQUITERPEN	ND	-	2.8	0.02	1	0.01	1.05	0.01	ND	-	ND	-
31,30	α–PATCHOULENOL	ND	-	0.75	0.01	ND	-	1.2	0.01	ND	-	2.55	0.21
31,79	β-IONONE	0.69	0.02	1.35	0.01	ND	-	ND	-	ND	-	ND	-
32,27	SESQUITERPEN	1.52	0.01	0.83	0.01	ND	-	ND	-	ND	-	ND	-
32,33	CARYOPHYLLENE OXIDE	ND	-	1.29	0.01	ND	-	ND	-	ND	-	ND	-
32,47	PHENOL	0.84	0.01	0.45	0.05	ND	-	ND	-	ND	-	ND	-
32,56	5,6-EPOXIDE–β-IONON	2.61	0.02	2.02	0.01	2.72	0.02	2.99	0.02	2.96	0.02	ND	-
32,76	SESQUITERPEN	0.44	0.05	1.13	0.01	ND	-	ND	-	ND	-	ND	-
33,00	CUBENOL	0.58	0	0.59	0.02	ND	-	ND	-	ND	-	ND	-
33,07	SESQUITERPEN	1.37	0.01	1.81	0.01	1.29	0.01	0.81	0.01	ND	-	ND	-
33,91	SESQUITERPEN	0.36	0.04	0.27	0.03	ND	-	ND	-	ND	-	ND	-
34,06	6-ALLYL-O-CRESOL	ND	-	1.84	0.01	1.72	0.01	11.84	0.08	2.21	0.02	1.69	0.01
34,21	SESQUITERPEN	1.31	0.01	0.46	0	ND	-	ND	-	ND	-	ND	-
34,26	CARVACROL	1.51	0.01	0.15	0.03	ND	-	ND	-	ND	-	ND	-
34,54	SESQUITERPEN	ND	-	0.46	0.05	ND	-	ND	-	ND	-	ND	-
34,72	SESQUITERPEN	1.03	0.01	0.15	0.02	0.63	0.04	0.92	0.01	ND	-	1.87	0.01
34,89	SESQUITERPEN	ND	-	0.56	0.03	0.59	0.01	0.36	0	ND	-	ND	-
35,83	SESQUITERPEN	0.83	0.01	0.17	0.2	0.78	0.01	0.59	0.02	ND	-	ND	-
38,89	SESQUITERPEN	1.18	0.01	3.7	0.03	ND	-	ND	-	ND	-	ND	-

40,16	THYMOL	0.67	0.03	0.68	0.02	ND	-	0.68	0.01	ND	-	ND	-
42,42	SESQUITERPEN	0.47	0.02	0.45	0.02	ND	-	ND	-	ND	-	ND	-
44,35	SESQUITERPEN	1.44	0.01	3.19	0.02	ND	-	ND	-	ND	-	ND	-
44,85	SESQUITERPEN	ND	-	0.33	0.02	ND	-	ND	-	ND	-	ND	-
TOT		**428.09**	**6.98**	**570.24**	**33.83**	**176.75**	**1.37**	**121.18**	**1.08**	**100.88**	**3.71**	**181.27**	**5.08**
	ACIDS												
21,36	ETHYLIC ACID	ND	-	ND	-	ND	-	ND	-	6.3	0.04	ND	-
30,25	HEXANOIC ACID	1.31	0.01	1.41	0.01	2.01	0.01	2.02	0.01	ND	-	ND	-
31,34	ACID	ND	-	0.81	0.01	1.28	0.01	0.03	0	2.22	0.02	3.22	0.02
33,25	ACID	ND	-	0.22	0.03	0.43	0.03	0.6	0.01	1.57	0.01	ND	-
33,50	ACID	1.75	0.01	1.56	0.01	3.94	0.03	2.26	0.02	3.5	0.02	3.69	0.03
34,6	DECANOIC ACID	0.47	0.02	0.44	0.05	1.64	0.01	0.17	0	ND	-	6.44	0.05
35,27	ACID	0.34	0.03	0.36	0.03	ND	-	ND	-	ND	-	ND	-
35,96	ARACHIDIC ACIDS	ND	-	ND	-	0.45	0	0.42	0.01	ND	-	ND	-
37,61	BENZOIC ACID	1.7	0.01	1.44	0.01	ND	-	ND	-	ND	-	ND	-
37,21	ACID	ND	-	ND	-	ND	-	ND	-	738.23	19.39	739.73	21.51
37,36	BENZENECARBOXYLIC ACID	0.3	0.02	0.56	0.02	ND	-	ND	-	ND	-	ND	-
37,73	DODECANOIC ACID	ND	-	ND	-	1.58	0.01	0.74	0.01	ND	-	4.49	0.03
38,93	ACID	4.43	0.03	6.88	0.05	ND	-	1.3	0.01	ND	-	2.17	0.02
39,33	ACID	ND	-	ND	-	ND	-	ND	-	127.03	13.24	74.43	6.19
39,59	ACID	ND	-	0.56	0.06	ND	-	ND	-	ND	-	ND	-
40,52	ACID	0.35	0.02	ND	-	0.56	0.02	1.55	0.01	ND	-	ND	-
41,71	HEXADECANOIC ACID	0.38	0.02	0.3	0.02	ND	-	ND	-	ND	-	ND	-
43,61	ACID	4.34	0.03	1.61	0.01	ND	-	ND	-	ND	-	4.85	0.03
44,34	ACID	0.28	0.02	4.11	0.03	ND	-	ND	-	48.93	1.76	ND	-
45,76	9-OCTADECENOIC ACID	ND	-	ND	-	ND	-	ND	-	184.15	12.83	363.43	2.58
TOT		**15.65**	**0.22**	**20.26**	**0.34**	**11.89**	**0.12**	**9.09**	**0.08**	**1111.93**	**47.31**	**1202.45**	**30.46**
	OTHERS												

21,28	UNKNOWN	ND	-	ND	-	12.35	0.09	ND	-	ND	-	3.3	0.02
21,00	UNKNOWN	ND	-	13.46	1.44	ND	ND	9.09	0.06	ND	ND	9.76	0.21
		0	**0**	**13.46**	**1.44**	**12.35**	**0.09**	**9.09**	**0.06**	**0**	**0**	**13.06**	**0.23**

[a]= Retention time

[b]=means value of area under the peak (n=3)

[c]Arbitary unit = Area under the peak /1000000

[d]= Standard deviation

3.5.1 Newly induced volatiles in *Achillea collina*, of yarrow (*Achillea collina)*, pea (*Pisum sativum*) and peach(*Prunus persica*) after infestation by the generalist aphid *Myzus persicae*

Newly induced volatiles in yarrow *(Achillea collina)*, pea *(Pisum sativum)* and peach*(Prunus persica)* plants, when they were stressed with infestations of the aphid *Myzus persicae* are shown in Fig.24. The volatiles were identified using the developed HS-SPME-GC/MS *in vivo* method as explained in (3.5.0.). The compounds which are to be identified are indicated with corresponding retention time in GC/MS with respect to the class of compounds, where they must be included.

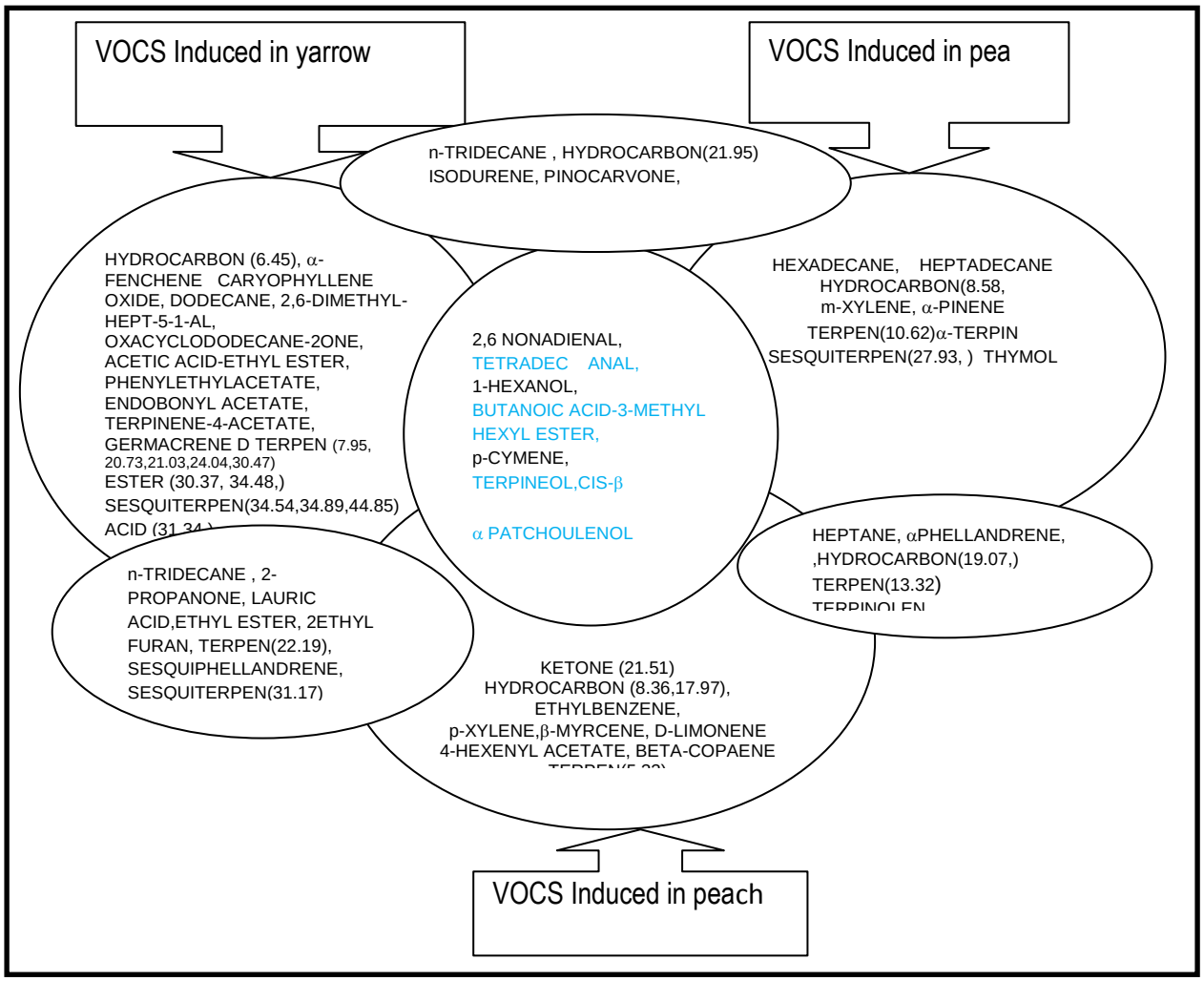

Fig. 24: The distribution of the newly induced volatiles of yarrow, pea and peach plants when infested by the generalist aphid *Myzus persica.* The numbers represent the retention time of corresponding compound in GC/MS.

3.6.0 *In vitro* study protein extracts from infested leaves of *Achillea collina* on transformation of farnesylpyrophosphate

Total protein extracts from leaves of control and infested by the generalist aphid *Myzus persicae* yarrow plants after adding 1μM of FDPP farnesylpyrophosphate) were analyzed with the developed *in vitro* method. The volatile fingerprint of the chromatograms were individually identified using a data base and the results were reported in Table 05. The amount of each compound was reported as an arbitrary value corresponding to the area under the peak /1000000.

Table- 05 Volatile organic compounds found in the head space of total protein extracts from the infested and control *A. collina* leaves after adding 1μM of FDPP (farnesylpyrophosphate).

		Control n=3	**Control n=3**	**Infested n=3**	**Infested n=3**
R.T.[a]	compounds	**mean** [bc]	**SD**[d]	**mean**	**SD**
	HYDROCARBONS				
1,37	PENTANE	2510,90	12,62	4201,25	56,29
1,62	1,4-PENTADIENE	ND	-	21,87	4.92
2,47	1-OCTENE	2,84	0,01	249,90	41,46
3,16	3-METHYLOCTANE	3,15	0,02	7,95	0.04
3,95	3-METHYLCYCLOOCTANE	658,81	3,31	36,70	2,19
4,20	HYDROCARBON	ND	-	13,53	11,04
4,32	HYDROCARBON	ND	-	83,39	2.43
4,64	HYDROCARBON	ND	-	108,83	88,86
4,94	HYDROCARBON	ND	-	74,41	3,89
5,53	DECANE	1,21	0,01	1,38	0.06
6,71	TOLUENE	17,89	0,09	1,06	0,87
8,09	HYDROCARBON	ND	-	1,23	0,06
8,36	HYDROCARBON	ND	-	16,95	1,09
8,58	HYDROCARBON	ND	-	103,13	24,36
10,34	m-XYLENE	ND	-	221,58	1,11
11,29	HYDROCARBON	ND	-	2,46	0,01
13,82	n-PENTYLCYCLOHEXANE	ND	-	94.40	3,99
17,97	HYDROCARBON	ND	-	6,71	0.13
18,41	HYDROCARBON	29,41	0,15	16,11	0,42
21,95	HYDROCARBON	ND	-	41.13	1,06
30,10	HYDROCARBON	ND	-	11,52	0,56

TOT		**3224,21**	**16,21**	**5179,96**	**237,26**
	ALDEHYDES				
2,07	2-METHYLPROPANAL	ND	-	255,17	51,53
3,04	2-METHYLBUTANAL	4,32	0,02	423,09	52,38
8,97	HEXANAL	16,23	0,08	149,12	58,18
12,26	LAURALDEHYDE	0,35	0,00	14,38	0,57
18,86	2-HEPTENAL	0,05	0,00	14,68	0,07
19,87	2,6-DIMETHYL-HEPT-5-1-AL	ND	-	973,07	4,89
19,89	ALDEHYDE	18,32	0,09	26,03	5,16
20,98	2,4-HEXADIENAL	ND	-	229,32	11,20
21,74	2-OCTENAL	ND	-	55,30	1,28
23,42	n-DECANAL	ND	-	527,88	2,65
23,84	BENZALDEHYDE	4,06	0,02	161,12	131,56
25,35	2,6-NONADIENAL	ND	-	396,32	1,99
26,00	β-CYCLOCITRAL	0,03	0,00	76,54	5,41
30,54	ALDEHYDE	1,61	0,01	17,49	0,09
32,82	TETRADECANAL	ND	-	17166,46	86,26
TOT		**44,97**	**0,22**	**20485,97**	**413,22**
	ALCOHOLS				
7,34	DECANOL	ND	-	8,23	0,19
12,06	CYCLOPENTADECANOL	ND	-	16,00	13,06
12,78	ETHYLHEXANOL	ND	-	12,68	2,07
14,13	9-TETRADECEN-1-OL	ND	-	36,26	5,71
20,01	1-HEXANOL	ND	-	11,42	0,56
20,76	3-HEXEN-1-OL	37,17	0,19	5497,70	27,63
21,36	2-HEXEN-1-OL	ND	ND	32,12	0,66
22,48	1-OCTENE-3-OL	10,24	0,05	ND	-
TOT		**47,41**	**0,24**	**5614,41**	**49,88**
	KETONES				
2,21	2- PROPANONE	ND	-	206799.37	1139,70
16,79	3-OCTANONE	ND	-	6,55	0,49
19,22	2,3-OCTANEDIONE	ND	-	39,29	0.20
19,43	6-METHYL-5-HEPTENE-2-ONE	ND	-	434,53	32,33
26,75	KETONE	6,07	0,03	37,14	0,19
29,22	2-PENTANONE	0,07	0,00	ND	-
33,73	KETONE	ND	-	1,29	0,14
TOT		**6,14**	**0,03**	**518,8**	**1172,85**
	ESTERS				
2,92	ACETIC ACID-2-METHYL ESTER	ND	-	1816,16	40,62
17,58	HEXYL ACETATE	ND	-	15,11	1,58
17,80	2-PENTANOL-4-METHYL-ACETATE	ND	-	6,11	1,09

22,38	BUTANOIC ACID-3-METHYL HEXYL ESTER	ND	-	2047,59	10,29
24,12	ESTER	0,01	0,00	57,83	1,79
25,28	ESTER	ND	-	137,56	3,71
28,96	2-HYDROXYBENZOIC ACID METHYL ESTER	ND	-	33,07	27,00
TOT		**0,01**	**0**	**4113,43**	**86,08**
	FURANS				
4,17	2-ETHYLFURAN	ND	-	11176,65	6373,60
4,71	2-METHYLFURAN	ND	-	142,29	139,44
TOT		**0**	**0**	**11318,94**	**6513,04**
	TERPENES				
6,01	α-PINENE	1,64	0,01	101,59	0,51
6,33	α-PHELLANDRENE	0,05	0,00	20,60	16,82
7,47	α-FENCHENE	ND	-	129,68	24,14
7,76	CAMPHENE	0,02	0,00	0,71	0,06
9,91	β-PINENE	6,08	0,03	72,13	1,87
10,80	β-PHELLANDRENE	0,86	0,00	4,68	3,82
11,66	TERPENE	0,83	0,00	52,39	51,34
13,37	β-MYRCENE	0,02	0,00	97,08	80,50
14,50	D-LIMONENE	2,22	0,01	5,44	0,08
14,78	EUCALYPTOL	56,72	0,29	3,36	0,12
16,36	TERPEN	0,04	0,00	33,26	2,18
16,46	γ-TERPINENE	0,01	0,00	9,55	1,05
16,90	γ -PINENE	0,02	0,00	2,66	2,61
17,27	o-CYMENE	0,08	0,00	6,32	6,20
17,46	TERPEN	ND	-	6,66	0,59
17,71	TERPEN	0,02	0,00	6,23	1,04
22,59	α-CUBEBENE	5,49	0,03	830,69	9,20
22,72	trans-SABINENE	0,07	0,00	12,70	2,13
22,97	γ-ELEMENE	3,90	0,02	3,67	3,60
23,13	α-YLANGENE	0,02	0,00	3,64	3,57
23,33	α-COPAENE	1,09	0,01	86,35	70,50
23,63	CAMPHOR	34,00	0,17	1,12	0,01
23,94	TERPEN	1,47	0,01	1,68	0,01
24,04	TERPEN	ND	-	41,39	0,79
24,24	VALENCENE	5,97	0,03	13,47	0,42
24,40	TERPEN	3,48	0,02	238,11	1,20
24,59	cis-β–TERPINEOL	ND	-	303,26	1,52
24,65	LINALOOL	0,01	0,00	72,48	1,86
24,87	PINOCARVONE	ND	-	25,64	5,59
25,19	β-CUBEBENE	0,05	0,00	3,62	3,02
25,45	SESQUITERPENE	0,02	0,00	9,99	0,05
25,60	β-CARYOPHYLLENE	0,04	0,00	54,63	44,79

25,69	SESQUITERPENE	3,13	0,02	33,18	2,18
25,84	AROMADENDRENE	ND	-	12,98	2,08
26,35	ISOLEDENE	0,08	0,00	5,54	1,49
26,51	τ-CADINENE	2,44	0,01	41,02	0,71
27,07	α–HUMULENE	0,01	0,00	26,46	0,13
27,10	SESQUITERPENE	0,07	0,00	49,44	0,75
27,17	trans-β-FARNESENE	1,69	0,01	34,03	5,30
27,40	TERPEN	8,87	0,04	75,12	0,38
27,52	α-AMORPHENE	5,40	0,03	ND	-
27,61	α-TERPINEOL	3,60	0,02	63,70	1,33
27,91	CAMAZULENE	1,92	0,01	45,52	0,23
27,93	SESQUITERPEN	ND	-	11,45	9,34
28,14	ISOCARYOPHILLENE	1,67	0,01	636,69	33,35
28,34	α-BERGAMOTENE	0,26	0,00	6,49	0,33
28,55	D-GERMACRENE	ND	-	769,38	3,89
28,74	α-FARNESENE	1,65	0,01	35,37	0,18
28,85	α-CADINENE	1,52	0,01	18,84	15,39
29,04	SESQUIPHELLANDRENE	0,01	0,00	1,22	0,41
29,32	TERPEN	0,05	0,00	111,04	9,49
29,47	NAPTHALENE	0,02	0,00	25,45	0,13
29,79	TERPEN	ND	-	12,12	2,07
29,90	SESQUITERPEN	ND	-	28,77	0,14
31,03	TERPEN	ND	-	33,09	2,93
31,30	α–PATCHOULENOL	ND	-	612,06	8,10
31,79	β-IONONE	0,01	0,00	54,27	0,27
32,27	SESQUITERPEN	0,05	0,00	102,03	5,54
32,33	CARYOPHYLLENE OXIDE	ND	-	758,78	618,74
32,47	PHENOL	7,29	0,04	5,92	5,80
32,76	SESQUITERPEN	342,84	1,72	62,91	2,83
32,89	SESQUITERPEN	0,01	0,00	22,15	18,09
33,00	CUBENOL	0,03	0,00	35,42	2,69
33,69	SPATHULENOL	ND	-	24,54	0,12
33,91	SESQUITERPEN	21,34	0,11	92,76	1,97
34,26	CARVACROL	ND	-	6,49	0,03
34,72	SESQUITERPEN	ND	-	21,24	0,61
TOT		**528,18**	**2,67**	**6130,25**	**1098,21**
30,25	n-HEXANOIC ACID	0,97	0,00	7,90	1,45
34,21	METHYLTESTOSTERONE	ND	-	7,55	0,29
34,91	ACID	ND	-	33,21	2,30
35,27	ACID	ND	-	5,45	0,43
TOT		**0,97**	**0**	**54,11**	**4,47**
	OTHERS				
1,82	UNKNOWN	ND	-	359,80	19,40

21,00	UNKNOWN	ND	-	1345,01	57,01

[a]= Retention time

[b]=means value of area under the peak (n=3)

[c]Arbitary unit = Area under the peak /1000000

[d]= Standard deviation

3.6.1 Most significant ten compounds in the *In vitro* study of total protein extracts from infested leaves of *Achillea collina* on transformation of farnesylpyrophosphate

The most significant 10 compound found in the *in vitro* study with the total protein extracts from *Achillea collina* leaves of control and infested by the generalist aphid *Myzus persicae*, were reported as the percentage of total volatile emitted by the control and infested conditions. Fig.25 is shown the distribution of the compounds between the control and infested plant extracts.

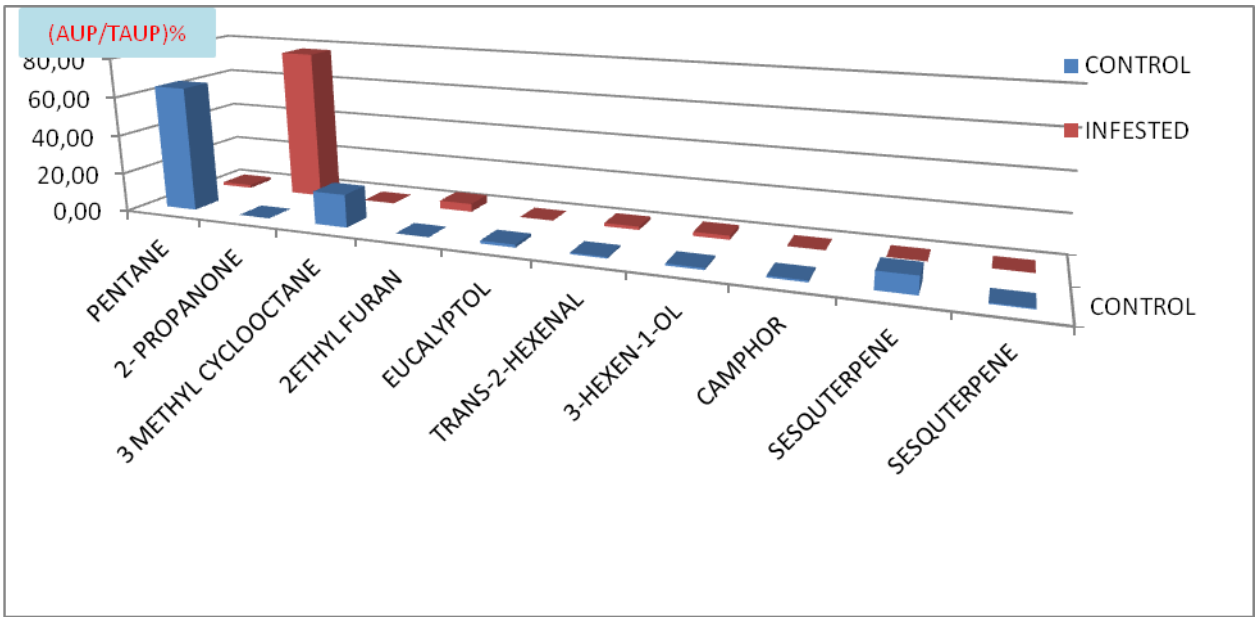

Fig. 25: The volatile emissions of most significant ten compounds in the protein extractions of infested and control plant leaves of *Achillea collina.*

3.7.0 Expression analysis of *patchoulol synthase* gene

A preliminary RT- PCR expression analysis was conducted. Since there were no literature data regarding the stress response genes in *Achillea collina* we used the *Pogostemon cablin, patchoulol synthase* gene sequence (DQ355151; Line et al., 2010) to design specific primers with the aim to detect the *patchoulol synthase* expression levels in *A. collina* infested plants vs control. The data obtained by RT-PCR, showed that *patchoulol synthase* gene expression was present in *A.collina* infested plant leaves (250bp) with respect to the control as shown in Fig.26.

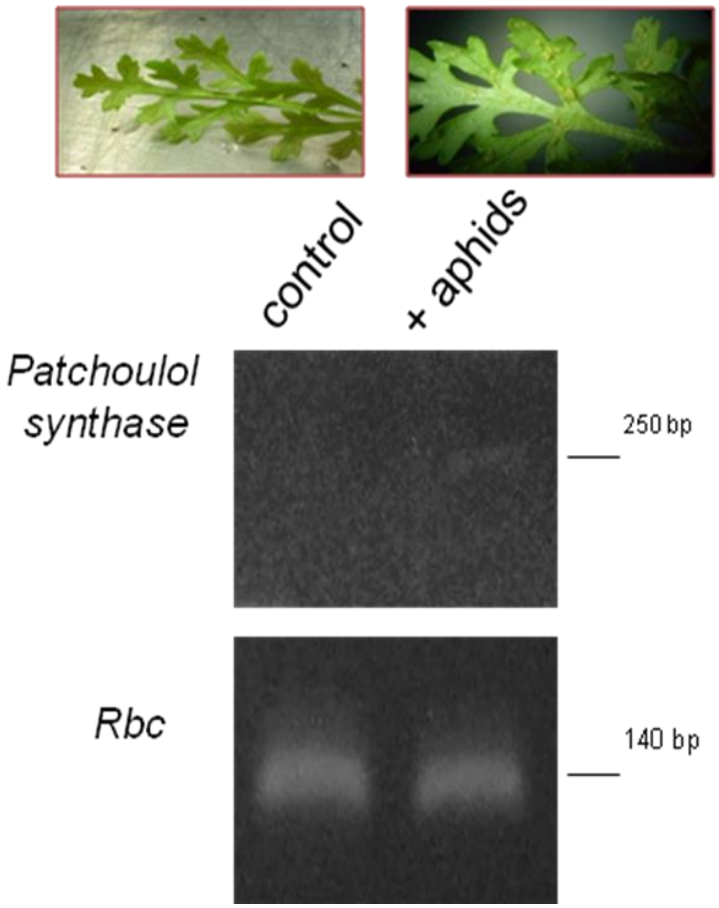

Fig. 26: RT-PCR analysis showing the expression of *patchoulol synthase* gene in *Achillea collina* infested plant while it was not present in control plant leaves.

4.DISCUSSION

The important physiological, ecological and agronomic roles of the interaction between plants and insect (Prado et al., 2007; Mooney et al., 2010) addressed my interest in the last part of my PhD activity in which I focused my studies on the effect of phloem feeder aphids on *Achillea collina*, a medicinal plant rich in bioactive volatile organic compounds (Giorgi et al., 2010a). In particular, the attention was addressed to evaluate the volatile compounds changings induced by a generalist and a specialist aphid species infestation, as compared to the normal volatile compounds fingerprint of *A. collina*,. Moreover a comparison with the volatiles fingerprint caused by mechanical damage provoked by two different of mechanical injuries.

The aphid species *Myzus persicae* (Sulzer), was used as the generalist aphid considering that it infests over than 40 different plant species. It has the ability to transmit 182 plant viruses including plum pox virus (Chan et al., 1991), while *Macrosiphoniella millefolii* (De Geer), and was used as the specialist aphids.

Aphids are typically considered as phloem-feeding insects with stylets which penetrate throughout different intercellular layers of the crop tissues, injecting saliva and causing necrosis, toxicosis and viruses transmission (Miles, 1999), causing economically destructive damages (Price et al., 1980). Aphids influence on plant growth have been studied and, commonly, a decrease in growth occurs depending on water loss, reduction of long term transport of mineral nutrient and sugar (Nanayakkarawasam et al, 2011a; Shannag et al, 2007). Johnson et al., (2009). It has reported that aphids did not affected soybean growth but caused significant reductions in most leaf mineral contents e.g., nitrogen, sulphur, calcium and phosphorus. Also a study on faba bean plants resulted in an excessive water loss due to aphid infestation (Shannag et al., 2007). The involvements of volatile secondary metabolites as insect repellents, attractant of parasitoids of the predators as well as plant communicator increase the attention on these compounds, lead my activity on the volatile organic compounds analyses (Mello et al., 2002; Fernandes et al., 2009; Stotz et al., 1999; Karimzadeh et al., 2008).

Our results on the influence of aphids infestation on yarrow growth, biomass and total volatile emissions indicated that in the 2nd and 3rd weeks after the infestation the biomass of the infested plants are reduced and the total volatile emission decreased (Fig.18) suggesting that a prolonged aphids attack cause a significant disturbance on the whole metabolism of the plant (Nanayakkarawasam et al, 2011a). Our study on total volatile emissions on daily basis for infested and control plants showed that the first week after the infestation is the best time to study the effect of the aphids attack on the total volatile emissions of *Achillea collina* (Fig.19).The same results suggested that the future concern on the natural compounds induced by the aphids must be focused on the youngest part of the plant. After 3 weeks of infestation the average fresh biomass decreased of 48.75% and 39.19% in plants infested by *M. persiceae* and *M. millefolii* respectively, as compared to the control (Nanayakkarawasam et al., 2011a). The results indicated that biomass reduction was higher in the plants infested by the generalist rather than by the specialist aphid, probably due to a better resistance of the plant to the specialist aggressor determined by co-evolutionary mechanisms.

The analyses of the volatile organic compounds fingerprints of *Achillea collina* plants in both the infested conditions showed that the emissions of terpenes was significantly greater than in the control, moreover a significantly higher value in infestation with *M. persiceae* rather than with *M. millefolii* was recorded. This result indicate terpenes as a chemical class highly sensitive to this kind of stress condition and suggest the possibility to find therein even the compounds differentiating the two types of insect attack.

Some compound levels were increased due to the infestation, in particular β-pinene, β-phellandrene, β-caryophyllene, chamazulene, α-bergamotene and α-farnesene showed an increase induced by both aphids. Some compound levels were significantly greater in the plant infested by the specialist aphids (eg. decanol) while some other were greater in the plants infested by the generalist aphids (eg. p-Xylene). Some compound levels decreased due to the infestation and a few

disappeared due to the infestation. Anyhow the variations were depending on the type of infestation. Our attention mostly focused on the newly induced volatiles in order to perceive secondary metabolic pathways specifically activated by the stress conditions.

The Fig.21shows the most induced volatiles are common for both the types of biotic stresses caused by the specialist and the generalist aphids (*M. persicae* and *M. millefolii)* over *A. collina* plant. A few compounds seem to be specifically induced by the particular species of aphid. Increasing of farnesene, caryophyllene, germacrene D levels in the infested plants were observed by many authors (Pare' et al., 1999; Kigathi et al., 2009; Guerrieri et al., 2008; Dancewicz et al., 2008). Alcohols involved in infested plant profiles are classified as herbivore-induced plant volatiles HIPVs. (Kigathi et al., 2009; Guerrieri et al., 2008; Dancewicz et al., 2008; Pare' et al., 1999). In cotton breakage of leaf glands causes both the release of stored terpenes and the corresponding increasing of "green-leaf volatiles" which are C6 aldehydes, alcohols, and their esters formed in oxylipin metabolism (Paré, &Tumlinson, 1996)produced through lipoxygenase pathway (Pare'et al., 1999; Guerrieri et al., 2008); those results were in agreement with ours.

Subsequently, our attention was focused on mechanical damages in order to evaluate which kind of compounds were induced by the insect attack and not by the mechanical damage it provoked. Using a specially built equipment and a needle, *A. collina* plants were subjected to pressure and needle-prick damages respectively. The identified volatiles were reported in the Table 2 and those newly induced by the mechanical stress conditions were shown in Fig. 22.

Distributions of induced volatiles between two types of mechanical damage are also shown in Fig. 22. The result shows most induced volatiles are common for both the types of mechanical stresses applied while only a few compounds are specifically induced by the type of mechanical stress applied. Plants respond to the

insect feeding damage by releasing a variety of volatiles from the damaged site, and the profile of the volatiles emitted is markedly different from those of undamaged or mechanically damaged plants in many species (Pare et al.,1999). In cotton, induced volatiles that are released in response to wounding are greater in caterpillar feeding than as a result of mechanical damage alone (Pare´et al.,1999). Our results also showed that the volatile composition of mechanical damage and infested plants are different. This observation suggested that volatile emissions and other plant defense responses can be potentiated by components associated specifically with the feeding herbivore that allows the plant to distinguish between a wounding mechanically provoked and damage due to insects' attack (Pare´et al., 1999). Many researchers (Pare'et al.,1999; Leìon et al., 2001) observed that both herbivore and mechanical damage induce an increase in plants jasmonic acid (JA) level, but Eric et al., 1997 observed that jasmonic acid increse due to herbivory was higher than that provoked by mechanical damage. Therefore our attention was mainly focused on jasmonic acid signaling pathways and the volatile emissions of the *A. collina* treated with jasmonic acids, was evaluated.

The volatile organic compounds variation of *Achillea collina* plants treated by jasmonic acids and control were reported in Table03. The distribution of volatiles induced by *A. collina* under the biotic, abiotic (mechanical) and jasmonic acid stressed were shown in Fig. 23. There are secondary metabolites common for all type of stresses (eg α-fenchene, pinocarvone, aromadendrene). While there are metabolites shared with the two type of stresses for example, p-cymene, 2, 6-nonadienal, acetic acid-ethyl ester and germacrene D shared between biotic and mechanical stresses. There are metabolites specific for the type of stress, for example terpinene-4-acetate, 3-ethylhexane are only induced by mechanical stresses. Our attention was mainly focussed on the secondary metabolites only induced by biotic stress. Tetradecanal, butanoic acid-3-methyl hexyl ester, terpineol cis-β and patchoulenol were identified as *A. collina* volatiles induced only by biotic stress conditions.

Pare´et al., (1999) reported that, both among individual plants of the same species and between different plants species, whether the blend of volatile compounds is induced through a common signaling pathway or if their emissions are triggered by different signaling mechanisms is not yet known. It is interesting to know whether the volatiles compounds specifically induced by biotic stresses in *A. collina* are specie – specific or are induced also in other plant species when infested by the same species of aphids. Hence HS-SPME-GC/MS *in vivo* experiments were conducted to evaluate the volatile emissions of yarrow *(Achillea collina)*, Pea (*Pisum sativum*) and Peach (*Prunus persica*) infested by the generalist aphid *M. persiceae* and the results were reported in Table 04. The distributions of induced volatiles among the species were shown in Fig. 24. The results indicated that there are both specie-specific and common induced volatiles. The same compounds: tetradecanal, butanoic acid-3-methyl hexyl ester, terpineol, cis-β and patchoulenol, which were only induced by the biotic stress in *A. collina* were common for pea and peach. The uniformity in the chemical emissions from different plant species when they were infested by the same aphid (*M. persiceae*) suggest the activation of a common set of biosynthetic pathways shared by different plant families.These compounds may be produced as bioactive agents against elicitors associated with aphid's saliva or pathogens attack associate with the infestation.

Phytohormones also play central roles in abiotic and biotic stress signaling (Leìon et al., 2001). These signals ultimately induce expression of specific sub-sets of defense genes that lead to the assembly of the overall defense reactions. Leìon et al., (2001) also indicated that powerful molecular tools, including transcriptome and proteome analysis, sequencing of entire genomes in plants, bioinformatic analysis and functional studies, are enabling the dissection of networks and identification of key factors in abiotic and biotic signaling and crosstalk.

The volatiles emitted by protein extracts of control and infested leaves of *A. collina* plants after adding farnesyl di phosphate (FDPP) gave a preliminary biochemical

confirmation regarding the activation of different pathways in the different experimental condition examined.

Volatiles need to be judiciously synthesized and safely stored by the plants, as increased synthesis can be costly and potentially toxic to the plant itself (Pare´et al, 1999). Hori (1998, 1999) reported that linalool and α-terpineol can repell aphids *Myzus persicae* . Gutierrez et al. (1997) found that geraniol inhibited *Myzus persicae* settling on host plants. α-patchoulene was reported as toxic compound to *Myzus persicae* and patchoulol (patchouli alcohol, 1) was identified as a good fungistatic (Aleu et al., 1999). Furthermore, production of the volatile patchoulol and 13 additional sesquiterpene products in transgenic tobacco overexpressing *patchoulol synthase* (PTS) deterred tobacco hornworms, a majority of which had migrated away from leaves of the transgenic plants to the leaves of wild-type plants (Wu, et al., 2006 ; Dancewicz et al.,2008).

RT- PCR analysis suggest the *patchoulol synthase* gene expression (250bp), in *A.collina* infested plants while no signal of gene expression in the control plants was recorded. Patchoulenol was induced in *Achillea collina* only under the biotic stresses and it was induced in all the plants species used: yarrow (*Achillea collina*), Pea (*Pisum sativum*) and Peach (*Prunus persica*) ,when they were infested by the aphid *Myzus persicae.* This preliminary finding suggested that patchoulenol is judiciously synthesized by the plants as a bioactive secondary volatile compound against the aphid infestations.

5.CONCLUSION

The proposed HS-SPME-GC/MS *in vivo* analysis method is proved to be successful in detecting and monitoring VOCs variations due to different stress conditions. *A. collina* shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a good model for future investigations. Preliminary molecular results seem to confirm pytochemical data, at least for patchoulenol, as the *patchoulol synthase* gene seems to be expressed only in the infested plants.The identification of tetradecanal, butanoic acid-3-methyl hexyl ester, terpineol cis-β and patchoulenol as compounds induced only by the aphids infestation in different plant species, suggests to further investigate their role in aphid-plant interaction, as specific bioactive compounds, i.e. able to interfere with the aphids life cycle, or as plant alert signals, i.e. able to induce further defense mechanisms in plants. Preliminary molecular results seem to confirm pytochemical data, at least for patchoulenol, as the *patchoulol synthase* gene seems to be expressed only in the infested plants.

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7.PUBLICATIONS

- **Articles for scientific journals**

Influence of aphids infestation on yarrow growth, biomass and total volatile emissions Nanayakkarawasam Masachchige Chandrika Nanayakkara N,-Giorgi A, Panseri S, Chiesa LM, Biondi PA', Lozzia GC, Cocucci M *Minerva Biotecnologica,* 28: 31(2011).

"*In vivo"* study on Terpene variations induced by Plant- Insect interaction using HS-SPME-GC/MS. Nanayakkarawasam Masachchige Chandrika Nanayakkara N,-Giorgi A, Panseri S, Chiesa LM, Biondi PA', Lozzia GC, Cocucci M *Minerva Biotecnologica,* 49:52 (2010).

Comparison of Volatile Compounds Induced by Aphids and Mechanical Damage in *Achillea collina*, Panseri S, Nanayakkarawasam Masachchige Chandrika Nanayakkara N, Chiesa LM, Biondi PA', Lozzia GC,-Giorgi A ***Acta Horticulurae*** (in press)

HS-SPME-GC/MS analysis of the volatile compounds of *Achillea collina* Becker ex Rchb.: evaluation of the volatile fingerprint induced by the aphids A. Giorgi, S. Panseri, N. N. M. C. Nanayakkara, L. M. Chiesa (2011) Journal of agricultural and food chemistry (submitted)

- **Abstract for poster presentations at International congresses**

Achillea collina response to biotic and abiotic stresses: a comparative evaluation of volatile emissions pathways. Nanayakkarawasam Masachchige Chandrika Nanayakkara N,-Giorgi A, Panseri S, Chiesa LM, Biondi PA', Lozzia GC, Cocucci M. *Planta medica,* 77: 1234-1235 (2011).

Comparison of Volatile Compounds Induced by Aphids and Mechanical Damage in *Achillea collina,* Giorgi A, Nanayakkarawasam Masachchige Chandrika Nanayakkara NPanseri S, Chiesa LM, Biondi PA', Lozzia GC, Cocucci M. *MAP-Mountain,* 91:(2011).

HS-SPME method to monitoring "*in vivo*" volatile compounds emitted from *Achillea collina* infested by aphids. Giorgi A, Panseri S, Nanayakkarawasam Masachchige Chandrika Niranjala N, Chiesa LM, Soncin S , Biondi P, Maurizio Cocucci. *Planta Medica,* 76: 1337 (2010).

HS-SPME-GC-MS method development of volatile constituents from *Achillea collina* Logo of the publication. Authers -Giorgi A, Panseri S, Nanayakkarawasam Masachchige Chandrika Niranjala N, Madeo, M, Mingozzi M, Morlacchi P, Chiesa LM, Biondi P. Planta Medica, 75: 1042 (2009)

Influenza degli attacchi entomatici sulla sintesi di metabolite secondary in *Achillea collina* Becker ex Rchb. Giorgi A, Madeo M, Nanayakkarawasam Masachchige Chandrika Niranjala N, Mingozzi M, Morlacchi P, Panseri S, Chiesa LM, Maurizio Cocucci *Convegno nazionale della societa'Italiana di chimica agraria XXVII* , 72 (2009)