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The chemical ecology of a model aphid pest, *Myzus persicae*, and its natural enemies

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DECLARATION

I hereby declare that I have composed my thesis and that it has not been accepted in for any degrees in past. All the work in this is done by me and any collaborative work has been specifically acknowledged.

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ABSTRACT

The peach potato aphid, *Myzus persicae*, is a serious crop pest with a worldwide distribution. It has short generation time, high reproductive rate and the ability to adapt to new hosts and develop resistance against major class of insecticides. There is an urgent need to develop eco-friendly and sustainable new ways to protect plants from this cosmopolitan pest.

The aim of this project was to study the chemical ecology of multitrophic interactions of *M. persicae* with its host plants and natural enemy, *Diaeretiella rapae*. Potato (wild *Solanum stoloniferum* vs. cultivated *Solanum tuberosum* Desiree) and brassica (*Brassica napus*, *Brassica rapae*) plants were used to study the behavioural responses and performance of insects. To explore plant defence induction, the effect of a plant defence elicitor, *cis*-Jasmone, was also tested on brassica plants. For this, we performed a series of insect performance and behavioral bioassays and headspace sampling of plants for volatile collection. In this project, *M. persicae* showed a negative response towards the wild potato plants in performance and behavioral bioassay. The wild accessions had a high reduction in aphid survival and larviposition after both time points 48 h and 96 h. A significant change in volatile emission was also recorded when volatile analysis was performed for wild and cultivated potatoes. The volatile compounds released by wild potato were highly repellent to aphid *M. persicae* but acted as an attractant to parasitoid *D. rapae*. Conclusively, wild potato showed a high potential of reducing *M. persicae* population by direct (high mortality and low larviposition) and indirect defenses (enhanced parasitoid foraging time).

We also tested the effect of CJ treatment on brassica crops, the obtained results showed that CJ treated brassica lines were highly resistant to *M. persicae* throughout the series of experiments. The performance of *M. persicae* was significantly reduced on CJ treated brassica lines in clip-cage and settlement bioassay. The volatile compounds collected from CJ treated brassica lines also had repellent effect on *M. persicae*. In contrast, CJ treatment had a positive effect on *D. rapae* and a positive behavioral response of *D. rapae* was recorded towards CJ treated plants and volatile compounds. A significant increase in parasitoid foraging time and parasitism behavior was recorded on all CJ treated brassica lines. In Olfactometer bioassay, *M. persicae* spent significant less time when exposed to CJ plant volatile samples while *D. rapae* spent significant longer time in the olfactometer arm treated with volatile sample collected from CJ treated brassica lines. Volatile analysis also revealed that there was a significant increase in quality and quantity of volatile compounds after brassicas treatment with CJ. Taking altogether, our results showed that CJ treatment induced defence in brassica crops against *M. persicae* by affecting pest performance and increasing biological control. These results could contribute towards developing novel management approaches for the pest.

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ACRONYMS

ANOVA	Analysis of variance
BF	Blank formulation
CJ	*cis*-Jasmone
Cont.	Control
RH	Relative humidity
DEE	Diethyl ether
EAG	Electroantennogram
EGBN	English giant *Brassica napus*
SBN	Samurai *Brassica napus*
TR	Turnip rutabaga
FID	Flame ionization detector
h	Time (hours)
VOCs	Volatile organic compounds
GC-MS	Gas chromatography - Mass spectrometry
ORNs	Olfactory receptor neurones
PTFE	Polytetrafluoroethylene
RI	Retention time
S.E.	Standard Error of the Difference

S.E.D.	Standard Error of the Difference
TMTT	(*E,E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene
HIPVs	Herbivore induced plant volatiles
OBPs	Odour-binding proteins
CSPs	Chemosensory proteins
ESTs	Expressed sequence tags
SA	Salicylic acid
JA	Jasmonic acid
OPDA	Oxophytodienoic acid
DMNT	(*E*)-4,8-dimethyl-1,3,7-nonatriene
MeSA	Methyl salicylate
MHO	6-methyl-5-hepten-2-one
ND	Not Detected
CAEP	Centre of Applied Entomology and Parasitology
LD	Light dark
GLM	Generalized linear model
BABA	ß-aminobutyric acid
MeJA	Methyl jasmonate
EPV	Entomopathogen Virus

EPF	Entomopathogen Fungi
EPN	Entomopathogen Nematodes
PDJ	Prohydrojasmon
IPM	Integrated pest management

Chapter 1. GENERAL INTRODUCTION

1.1 Introduction

Aphids are serious sap-sucking pests that affect plants growth and causing a considerable damage to crop plants worldwide (Pickett *et al.*, 1992; Van Emden and Harrington, 2017). Aphid populations increase explosively due to their short maturation period, telescoping generations and high fecundity (Dreyer and Campbell, 1987; Kindlmann and Dixon, 1989; Mohammed and Hatcher, 2016). However, abiotic and biotic factors such as extreme weather and natural enemies (predators and parasitoids) control aphid populations (Carter, Dixon and Rabbinge, 1982; Morris, 1992; Schmidt *et al.*, 2004; Gontijo, Beers and Snyder, 2015). In addition to this, host-plant resistance is one of the main factors that slows down the reproduction rate of aphids and extends the duration of development (Starks, Muniappan and Eikenbary, 1972; Dreyer and Campbell, 1987; Züst and Agrawal, 2016). *M. persicae* is one of the most serious aphid pest species due to its high reproduction rate, high adaptability to new hosts and capacity to develop resistance to insecticides (Blackman and Eastop, 2000; Margaritopoulos *et al.*, 2009; Puinean, *et al.*, 2014).

Biotype (morphologically indistinguishable subspecies that are different in behaviour or physiology) formation in *M. persicae* permits it to extend its host range and quickly occupy new ecological niches (Ramsey *et al.*, 2014). The formation of biotypes means that developing insect-resistant crop varieties is a complicated and challenging process against this pest. In potato crop, it was observed that plant resistance is frequently overcome after a few years because of the appearance of new aphid biotypes (Alvarez *et al.*, 2006).. One of the main significant differences between aphid-resistant and aphid-susceptible lines of the same crop is the length of probing

time taking by the aphid in reaching up to the phloem (Dreyer and Campbell, 1987; Klingler *et al.*, 2005; Tetreault *et al.*, 2019). On resistant lines aphids take a long time reaching the phloem and cannot feed successfully on it (Dreyer and Campbell, 1987). In contrast, aphids take a relatively short probing time before reaching the phloem on susceptible lines and then feed on the phloem for prolonged periods of time (Klingler *et al.*, 2005; Tetreault *et al.*, 2019). Aphids have difficulty in feeding on resistant lines and in locating the phloem during probing (Ponder *et al.*, 2000; Alvarez *et al.*, 2006).

The peach-potato aphid, *Myzus persicae* (Hemiptera; Aphididae), is a highly polyphagous and cosmopolitan pest with a global distribution, including significant portions of North America, Europe and Asia (Blackman and Eastop, 2000; Van Emden and Harrington, 2017). *Myzus persicae* colonises many economically important crops including potato, pumpkin, peach, squash, lettuce, beet, bean and melon (McKinlay, 1992; Capinera, 2012, 2020). It has a very short generation time and a high reproductive rate and reach densities that are sufficient to cause noticeable crop injury in a short time (Dreyer and Campbell, 1987). *Myzus persicae* is also responsible for elevated transmitting the plant viruses and act as vector for many species of serious plant viruses such as alfalfa mosaic virus, bean common mosaic necrosis virus, East Asian Passiflora virus, potato leafroll and potato virus Y (Martin *et al.*, 1997; Eigenbrode *et al.*, 2002). As mentioned earlier, *M. persicae* has wide range of host plants including vegetables, ornamental, plants grown in greenhouses and in the field (Van Lenteren and Woets, 1988; Payton Miller and Rebek, 2018)

The main symptoms of aphid attack are dwarfing, curling or wilting of leaves. It also leads yellowing of leaves and chlorotic spots. A high density of aphids causes a reduction in growth and water stress that results in yield reduction. Early infestation is especially damaging to potato, even if the aphids are removed later (Petitt and

Smilowitz, 1982). Contamination of harvested plant material with aphids or with aphid honeydew also causes further damage, for instance; presence of honeydew on leaves inhibits photosynthesis, and provide good breeding ground for fungi; sooty molds and causes secondary damage (Puri and Hall, 1998; Mathulwe, Malan and Stokwe, 2021). To avoid contamination of vegetables with aphids, precautionary measures such as quarantine (to prevent the spread of the disease) or irradiation strategies to kill the insects without affecting the vegetables may be needed (Stewart *et al.*, 1980; Farkas, 2006; Molnár, 2009). For fumigation (acetaldehyde fumigation), a proper procedure needs to be followed, for example, plants should be wrapped in polyethylene film and packed in cartons before experimental treatments application (Stewart *et al.*, 1980).

M. persicae causes serious harm to plant health by transmitting plant infections and is considered as an important vector of plant viruses and transmits both persistent and non-persistent viruses viably (Capinera, 2012). Both nymphs and adults are responsible for disease transmission, winged adults also play a prominent role in spreading diseases due to their high mobility (Namba and Sylvester, 1981; Capinera, 2012; Xu and Gray, 2020). Kennedy, Day and Eastop, (1962) recorded more than 100 viruses spread by *M. persicae*. Some of serious diseases transmitted by *M. persicae* include potato leafroll virus and potato virus Y, beet western yellows viruses, beet yellows viruses, lettuce mosaic virus, cauliflower mosaic, turnip mosaic virus, cucumber mosaic and watermelon mosaic viruses (Capinera, 2012, 2020). Transmission of potato leafroll virus in potato causes discolouration in potato tubers, called net necrosis (Heuvel and Peters, 1990; Naga *et al.*, 2020).

1.2 Aphid biology

The majority of aphid species spend their entire life cycle on the same plant species or closely related group of plants species (Moran, 1989; Peccoud *et al.*, 2010). They produce eggs on the same host plant and obtain all their nutrition from the host throughout all their parthenogenetic generations. Approximately 450 aphid species exhibit host alternation (heteroecious or dioecious) in their life cycle (Dixon, 1987; Powell and Hardie, 2001). Aphids that have heteroecious life cycles colonise two unrelated plant species: a woody plant as a primary winter host and a herbaceous plant species as a secondary host in the summer (Blackman and Blackman, 1974; Van Emden and Harrington, 2017). Host alternating aphids are more economically significant than non-host alternating species because the secondary hosts are typically nonwoody species and frequently crop plants (Dixon and Kundu, 1994; Powell and Hardie, 2001).

Aphid annual life cycles include many generations of female parthenogenetic (asexual) morphs, followed by males and oviparous aphids in a sexual generation which produces overwintering eggs (Fig 1.1). Aphids that exhibit polymorphic (sexual/asexual) patterns of reproduction are called holocyclic. While species that have lost the sexual phase are called anholocyclic. Some species have both forms: holocyclic and anholocyclic. In Aphidinae subfamily, all genera exhibit anholocyclic and holocyclic forms (Blackman and Eastop, 2000; Powell and Hardie, 2001). In the holocyclic life cycle, the aphid starts as an egg which is usually laid before winter. The egg hatches into a Fundatrix (a wingless female) that then produces spring migrants that give rise to apterous and alate virginoparae. In addition to this, there can be an extra generation, the fundatrigenae before emigration (Gratwick, 1992). Asexual generations form by parthenogenesis on the summer host. Virginoparae (on the

secondary host) give rise to gynoparae (also produced on the secondary host) which are winged forms that migrate to the primary host. The gynoparae then produce wingless oviparae (on the primary host). Meanwhile the virginoparae on the secondary host produce males, which fly to the primary host, mate with the oviparae, and the latter lay their overwintering eggs on the primary host. In an anholocyclic life cycle, female aphids produce clones of females asexually for the whole year.

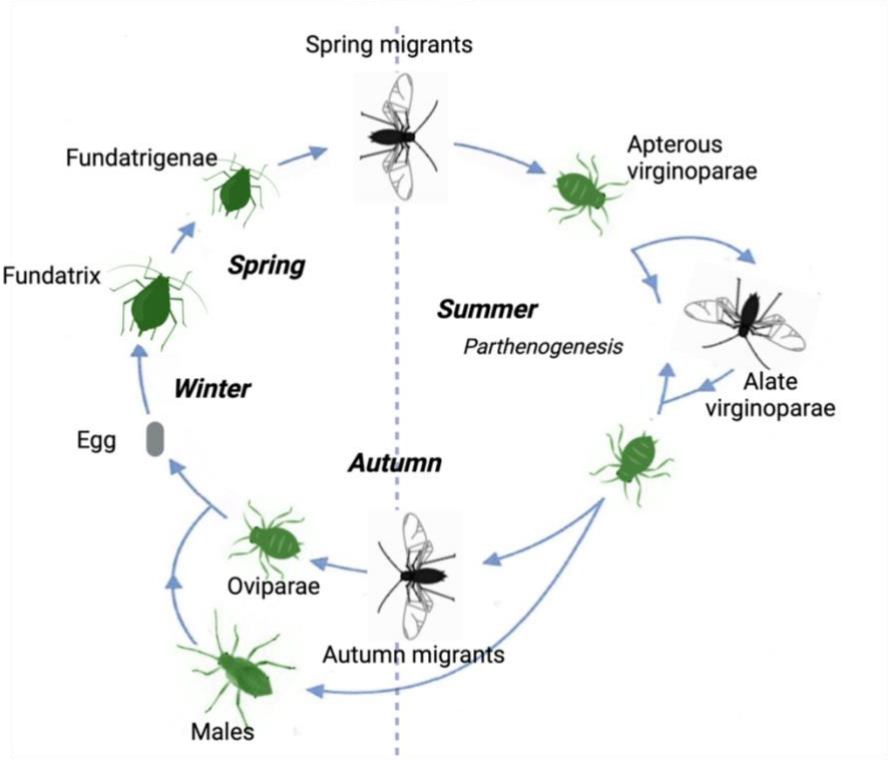

FIGURE 1.1 | an arrow chart showing the general life cycle of aphid (holocyclic, heteroecious) adapted from Blackman (1974).

Aphids are one of the most devastating herbivorous insect pests,. They have short generation time, quickly develop resistance against available insecticide and also act as a vector of plant virus (Devonshire *et al.*, 1998; Puinean, *et al.*, 2014). The association of aphids with the host plant brings several changes in plant physiology such as blockage of phloem sieve elements and suppression of callose (plant polysaccharide) formation (Will *et al.*, 2007). The secretion of salivary proteins causes

these changes in the host plant (Elzinga, De Vos and Jander, 2014; Ramsey *et al.*, 2014). The saliva secreted by aphids are of two types: one is gelling saliva, and another one is watery saliva (Van Bel and Will, 2016). Gelling saliva helps to protect the stylet at the time of penetration while watery saliva reaches into various cells of the body and phloem of the host plant (Moreno *et al.*, 2011) and also responsible for the modulation of cell processes, suppressing host defence mechanisms and enabling the colonisation of the host plant (Voelckel and Jander, 2014). It has also been reported that in some cases aphids can change the morphology of the plant by forming galls in leaves and curling of leaves (Blackman and Eastop, 2000).

Aphids also deliver effector proteins inside the host plant to suppress the plant defence system (Pitino and Hogenhout, 2013). In tobacco, eleven *M. persicae* salivary proteins were expressed transiently while *Arabidopsis thaliana* showed the presence of aphid released salivary protein Mp55 (Elzinga, De Vos and Jander, 2014). Salivary gland expressed sequence tags (ESTs) can be used in a functional genomics approach to identify effector proteins from *M. persicae* that depend upon sequence similarities with plant pathogen effectors (Bos *et al.*, 2010). The genomic approach revealed three candidate effectors, i.e. Mpc002 (Mutti *et al.*, 2008), Mp10 and Mp42 (Mutti *et al.*, 2008). These effectors modulate host cell processes (Harmel *et al.*, 2008) and affect aphid performance. Effector protein MpC002 plays a vital role in host plant colonisation, as shown by gene silencing experiments. Knock-down of the MpC002 gene present in the saliva of *Acrythosiphon pisum* causes a reduction in the survival rate of the aphid on the host plant (Mutti *et al.*, 2008).

1.2.1 Life cycle of *Myzus persicae*

M. persicae varies according to local climatic conditions, mainly depending on how cold the winter is. Van Emden *et al.* (1969) reviewed the life cycle and found that

development can be varied: it may be rapid with over 20 generations in a season in moderate climate, with each completed in 10-12 days. Based on experiments in Greece, regional variation in the life cycle of *M. persicae* has been reported (Margaritopoulos *et al.*, 2002; Capinera, 2012). In case of unavailability of the summer host plant and cold climate, aphids overwinter on *Prunus* spp. in the egg stage. In the spring, the plant comes out from the dormancy stage and starts to grow, eggs hatch and nymphs start feeding on flowers, stems and young foliage. In a cold climate, adults return to *Prunus* spp. where mating happens, and eggs are laid. Except autumn generation all generations culminating in egg production are non-sexual (parthenogenetic) (Van Emden *et al.*, 1969; Margaritopoulos *et al.*, 2002; Capinera, 2012) (Fig. 1.2).

Eggs: Eggs are yellow green in beginning which soon turn into black. They are elliptical in shape and size ranges from 0.3 mm to 0.6 mm, high mortality is recorded in this stage (Capinera, 2012).

Nymphs: Egg hatches into nymph, which is initially greenish but become yellowish and resemble viviparous (parthenogenetic, nymph-producing) adults. There are two assumptions about the number the developmental stage of *M. persicae*. According to Horsfall (1924), *M. persicae* has four instars in its developmental stage with the duration of each averaging 2.0, 2.1, 2.3, and 2.0 days respectively (Capinera, 2001, 2004). While according to MacGillivray and Anderson (1958), *M. persicae* has life cycle with five instars stages with a mean developmental time of 2.4, 1.8 2.0, 2.1 and 0.7 days respectively, assumption with five instar stages is well documented compare to four instars one (Ramireddy and Dwivedi, 2021; Perdikis, Lykouressis and Economou, 1999; Capinera, 2001, 2004).

Adults: adult *M. persicae* can occupy up to 8 generations on its winter host prunus, but due to high density winged (alate) forms are produced that migrate on to summer hosts. winged forms are entirely different to wingless form of *M. persicae*, it has black head and thorax, yellowish abdomens with dark patches on dorsal side and body length ranges from 1.8 to 2.1 mm (Capinera, 2001; Jung *et al.*, 2021). Later winged forms produce wingless offspring on overwintering host plant and produces young ones (Van Emden *et al.*, 1969; Capinera, 2001; Mauck, De Moraes and Mescher, 2010). Wing dimorphism has been proposed as a strategy to face trade-offs between flight capability and fecundity. In aphids, individuals with functional wings have slower development and lower fecundity compared with wingless individuals (Castañeda *et al.*, 2010). Besides, morphological differences in winged (alatae) and wingless (apterous) forms, there are several biological importance associated with these forms, for instance; Winged forms are economically more important because of their dispersive nature, they colonise a wide range of plants and serve as an effective vector for plant viruses (Capinera, 2001). It has been reported that clones which have higher fecundity tend to produce more winged (dispersing) offspring, thus reducing their ability to compete on a single host plant (Hazell *et al.*, 2005).

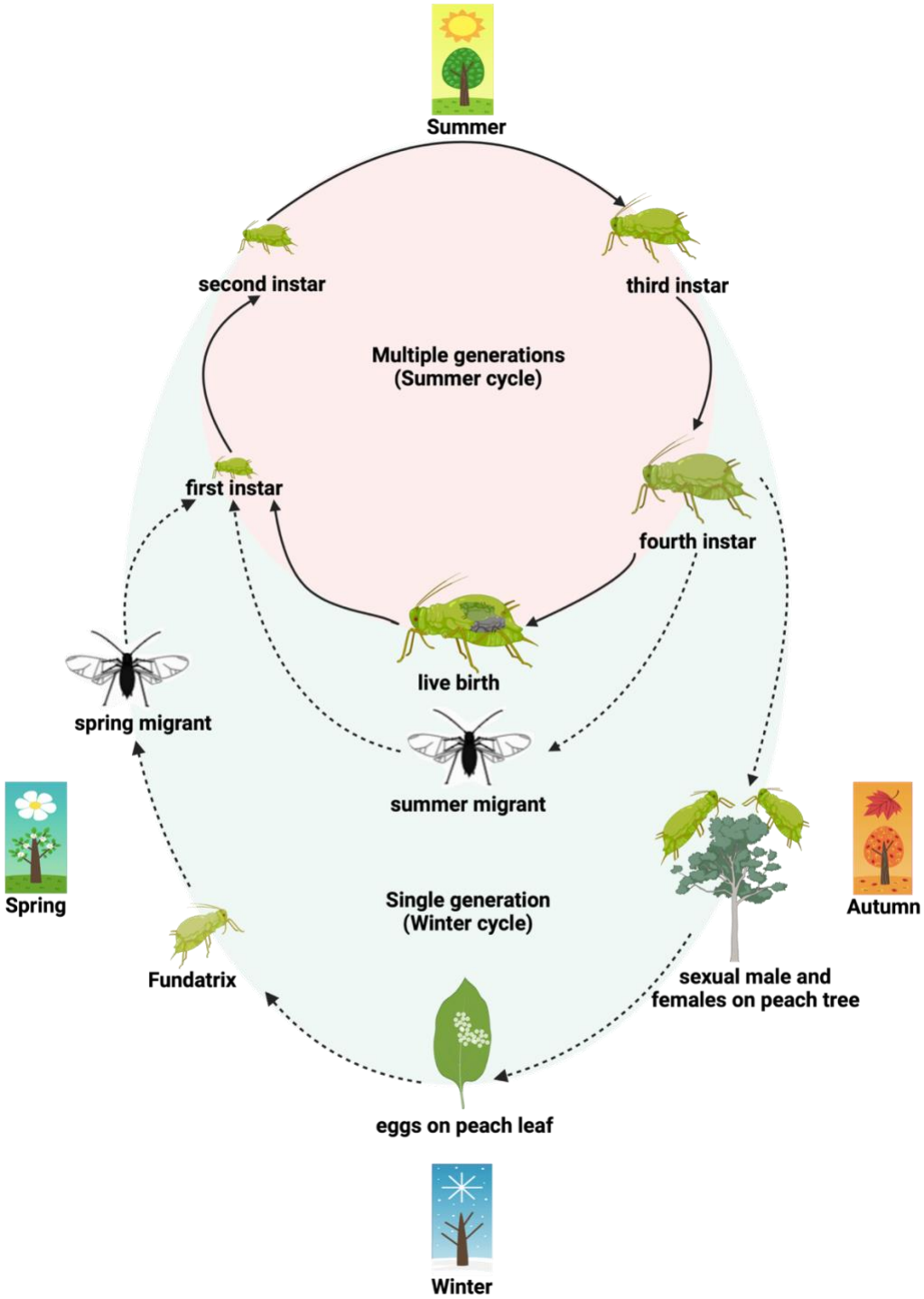

FIGURE 1.2 | Illustration showing a life cycle of *Myzus persicae* in the presence of its host peach tree (winter) and vegetables (summer).

In the winter part of the life cycle**,** sexual forms male and female aphid *M. persicae* mate on primary host plant and produce eggs to overwinter. Females (Fundatrix) hatch from the eggs and a later generation develop into spring migrants which give rise to

apterous and alate virginoparae daughter clones which are reproduce asexually on the summer host plant. Later, after return migration to the winter host, these daughter clones develop into males and females (Fig. 1.2). In case of oviparae, there is an intervening generation, gynoparae that develop into males and females (Hales, Wellings and Parkes, 1989). The Winter life cycle of *M. persicae* has a single generation. In contrast, the summer cycle has multiple generation that are produced asexually.

Host plants

M. persicae feeds on a wide range of host plants from more than 40 families of plants (Bass *et al.*, 2014; Ali *et al.*, 2021). However, only the viviparous (giving birth to living young) summer stages exist on this wide range of plants; the oviparous (egg-producing) winter stages are much more restrictive in their diet choice (Capinera, 2001, 2012). In temperate latitudes, the primary hosts are trees of the genus *Prunus,* while during summer months, aphids abandon their woody hosts for secondary or herbaceous plants including, vegetable crops of families Solanaceae, Chenopodiaceae, Compositae, Brassicaceae, and Cucurbitaceae (Capinera, 2012, 2020). Thus, *M. persicae* is known as the peach-potato aphid, reflecting two of its most common hosts peach and potato. Furthermore, *M. persicae* attacks a wide range of host plants that include artichoke, asparagus, bean, beets, broccoli, Brussels sprouts, cabbage, carrot, cauliflower, cantaloupe, celery, cucumber, fennel, kale, kohlrabi, turnip, eggplant, lettuce, mustard, okra, parsley, parsnip, pea, pepper, potato, radish, spinach, squash, tomato, turnip, watercress, watermelon and sugar beet (Capinera, 2020). Besides, numerous flower crops and other ornamental plants are also attacked by this pest (Capinera, 2001). However damage to these crops differ due to difference in their susceptibility to the peach-potato aphid, generally this pest prefers growing

and young plant tissue, which most often harbours large aphid populations (Heathcote, 1962).

1.2.2 Challenges with conventional insecticide treatments

The excessive use of synthetic chemical pesticides to control peach-potato aphid over the years has led to evolution of resistant biotypes (Anthon, 1955; Devonshire, 1989; Soderlund and Bloomquist, 1990; Anstead, Williamson and Denholm, 2005; Margaritopoulos *et al.*, 2007; Bass, *et al.*, 2014). To date *M. persicae* has developed resistance to the many classes of insecticides (Anstead, Williamson and Denholm, 2005; Margaritopoulos *et al.*, 2007, 2021; Papadimitriou *et al.*, 2022). Selection pressure from insecticide use against *M. persicae* has led to development of mechanisms to avoid or detoxify the synthetic chemicals (Bass, *et al.*, 2014). Bass, *et al.*, (2014) have classified the evolution of insecticide resistance in *M. persicae* under several mechanisms (Fig. 1.3) that include; mutation of the insecticide target site and production of catabolic enzymes that detoxify the insecticide. These mechanisms are responsible for resistance to specific chemicals.

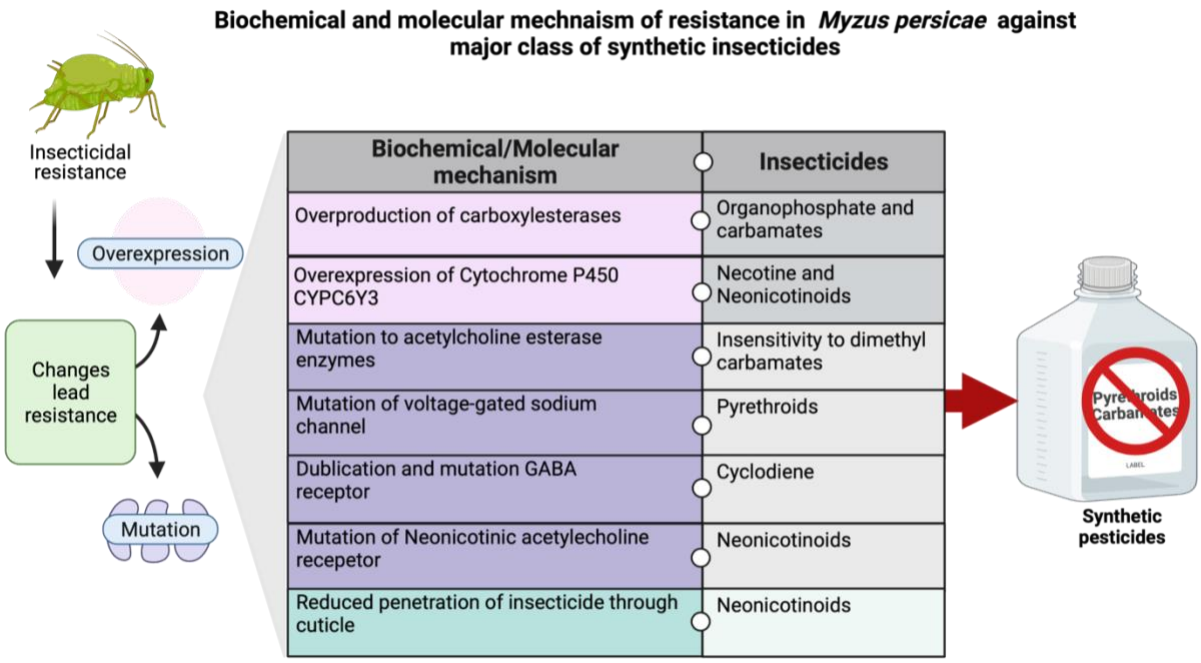

FIGURE 1.3 | Biochemical and molecular mechanism of resistance in *Myzus persicae* against major class of synthetic insecticides

1.2.3 Biological control as an alternative control method for peach-potato aphid

Biological control is an alternative to chemical pest management approaches and can involve a number of living organisms belonging to different phylums and kingdoms such as microbes (fungi, bacteria and viruses), nematodes and insects (parasitoids and predators belonging to the orders Hymenoptera and Coleoptera) (Fig. 1.4). These control methods are unique in their function and have specific mode of application, however implication of one method can be helpful for another and can be used in combination.

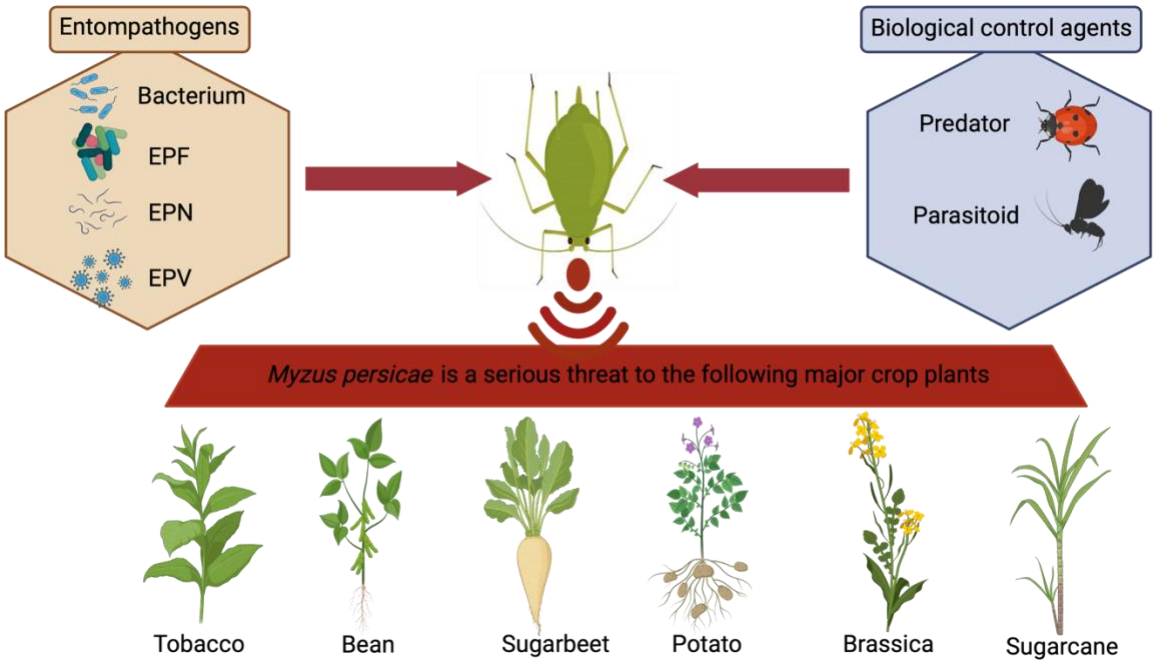

FIGURE 1.4 | Biological control methods used for *Myzus persicae*: EPF entomopathogens fungi; EPN (entomopathogens nematodes); EPV: entomopathogens viruses)

1.2.3.1 Entomopathogenic Fungi

More than 750 species of EPF belonging to 85 genera are functionally known for spreading infection in arthropods (Roberts and Hajek, 1992; Khachatourians and Qazi, 2008; Mora, Rouws and Fraga, 2016; Bamisile *et al.*, 2021; Paschapur *et al.*, 2021), but most of them have not used for commercially to manage plant pests yet. They are naturally present in agricultural soil but their efficacy in nature is not so high because of low spore numbers, but can be improved through inundative release of EPF. The most studied species of EPF belong to the genera *Metarhizium, Beauveria, Hirsutella, Isaria and Lecancillium* (Bamisile *et al.*, 2021). The genus *Metarhizium* has negative effects on the arthropods belonging to more than 150 families while *Beauveria* has been found effective against 200 species of insects and mites (De Barros, Fronza and Bertholdo-Vargas, 2015). Species *Beauveria bassiana* and *Metarhizium anisopliae*

both have been reported as a potential control method for more than 300 species of arthropods including aphids (*M. persicae*) (De Barros, Fronza and Bertholdo-Vargas, 2015; Yun *et al.*, 2017). Entomopathogenic fungi (EPF) play an important role as a potential biocontrol in pest management programs (Jandricic *et al.*, 2014; Mohammed and Hatcher, 2017). Extensive research has been done to evaluate their insecticidal properties against herbivorous pests belonging to different orders such as; Hemiptera, Hymenoptera, Lepidoptera and Coleoptera orders (Roberts and Hajek, 1992; Shah and Pell, 2003; Yun *et al.*, 2017). In particular, EPF reduce insect pest populations by reducing their performance, penetrating their body and feeding on them (Dara, Dara and Dara, 2017; Manoussopoulos *et al.*, 2019).

An accumulating body of evidence has shown that EPF have potential to control *M. persicae* populations by affecting their prefeeding behavior (Manoussopoulos *et al.*, 2019), reducing development and lowering fecundity rate (Yinquan, Shusheng and Mingguang, 1999; Xu and Feng, 2002; Jaber and Araj, 2018). High mortality of peach-potato aphids was reported in bioassay using cultural filtrates of entomopathogenic fungi (Lee *et al.*, 2015; Yun *et al.*, 2017). In addition, other deleterious insect pests such as wheat aphid (Ali *et al.*, 2018), soybean aphid (Clifton *et al.*, 2018) and cowpea aphid (Saranya *et al.*, 2010) are negatively influenced by infection with entomopathogenic fungal strains. Similarly, chewing pests such as lepidopteran caterpillars are adversely affected by EPF application (Carrillo *et al.*, 2015; Erler and Ates, 2015; Mohammed, Kadhim and Hasan, 2019; Mann and Davis, 2021).

1.2.3.2 Entomopathogenic Viruses

Plants come in contact with viruses because of interaction with herbivores and this plant-virus infection can affect the performance of the associated herbivores. Plants use these entomopathogenic viruses as a defence against a number of plant pests

(Der Geest, 2000). For example, *M. persicae* spreads entomopathogenic parvovirus (*M. persicae* densovirus MpDNV) during feeding on plants. The infected host plants use this entomopathogenic virus (EPV) as a part of their defense strategies and spread infection to non-infected subsequent future visiting aphids. EPV has negative effects on peach-potato aphid such as; reduced growth of *M. persicae* has been recorded on tobacco plant infested with Potato virus-Y (PVY) (Ren *et al.*, 2015), increased susceptibility to parasitoid (Mauck, De Moraes and Mescher, 2015). However, a positive effect of EPV on *M. persicae* has also been reported, where virus infested plants were preferred by *M. persicae* and improved growth, feeding and reproduction behaviour of aphid was recorded (Casteel *et al.*, 2014; Ren *et al.*, 2015; Liu *et al.*, 2019).

1.2.3.3 Entomopathogenic Nematodes

The entomopathogenic nematodes (EPN) are important entomopathogens that affect the performance of herbivores existing on the associated plant. However only limited work has been done to test their efficacy against aphids (*M. persicae*), EPN *Steinernema carpocapsae* showed low infectivity when tested on *M. persicae* (Park *et al.*, 2013).

Entomopathogens have been studied extensively to control herbivorous pest in crop fields with successful use of both augmentation and classical biological control strategies to apply or introduce bacteria, baculoviruses, fungi, and nematodes (Lacey *et al.*, 2001; Shah and Pell, 2003; Grewal, Ehlers and Shapiro-Ilan, 2005; Abd-Alla, Meki and Demirbas-Uzel, 2020). In particular, *Bacillus thuringiensis* var. *kurstaki* has been used to effectively control numerous defoliators in forests (Frankenhuyzen, 2000). Baculoviruses provide excellent examples of successful classical biological control and augmentative introductions, also for the control of defoliators.

Entomopathogens can serve as alternatives to broad-spectrum chemical insecticides. Entomopathogenic microbes serve as better alternatives to pesticides since they contribute to the natural regulation of arthropod populations (Butt and Goettel, 2000; Nguyen *et al.*, 2007). Numerous advantages can be found in the utilization of entomopathogens, in addition to efficacy. These include nonrepeat applications due to wide-spray coverage, safety for humans and other nontarget organisms, reduction of pesticide residues in food, preservation of other natural enemies and increased biodiversity in managed ecosystems. However, there are few disadvantages with the use of entomopathogens that include; slow killing time (Bonning and Hammock, 1996; Inceoglu, Kamita and Hammock, 2006), short shelf life, The pest must be present before the pathogen can be usefully applied thus making preventative treatment difficult, can be very costly to produce for commercial use and in quantity (Hall and Papierok, 1982; Maina *et al.*, 2018).

1.2.3.4 Parasitoids and predators

Biocontrol agents are widely used in pest management (Bale, Van Lenteren and Bigler, 2008; Van Lenteren, 2012; Barratt *et al.*, 2018). Extensive research has been done to test the efficacy of such biological control agents on *M. persicae* in the laboratory and field (Scopes, 1969; Wei *et al.*, 2003; Andorno and López, 2014). Approximately 200 biocontrol agents belonging to different families (*Coccinellidae, Cantharidae, Syrphidae, Anthocoridae, Pentatomidae, Aphidae, Aphelinidae, Acaridae*) have been found as a potential insects responsible for *M. persicae* population management (Van Emden *et al.*, 1969; Kavallieratos *et al.*, 2004; Zamani *et al.*, 2007; Cabral, Soares and Garcia, 2009; Acheampong, Gillespie and Quiring, 2012; Mohammed and Hatcher, 2017). The host plant plays a central role in implementing the biocontrol approach of naturally occurring biological control agents,

however it is not always valid. The effectiveness of biocontrol agents depends on species and physical status of host plants. Plants release volatile compounds which can be used as a cue by pollinators and natural enemies of pests to locate the host plants (Clavijo Mccormick, Gershenzon and Unsicker, 2014; Xu and Turlings, 2018). Previous studies have reported that plants infested with *M. persicae* release an increased amount of volatile compounds (Herbivore induced plant volatiles; HIPVs) that recruit parasitoids and predators (Gosset *et al.*, 2009; De Vos and Jander, 2010; Ahmed *et al.*, 2022). A wide range of natural enemies has been recorded from *M. persicae* infested crops such as, *Aphidius ervi*, *Diaeretiella rapae*, *Aphidius chelomani*, *Coccinella septempunctata*. Van Emden has provided a long list of biocontrol agents of *M. persicae* (Van Emden *et al.*, 1969).

Based on preference for target insects, parasitoids can be further classified as generalist and specialist. The specialist parasitoids *Diaeretiella rapae, Aphidius ervi* are well tested natural enemies against peach-potato aphid. According to an ADAS survey (Jude Bennison, *pers. comm.*) *D. rapae* is one of the common parasitoids of *M. persicae* in the UK. It has been reported that *M. persicae* infestation was responsible for the release of volatile blend rich in compounds that were responsible for the recruitment of parasitoid (De Vos and Jander, 2010; Aparicio *et al.*, 2019; Song *et al.*, 2021). Similarly, plants infested with *M. persicae* were also found attractive to predators such as; *Coccinella septempunctata, Hippodamia undecimnotata, Adalia bipunctata,* and *Adonia variegata* (Kavallieratos *et al.*, 2004; Li *et al.*, 2019). To augment the effect of predators and parasitoids various studies have been done using a number of natural defense elicitor compounds, which help the plants in releasing a plethora of volatile organic compounds attractive to these biocontrol agents.

1.2.3.5 Botanical biopesticides

Due to high environmental concern and adverse effect of synthetic chemical insecticides on non-target organisms and development of resistance by a number of herbivorous pests, there is a growing demand to develop biopesticides that have high insecticidal effect but negligible side effect to environment and non-target organisms (Chandler *et al.*, 2011; Kim *et al.*, 2018). Natural compound treatment of host plants can reduce growth, fecundity and foraging activity of pests (Paprocka *et al.*, 2018), settling and feeding preferences and causing high mortality on treated plants (Dreyer and Jones, 1981; Kim *et al.*, 2018). Plant natural compounds, such as linalool and thymol, can be used as a synergist to increase the efficacy of conventional insecticide and decrease the amount of synthetic insecticide (Faraone, Hillier and Cutler, 2015).

Plant derived natural compounds can play an important role as an alternative for toxic synthetic compounds (Jiménez-Reyes *et al.*, 2019; Saroj *et al.*, 2020; Senthil-Nathan, 2020; Souto *et al.*, 2021). Natural compounds have also been used in eliciting the plant defence system against a variety of pests. Besides induced defense, it is more likely that these compounds are also safe for environment and other beneficial organisms (Sobhy *et al.*, 2017). However, there may be non-target/environmental risks associated with using natural compounds (Bucheli, 2014; Hansen, Hilscherova and Bucheli, 2021). Previous studies showed that some natural compounds induced a considerable level of defence in plants against sucking insects particularly aphids (Sobhy *et al.*, 2017). To date several natural compounds have been tested against *M. persicae* that include flavonoids, methyl salicylate (MeSA), benzothiazole, dihydrojasmone and *cis*-jasmone. The history and use of these plant natural defence elicitors is discussed later in this chapter.

1.3 Semiochemicals

Aphids use their sense of smell to recognise host plants (Webster *et al.*, 2008). Olfactory receptors, present in the insects' antennae, detect volatile compounds, which are processed in the central nervous system (Bruce, Wadhams and Woodcock, 2005; De Bruyne and Baker, 2008). An appropriate stimulation with detected odorant molecules, elicits behavioural responses (Bruce and Pickett, 2011). Understanding of volatile compounds and their effect on insect behaviour can be a valuable tool in integrated pest control, where behaviourally active compounds can be used to manipulate the behaviours of insect pests to protect crops (Cook, Khan and Pickett, 2007; Mauchline, Hervé and Cook, 2018). These compounds help in transmitting the information between inter and intraspecies (Howse, Stevens and Jones, 1998; Heuskin *et al.*, 2011; El-Shafie and Faleiro, 2017). Volatile chemicals play a vital role in insect-plant interactions. Earlier studies done on semiochemicals provide strong evidences about their potentials to use them in pest management strategies (Cook, Khan and Pickett, 2007; Mauchline, Hervé and Cook, 2018).

Semiochemicals are signalling chemicals that play an important role in conveying the information between species (Dicke and Sabelis, 1988). These chemicals are critically important in insect-plant interactions, however, combination and ratio of chemical compounds affect the behavioural responses of insects (Bruce, Wadhams and Woodcock, 2005; Bruce and Pickett, 2011; Karban, 2015). The specificity of combinations and ratio of different compounds can be utilised to develop non-toxic approaches in integrated pest management. After knowing appropriate combinations of these compounds, they can be used as an insect deterrent or attractant (to traps the insects) (Pickett, Wadhams and Woodcock, 1997; Renwick, 2002). Semiochemicals have been employed against a number of insect pests (Pickett,

Wadhams and Woodcock, 1997; Agelopoulos *et al.*, 1999; Witzgall, Kirsch and Cork, 2010). These include attractants for mass trapping (Logan et al. 2007), repellents as protective bands (Xu *et al.*, 2018) and flushing agents (Rojas de Arias *et al.*, 2012), synchronised use of attractants and repellents to manoeuvre pests away from plants such as push-pull or stimulo-deterrent diversionary strategies (SDDS) (Agelopoulos *et al.*, 1999) and to enhance the effectiveness of biocontrol agents such as parasitoids (Lewis and Martin, 1990; Rodriguez-Saona, Blaauw and Isaacs, 2012; El-Ghany, 2019; Ayelo *et al.*, 2022). Insects use these chemicals to locate ovipositional sites, shelter and mates (Bell, 1990; Xu and Turlings, 2018).

Semiochemicals are categorised into different groups based on their effects on the receiver/emitter or interspecific or intraspecific. Some semiochemicals are beneficial for emitter while some have advantages for the receiver (Fig. 1.5). s

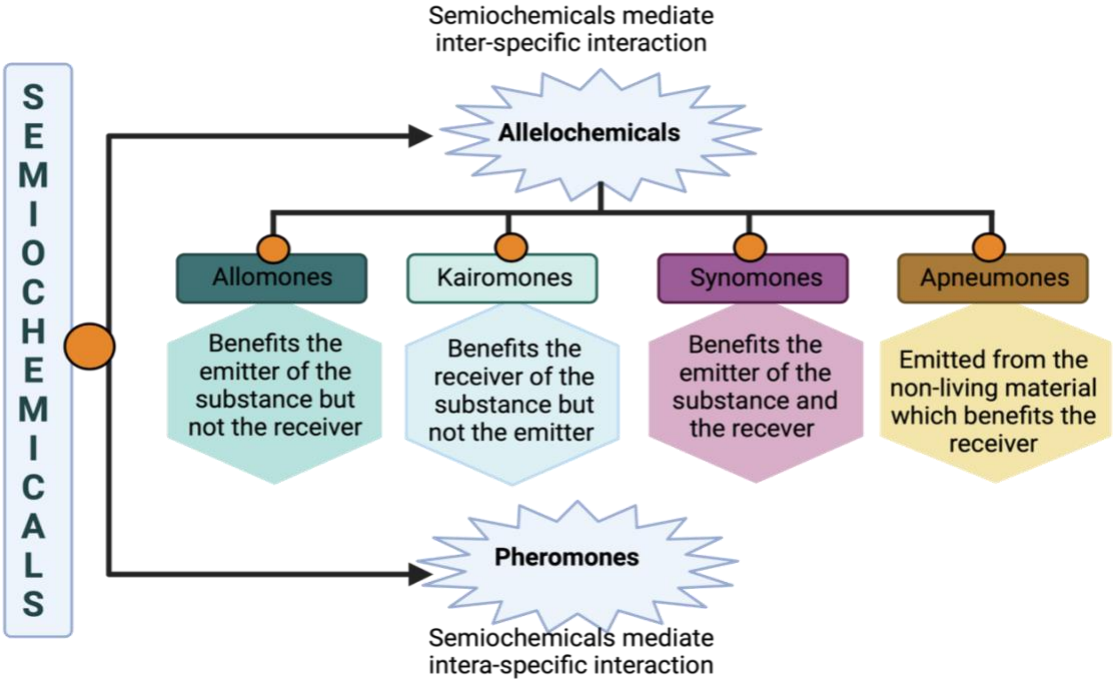

FIGURE 1.5 | A brief classification of semiochemicals with definition.

Individual volatile compounds may belong to more than one of the above groups. The same molecule can act as a pheromone for one species and as an allelochemical for another species. For example, aphids release (*E*)-β-farnesene under the attack of parasitoids. This compound elicits a repellent response and disperses nearby aphids (Nault, Edwards and Styer, 1973; Dicke and Sabelis, 1988). In this situation (*E*)- β-farnesene acts as a pheromone because it is used for intraspecific communication. However, when this compound exhibits the same function between different species of aphids, it is called allelochemical, for instance; (*E*)-β-farnesene being an allelochemical attracting natural enemies to caterpillar damaged plants (Christiane Schnee *et al.*, 2006). Plants produce a plethora of volatile compounds that allow quick defence signalling between distant plant organs (Heil and Bueno, 2007), communication between plants (Baldwin *et al.*, 2006) and recruitment of natural enemies (Kessler and Baldwin, 2001). Plant volatiles may be released due to plant tissue damage or induction by an elicitor upon insect feeding. Phytophagous insects damage plant tissue with their mouthparts, secrete elicitors in their saliva and cause the release of herbivore-induced plant volatiles (De Moraes *et al.*, 1998; Paré and Tumlinson, 1999; Engelberth *et al.*, 2004; Mithöfer, Boland and Maffei, 2009).

1.3.1 Identification of semiochemicals

A series of experimental steps are required to identify plant semiochemicals. The first step is the collection of volatile compounds from plants. The second step is to verify it is bioactive by recording of an insect behavioural response to it. The third step is analysis of the collected volatile sample in Gas chromatography (GC) coupled with electrophysiology to identify the electrophysiologically active volatile compounds for identification by mass spectrometry and GC analysis. The last step is to compare the behavioural responses of insects to the identified volatile compounds and the

biological sample to confirm the identification. These steps are discussed in more detail below.

1.3.2 Semiochemicals in Insect plant interaction

Organisms can detect chemical cues and signals from other organisms in the ecosystem and these chemicals help the insects and plants in interacting with each other (Paré and Tumlinson, 1999). Semiochemicals play a critical role throughout the life of insects since insects use chemical information from their environment for locating food, oviposition, aggregation, finding a sexual partner and avoiding adverse situations (Gut *et al.*, 2004). These chemicals are capable of repelling or attracting insects, masking the effect of other chemicals and are used in pest control strategies such as mating disruption, anti-oviposition or mass trapping (El-Shafie and Faleiro, 2017). Additionally, semiochemicals can also affect the behaviour of natural enemies and consequently affect tritrophic interactions in food webs (Carde, 1990; Cook *et al.*, 2007). As mentioned above, some semiochemicals regulate interactions within species (pheromones), while some act between species (allelochemicals).

It is well documented that volatile release from herbivore damaged plants is responsible for attracting natural enemies of the pests (Turlings and Wäckers, 2004; Mumm and Hilker, 2006; Aljbory and Chen, 2018) (Fig. 1.6). Natural enemies use plant odours as a signal to search for their prey. However, these interactions are specific to the plant and insect. For example, heterologous expression in Arabidopsis of a herbivore-induced terpene synthase, TPS10 from *Zea mays*, forms (*E*)-β-farnesene, (*E*)-bergamotene and other sesquiterpenes was shown to attract an insect associated with *Z. mays* (Schnee *et al.*, 2006). Plants overexpressing TPS10 in *Arabidopsis thaliana* produced large amounts of TPS10 sesquiterpene compounds identical to those produced by maize which indicates that activation of a single gene (herbivore-

induced) from *Z. mays* is enough to activate indirect defence (Christiane Schnee *et al.*, 2006). The volatiles produced by TPS10 overexpressing plants were found to attract *Cotesia marginiventris*, a parasitoid of *Spodoptera litura* (Köllner *et al.*, 2013). Infestation of maize roots with corn rootworm (*Diabrotica virgifera*) larvae induces (*E*)-β-caryophyllene emission, which attracts *Heterorhabditis megidis* (a nematode) that feeds on *D. vigifera* larvae (Rasmann *et al.*, 2005). Several volatile compounds, including linalool and farnesenes induced by gypsy moth in *Vaccinium corymbosum*, are repellent against many caterpillars (Markovic *et al.*, 1996; Rodriguez-Saona, Rodriguez-Saona and Frost, 2009). On exposure to HIPV, a 70% reduction in weight of *Lymantria dispar* was observed, while a positive effect was recorded in attraction of natural enemies. Earlier studies suggested that in field, an increase in volatiles emission is responsible for the abundance of increase in natural enemies (Rodriguez-Saona, Rodriguez-Saona and Frost, 2009; McCormick *et al.*, 2019).

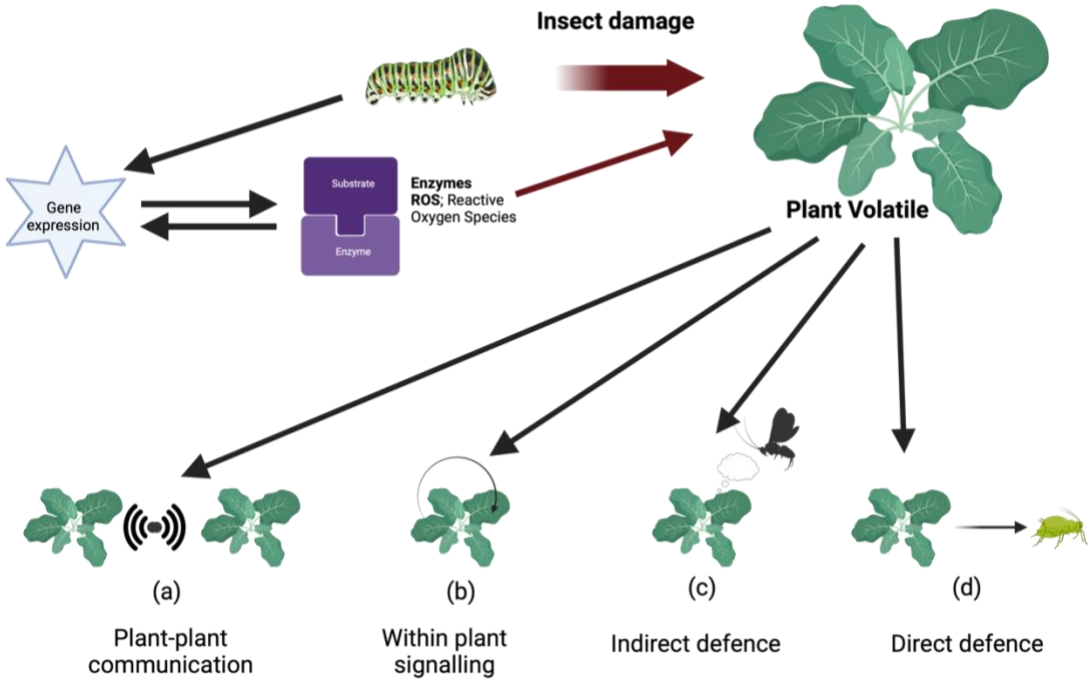

FIGURE 1.6 | Role of insect damage and HIPVs in plant defence. Insect damage

causes release of HIPVs that induces direct and indirect defence by repelling pest, attracting natural enemies, or priming within or between plants.

1.4 Chemoreception in insects

Insects generally rely more on chemicals for communication compare to vertebrates (Stotz, Kroymann and Mitchell-Olds, 1999; Wyatt, 2014). Insects produce chemicals for many reasons, for example mating, territory defence, and protection against predators(Čokl and Millar, 2009; Wong, Meunier and Koelliker, 2013). These chemicals are detected using specific chemoreceptors (Blomquist and Bagnères, 2010). Sensing chemicals may be divided into taste (detection of aqueous chemicals) and smell (detection of airborne chemicals (Chapman, 2003; Pearce *et al.*, 2006; Mollo *et al.*, 2017). Alternatively these modalities can be referred to as contact and distant chemoreception. Contact chemoreception is for taste (gustation), while distant chemoreception is for smell (olfaction)(Chapman, 2003). Chemical molecules attach to binding proteins and are transferred to receptors which is the site of recognition.

Chemical detection and nerve stimulation leads to the depolarization of the receptor neuron at the recognition site. The capacity of chemical detection depends upon the localization and type of chemoreceptors (Cocroft and Rodríguez, 2005). A large number of contact receptors are present on mouthparts, ovipositors and antennae (Renwick, 1989; Van Der Goes Van Naters and Carlson, 2006; Seada and Hamza, 2018). These receptors are associated with specific functions and respond to specific chemicals. For instance, the labella (a mouthpart) of Diptera have receptors for salt and sugar, while ovipositor bears receptors responsible for recognising the ovipositional site (Drosopoulos and Claridge, 2005). The most prominent part of the insect chemoreception system consists of the antennae which have many distant and

contact chemoreceptors and mechanoreceptors. In some insects, tarsi also have chemoreceptors (Yack, 2004).

A sensillum (plural; sensilla) is a unit of the insect chemoreception system and based on the number of pores, sensilla may be uniporous (with one pore) or multiporous (with more than one pore) (Schneider, 1964). Uniporous sensilla have thick walls and permeable pore and occur in different forms such as hair, peg, plates or simple pore as a depression in the cuticle (Zacharuk, 1980; Ryan, 2002). Peg like structure contains a basal chamber that remains in contact with dendritic chamber lies beneath the cuticle. It extrudes a viscous liquid to trap and transfer chemicals to dendrites. Uniporous sensilla detect chemicals by (contact chemoreception) (Zacharuk, 1980). While multiporous sensilla are primarily responsible for olfaction (Thurm and Küppers, 1980; Hunger and Steinbrecht, 1998; Nowińska and Brożek, 2021). Multiporous sensilla can also be hair or peg-like in appearance and have many circular pores or slits (Hunger and Steinbrecht, 1998). The walls of the sensilla are thin and fused with a chamber called the pore kettle. These pores are interconnected with multi-branched dendrites present beneath the cuticle (O'Connell *et al.*, 1983; Hunger and Steinbrecht, 1998; Nowińska and Brożek, 2021). The electrophysiology techniques determine which chemicals insect olfactory system can detect (Gitau *et al.*, 2013). Electroantennogram (EAG) recording is a well-known and influential technique that calculates the total response of an antenna to particular stimuli (Park *et al.*, 2002; Jacob, 2018).

1.4.1 Insect behaviour

Determination of the behavioural response of an insect is one of the first and most essential steps in the identification of semiochemicals. Behavioural responses can be

categorised as either repulsion or attraction. However, these terminologies are simple that can easily lead to confusion because these two terms do not cover all possible types of reactions of insects to chemicals (Table 1.1). To solve this problem, some authors have been led to use new terms, and others elaborated the terms attractant and repellent (Dethier, 1947; Kennedy, 1947; Dethier, Browne and Smith, 1960). Table 1.2. summarises the definitions of behavioural responses exhibited by the insects to chemicals (Dethier, Browne and Smith, 1960).

Types of behaviour	Description
Attractant	Insect moves towards the odour source
Locomotor stimulant	Increases locomotary activities
Feeding, mating or oviposition stimulant	Elicits feeding or ovipostion in insects
Arrestant	Reduces actual speed of locomotion
Repellent	Insects to move away from odour source
Deterrent	Inhibits feeding or oviposition

Table 1.1 | Terminologies used for chemical compounds to define insect behaviour and their description

These terms have significant importance in pest management; different terms describe different behaviours that may impact the application of semiochemicals in the field. Each term represents a specific type of behaviour, which differs significantly from the others. A chemical or odour source that acts as an attractant has a different impact on insects from the chemicals which acts as an arrestant. For instance, an attractant is more effective in catching insects than an arrestant because an attractant works as

a lure for insects that attracts the insects from a long distance, whereas an arrestant works when insects have already arrived in the place or trap.

To test the insect response to an odour a device called olfactometer was invented by Pettersson in 1970. An olfactometer is an important instrument to identify the behavioural response of insects to volatile compounds. Based on the shape, olfactometers may be Y-tube, linear track, four-arm olfactometer or a locomotion compensator (Thorpe and Jones, 1937; Dwumfour, 1992; Cook *et al.*, 2002; Girling *et al.*, 2006). The four-arm olfactometer bioassay is useful instrument for studying aphid behaviour. An insect is placed inside through a central opening at the top of the olfactometer in this olfactometer. The container is sealed except for four side openings through which odour carrying air is passed. Inside the olfactometer, insects are allowed to move freely and choose the odour source (Pettersson, 1970; Visser and Taanman, 1987; Nottingham *et al.*, 1991; Girling *et al.*, 2006; Dewhirst, 2007). Y-tube and linear olfactometers are designed in a way where two odour sources can be tested to measure attraction and repulsion (Dethier, Browne and Smith, 1960). An insect walks towards a junction where two different odours meet, and then the insect responds to any of them by making an oriented movement either towards the odour source or away from the odour source.

In a four-arm olfactometer, the insect is enclosed in an area divided into four regions as the name indicated. Each region has its opening for the odour source. In a four-arm olfactometer, total time spent by an insect in each arm is recorded to determine the insects' preference for different odour sources. A four-arm olfactometer does not distinguish between attraction and arrestant or between repulsion and locomotion stimulation because it is designed to record the time spent by the insects, even it does not tell how the insect has moved in response to the odour source. In contrast, four-

arm olfactometer has several advantages, such as; allowing insects to make multiple choices and avoid initial behaviour responses that may be due to the stress of being placed into an olfactometer. While in locomotion compensator olfactometer, insects are placed on a sphere, and the odour source is blown over the insect; when the insect moves in response to the odour source, it causes the rotation of the sphere. The sphere's rotation is then recorded and describes the direction of movement, speed of movement, and rate of turning of the insects, which allows the recording of different behaviour.

Olfactometers are commonly used for the study of insect behaviour. However, it is vital to be aware of the type of behaviour associated with the olfactometer. Each olfactometer is capable of measuring specific behaviour. A four-arm olfactometer is an extensively used device to test the preference of the insects to odour sources. The locomotion compensator can record a range of different behavioural responses that can be useful to describe the insects' behaviour.

1.4.2 Detection of semiochemicals by aphids

Like many other insects, aphids also detect volatiles via olfactory sensilla located on the antennae. The antennae of an aphid is a thin, and five or six segmented structure (Visser, 1986; Zhang *et al.*, 2017). Primary and secondary olfactory receptors are located on the base of the terminal and distal end of the penultimate segment and are collectively called primary rhinaria, which is common in all morphs of aphids (Zhang *et al.*, 2017). In addition to primary rhinaria, alate and some apterous adult morphs also have secondary rhinaria, generally present on the third segment and maybe on segments four and five (Hardie, 1980; Visser, Piron and Hardie, 1996; Park and Hardie, 2002).

Aphid antenna is divided into proximal and distal primary rhinaria (Visser and Piron, 1995). Proximal primary rhinaria contain single placoid sensilla, while distal primary rhinarium contains three placoid sensilla (one large and two small) and four coeloconic sensilla. Secondary rhinaria contain single small placoid sensilla that can be consists of one or more bipolar neurones (Bromley, Dunn and Anderson, 1979; Park and Hardie, 2004). When insects are exposed to airborne volatile, the volatile molecule absorbs on the surface of the sensilla and diffuses into many pores. Transfer of these odour molecules to the odorant receptor is achieved by odour-binding proteins (OBPs) or chemosensory proteins (CSPs) (Pernollet and Briand, 2004; Hilker and Meiners, 2010; Beyaert and Hilker, 2014; Reinecke and Hilker, 2018). Depolarisation of the dendritic membrane and stimulation of nerve impulses in the body cell occurs when odour molecules bind with a G-protein-coupled receptor (Pernollet and Briand, 2004; Hilker and Meiners, 2010). Dendritic depolarisation and nerve stimulation pass the information back to the olfactory lobe in the brain. Activation and degradation of the olfactory molecules happens in a way that prevents interference with the recognition system. Odour-binding proteins and G-protein-coupled receptors achieve recognition of odour molecules (Firestein, 2001; Pernollet and Briand, 2004; Reinecke and Hilker, 2018; Wasilewski, Gębicki and Kamysz, 2018).

1.5 Plant volatile compounds

The number of identified plant volatiles is approximately 1700, which will likely increase as the number of studies on plant defence systems is increasing (Knudsen *et al.*, 2006; Shrivastava *et al.*, 2010; Ramya *et al.*, 2017). Plants use volatile compounds for various purposes, including direct and indirect defence, insect-plant and plant-plant communication and, when exposed to abiotic stress conditions, for heat-tolerance and environmental adaptation (Heil and Karban, 2010; Holopainen and

Gershenzon, 2010; Ninkovic *et al.*, 2013; Sharma, Malthankar and Mathur, 2021). Volatile organic compounds (VOCs) are released into the air and their emission differs according to the plant species (Llusià, Peñuelas and Gimeno, 2002; Vivaldo *et al.*, 2017). Different plant lineages may produce different chemical odours to resolve similar ecological challenges and may be exposed to different challenges (Pichersky and Gang, 2000; Wright and Schiestl, 2009; Thompson *et al.*, 2022). The emission rate and composition of these chemicals differ according to various factors discussed later in this chapter.

Plant volatiles mainly comprise of terpenoids, phenylpropanoids/benzenoids, fatty acid and amino acid derivatives that constitute approximately 1% of total plant secondary metabolites (Dudareva, Pichersky and Gershenzon, 2004; Dudareva, *et al.*, 2013; Derbassi *et al.*, 2022). These volatiles can be released in the absence of a diffusion barrier due to their lipophilic nature and high vapour pressure (Pichersky, Noel and Dudareva, 2006; Dudareva, *et al.*, 2013). One of the primary functions of plant volatiles is to defend the plants from herbivorous insects but specialized insects may use them for host recognition (Baldwin, Kessler and Halitschke, 2002; Baldwin, 2010; Belete, 2018). The emitted odours can indicate the physiological status of plants and the stress level that they are suffering (Pichersky and Gershenzon, 2002; Belete, 2018). After herbivore attack, plants emit herbivore induced plant volatiles (HIPVs).

Herbivore attack can induce plant volatiles that directly repel herbivores and recruit natural enemies to defend the pest (Turlings and Erb, 2018; Gebreziher, 2020). This recruitment process of natural enemies through VOCs is known as indirect defence and establishes tritrophic interactions between plants and insects (Arimura *et al.*, 2004; Degen *et al.*, 2004; Mercke *et al.*, 2004; Heil and Ton, 2008; Turlings and Erb, 2018). Emission of volatiles can also transmit danger signal that activates defence

genes within the plant and/or in neighboring plants and makes them resistant against attack (Arimura *et al.*, 2000; Birkett *et al.*, 2000; Farag *et al.*, 2005; Ruther and Kleier, 2005; Belete, 2018; Turlings and Erb, 2018). Apart from these airborne signals, roots also produce volatiles responsible for belowground defence by functioning as antimicrobial agents or attracting natural enemies to attack root-feeding insects (Rasmann *et al.*, 2005). In the last ten years, advancements in research on plant volatiles have been facilitated by improvements in analytical instrumentation and advancements in approaches for a better understanding of the origin, function, and metabolic engineering of plant volatiles (Bouwmeester *et al.*, 2019; Nagegowda and Gupta, 2020; Tholl *et al.*, 2021).

Plant volatiles play a crucial role in defence mechanisms against herbivores by acting directly as a repellent to the attacking insects (Agelopoulos *et al.*, 1999; Sobhy, Erb and Turlings, 2015; Turlings and Erb, 2018), or indirectly by attracting the natural enemies, i.e. predators and parasitoids of the insect pest (Dicke *et al.*, 1990; Turlings, Tumlinson and Lewis, 1990). The plant volatiles released in response to herbivore feeding are called herbivore-induced plant volatiles (HIPVs), which act as indirect defence signals. The production of inducible defences against the pest is regulated by signalling pathways of plant hormones, including salicylic acid (SA) and jasmonic acid (JA) (Erb, Meldau and Howe, 2012; Dudareva *et al.*, 2013). The induction of plant volatiles can also be triggered by certain chemicals known as plant elicitors (Sobhy *et al.*, 2020). Phenotypic manipulation of HIPVs can be achieved by the application of plant elicitors (Sobhy, Matthias Erb, *et al.*, 2012; Sobhy, Bruce and Turlings, 2018), these elicitors do not have a direct toxic impact on the target pest (Sobhy, Erb and Turlings, 2015) and play a vital role in defence signalling pathways that could be utilised in pest management programs (Bruce *et al.*, 2017). Chemicals responsible for

priming plant defence systems against pests and pathogens are being applied commercially; however, their development and application are still at the experimental stage. This is due to the fact that plant genotype, environmental factors, and other stressors significantly influence any subsequent progress in plant defence against pests caused by the application of priming chemicals (Bruce, 2014). A simple classification of plant volatiles is given below (Fig. 1.7).

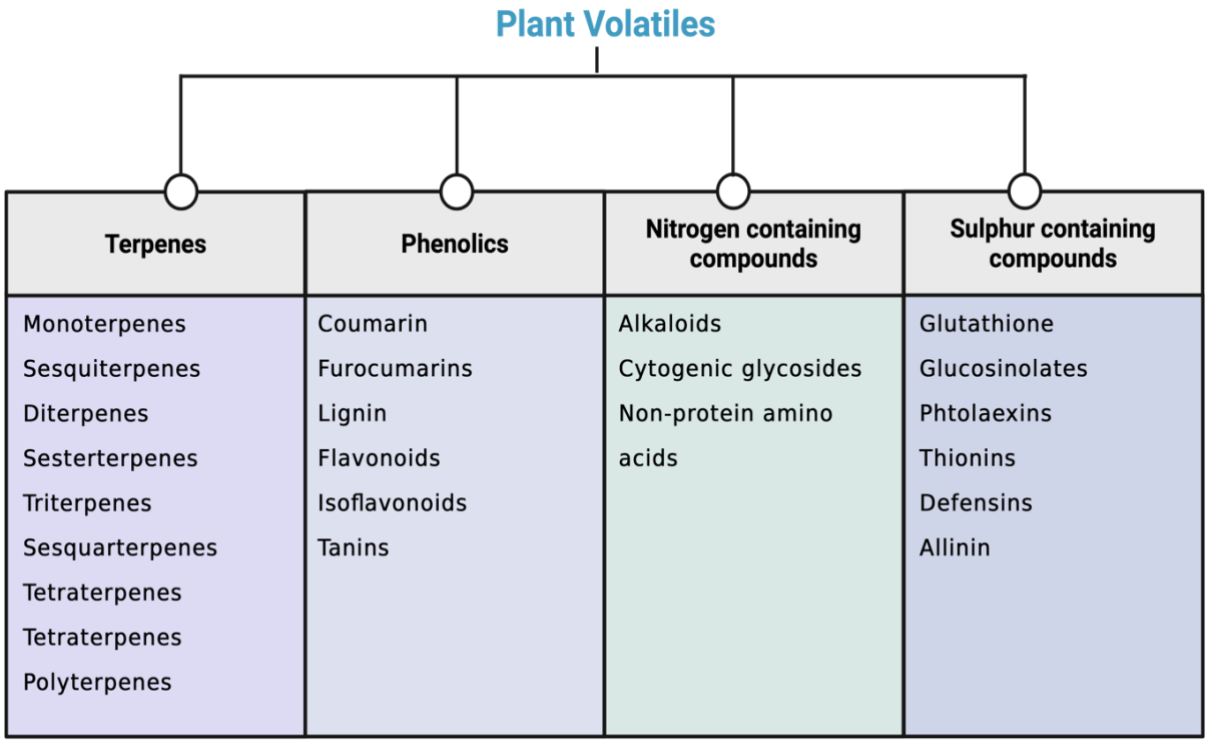

FIGURE 1.7 | Major classes of plants produced secondary metabolites

1.5.1 Regulation of volatile emission in plants

Volatile compound production is structurally regulated; different parts of plants release volatiles. An abundant and diverse blend of volatiles is released from flowers (Knudsen *et al.*, 2006). Vegetative parts can also produce a considerable amount of volatiles, for example, stems and foliage of conifers and leaves of peppermint and basil (McConkey, Gershenzon and Croteau, 2000; Vassão *et al.*, 2006). In general, volatiles released from vegetative tissue can be induced by an injury caused by mechanical

damage, herbivory or microbial infection (Arimura *et al.*, 2004). The amount of volatile emission depends upon the plants' age, response to endogenous diurnal rhythms and environmental conditions such as light, humidity and temperature (Dudareva, Pichersky and Gershenzon, 2004). Previous studies suggest that volatile production and emission increases during the early stages of plant development, especially during young leaves and immature fruits, and starts to decrease steadily thereafter (Dudareva and Pichersky, 2000; Vassão *et al.*, 2006). A plant emits VOCs through several biochemical pathways which are interconnected to each other. Biosynthesis of volatiles requires activation of enzymes and expression of specific genes; evidence supports the idea of transcriptional regulation of one or multiple intermediate steps (Dudareva and Pichersky, 2000; McConkey, Gershenzon and Croteau, 2000; Muhlemann *et al.*, 2012). The emission of volatile compounds can be natural or induced by biotic or abiotic factors. The release of plant volatiles in response to herbivory is known as herbivore induced plant volatile (HIPV) production, which depends on a complex process of upregulation of gene expression (Ament *et al.*, 2004; Ralph *et al.*, 2006).

Generally, plants release volatiles in five different ways: (1) diffusion; through subterranean and aerial surfaces, (2) leaching; (dew and rainwater), (3) exudation, (4) damage caused by biotic/abiotic factors, and (5) by the decay of plant material (Rice, 2012). The storage of plant volatiles occurs in different structures; monoterpenes, sesquiterpenes and aromatics compounds are stored in trichomes while blends of green leaf volatiles (GLVs) (consist of saturated and unsaturated six-carbon alcohols, aldehydes and esters) are quickly produced in the parts of leaves other than trichomes and emitted after damage (Paré and Tumlinson, 1999; Scala *et al.*, 2013). The patterns of plant volatile release have been studied extensively (Dudareva *et al.*, 2006;

Niinemets, Kännaste and Copolovici, 2013; Ni *et al.*, 2021). Plant damage is one of the main factors causing the release of volatiles that includes the damages caused by carnivores and herbivores (Mattiacci, Dicke and Posthumus, 1994; Takabayashi, Dicke and Posthumus, 1994; Agrawal, 2002; Rodriguez-Saona, Rodriguez-Saona and Frost, 2009; Mitchell *et al.*, 2016). Plant volatiles play a critical role in establishing the interactions between biotic and abiotic factor and the host plant, a brief summary of these entities is given in (Fig 1.8) .

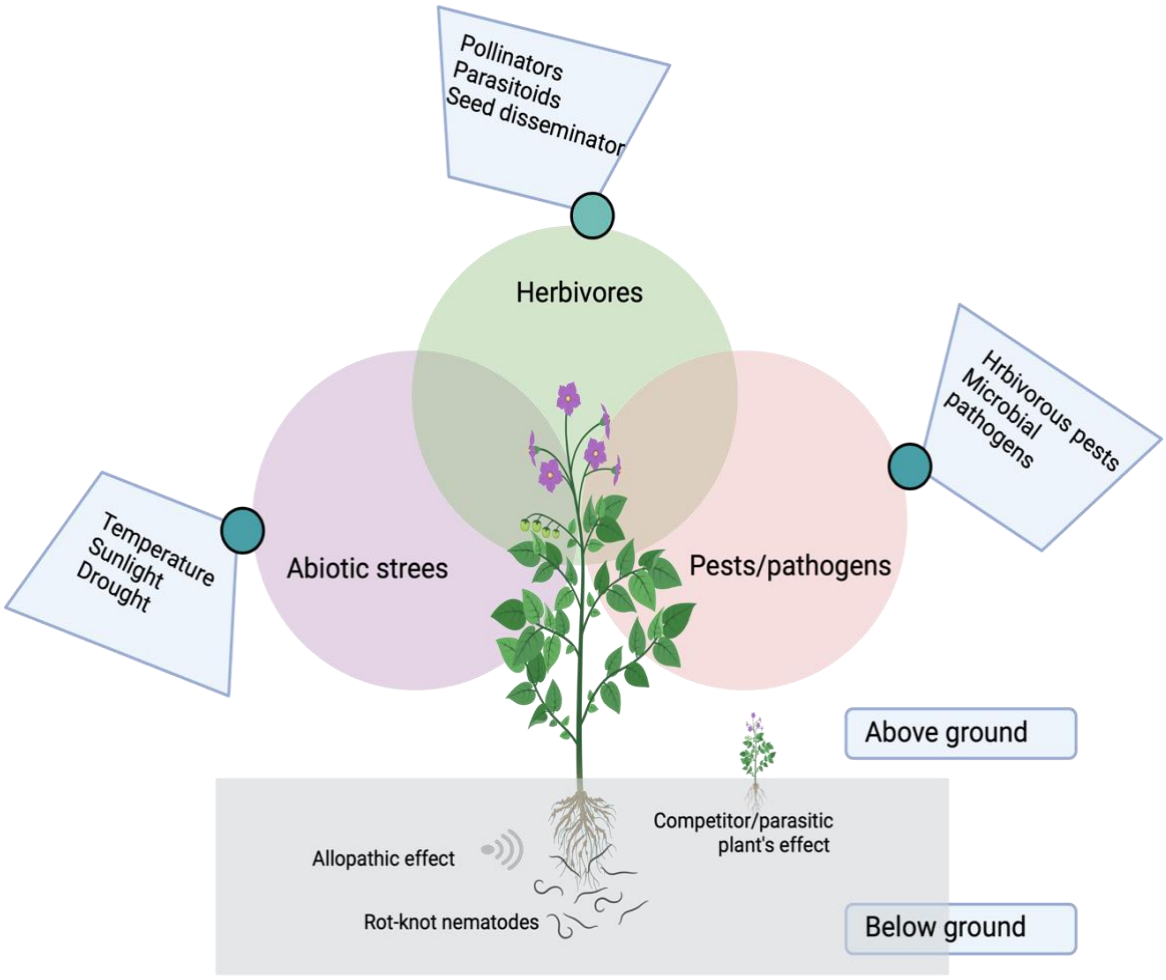

FIGURE 1.8 | A summary of roles of plant volatiles in plant interactions with its surrounding environment.

Above-ground interactions can be affected by priming or elicitation of the defense responses of nearby unattacked plants through the leaves of the same plant (Kessler

et al., 2006). Moreover, the below-ground interactions also exhibit allelopathic effect and affect germination, growth and development of nearby competing plants (Igiehon and Babalola, 2018). Terpenoid volatiles emitted from roots and reproductive organs also possess antimicrobial activity that protect the plants against pathogen attack (Dudareva *et al.*, 2006). Furthermore, leaf volatiles help the plant against thermotolerance and photoprotection (Dudareva *et al.*, 2006). Plants can distinguish between the stress caused by biotic or abiotic factors; they can even differentiate between general mechanical wounding and damage caused by the herbivores (Paré and Tumlinson, 1999). Volatile blends emitted from undamaged, artificially damaged, or herbivore damaged plants show qualitative and quantitative differences (Paré and Tumlinson, 1999; Niinemets, Kännaste and Copolovici, 2013). The presence of associated components, for example elicitors or proteins in insect saliva, at the injury site leads to the activation of different plant defence pathways (Erb, Meldau and Howe, 2012).

Plants respond against pests through a number of defence pathways, including inducible and systemic defence (Bruce and Pickett, 2007). Plants release a qualitatively and quantitatively different blends of volatiles in response to damage. This volatile profile also varies according to the type and source of injury; for instance, a caterpillar infested cotton plant releases higher levels of induced volatiles than a mechanically damaged plant (Paré and Tumlinson, 1996). Presence of elicitors in an insect's saliva allow the plant to discriminate between damage caused by general wounding or insect (Fig. 1.9) (Freeman, 2008). In response, plants release volatile organic compounds that differ both qualitatively and quantitatively depending on the source of damage (Paré and Tumlinson, 1996; Moraes *et al.*, 2008; Tiwari *et al.*, 2020; Amo *et al.*, 2022).

HIPVs can have different effects on herbivores, they can act as a repellent for one species but as an attractant for another. Field studies on native habitat of tobacco (*Nicotiana attenuata*), showed that silencing of green leaf volatiles (GLVs) increased the herbivore burden on the modified plants (Halitschke *et al.*, 2008; Meldau, Wu and Baldwin, 2009). The quantity and quality of HIPVs released depends upon the diversity of associated herbivores and the intensity of damage that comes from each of these insects (Fig. 1.9) (Poelman *et al.*, 2008; Dicke and Baldwin, 2010; Rowen and Kaplan, 2016).

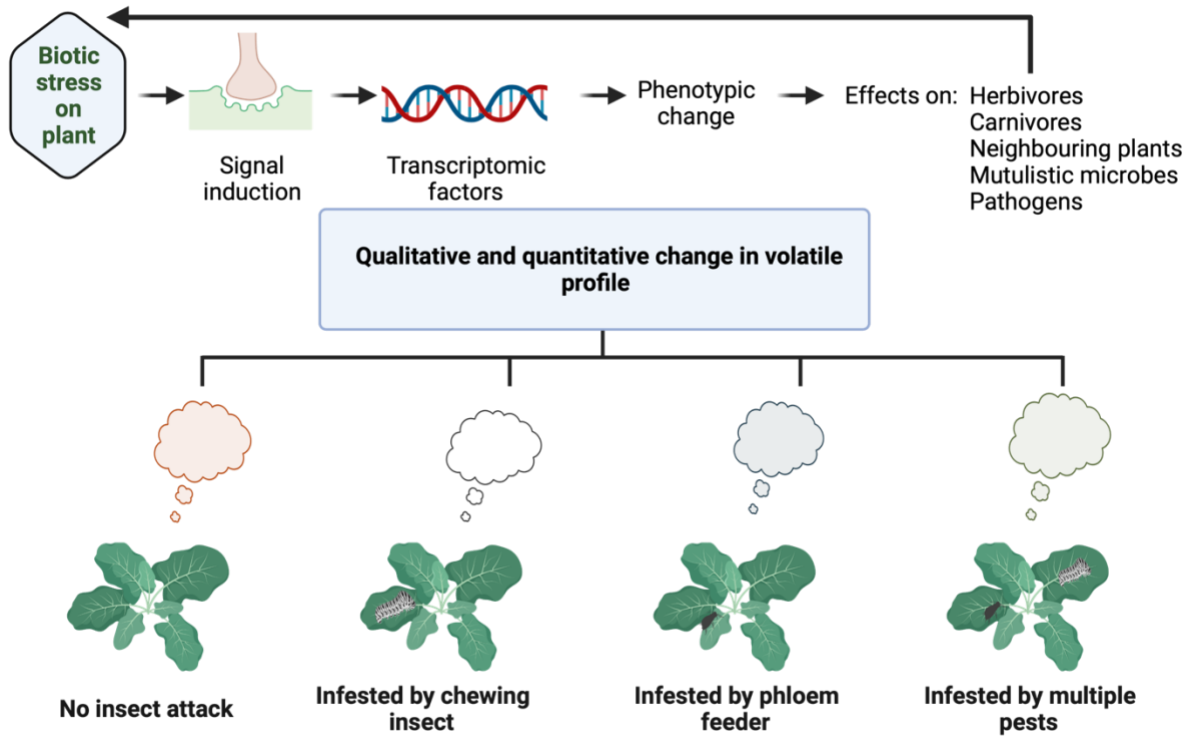

FIGURE 1.9 | Insect herbivory affects the biosynthesis and emission of volatile compounds in terms of quality and quantity. Plant volatile blend differs in their composition according to the herbivores feeding on the plant.

1.5.2 Collection of plant volatile compounds

Increasing scientific interest in the chemistry of plant volatile organic compounds (VOCs) has led to the development of various systems for the collection of volatiles

(Linskens and Jackson, 1999). Volatile compounds can be collected from living odour sources by headspace sampling that can be static or dynamic. Static sampling enables the sample to occur into equilibrium with its vapours which are then collected with an absorbent filter. In dynamic headspace sampling, clear air is drawn through one end into the chamber where the odour source is present, and on the other end, an adsorbent is connected that collects the volatile compounds. Volatile compounds can be extracted from the adsorbent by thermal desorption or by eluting with solvent. However, in thermal desorption, the whole sample is used at once, so it cannot be used again. While through elution, we can use our sample in multiple chemical analyses, bioassays and electrophysiological experiments. Different sampling techniques result in different isolation of the volatile compounds; the ratio of volatile compounds depends on the method used for sampling. Generally, dynamic headspace sampling is the best in the conservation of the ratio of compounds. Differential adsorption may result from other factors such as; breaking through of some compounds in the filter without being trapped, or the adsorption of compounds onto the surface of the container (dome/bag) that encloses the odour source and the affinity of the material used for adsorption.

In addition to static and dynamic sampling, other sampling methods can also be employed. For example, steam distillation is another method for volatile collection that lowers the boiling point of compounds by adding steam so that volatile compounds start to evaporate without deterioration. However, there may be an increase in the extraction of specific volatile compounds due to the breakdown of plant tissue by the steam and this may change the quality and quantity of the volatile compounds. Vacuum distillation is another method that uses vacuum to lower the boiling points of the volatile compounds to collect them in solution. Steam and vacuum distillation

methods allow large quantities of volatile plant compounds to collect in a short space of time. However, it is not easy to know the accuracy of quantity and ratio of the compounds emitted naturally by the plant because it is a destructive method that could easily release additional compounds from/within the plant tissue by destruction.

1.5.3 Identification of volatile compounds

Identification of semiochemicals can be achieved using mass spectrometry and comparison of GC retention times. Mass spectrometry is used to determine the chemical composition and molecular structure of the sample. In mass spectrometry, electron bombardment makes the sample lose electrons and breaks it into ions. With the help of high voltage, ions are then accelerated onto a detector in the presence of a magnetic field. The GC works on the principle that involves the separation of the mixture into individual components when heated. The heated gas passes through a column with an inert gas. Separated substances release from the column opening enter into the mass spectrometry (MS). MS spectrometry then identifies the compounds based on the mass, charge and abundance of ions obtained from them in their mass spectra. These compounds can be compared with the library of known mass spectra stored on a computer covering several thousand compounds. However, this database provides a tentative identification. Mass spectra only provide tentative identification of a compound that can be confirmed by comparing the compound's retention time with the authentic standards since each compound has a consistent retention time. A compound's retention time is the time at which it elutes from the GC column under the conditions used i.e. when it converts from a liquid or solid stationary phase into a mobile gas phase.

A flame ionization detector (FID) combined with GC may quantify the compounds present in a sample. FID works on the principle that involves the number of carbon

atoms reaching the detector. A Flame-ionisation detector (FID) is a good detector for natural compounds. FID fitted with GC detects the amount of carbon in a sample. In the column, the sample produced carbon ions after burning in a hot hydrogen-air flame. While the overall efficiency of carbon ions remains low, the total amount of ions is directly proportional to the amount of carbon in the sample, and the magnitude of the FID is proportional to the number of carbon atoms reaching the detector. Known concentrations of a compound can be used to plot a calibration curve of FID response against the quantity of a compound. Since the FID responds differently to different chemical structures, separate calibration curves are usually constructed for each compound that needs to be identified if the accurate determination of quantities is required (Bartlet *et al.*, 1993).

1.6 Wild crop relatives

Plants are subjected to environmental stresses that affect their growth, development and productivity. There are different categories of stress; the stress can be abiotic and/or biotic (Fujita *et al.*, 2006; Rejeb, Pastor and Mauch-Mani, 2014; Suzuki *et al.*, 2014; Gull, Lone and Wani, 2019). Biotic stress includes pests and pathogens causes a significant loss to crop plants that reaches up to 30% (Lenné, 2000; Oerke, 2006). In contrast to crops, which have been selected for yield and quality in less austere conditions, wild relatives of crop plants are often more resistant to biotic and abiotic stress (Hajjar and Hodgkin, 2007; Zhang *et al.*, 2017; Muñoz *et al.*, 2017; Mammadov *et al.*, 2018). It is thought that wild plants adapt to a wide range of habitats that make them genetically more diverse and leads to the production of high amounts of protective secondary plant metabolites (Wink, 1988, 2009; Anderson, Willis and Mitchell-Olds, 2011). Environmentally induced physical, chemical and physiological

changes in wild plants may offer an increased level of resistance to pest and pathogens (Xie *et al.*, 2019).

Cultivated crop plants grow in a less diverse environment and are often planted as monocultures. They often possess a lower amount of secondary plant metabolites compared to wild relatives (Smýkal *et al.*, 2018). Furthermore, domestication of crop plants alters their genetic makeup by sweeping selective genes as per human preferences. These imposed changes lead to a genetic bottleneck effect during domestication that further restricts the gene pool (Gross and Olsen, 2010; Olsen and Wendel, 2013). After domestication a selected set of genes remains active that creates a genetic valley with an extremely low genetic diversity (Cowling, Buirchell and Falk, 2009; Gross and Olsen, 2010; Olsen and Wendel, 2013). This altered genetic diversity affects the plant-herbivore interaction and can have effects on both herbivore pests and their natural enemies (Chen, Gols and Benrey, 2015; Whitehead, Turcotte and Poveda, 2017). Crops plants that are subjected to breeding programme subsequently have manipulated germplasm that made them less tolerant to biotic and abiotic stresses (Bleeker *et al.*, 2012). Domestication of crop plants often focuses on increasing yield from crop plants and this could compromise plant defence especially if defences have a yield penalty and are therefore selected against (Rodriguez-Saona *et al.*, 2011). Previous studies have shown that breeding programmes (with primary goal of achieving high yield) have a negative impact on host plant resistance that make crops more susceptible to pest due to manipulation of its genetics to produce high yield (Gepts, 2002; Mishra *et al.*, 2015).

1.7 A brief history of research on plant defence elicitors

Almost 60 years ago, upon Fraenkel's (1959) compelling arguments on the raison d'être of secondary plant metabolites as a means of protecting plants from insects,

attention was first drawn to exploit the elicitors of defense plant and their role in boosting the plant overall resistance. Salicylic acid was first isolated as a growth-inhibiting factor for the rice stem borer (*Chilo suppressalis* W.) in Japan (Ishii *et al.*, 1962). Then, the role of non-protein amino acids in the protection pea plants against the oomycete pathogen was reported (Davey and Papavizas, 1962). Subsequently, Uchiyama et al.(1973) identified 3-allyloxy-l,2-benzisothiazole 1, 1-dioxide as a new rice blast controlling agent. Few years later, Probenazole, which is a saccharin derivative, was first registered in Japan as a defense chemical activator to control rice blast (Watanabe *et al.*, 1977). Exogenous application of SA and other benzoic acid derivatives, such as acetylsalicylic acid (Aspirin), was reported to induce resistance of tobacco against Tobacco Mosaic Virus (TMV) (White, 1979). The first work on physiological effects attributed to Jasmonic acid (JA) and methyl jasmonate appeared, describing activities related to senescence in *Artemisia absinthium* during the 1980's (Ueda and Kato, 1980).

In early 1990s, the synthetic compounds 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3) -thiadiazole-7-carbothioic acid S-methyl ester (BTH) were identified as potent systemic acquired resistance (SAR)-inducers in plants (Metraux *et al.*, 1990; Ward *et al.*, 1991). Afterwards, BTH was commercially sold as an elicitor of SAR in various crops (Kunz, Schurter and Maetzke, 1997). In addition, during this period, an increasing regard has been given to explore jasmonate-related defence inducers against insect herbivores. For example, Farmer and Ryan (1990) found that methyl jasmonate (MeJA) induces the synthesis of defensive proteinase inhibitor proteins both treated and nearby untreated plants. Then, Gundlach et al. (1992) demonstrated the integral role of jasmonic acid (JA) and MeJA in the intracellular signal cascade

which ultimately results in the accumulation of secondary compounds upon the interaction of an elicitor molecule with the plant cell surface.

An extensive literature is available on the effect of these natural compounds as a defence elicitors for instance; exogenous application of MeJA induces formation of defence enzymes and reduce pupal/larval weights, performance, population densities and feeding behaviour (Erbilgin *et al.*, 2006; Yang *et al.*, 2013; Cao, Wang and Liu, 2014; Paudel, Rajotte and Felton, 2014; Jiang and Yan, 2018; Xiao *et al.*, 2019; T. Wei *et al.*, 2021); Prohydrojasmon is a functional analogue of jasmonic acid responsible for induced long-lasting and systemic disease resistance against two key pathogens of tea plants (Yoshida *et al.*, 2010). It is also reported that plant treatment with Prohydrojasmon (PDJ) induced direct plant defence against both chewing and sucking insect herbivores. Specifically, PDJ negatively affected the oviposition and fecundity of two-spotted spider mites (Uefune, Ozawa and Takabayashi, 2014) and supressed the infestation and feeding damage of western flower thrips (Matsuura *et al.*, 2020). In respect to chewing insects, PDJ reduced the weight and survival of Noctuid larvae (Mandour *et al.*, 2013; Sobhy, Mandour and Sarhan, 2015). Interestingly, PDJ also induced the emission of key volatiles which increased parasitoid attraction (Mandour *et al.*, 2013).

Salicylic acid exogenous application on plants makes the plant less suitable for whiteflies (Shi *et al.*, 2016; Jafarbeigi *et al.*, 2020), aphids (Mohase and van der Westhuizen, 2002; Rodríguez-Álvarez *et al.*, 2015), stink bug (Stella de Freitas, Stout and Sant'Ana, 2019), cotton bollworm (War *et al.*, 2015), thrips (Wei *et al.*, 2021). Methyl salicylate (MeSA) acts as a critical part of a signal transduction chain that triggers the response of plant defence and induces an oxidative burst in the roots of the sunflower seedling (Palma *et al.*, 2009). MeSA is a herbivore-induced plant volatile

which releases in response to pests damage. It has been reported that the release of MeSA attract natural enemies and affect the herbivore performance on the plant (Mallinger, Hogg and Gratton, 2011; Gadino, Walton and Lee, 2012). MeSA enhances the biological control by recruiting arthropod natural enemies of the attacking herbivores (Orre *et al.*, 2010). Laboratory studies have demonstrated the positive response of the beneficial arthropods to MeSA (De Boer and Dicke, 2004a; Katsuragi *et al.*, 2010). For example, ladybird beetle (*Coccinella septempunctata)* uses aphid induced soybean MeSA as a signal to foraging the location of prey (Zhu and Park, 2005).

1.8 Induced defence

Plants exhibit a range of defence responses to avoid herbivory (Bandoly, Hilker and Steppuhn, 2015). Plant defense responses are broadly categorised into constitutive and induced defense. Constitutive defense includes physical barriers; cell wall, epidermal cuticle wax, and bark, which provides strength, rigidity and protection against pest invasion (Freeman, 2008). Like all other living organisms, plants can also detect attacking pathogens and defend themselves by inducible defense by producing toxic chemicals and defense related proteins (Van Loon, Rep and Pieterse, 2006). Domestication of crop plants affects the defense capacity of plant and may increase the dependency on synthetic chemicals (Chen, Gols and Benrey, 2015). In terms of host plants, herbivorous insects include generalists that can feed on diverse plant species, or specialists that consume a restricted range of phylogenetically related plants (Rowen and Kaplan, 2016), each strategy providing certain benefits to herbivores. The phytophagous insects existing today with plants are the consequence of a continuous coevolutionary process that has been ongoing for 400 million years (Labandeira, 2013). During the era, insects adjust promptly to the adaptive defense

responses of plants that allow them to feed successfully on the unpredictable and hostile plant host (Després, David and Gallet, 2007).

On the other hand, being in continuous co-evolution with insects, plants have evolved a broad armoury of effective and sophisticated defence mechanisms that enable plants to defend themselves against these invading insects accordingly before they cause significant damage (Howe and Jander, 2008). Such array of defensive strategies involve the production of a plethora of different chemical compounds covering numerous classes of toxic and/or repellent secondary metabolites, digestibility reducing proteins, and antinutritive enzymes that either kill or severely affect the herbivore's growth (Mithöfer and Boland, 2012). As such, plants tackle the herbivores directly by negatively influencing their preference (host plant selection, oviposition, feeding behaviour) or performance (growth rate, development, reproductive success) resulting in an improved plant fitness. Examples of defensive secondary metabolites comprise alkaloids, cardenolides, glycosides, glucosinolates, flavonoids and phenolic acids, coumarins and furanocoumarins, protease inhibitors, terpenoids and tannins (Després, David and Gallet, 2007).

Some of these chemical defences are constitutive but the majority is induced soon after the invasion of herbivores (Mithöfer and Boland, 2012). The cost of induced defence responses in plants is much lower than that of constitutive resistance (Lu *et al.*, 2015). Given that many defence chemicals are of high energy cost and have nutrient requirements associated with their production (Cipollini and Heil, 2010), plants thus only produce these chemicals once they discern any stimuli of invading insects, this phenomenon is known as induced plant defence (Karban and Myers, 1989). In this regard, plants also defend themselves indirectly against herbivores by releasing a complex bouquet of volatiles that recruit the natural enemies (parasitoid and

predators) and/or providing reward (extra floral nectar) and shelter to enhance the foraging success of natural enemies (Heil, 2008). Furthermore, plant volatiles can also affect the herbivores themselves by repelling further colonization (De Moraes, Mescher and Tumlinson, 2001).

The production of plant volatiles varies with type, developmental stage, condition of plant and insect type as well and therefore volatile blends are specific for the particular insect-plant system (Hare, 2011). These inducible defences that render the host plant phenotypically less favourable to insects are complex according to the feeding nature of attackers (e.g. chewing herbivores, piercing/sucking herbivores or pathogens) because different types of attackers elicit different types of defence reactions (Mithöfer and Boland, 2012) and there can be trade-offs between the defence pathways they elicit. Specifically, the salicylic acid (SA) pathway is mostly associated with induced defence against biotrophic pathogens and piercing/sucking insects, while Jasmonic acid (JA) pathway regulates plant defence against chewing insects and necrotrophic pathogens (Erb, Meldau and Howe, 2012). However, the interactions among JA and SA support plants to reduce energy expenditures by making plant defence responses more precise to diverse attackers (Koornneef and Pieterse, 2008).

Interestingly, inducible defences can be also activated using particular bioactive chemical compounds that are called elicitors or inducers of plant defence (Vallad and Goodman, 2004; Holopainen *et al.*, 2009). These findings have promoted the notion that plant inducible defences can be further enhanced or manipulated by switching on defence genes responsible for a key defence compounds (Degenhardt *et al.*, 2003; Kappers *et al.*, 2005). Genetic manipulation of induced plant defence has also been used successfully to alter the emissions of several volatile compounds and thereby

enhance the attraction of natural enemies (Schnee *et al.*, 2006; Degenhardt *et al.*, 2009; Brillada *et al.*, 2013). This approach may offer future solutions, but in most systems, we still miss fundamental knowledge on which key attractants should be targeted to achieve this approach (Sobhy *et al.*, 2014) and there could be costs if induced defences are expressed constitutively.

Other options, such as phenotypic manipulation of plant defence via application of chemical elicitors has received considerable research attention to combat insect pests, particularly in the context of indirect plant defence and tritrophic interaction (Pickett and Poppy, 2001; Stout, Zehnder and Baur, 2002; Sobhy *et al.*, 2014; Turlings and Erb, 2018). During the last two decades, an accumulated body of investigations has been carried out to exploit the potential of using defence inducers to enhance plant immunity in general (Bektas and Eulgem, 2015; Zhou and Wang, 2018). Nevertheless, while major advances have now been made in the application of plant inducers that activate resistance against plant pathogens (Gozzo and Faoro, 2013; Walters, Ratsep and Havis, 2013), most of the inducers promoting resistance to herbivores are still at the experimental level. Sustainable agriculture encourages reductions in toxic chemical pesticide use. To increase the plant defence capability and reduce dependency on synthetic chemical inputs, there is an urgent need of new defensive strategies for crop protection. New options are also needed because resistance has evolved to conventional pesticides.

1.8.1 Use of *cis*-Jasmone in pest management

cis-Jasmone is a natural plant-derived chemical, which is synthesised by the isomerization of *cis*-OPDA to iso-oxophytodienoic acid (iso-OPDA) following the cleavage of oxidative side-chain of oxophytodienoic acid (*cis*-OPDA) (Dabrowska and Boland, 2007). *cis*-Jasmone is a vital component of floral volatiles. However, it can

also be released by damaged vegetative parts of the plant (J. H. Loughrin *et al.*, 1995). Plants release CJ under various circumstances such as herbivory (Birkett *et al.*, 2000; Röse and Tumlinson, 2004), application of insect saliva (Ursula S.R. Röse and Tumlinson, 2005; Sobhy, Erb and Turlings, 2015), treatment with jasmonic acid (JA) (Heil, 2004), or inoculation with nitrogen-fixing rhizobia (Ballhorn, Kautz and Schädler, 2013). It has been found that *cis*-Jasmone acts as a repellent against pests; e.g. in olfactometer and field studies, *cis*-Jasmone was directly repellent to the lettuce aphid, *Nasonovia ribis-nigri* Mosh, damson-hop aphid, *Phorodon humuli* Schrank, respectively (Birkett *et al.*, 2000). Besides, a fewer number of aphids were found on *cis*-Jasmone treated wheat crops than the untreated plant. Furthermore, predacious seven-spot ladybird, *Coccinella septempunctata* L, and aphid parasitoid *Aphidius ervi* Haliday, were directly attractive in an olfactometer and wind tunnel studies (Birkett *et al.*, 2000). Previous studies supported the role of *cis*-Jasmone to elicit plant defence in crop plants, e.g. cereals (Bruce *et al.*, 2003; Moraes *et al.*, 2008), soybean (Moraes *et al.*, 2009), cotton (Hegde *et al.*, 2012) and potato (Sobhy *et al.*, 2017). However repellent responses of insect pests to CJ treated plants is not just a response to CJ itself because CJ treatment induces changes in plant volatile emission.

1.9 Hypothesis

We have two hypothesis behind this research work, one hypothesis is for potato work while another one is for brassica work.

- Do wild potato lines are more resistant to *M. persicae* compared to desiree?
- Does CJ treatment of brassica activates plant defense system as it activated in brassica model plant?

1.10 Aims and objectives

The overall aim of this research is to gain an in depth understanding of the chemical ecology of a serious aphid pest, *Myzus persicae*, and its natural enemy, *Diaeretiella rapae*, on potato and brassica crop lines. This included an investigation of the potential for use of a plant defence elicitor, *cis*-Jasmone (CJ), to treat brassica crops. To achieve this aim, the study had the following specific objectives:

- **Conduct bioassays to record the performance and behavioural responses of *M. persicae* and *D. rapae* to wild and cultivated cultivars of potato.** It was hypothesised that crop wild relative plants contain a greater diversity of secondary plant metabolites than modern cultivated crops. We hypothesise that particular plant secondary metabolites will repel aphids and attract their natural enemies. (Chapter 2)
- **Design and conduct a performance bioassay to determine the survival, fecundity and settlement of *Myzus persicae* on brassica plants.** It was hypothesised that CJ treatment of brassica crop plants make them less favourable for aphids. (Chapter 3)
- **Determine the behavioural responses of *Myzus persicae* to host plant volatiles.** A series of four-arm olfactometer bioassay was performed to investigate responses of aphids to odour collected from treated and control plant. It was hypothesised that reduced performance of *Myzus persicae* on plant is due to changes induced by CJ. (Chapter 3)
- **Determine the preference and foraging behaviour of natural enemies *Diaeretiella rapae*.** A number of parasitoid foraging bioassays were used to investigate foraging on CJ treated and control plants. It was hypothesised that

CJ treatment makes plant favourable to natural enemies and parasitoid would spend more time on CJ treated plant. (Chapter 4)

- **Determine the behavioural responses of *Diaeretiella rapae* to host plant volatiles.** A series of four-arm olfactometer bioassay was used to record the behavioural response of *D. rapae* to host plant volatiles. It was hypothesised that volatiles released from treated plants act as an attractant natural enemies *D. rapae*. (Chapter 5)
- **Identify plant volatiles by GC-MS analysis.** This was carried out to investigate quantitative and qualitative differences in volatile emission of CJ treated and blank formulation treated plants. It was hypothesised that CJ treatment induces changes in plant that leads high emission of plant volatiles, in terms of quality and quantity. To test the effect of individual volatile compounds released by brassica lines on the behaviour of *M. persicae* and *D. rapae*, synthetic analogues of identified volatile compounds were used in olfactometer bioassay. (Chapter 6)

Chapter 2. WILD SPECIES OF POTATO AS POTENTIAL SOURCES OF RESISTANCE AGAINST THE APHID *Myzus persicae*

2.1 Introduction

Considerable variation exists in plant defence mechanisms between different genotypes and this has been shaped by differences in natural variation of plant habitat (Foster, Denholm and Thompson, 2003; Colette *et al.*, 2011). Due to the difference in natural selection pressure, plant populations possess different resistance levels against pest species (Kessler and Heil, 2011). Genetic resources for resistance against pests are limited within the current cultivars of potato, resulting in susceptibility of these cultivars to pests and disease. In contrast, wild relatives of potato grow in a wide range of environments that make them genetically more diverse than the domesticated cultivars of *Solanum* species. Furthermore, they have not been through genetic bottlenecks that restrict genetic variation when plant material is selected for breeding. Commercially available potato cultivars are susceptible to a wide range of insect pests. Factors responsible for plant growth and development can affect the level of resistance exhibited by the plant species (Panda and Khush, 1995; Smith, 2005; Gaillard *et al.*, 2018). Plants growing in fluctuating environmental conditions, face difficulties attaining the full genetic potential required for growth and defense (Iqbal *et al.*, 2021). For example; presence or absence of light not only affects the growth of plants but also has impact on plant defense by affecting the biochemistry of plants and defense against attackers.

Deployment of defense strategy is critical for plant survival but plant defense activation comes at the expense of plant growth (Huot *et al.*, 2014). In nature, plants encounter a large number of pests; in order to grow and combat the pests, the plant has evolved a sophisticated mechanism to adjust its resources to both grow and defend the pests.

However, most crop breeding programs focus on growth-related traits that lead to loss of genetic diversity and make them vulnerable to pests by compromising their defense capacity. Factors that make commercially available crops susceptible to insect pests and diseases include lack of stability in a changing environment, downregulation of defence-related genes and where there has been intense use of insecticides crop resistance traits are not needed and therefore they may have been lost (Peterson *et al.*, 1992; Chaudhary *et al.*, 2008). Wild relatives of crop plants develop in a comprehensive environment that makes them genetically more diverse (Bradshaw, Bryan and Ramsay, 2006; HeřmanoVá, Bárta and Čurn, 2007). These wild cultivars could be a potential source of resistance genes and can be utilised in plant breeding programs (Leppik, 1970; Lenne and Wood, 1991; Hajjar and Hodgkin, 2007). Genetic and environmental factors regulate the content of secondary metabolites in different wild and cultivated species of plants. Secondary metabolites play a significant role in plant resistance and genes encoding that can be transferred to progenies during crop breeding (Wink, 1988; Ode, 2006; Züst and Agrawal, 2016). Phytochemicals can be used in strategies to lower the attractiveness such as changes in the taste of the plant (make it unpalatable for herbivores) that consequently reduce the level of herbivores attack (El-Sayed *et al.*, 2006; Koul, 2008; Ratnadass *et al.*, 2012; Gregg, Del Socorro and Landolt, 2018).

In this chapter, we aimed to identify the resistance level of wild accessions of *Solanum stoloniferum* potatoes, compared with cultivated ones, against the aphid *M. persicae*. Besides, we also recorded the behavioural responses of parasitoid *Diaeretiella rapae* a common natural enemy of peach-potato aphid to odours collected from the potato plants. The resistance of wild relatives of several crop plant species is well documented (Prescott-Allen and Prescott-Allen, 1986; Cooper, Spillane and

Hodgkin, 2001; Hajjar and Hodgkin, 2007; Powles and Yu, 2010; Moore, 2015), however, previous studies have not investigated the lines that we tested here.

2.1.1 Aim and Objectives

- To test the performance of *M. persicae* on different lines of potato species.
- To identify the difference between the blend of volatile compounds released from different lines of potato species.
- To test the behavioural response of *M. persicae* and *D. rapae* to odour collected from different lines of potato species.

2.2 Methods and materials

2.2.1 Insects

Myzus persicae aphids, originally obtained from Harper Adam University, were reared in the insectary in the Centre of Applied Entomology and Parasitology (CAEP) at Keele University. *M. persicae* clone O was reared on Pak choi *Brassica chinensis*, commonly known as Chinese cabbage, in Bugdorm cages (46 cm x 46 cm x 46 cm; NHBS ltd, Devon, UK) under controlled conditions (24 °C, 38 % RH, 16 h L: 8 h D photoperiod). The aphid parasitoid *Diaeretiella rapae* (obtained from Harper Adam University, UK) was reared on *M. persicae*. Parasitoids were kept under controlled condition (20 °C, 40 % RH, 16 h L: 8 h D photoperiod).

2.2.2 Plants

Wild potato, *Solanum stoloniferum* seeds (accessions 18333, 22718, 23072) used in the experiments were received from Wageningen Centre for Genetic Resources (Wageningen University and Research, The Netherlands). The reason for selecting these lines was the results obtained at Rothamsted research institute, in initial screening these lines were found highly resistant, but no further work was done on

these lines. While *Solanum tuberosum* cv. Desirée tubers (grown by Nick Crane, Norfolk, UK) were purchased (Sainsburys supermarket, UK). All plants were grown under controlled environment conditions (20 °C, 37 % RH, 16 h L: 8 h D photoperiod) in a growth chamber (MLR-352-PE, Panasonic, The Netherlands). Potato plants were grown individually in 7.5 cm pots in John Innes No. 2 compost (Westland Horticulture Limited, Tyrone, UK). Four-five week old plants were used for the experiments.

2.2.3 Aphid performance clip-cage bioassay

Performance of *M. persicae* was assessed on the wild potato accessions and compared with cultivated potato species. There were two separate series of experiments with different plants; the first series recorded observation after 48 h and the second recorded observations after 96 h. Fresh plants and aphids were used in each replicate observation in each experiment. In each replicate 10 adult alate *M. persicae* were placed in a clip cage (2.5 cm diameter, Bioquip Product Inc. USA), which was attached to the lower surface of plant leaves (Sobhy *et al.*, 2020). Two clip cages were placed on each plant. Ten replicates, on separate plants, were performed for each genotype. To assess the survival and fecundity of aphids, plants were left undisturbed in a controlled environment room (25 °C ± 2 °C, 37 % RH, 16 h L: 8 h D photoperiod). Plants were assessed after the 48 h (series 1) or 96 h (series 2) period. For assessment, leaves containing the cages were cut and cages were removed without losing any aphids. The number of live adults and nymphs produced were recorded.

2.2.4 Volatile collection

Headspace sampling allows samples to be collected with the chemicals released by the plant in ratios similar to those found in nature. In this project, an air entrainment was carried out following a procedure adapted from (Agelopoulos et al. 1999). During

volatile collection, the plant was kept inside a bag (35 x 43 cm; Bacofoil, UK) to collect the plant volatiles. The bag was partially sealed, so that only the volatiles produced by the plants were collected. Prior to entrainments, bags were baked in an oven (Heraeus, Thermo Electron corporation, Mark Biosciences, UK) at 120℃ overnight. The Porapak Q filters (0.05 g, 60/80 mesh; Supelco, Bellefonte, PA, USA) were rinsed with diethyl ether and conditioned before use. Plants with five true leaves were enclosed in bags individually. Each bag was open at the bottom and closed at the top. An outlet hole was made in the upper part of the bag to connect the Porapak Q filter. Whereas the bag was closed by attaching a rubber band around the pot. Charcoal filtered air was pumped in at 600 ml min^{-1}, and sampled air was pulled out at 400 ml min^{-1} through a Porapak Q filter in which the plant volatiles were trapped. To avoid the entry of unfiltered air, positive pressure was maintained by using differing in flows rates. Connections were made with 1.6 mm (i.d.) polytetrafluoroethylene (PTFE) tubing (Alltech Associates Inc., Lancashire, UK) with Swagelok brass ferrules and fitting (North London Valve Co., London, UK) and sealed with PTFE tape (Gibbs & Dandy Ltd., Luton, UK). Volatile collection was done for a period of 48 h, after which the Porapak filters were eluted with 0.5 ml of diethyl ether, into sample vials (Supelco, 2 ml, PTFE/silicone) and stored at -20℃ in a freezer (Lec Medical, UK) for use in olfactometer bioassays and chemical analysis.

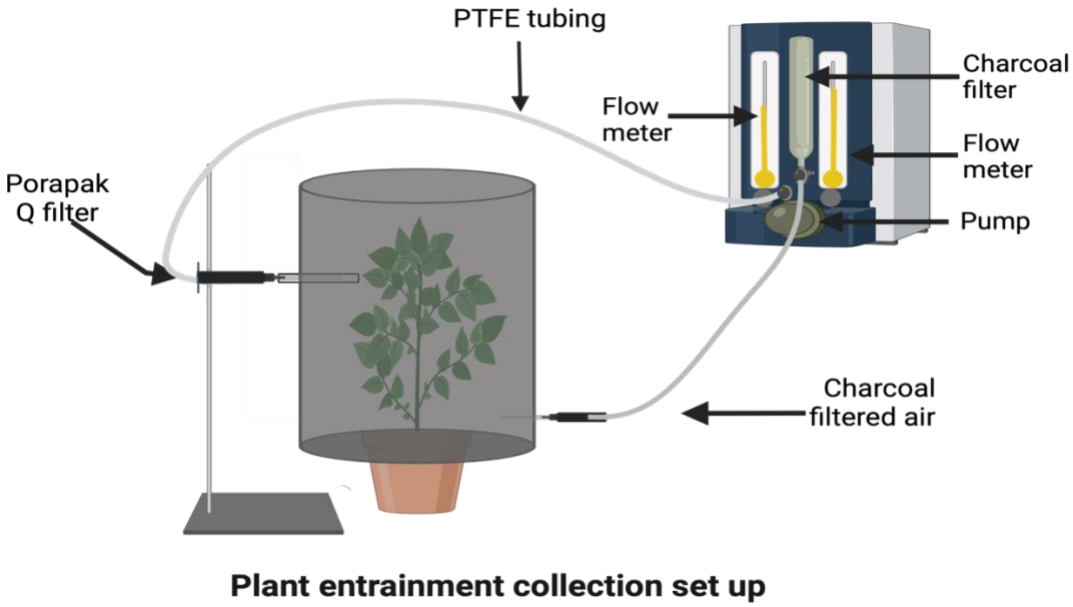

FIGURE 2.1 | An illustration of an air entrainment of a plant.

2.2.5 Behavioural bioassay

An olfactometer (four-arm) bioassay was performed in order to assess the behavioural responses of insects to plant volatiles. All insect bioassays were performed in a room (24℃, 30 ± 2 % RH). After each bioassay, all parts of the olfactometer were washed with an aqueous solution of Teepol, 80% ethanol and tap water, then air dried. No choice (one treatment vs. three solvent) olfactometer bioassay was performed. The protocol followed for olfactometer bioassay described here was also used in other chapters of this thesis.

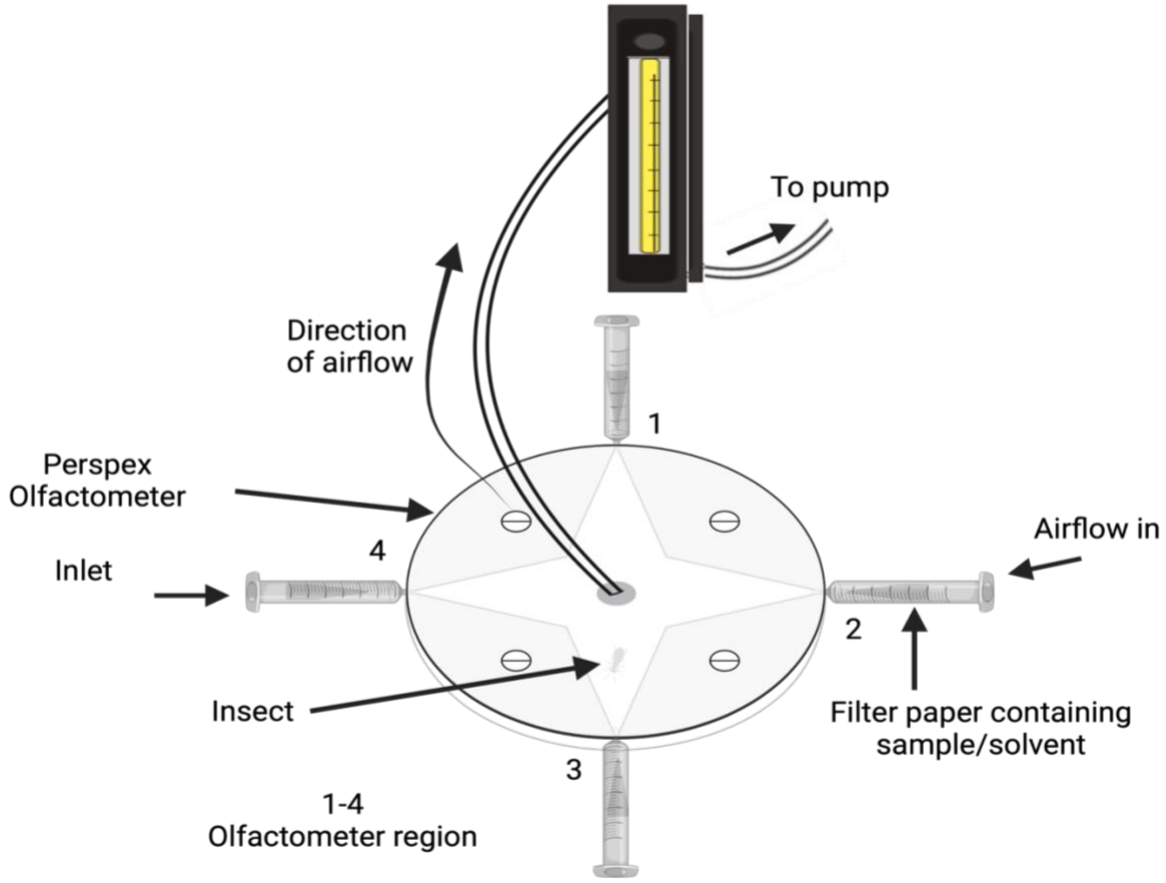

FIGURE 2.2 | A generalised illustration showing four-arm olfactometer with odour inlets

2.2.6 Aphid and parasitoid olfactometer bioassay

The behavioural response of alate *M. persicae* and *D. rapae* to potato volatile organic compounds (VOCs) was investigated using a Perspex 4-arm olfactometer in a controlled environment room (24 ºC ± 2, 30 ± 2 % RH). At the top of the olfactometer, the central area contained a hole into which a single alate *M. persicae* or female *D. rapae* were introduced, which was connected to a low-pressure air pump. Air was pulled out at 200 ml/min from the centre of the olfactometer by a vacuum pump with a layer of muslin to prevent access by an aphid during the bioassays. All replicates were done under uniform illumination. The olfactometer arena was split into five areas; four areas by each arm (one or two treatment(s) and three, or two, control arms) and a

central area (Webster *et al.*, 2010). Each replicate was run for 12 min, and after every 3 min, the position of the olfactometer was rotated clockwise by 90° to eliminate bias. Time spent by the insect in each arm was recorded using a software program OLFA, F. Nazzi Udine, Italy). Ten replicates were done for each insect. Filter paper (Whatman Filter Paper, Buckinghamshire, UK) strips (cut to 5 x 20 mm) were treated with an aliquot (10 µl) of the test solution, applied using a micropipette (Drummond 'microcaps'; Drummond Scientific Co., USA). One arm was assigned to the collected VOCs from the potato plants, whereas three control vessels were treated similarly with the same volume of solvent (diethyl ether) on the filter paper strips (wild potato vs solvent and Desirée vs solvent). If an insect remained motionless for the first 2 min of a replicate, that replicate was discarded. All bioassays were performed between 10:00 and 13:00.

2.2.7 Plant-plant communication: entrainment collection

This study is based on the theme of plant-plant communication. An experiment was designed to investigate the effect of a neighbouring plant (emitter) on the performance of receiver plants. Two plants were connected with the help of a sterile glass test tube (75 mm x 12 mm; Fisher Scientific, UK), (Fig. 2.3). A Desiree plant was used as a receiver throughout the experiment while the emitter plant was replaced with different treatments consisting of non-infested and infested Desiree and wild plants. There was no aphid inoculation on the receiver plant. The accession 22718, was the only wild cultivar that was used in the experiment because of the availability of seeds. Infested plants were inoculated with 100 adult alates 24 h prior to entrainment collection. Volatiles were collected from four different treatments; Treatment A: Desiree receiver + Desiree emitter (DD), treatment B: Desiree receiver + Infested Desiree emitter (DID); treatment C: Desiree receiver + Wild potato emitter (DW); and treatment D: Desiree

receiver + Infested wild emitter (DIW). The volatile entrainment collection was carried out as described in section (2.2.4). Following entrainment collection, olfactometer bioassay (described in section 2.2.5) was performed to study the behavioural responses of *M. persicae* and *D. rapae*. Five replicates were performed for each combination and fresh plants were used each time.

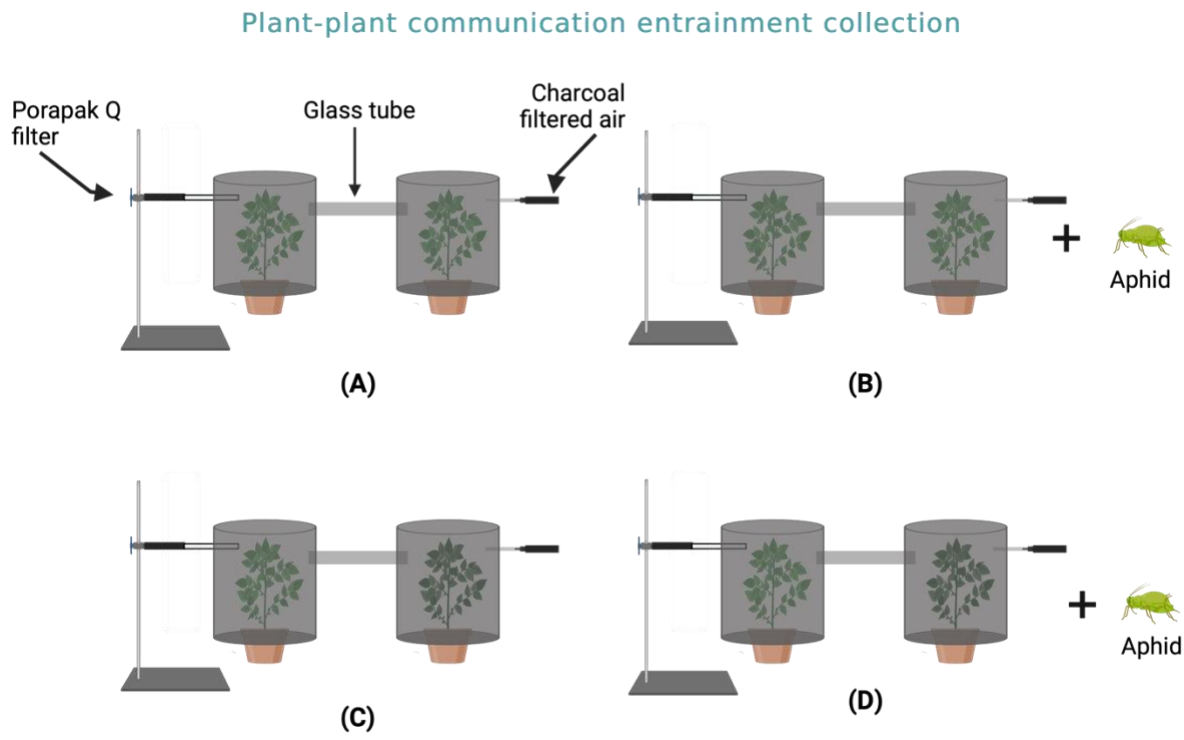

FIGURE 2.3 | plant-plant communication entrainment collection set-up: ((A) Desiree (receiver) + Desiree (emitter) (DD); (B) Desiree (receiver) + Infested Desiree (emitter) (DID); (C) Desiree (receiver) + Wild potato (emitter) (DW); and (D) Desiree (receiver) + Infested Wild potato (emitter) (DIW). Entrainment collection was carried out for 48 h.

2.2.8 Plant-plant communication: clip-cage bioassay

Aphid performance bioassays were performed after entrainment collection. The procedure followed plant-plant communication entrainment collection which was

repeated with some modifications. Two plants were connected through an open-ended glass tube, and filtered air was drawn inside the bag. PTFE tube was attached to the bag containing the receiver plant instead of the Porapak Q filter to maintain the airflow inside the bags (Fig 2.4). Two clip cages (each containing ten adult aphids) were attached to the receiver plant. To assess the survival and fecundity of aphids, the experimental setup was left undisturbed in a controlled environment room (25 °C ± 2 °C , 37 % RH, 16 h L: 8 h D photoperiod) and plants were assessed after the 48 h. For assessment, leaves containing the cages were cut, and cages were removed without losing any aphids. The number of live adults and nymphs produced were recorded.

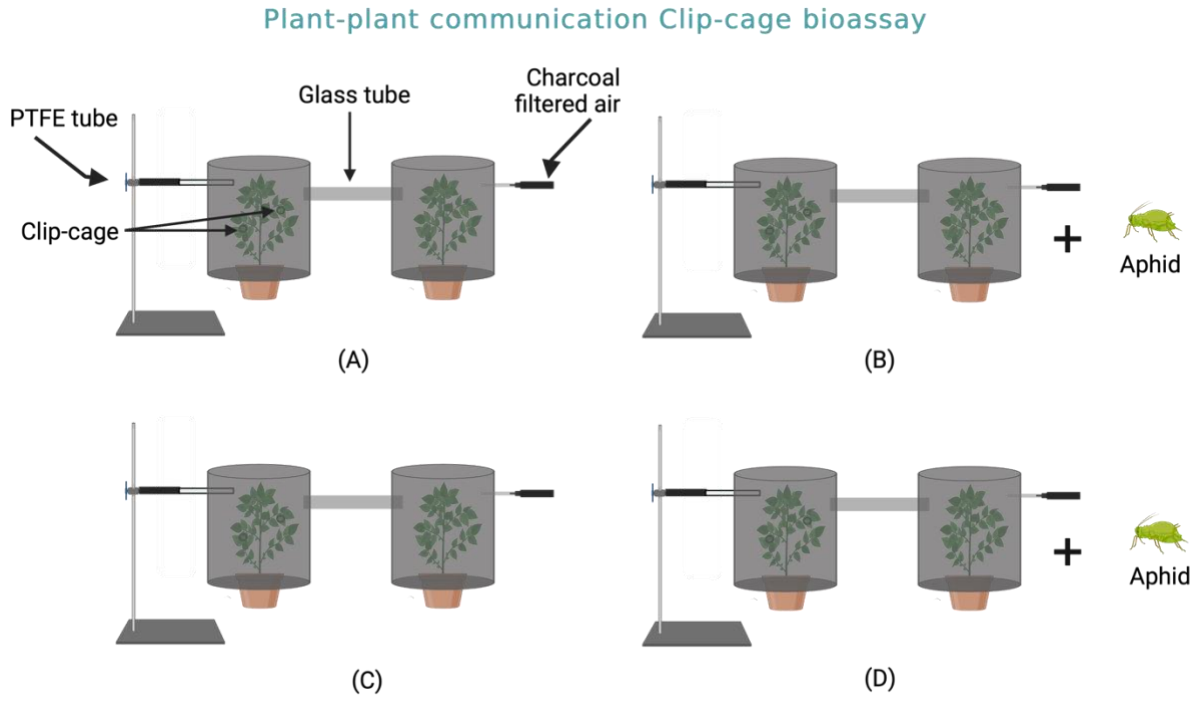

FIGURE 2.4 | Adult *Myzus persicae* performance bioassay on four different combinations: (A) Desiree (receiver) + Desiree (emitter) (DD); (B) Desiree (receiver) + Infested Desiree (emitter) (DID); (C) Desiree (receiver) + Wild potato (emitter) (DW);

and (D) Desiree (receiver) + Infested Wild potato (emitter) (DIW).Ten adult aphids were enclosed in one cage and two clip-cages were attached on each plant.

2.2.9 Volatile analysis

Analyses were carried out on a 7820A GC coupled to a 5977B single quad mass selective detector (Agilent Technologies, Cheadle, UK). The GC was fitted with a non-polar HP5-MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness) coated with (5%-Phenyl)-methylpolysiloxane (Agilent Technologies) and used hydrogen carrier gas at a constant flow rate of 1.2 ml/min. Automated injections of 1 µl were made using a G4513A autosampler (Agilent Technologies) in splitless mode (285 °C), with oven temperature programmed from 35 °C for 5 min then at 10 °C/min to 285 °C. Compounds were identified according to their mass spectrum, linear retention index relative to retention times of n-alkanes, and co-chromatography with authentic compounds.

2.3 Statistical analysis

Aphid clip cage bioassay

Differences in the mean number of live adult aphids and produced nymphs on wild (18333, 22718, 23072) and cultivated (Desiree) potato plants were compared at two timepoints (48 and 96 h) by one-way analysis of variance (ANOVA). Prior to analysis, data were examined for a normal distribution using the Shapiro–Wilk test. Comparisons among means were performed using Holm–Sidak method ($P < 0.05$).

Olfactometer bioassay

Data on the behavioural response of *M. persicae* and *D. rapae* were analysed by a paired *t*-test (one tail). In this analysis, the time spent by the tested individuals in treated and the average of three control arms in the four-arm olfactometer were compared (Bruce *et al.*, 2003).

Volatile profiling

To visualize the overall differences in volatile profiles emitted from wild (18333, 22718, 23072) and cultivated (Desiree) potato plants, a principal component analysis (PCA) was performed using the concentrations of the detected volatiles as dependent variables. Loading and score plots were derived after mean-centring and log transformation of volatile data. VOC visualization was done using the MetaboAnalyst online tool suite (Chong *et al.*, 2018). Subsequently, univariate analysis (F-test) of variances was performed to investigate whether the concentrations of individual volatile compounds differed between wild and cultivated potato plants. All univariate analyses were performed using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA, USA).

Plant-plant communication: clip-cage bioassay

Differences in the mean number of live adult aphids and produced nymphs on four combinations (DD, DID, DW, and DIW) of plants were compared after (48 h) by one-way analysis of variance (ANOVA). Prior to analysis, data were examined for a normal distribution using the Shapiro–Wilk test. Comparisons among means were performed using Holm–Sidak method ($P < 0.05$).

Olfactometer bioassay

Data on the behavioural response of *M. persicae* and *D. rapae* were analysed by a paired t-test (one tail). In this analysis, the time spent by the tested individuals in treated and the average of three control arms in the four-arm olfactometer were compared (Bruce *et al.*, 2003).

2.4 Results

2.4.1 Aphid performance clip-cage bioassay

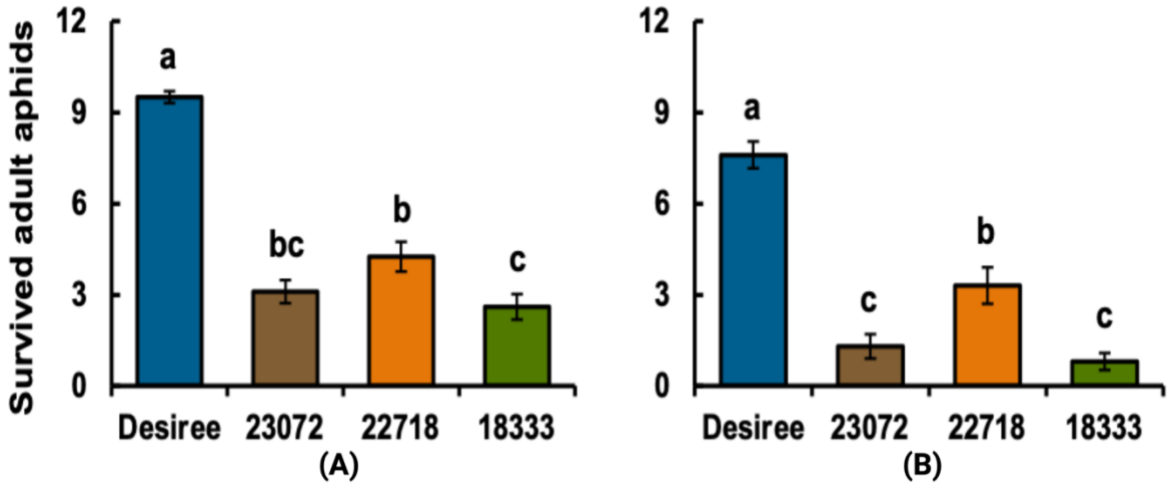

FIGURE 2.5 | Performance of *Myzus persicae* on cultivated (*Solanum tuberosum*. cv. Desiree) and wild (*Solanum stoloniferum*) potato lines. Mean number (± standard error) of surviving adult aphids *M. persicae* after 48 h (A) and 96 h (B). Different letters indicate statistically significant differences among plant species (F-test; $P < 0.05$), based on the Holm–Sidak method (one-way ANOVA).

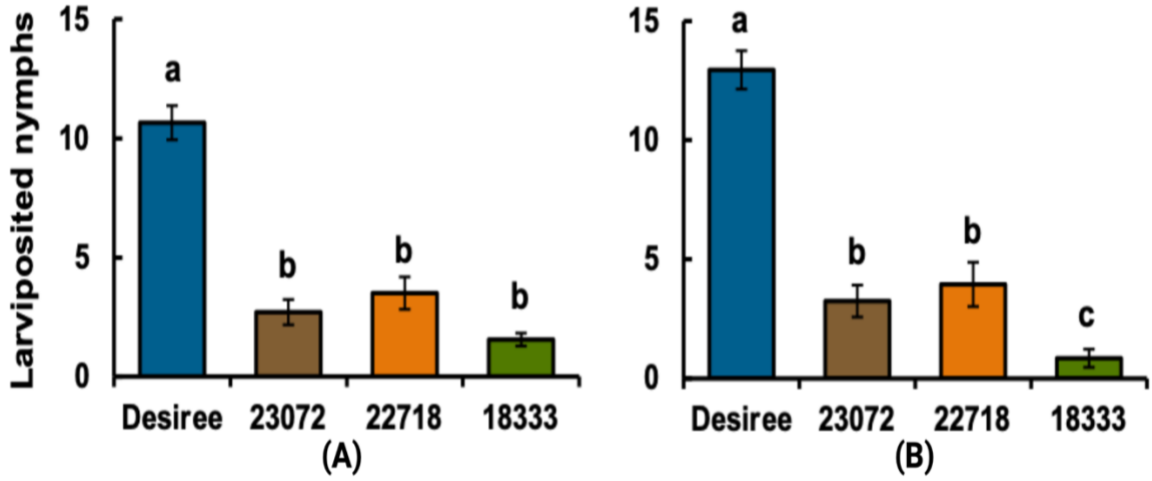

FIGURE 2.6 | Performance of *Myzus persicae* on cultivated (*Solanum tuberosum*. cv. Desiree) and wild (*Solanum stoloniferum*) potato lines. Mean number (± SE) of

larviposited nymphs of *M. persicae* after 48 h (A) and 96 h (B). Different letters indicate statistically significant differences among plant species (F-test; P < 0.05), based on the Holm–Sidak method (one-way ANOVA).

After 48 h, there was a significant reduction in adult *M. persicae* survival on wild potato accessions in clip cage experiments. The number of live adult *M. persicae* on Desiree (mean = 9.5) was up to more than three-fold higher compared to the number on wild plants (18333 mean = 2.6) ($F_{3,76}$ = 63.732; P < 0.001; Fig. 2.5(A)). A similar pattern was observed in clip cage experiments after 96 h. All wild accessions had high aphid mortality with less than 35% rate of survival. Accessions 18333 and 23072 had 8 % and 13 % survival of aphids respectively ($F_{3,76}$ = 46.299; P < 0.001; Fig. 2.5(B)). In contrast, Desiree had the least mortality (mean = 7.60) with more than 75% survival rate after 96 h.

There was a significant reduction in nymph production on wild accessions of *Solanum stoloniferum* across both time points compared to cultivated potato. Mean larviposition on wild accessions was significantly lower after 48 h, decreasing by 85% from 10.65 on Desiree plants to 1.55 on wild plants ($F_{3,76}$ = 48.428; P < 0.001; Fig. 2.6(A). In wild accessions, larviposition was reduced most on 18333 and least on 22718. Similarly, mean larviposition on all wild accessions was significantly reduced after 96 h, decreasing by 93% from 12.95 on Desiree to 0.85 on 18333 ($F_{3,76}$ = 50.739; P < 0.001; Fig. 2.5(B).

2.4.2 Volatile analysis

GC-MS analysis of entrainment samples of wild (18333, 22718, 23072) and cultivated accessions of potato revealed 23 detectable organic compounds belonging to different functional classes (alcohol, aldehydes, benzenoids, ketones, terpenes). There were significant quantitative differences (3-7 fold) in total emitted volatiles of

compared to Desiree plants ($F_{3, 12}$ = 61.20; P <0.001) (Fig. 2.7). In addition, most volatile compounds in the above-mentioned VOC groups were emitted from wild accessions in significantly higher amounts compared to Desiree plants. The difference in volatile emissions from wild and Desiree accessions may impact the behavioural responses of *M. persicae* and its natural enemy *D. rapae*.

Table 2.1 shows the details of the compounds identified from wild potato and cultivated accessions of potato. Among wild accessions, 23072 and 18333 accessions released high amount of volatile compounds while 22718 showed comparatively low volatile emission. Volatile compounds p-Cymon-7-ol, 4-ethyl-benzaldehyde, MeSA, D-Limonene, Benzothiazole, MHO, α-Copaene, β-elemene, trans-α-Bergamotene, (*E*)-β-Farnesene, and Germacrene D were the main compounds released by wild potato lines, while DMNT was the main compound released by Desiree in significant high amount. In particular, main compounds released by 23072 were p-Cymon-7-ol, 4-ethyl-benzaldehyde, D-Limonene, and Germacrene D. Accession 18333 released 11 main compounds that include p-Cymon-7-ol, 4-ethyl-benzaldehyde, Benzothiazole, D-Limonene, TMTT, α-Copaene, β-elemene, trans-α-Bergamotene and (*E*)-β-Farnesene. While a marked increase in emission of about 4 volatiles including MeSA, β-Myrcene, D-Limonene and Caryophyllene was recorded in 22718.

Table 2.1 Emission (in ng per plant $^{-1}$ h^{-1}; mean ± SE; *n* = 3) of volatiles released by cultivated (*Solanum tuberosum.* cv. Desiree) and wild (*Solanum stoloniferum)* potatoes lines.

Plant volatiles	KI	*Solanum stoloniferum*			*S. tuberosum*	*P*
		23072	22718	18333	Desiree	
Alcohols						
Phenylethyl alcohol	1116	3.89±1.26	4.25±0.87	2.63±0.58	2.59±0.82	0.688

p-Cymen-7-ol	1289	119.27±11.45[a]	17.52±6.48[c]	64.32±19.07[b]	1.37±0.38[d]	**<0.001**
Aldehydes						
4-ethyl-benzaldehyde	1122	63.49±8.63[a]	7.31±3.19[b]	21.60±6.47[b]	1.11±0.10[b]	**<0.001**
Benzenoids						
MeSA [#]	1192	7.81±0.93[b]	26.08±6.08[a]	4.39±0.51[b]	3.25±0.37[b]	**0.002**
Benzothiazole	1229	1.05±0.52[b]	7.59±1.42[a]	10.07±0.93[a]	1.00±0.09[b]	**0.002**
Ketones						
MHO	989	13.15±0.65	4.39±1.66	10.69±2.79	0.49±0.24	**0.024**
Monoterpenes						
β-Myrcene	992	3.65±0.41	5.95±1.38	3.75±0.42	1.84±0.61	0.062
p-Cymene	1026	1.79±0.14	1.86±0.20	1.78±0.17	2.56±0.19	0.104
D-Limonene	1030	20.39±2.09[a]	23.99±2.86[a]	19.83±1.98[a]	0.71±0.25[b]	**0.003**
Linalool	1099	8.71±2.76[a]	9.37±1.58[a]	8.83±0.60[a]	2.52±0.76[b]	0.083
Homoterpenes						
DMNT[#]	1116	0.76±0.08[b]	2.15±0.18[b]	1.15±0.22[b]	11.26±3.24[a]	**0.045**
TMTT[#]	1577	2.62±0.38[b]	4.10±0.84[b]	23.68±3.89[a]	0.79±0.08[b]	**<0.001**
Sesquiterpenes						
β-Cubebene	1351	10.27±2.46	5.62±2.17	4.09±0.86	7.38±3.86	0.497
α-Copaene	1376	3.70±0.58[c]	7.34±0.64[b]	15.12±1.08[a]	1.89±0.59[c]	**<0.001**
β-elemene	1391	4.07±1.67[b]	2.67±0.18[b]	40.52±14.16[a]	1.02±0.04[b]	**0.014**
Longifolene	1402	3.29±0.72	2.72±0.76	2.72±0.69	ND	0.858
Caryophyllene	1419	5.49±0.67	6.89±0.59	4.72±0.28	7.92±2.47	0.485
trans-α-Bergamotene	1435	1.35±0.14[b]	3.54±0.32[b]	20.69±7.69[a]	0.81±0.08[b]	**0.024**
(*E*)-β-Farnesene	1457	1.61±0.11[b]	4.34±0.38[ab]	10.13±3.68[a]	1.31±0.13[b]	**0.045**
Germacrene D	1481	21.23±5.45[a]	1.49±0.08[c]	6.68±1.70[b]	1.25±0.21[c]	**0.005**
β-Selinene	1486	1.41±0.09[b]	6.31±1.21[a]	7.27±0.63[a]	0.97±0.16[b]	**<0.001**
β-Bisabolene	1509	1.59±0.20[c]	2.78±0.26[b]	5.07±0.45[a]	0.83±0.05[c]	**<0.001**
Nerolidol	1566	1.68±0.05	2.11±0.07	6.49±2.71	0.87±0.15	0.101
Total emitted volatiles		**300.68±18.48[a]**	**157.19±13.89[b]**	**294.32±7.91[a]**	**49.42±9.76[c]**	**<0.001**

Under each chemical class, VOCs are ordered in accordance with their increasing retention time in a gas chromatograph and Kovats index. # [DMNT: (*E*)-4,8-dimethyl-1,3,7-nonatriene; MeSA: methyl salicylate; MHO: 6-methyl-5-hepten-2-one: ND: Not Detected]. VOCs were tentatively identified based on spectra, Kovats retention index and NIST 17 library matches. KI: Kovats index determined on the intermediately nonpolar HP5-MS column (https://webbook.nist.gov/; http://www.pherobase.com/). Different letters in the same row indicate significant differences between potato lines (One way ANOVA; $P < 0.05$). *P*-values in bold indicate significant difference.

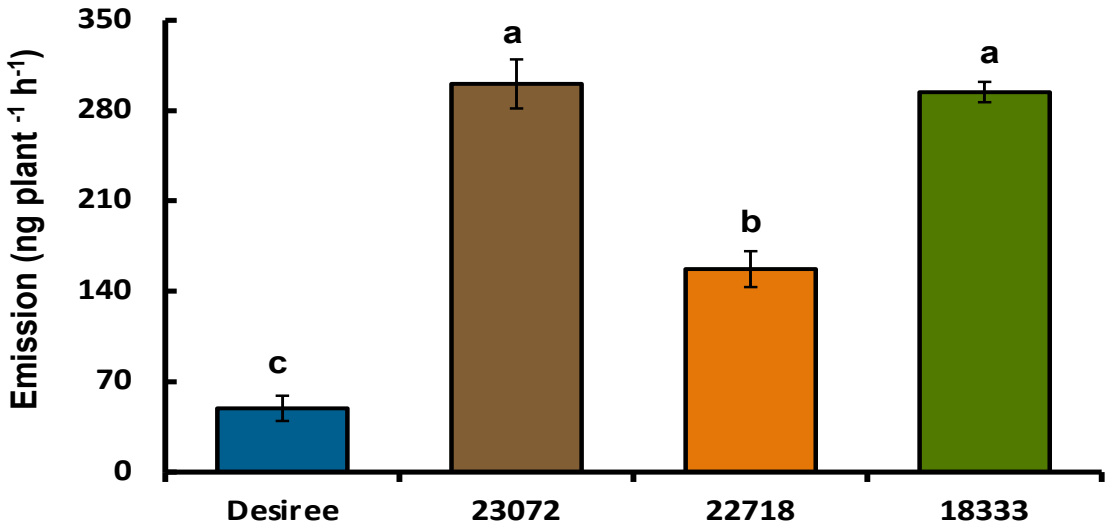

FIGURE 2.7 | Total amounts (mean nanograms plant^{-1} h^{-1} ± SE) of identified volatile organic compounds (VOCs) emitted from cultivated (*Solanum tuberosum*. cv. *Desiree*) and wild *(Solanum stoloniferum)* potato lines. Different letters indicate statistically significant differences among plant species (*F*-test; $P < 0.05$), based on the Holm-Sidak method (one-way ANOVA).

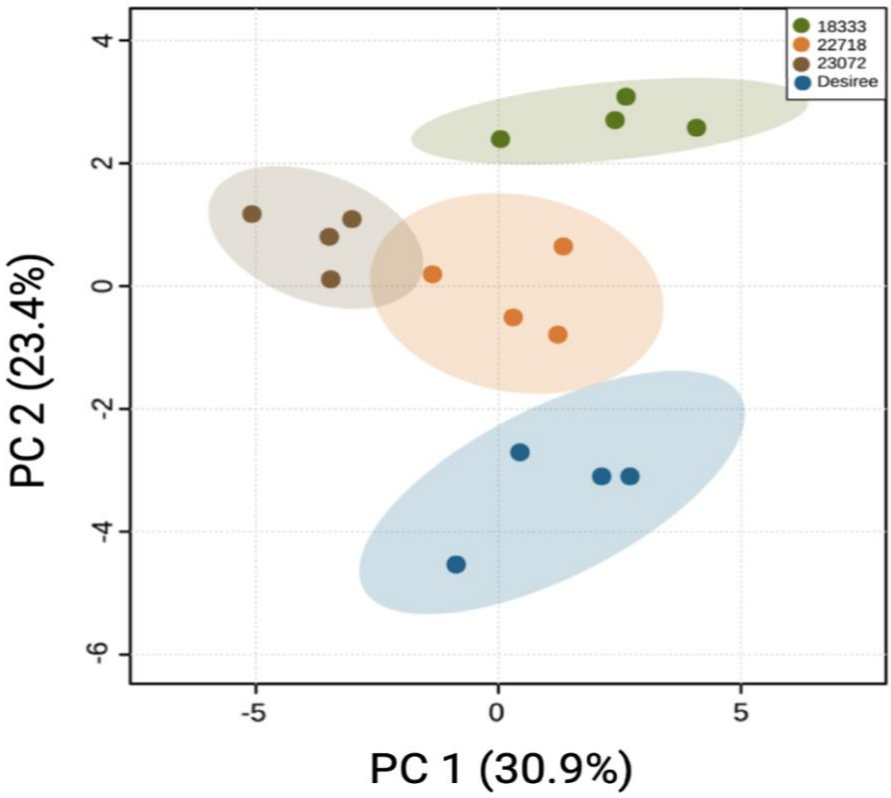

FIGURE 2.8 | PCA of volatile compounds emitted by cultivated (*Solanum tuberosum*. cv. Desiree) and wild (*Solanum stoloniferum*) potato lines (n = 4) sampled for 48 h. The score plot visualizes the ordination of collected samples according to the first two PCs based on the quantity of different volatiles emitted from different potato lines, with the percentage of the variation explained in parentheses. The ellipses show 95% confidence regions.

PCA of the VOCs showed that the first two principal components (PCs) accounted for 54.3% of the total variation in the volatile data (Fig. 2.8). Hence, these two PCs illustrated most of the variation in the data of likely biological relevance. A clear separation based on the second principal component (PC2) is visible between the volatile profiles of wild (18333, 22718, 23072) in one cluster and cultivated (Desiree) potato plants, whereas another separation but based on the first principal component (PC1) is obvious for the volatile profiles of 23072 and a cluster of 18333, 22718 and

Desiree plants. The greatest loadings of PC2, in descending order, were for D-limonene (0.285), (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (0.272), and p-cymen-7-ol (0.255), whereas the major loadings of PC1 were for ß-bisabolene (0.293), (E)-ß-farnesene (0.288), and trans-ω-bergamotene (0.285). This suggests that these VOCs, shown to contribute to PC1 and PC2, may impact the behaviour response of both *M. persicae* and *D. rapae*.

The compounds identified from wild potato lines volatile blends have been tested against various species of herbivore pests in previous studies. These studies suggested that the induced volatiles compounds have potential to negatively affect the plant pests, for instance; p-Cymon-7-ol found behaviourally active compounds when tested against tephritid pests and spring aphid *Kaburagia rhusicola* (Aluja *et al.*, 2020; Zhu *et al.*, 2020). The star anise *Illicium verum* extract with 4-ethyl-benzaldehyde as a component showed repellent effect against maize weevil *Sitophilus zeamais* and *M. persicae* (Wei *et al.*, 2014; Shi-Guang *et al.*, 2017). Earlier studies reported that MeSA as an repellent compound that affects behaviour and oviposition of herbivorous pests (Ulland *et al.*, 2008; Snoeren *et al.*, 2010). Additionally, MeSA treatment of plant activates defensive signaling pathways (Riahi *et al.*, 2022). D-Limonene was effective against whiteflies, mealybugs and scale insects (Hollingsworth, 2005; Conboy *et al.*, 2019), β-elemene as a constituents of essential oil (*Tetradium glabrifolium*, Evodia rutaecarpa and *Zanthoxylum rhoifolium*) showed strong repellency and larvicidal activity against Asian tiger mosquito (*Aedes albopticus*), *Triboilum castaneum, Lesioderma serricorne Liposcelis bostrychophila and Bemisia tabaci* (Christofoli *et al.*, 2015; Liu *et al.*, 2015; Cao *et al.*, 2018), (*E*)-β-Farnesene induces changes in behaviour of codling moth (Sutherland, Hutchins and Wearing, 1974; Yan *et al.*, 2003),

In olfactometer bioassay, Germacrene D as a component of *Hemizigiya petiolate* affects *S. avenae* and *M. persicae* behaviour negatively (Bruce *et al.*, 2005). Furthermore, the compounds released from wild potato accessions were also effective when tested with biocontrol agents i.e., predators and parasitoids. For example, 4-ethyl-benzaldehyde showed a positive effect on several natural enemies including *Stethorus punctum*, *Cotesia plutellae* and *Trichogramma dendrolomi* (James, 2005; Yang *et al.*, 2016; Zhao *et al.*, 2022), Germacrene D was found attractive to predatory mite *Amblysehis cucumer* in olfactometer bioassay (Manjunatha *et al.*, 1998).

2.4.3 Aphid and parasitoid olfactometer bioassay

In an olfactometer bioassay, *M. persicae* were repelled by the volatiles of wild accessions, whereas they spent more time in the treated zone for Desiree ($P = 0.19$). Wild accessions had a significant repellent effect on *M. persicae*. Accessions 18333 and 23072 showed a significant repellent effect on *M. persicae* with *P* values of 0.013 and 0.018, respectively (Fig. 2.9).

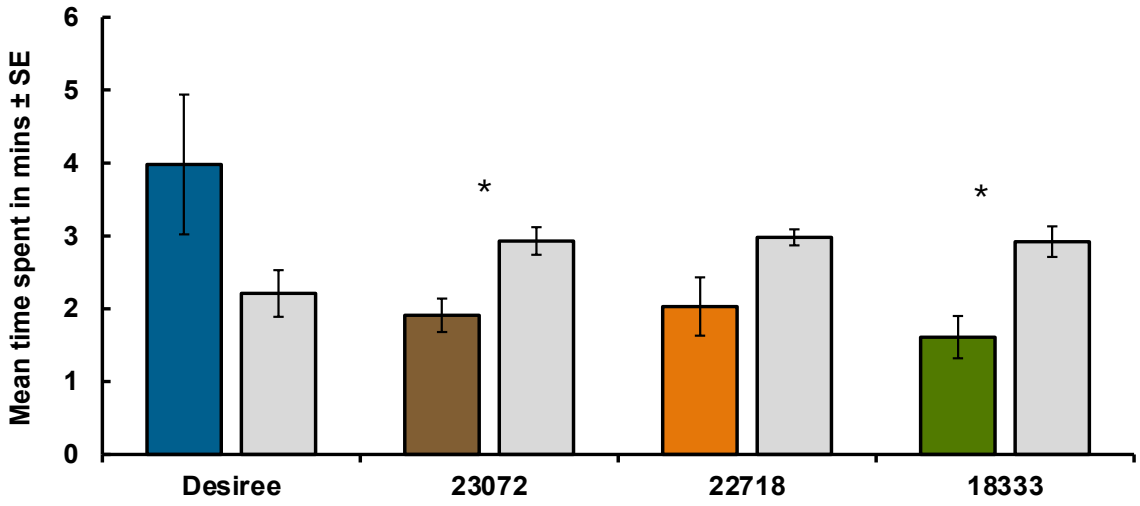

FIGURE 2.9 | Behavioural responses of *Myzus persicae* to headspace volatiles from cultivated (*Solanum tuberosum*. cv. Desiree) and wild (*Solanum stoloniferum*) potato

lines in a four-arm olfactometer bioassay. Each insect was given 12 min to make a choice between one arm of plant volatiles (coloured bars) versus three solvent diethyl ether (DEE) arms (grey bars). The values shown are mean time spent in the arm ± SE (n = 10). Asterisks (*0.01 ≤ P ≤ 0.05) above bars indicate statistically significant differences based on a paired t-test (one tail).

In contrast to *M. persicae*, odour collected from wild accessions attracted parasitoids and odour collected from Desiree repelled the parasitoid. *D. rapae* showed a preference for the blend of volatiles emitted from wild accessions and spent more time in the zone treated with volatiles collected from wild accessions. Accession 18333 was the only wild accession that had a significant attractant effect on *D. rapae (P* = 0.012). *D. rapae* spent significantly less time (*P* = 0.016) in the olfactometer zone treated with volatiles collected from Desiree (Fig. 2.10)**.**

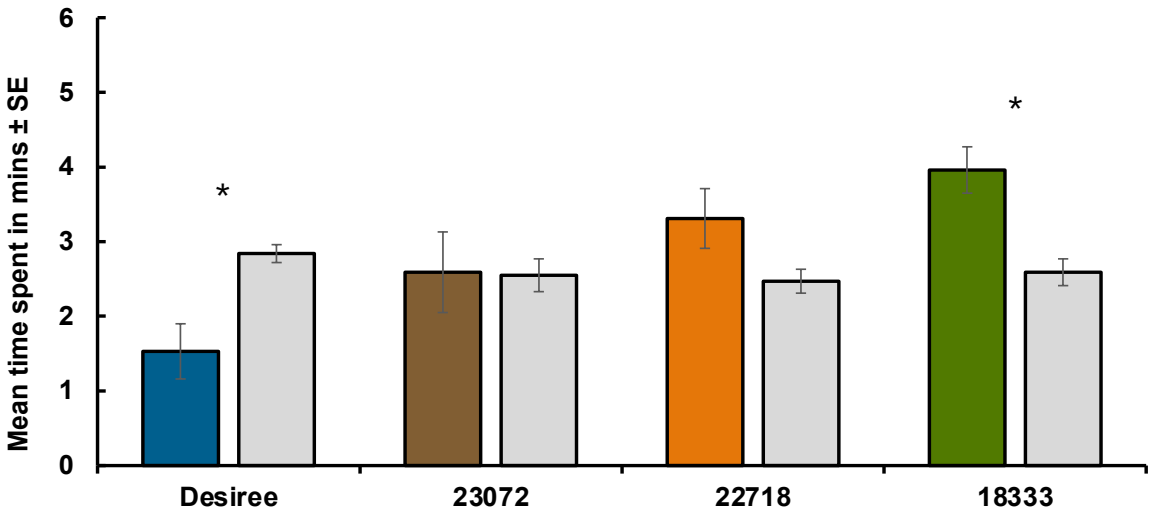

FIGURE 2.10 | Behavioural responses of *Myzus persicae* to headspace volatiles from cultivated (*Solanum tuberosum*. cv. Desiree) and wild (*Solanum stoloniferum*) potato lines in a four-arm olfactometer bioassay. Each insect was given 12 min to make a choice between one arm of plant volatiles (coloured bars) versus three solvent diethyl ether (DEE) arms (grey bars). The values shown are mean time spent in the arm ± SE

(n = 10). Asterisks (*0.01 ≤ P ≤ 0.05) above bars indicate statistically significant differences based on a paired t-test (one tail).

2.4.4 Plant-plant communication: clip-cage bioassay

In the clip-cage bioassay there was a significant difference (*P* = 0.006, One-way ANOVA) in adult survival on different plant combinations. Higher mortality was recorded on combinations that included infested plants as the emitter treatment. Combinations (DD) and (DW) had fewer dead adults with mean value of surviving adults 9.6 and 8.1 respectively (Fig 2.11). A similar pattern was observed for nymph production with a significant difference (*P* = 0.04, One-way ANOVA), combinations with infested plants had less larviposition of *M. persicae*. Plant treatment Desiree + Infested Desiree (DID) showed (mean = 11) least number of nymph after 48 h compared to DD (mean = 20.4), DW (mean = 16.5) and DIW (mean = 15.4) treatments.

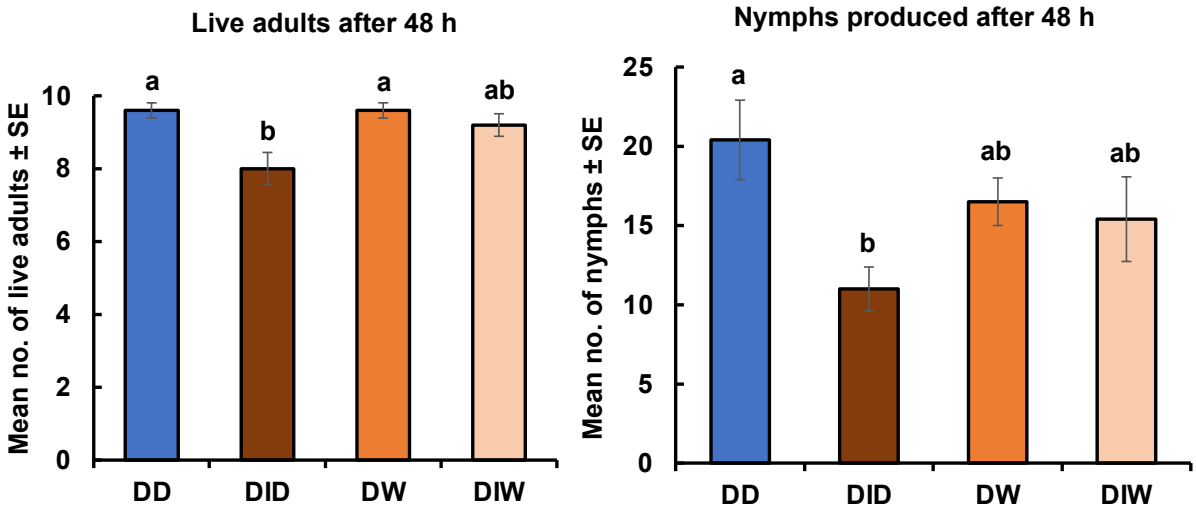

FIGURE 2.11 | Adult *Myzus persicae* **(A)** survival (Mean ± SE) out of original 10 individuals and **(B)** larviposition (Mean ± SE) after 48 h in clip cages on four different combinations of *Desiree* and wild cultivars (*n* = 10). Different letters indicate statistically significant differences among combinations (*P* < 0.05) (one-way ANOVA).

(DD: Desiree + Desiree; DID: Desiree + Infested Desiree; DW: Desiree + Wild potato; and DIW: Desiree + Infested Wild potato).

2.4.5 Plant-plant communication: olfactometer bioassay

Behavioral response of adult *D. rapae* to volatile samples collected from four different treatments of desiree and wild accessions.

M. persicae spent significantly more time ($P = 0.0382$) in the olfactometer arm treated with volatiles collected from treatment DD (uninfested Desiree plants). However, the time spent by *M. persicae* was lower when it was exposed to volatiles collected from treatments DID, DW and DIW, with the lowest amount of time being spent in the arm treated with volatiles of treatment DW ($P = 0.0245$). Interestingly, volatiles collected from treatments DID, DW and DIW, with receiver plants exposed to infested Desiree or wild plants, had a repellent effect on *M. persicae* (Fig. 2.12).

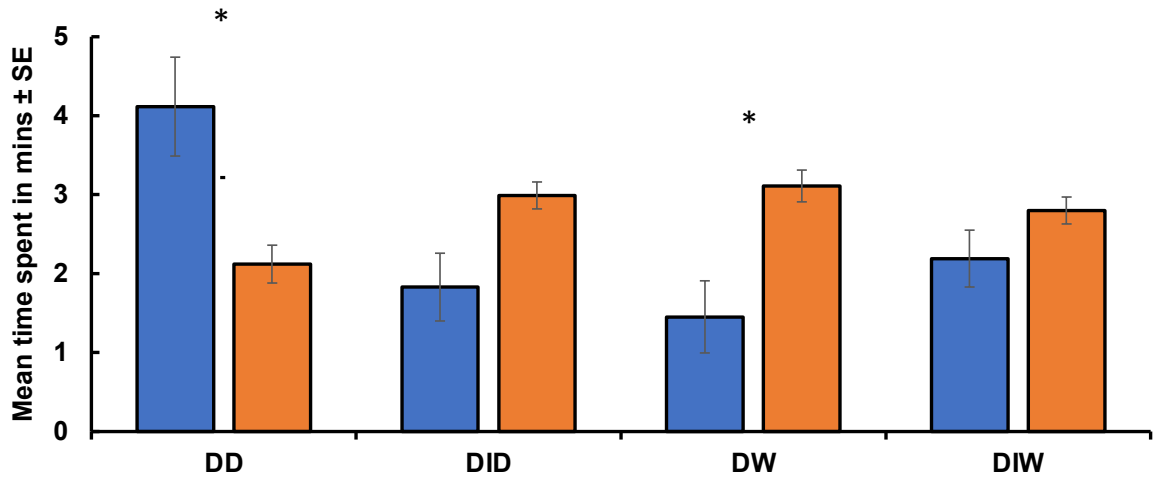

FIGURE 2.12 | Behavioural responses of *Myzus persicae* to volatiles from four combinations of Desiree and wild cultivars in an olfactometer bioassay. Individual aphids were given 12 min to make a choice between one arm of plant volatile (blue bars) vs. three solvent (DEE) arms (orange bars). The shown values are the mean time spent in arm ± SE ($n = 15$). Asterisks (*$0.01 \leq P \leq 0.05$) above bars indicate

statistically significant differences based on paired *t*-test. (DD: Desiree + Desiree; DID: Desiree + Infested Desiree; DW: Desiree + Wild potato; and DIW: Desiree + Infested Wild potato).

Behavioral response of adult *D. rapae* to volatile samples collected from four different treatments of desiree and wild accessions.

In contrast to *M. persicae*, the parasitoid *D. rapae* spent less time when exposed to volatiles collected from treatment DD. However, *D. rapae* spent more time in the olfactometer arms treated with volatiles collected from the treatments (DID, DW and DIW) containing wild and infested plants. Both treatments (DID and DIW) with infested plants, significantly affected the behavioural responses of *D. rapae* with $P = 0.043$ and $P = 0.0183$ respectively (Fig. 2.13).

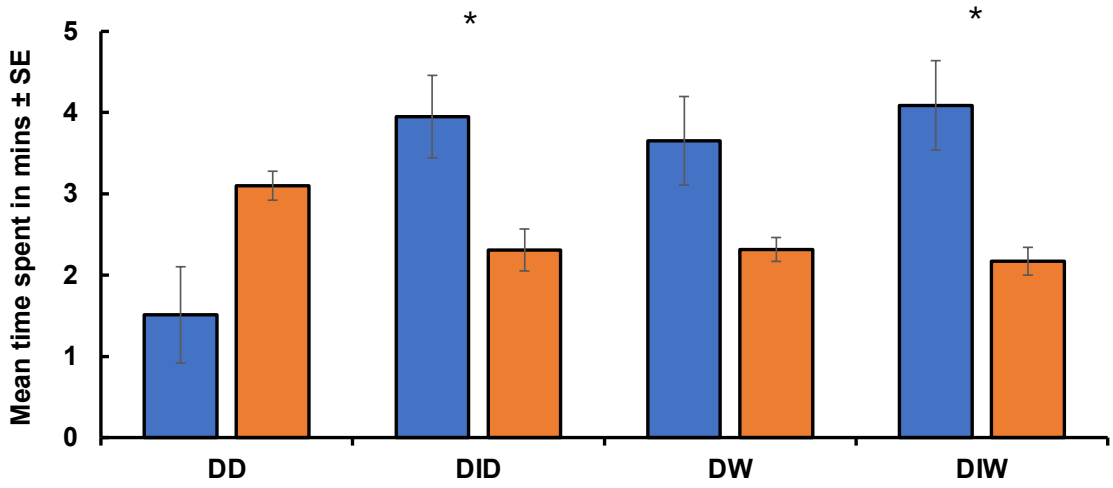

FIGURE 2.13 | Behavioural responses of *Diaeretiella rapae* to volatiles from four combinations of Desiree and wild cultivars in an olfactometer. Individual parasitoids were given 12 min to make a choice between one arm of plant volatile (blue bars) vs. three solvent (DEE) arms (orange bars). The values shown are the mean time spent in arm ± SE ($n = 15$). Asterisks (*$0.01 \leq P \leq 0.05$) above bars indicate statistically significant differences based on paired *t*-test. (DD: Desiree + Desiree; DID: Desiree +

Infested Desiree; DW: Desiree + Wild potato; and DIW: Desiree + Infested Wild potato).

2.5 Discussion

The peach potato aphid *M. persicae* is a globally important pest of many economically important crops (Van Emden *et al.*, 1969; Alyokhin *et al.*, 2013; Van Emden and Harrington, 2017). Synthetic chemical pesticides used to be the main control option for this species, but increasing levels of pest resistance to these chemicals and safety concerns about food and the environment mean that new solutions are needed (Alyokhin *et al.*, 2013; Bass, *et al.*, 2014; Singh *et al.*, 2021). Current research is focused on finding alternative methods to replace the use of traditional insecticides. Previous studies reported that plant secondary metabolites could provide a way to enhance plant resistance (Bennett and Wallsgrove, 1994; Schoonhoven *et al.*, 2005; Guerriero *et al.*, 2018). Plant resistance could be an important aspect in integrated pest management. The presence of secondary metabolites in a plant affects the survival and reproduction rate of insects (Schoonhoven *et al.*, 2005). Plants producing high level of secondary metabolites have better defense against insect and pathogens and can be used to establish a sustainable agricultural system. Higher levels of disease and pest resistance are highly recommended in potato cultivars, but of course, further to these properties, they must also retain the marketable yield and quality required for a modern cultivar to be successful (Bradshaw & Mackey 1994).

The current experiments showed good levels of resistance in the wild potato lines tested. There was a significant difference in the susceptibility of wild and cultivated species of *Solanum*. The most resistant accession of wild potato in these experiments was 18333, followed by 23072 and 22718. The cultivated potato *S. tuberosum* cv. Desiree, used as a standard for comparison, had substantial nymph

production after 96H meaning it is much more susceptible. The wild accessions were shown to be resistant. Among these two *Solanum* species, *S. tuberosum* (Desiree) was less resistant. Nymph production was reduced in wild accessions because there was high mortality of adults and no more adults to produce the nymphs. In accessions 18333 and 23072, a significant number of adults was dead after 96 h.

The parasitoid *D. rapae* spent more time in the olfactometer arm treated with volatile samples collected from wild plants. In contrast, parasitoids spent significantly less time on Desiree plants. The olfactometer studies thus suggest that wild accessions help in recruiting natural enemies of aphids, however field experiments are needed to fully confirm this. Recruitment of natural enemies is an important approach exhibited by the plants in defending the herbivore's attack (Price, 1987; Vet and Dicke, 1992; Gols, 2014). Plants that produce a large plethora of appropriate volatile compounds can successfully recruit a wide range of natural enemies (Turlings and Wäckers, 2004). However, the quantity and quality of released plant volatile are critical. Sometimes compounds present in small quantities are more biologically active despite small quantities (Vet and Dicke, 1992). Quality, quantity, and the ratio of volatile compounds all play a crucial role in plant-insect interactions (Bruce *et al.*, 2010). Commercially available crop plants have been selected primarily to obtain more yield. This genetic manipulation through selection compromises their defence capacity by altering the interaction between plants, herbivores, and natural enemies (Yolanda, Gols and Benrey, 2015).

In a plant-plant communication cage bioassay, the performance of *M. persicae* was assessed on four different plant-plant combinations. Plant combinations exposed to volatiles from infested Desiree and wild plants showed high aphid mortality and low nymph production. However, significant difference was observed in mortality and

fecundity of aphids. High mortality and low larviposition could be due to exposure of receiver plant to the volatiles release from infested emitter plant. It is well documented that infested plants released volatiles to alert their neighbouring plants of further attack (Arimura *et al.*, 2000; Howe and Jander, 2008; Li, 2016)

The volatiles collected from plant-plant communication setup were used to study behavioural bioassay of aphids and parasitoid. We found that the plant receives information from its surroundings and responds to that information accordingly (Baldwin, Kessler and Halitschke, 2002; Karban and Shiojiri, 2009; Heil and Karban, 2010). Insects show the same behavioral response to the volatiles collected from treatment DD (Desiree vs. Desiree) similar to the volatiles collected from individual Desiree plants. However, insects' behavioural responses were changed when they were exposed to the volatiles collected from treatments Desiree (receiver)+ Infested Desiree (emitter), Desiree (receiver) + Wild potato (emitter), and Desiree (receiver) + Infested Wild (emitter). This behavioural change of insects is most probably due to the change in volatile profile of receiver plant. Which is a consequence of change of neighbouring (emitter) plant or change in the physiology of neighbouring plants that induce the changes in the receiver plant (Baldwin *et al.*, 2006; Heil and Bueno, 2007; Heil, 2014; Ninkovic, Markovic and Rensing, 2021).

The current study shows that there are promising sources of direct aphid resistance in wild potato germplasm. Furthermore, there is also evidence they could provide indirect resistance via recruitment of natural enemies. The genetic makeup of these accessions creates chemical diversity in plants that affect the development of the aphid (Manrique-Carpintero *et al.*, 2013). In both *Solanum* species which were used in experiments, morphological differences could be seen; wild accessions had smaller leaves compared to Desiree. Although there could be some relation between aphids

and leaf size it is unlikely to explain the high mortality observed in the current study which is more likely due the presence of toxic phytochemicals. The current research findings open up the prospect of breeding for aphid resistance by crossing cultivated and wild potatoes. However, we need to know what the chemicals conferring resistance are and if they are safe for human consumption. Also, is it possible to have higher concentrations of protective chemicals in the leaves but not in the tubers which are eaten.

Chapter 3. EFFECT OF *cis*-Jasmone ON THE PERFORMANCE AND BEHAVIOUR OF *Myzus persicae*

3.1 Introduction

Plants have developed different defence mechanisms against herbivorous insects, including release of a plethora of volatile organic compounds (VOCs) that directly repel the pests (Bruce and Pickett, 2011; Mithöfer and Boland, 2012). This implies that it might be possible to manipulate the emission of VOCs to enhance the crop protection against insect pests (Turlings and Ton, 2006; Heil, 2014). By boosting signal transduction pathways for VOCs emission, qualitative and quantitative manipulation can be achieved (Thaler, Humphrey and Whiteman, 2012). VOCs emission can be increased and/or decreased by elicitors involved in these signalling pathways (Smith *et al.*, 2009). (Loughrin *et al.*, 1995; Birkett *et al.*, 2000; Röse and Tumlinson, 2004)

cis-Jasmone is natural plant derived compound which is biosynthesized via isomerization of *cis*-oxophytodienoic acid (*cis*-OPDA) to iso-oxophytodienoic acid (iso-OPDA) following oxidative side-chain cleavage (Dabrowska and Boland, 2007). Factors such as herbivory (Loughrin *et al.*, 1995; Birkett *et al.*, 2000; Röse and Tumlinson, 2004), insect saliva application (Lou and Baldwin, 2003; Röse and Tumlinson, 2005; Sobhy, Erb and Turlings, 2015), spray with jasmonic acid (JA) (Heil, 2004), or introduction of nitrogen-fixing rhizobia (Ballhorn, Kautz and Schädler, 2013) can cause plants to release CJ (Loughrin *et al.*, 1995; Birkett *et al.*, 2000; Röse and Tumlinson, 2004). In addition to these factors some plants also constitutively release *cis*-Jasmone through flowers and leaves (Tanaka *et al.*, 2009), that could be used as a cue for host location by some herbivores although the plant may produce it as a signal to attract pollinators (El-Sayed *et al.*, 2009). *cis*-Jasmone has been used artificially to induce plant defence. In *Arabidopsis*, *cis*-Jasmone application increased

expression of a number of genes, in particular CYP81D11 which is a Cytochrome P450. Volatiles emitted from Arabidopsis plants with overexpressed CYP81D11 were repellent to the *Myzus persicae* (Bruce and Pickett, 2007).

Volatiles induced by plants under herbivore attack can directly repel the herbivores (Agelopoulos *et al.*, 1999) and also attract natural enemies (predators and parasitoids) of the attacking herbivores (Turlings, Tumlinson and Lewis, 1990; De Boer and Dicke, 2004b). Plants produce *cis*-Jasmone as a component of floral volatiles. In addition to this plants release *cis*-Jasmone after damage of vegetative tissues (Loughrin *et al.*, 1995). It is already proved that release of *cis*-Jasmone can be used to induce plant defence (Birkett *et al.*, 2000) but these effects have not yet been studied in Brassica crops. Previous studies showed that *cis*-Jasmone act as a repellent to lettuce aphid *Nasonovia ribis-nigri* Mosh and damson-hop aphid, *Phorodon humuli* Schrank (Birkett *et al.*, 2000) in olfactometer and field studies respectively. While in case of seven-spot ladybird beetle *Coccinella septempunctata* L, and aphid parasitoid, *Aphidius ervi cis*-Jasmone was attractive in olfactometer and in wind tunnel studies respectively (Birkett *et al.*, 2000).

Biosynthesis of methyl jasmonate, a catabolite of jasmonic acid, during herbivory activates defence mechanisms in tomato plants, *Lycopersicon esculentum* Mill. (Farmer and Ryan, 1990) while exogenous spray of jasmonic and methyl jasmonate imitate the wound response similar to attack of lepidopterous larvae (Tanaka *et al.*, 2009). In field conditions, application of jasmonic acid to tomatoes is effective and made them more resistant against a number of pests (Tanaka *et al.*, 2009). *cis*-Jasmone is another catabolite of jasmonic acid and biosynthetically related to this and has been considered as single biological sink in the jasmonate pathway (Koch, Bandemer and Boland, 1997). *cis*-Jasmone could be a good elicitor of plant

defence system by acting as an external signal that can alert the receiver (plant) when their neighbours are under attack by the pests and activate their defence system prior to the pest attacks (Chamberlain, Pickett and Woodcock, 2000; Khan *et al.*, 2008). Application of *cis*-Jasmone changes gene expression and makes bean plants more attractive to *A. ervi* and these changes were observed long after the dissemination of *cis*-Jasmone from the air around the treated bean plants (Birkett *et al.*, 2000).

3.1.1 Aims and objectives

The overall aim of this chapter is to assess the effect of CJ on brassica genotypes: *Brassica rapa* subspecies *chinensis*, (Pak choi), *Brassica napus* (Samurai, Wesway, English Giant, Turnip rutabaga) in regards to aphid performance on treated plants. Within this aim there are three objectives:

- To investigate the performance of *M. persicae* on CJ treated brassica plants using a clip-cage bioassay
- To investigate the settlement preference of *M. persicae* on CJ treated brassica plants in a choice test bioassay
- To investigate the behavioural response of *M. persicae* to the volatiles collected from CJ treated brassica plants

3.2 Materials and Methods

3.2.1 Insects

Myzus persicae aphids were collected from the well-established aphid rearing lab, Centre of Applied Entomology and Parasitology (CAEP) at Keele University. The *M. persicae* clone O was reared on Pak choi, commonly known as Chinese cabbage, in Bugdorm cages (46 x 46 x 46 cm; NHBS Ltd, Devon, UK) under controlled conditions (24 °C, 38 % RH, 16 h L: 8 h D photoperiod).

3.2.2 Plants

Five *Brassica* lines: *B. napus* cv. 'Samurai'*, B. napus* cv. 'Wesway'*, B. napus* cv. English giant*, B. napus* cv. 'Turnip rutabaga 57' and *Brassica rapa* subsp. *chinensis* cv. Hanakan (Pak choi) were obtained from Warwick University, UK. All plants used were grown in a plant growth chamber (22 °C, 16 h L: 8 h D photoperiod). Brassica plants were grown individually in 7.5 cm pots in James magic compost (Westland Horticulture Limited, UK). Plants with five true leaves plants were used for entrainment.

3.2.3 Plant treatment

Plants were sprayed with an aqueous emulsion of *cis*-Jasmone (CJ) (Sigma Aldrich, Buchs, Switzerland) as described in Bruce *et al.* (2003). A stock CJ emulsion was formulated by mixing 25 µl of CJ with 100 µl of Tween 80 (Sigma Aldrich) in 100 ml of deionised water, while a blank formulation to act as a control was formulated by mixing 100 µl of Tween 80 in 100 ml of deionised water. Spray treatment was carried out using an Oshide spray bottle (100 ml; Zhengzhou Xinrui Tongda Metal and Material Co., Ltd. Henan Sheng, China) by applying three triggers pulls of spray formulation (250 µl) to each plant at a distance of 30 cm. Sprayed plants were left for 24 h and then used for experiments. Control plants and CJ treated plants were placed in different compartments to avoid any plant-plant interaction.

3.2.4 Clip-cage bioassay

Performance of *M. persicae* was assessed on brassica genotypes: *Brassica rapa* subspecies *chinensis* (Pak choi), *Brassica napus* (Samurai, Wesway, English Giant, Turnip rutabaga). There were two separate series of experiments with different plants: the first series recorded observations after 48 h and the second recorded observations after 96 h. Fresh plants and aphids were used in each replicate observation in each experiment. In each replicate, 10 adult alate *M. persicae* were placed in a clip cage

(2.5 cm diameter, Bioquip Products Inc. USA), which was attached to the lower surface of plant leaves (Sobhy *et al.*, 2020) (Fig. 3.1). Two clip cages were placed on each plant. Ten replicates (control and CJ treated) were performed for each genotype. To assess the survival and fecundity of aphids, plants were left undisturbed in a controlled environment room (25 °C ± 2 °C, 37 % RH, 16 h L: 8 h D photoperiod). Plants were assessed after the 48 h (series 1) or 96 h (series 2) period. For assessment, leaves containing the cages were cut and cages were removed without losing any aphid. Numbers of live adults and newly produced nymphs were recorded.

FIGURE 3.1 | Clip-cage bioassay, two clip-cages were placed on each plant, ten alate adult aphids were enclosed in each clip cage. Bioassays were run for 48 h and 96 h.

3.2.5 Settlement bioassay

Brassica plants were sprayed with *cis*-Jasmone and control plants sprayed with Tween 80 and tested under choice conditions in a Bug dorm-6E insect rearing cage (60 X 60 X 60cm, 25 °C ± 2 °C, 16 h L: 8 h D photoperiod). Treatment was applied 24

h before the start of the experiment. Four plants; two treated and two control, were put inside the bug dorm at alternate positions (Fig. 3.2). A vial (9 cm L X 2.5 cm D) containing 50 alate *M. persicae* was placed in the bug dorm, opened and then the bug dorm was left undisturbed for the next 24 h. Counts of settled aphids were made after 24 h after release, and settlement on treated plants was compared with settlement on control plants using a *t*-test.

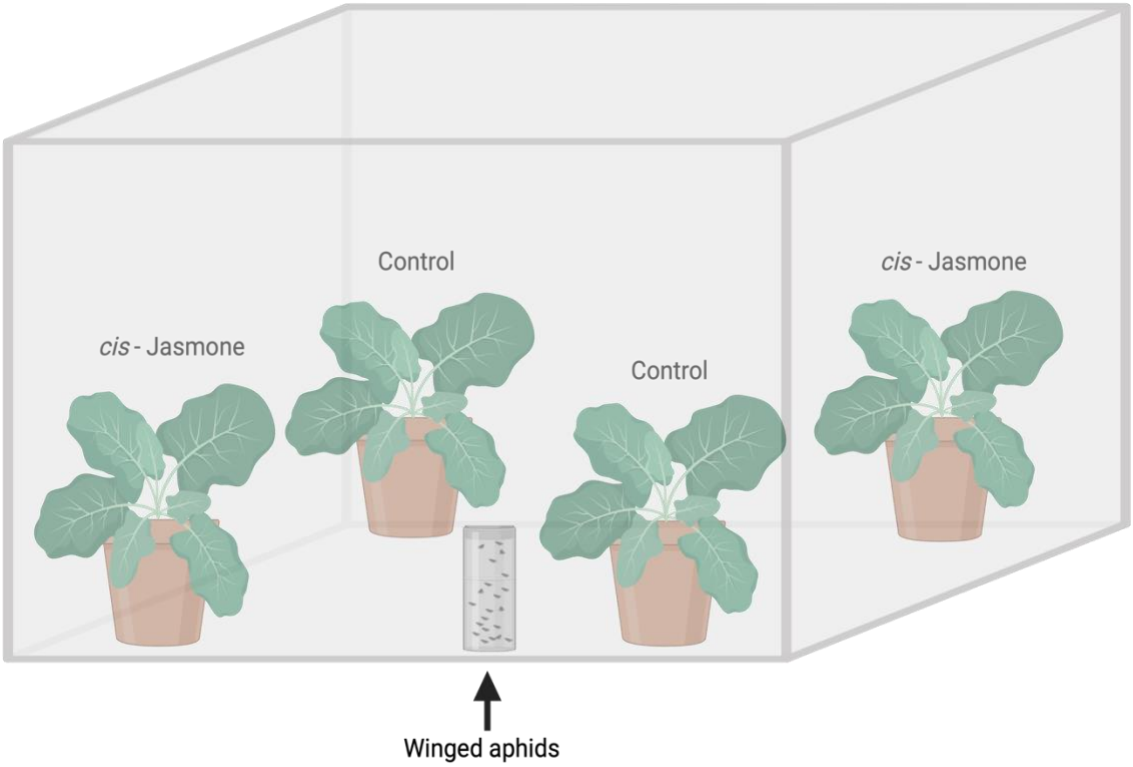

FIGURE 3.2 | Settlement bioassay; two control and two CJ treated plants were placed in a Bugdorm as shown in the figure. A vial containing 50 winged aphids was placed in the middle.

3.2.6 Olfactometer bioassay

A Perspex four-arm olfactometer (Pettersson, 1970) was used to determine behavioural responses of *M. persicae* to host volatiles in a controlled environment room (24 °C ± 2 °C, 30 % RH). Prior to each experiment, all Perspex components

were washed with Teepol solution and then rinsed with 80% ethanol solution and distilled water and left to air dry. Filter paper (Whatman No. 1, Buckinghamshire, UK), was fitted at the base of the olfactometer to provide purchase for the walking insects. Air was drawn out through the central hole on top of olfactometer by PTFE tubing. A single aphid was introduced through a central hole at the top of the olfactometer. The air was drawn out through the central hole at a rate of 200 ml/min. Aphids were acclimatised in the room for two h, and each replicate of the experiment was run for 12 min. After every 3 min, the olfactometer was rotated 90 degrees to avoid any directional bias in the experiment. Volatile samples were collected from brassica plant lines that were used as odour sources. Odour sources were prepared by putting an aliquot (10 µl) of sample on the filter paper, inserted into odour source side arms. The olfactometer was divided into five regions. Regions 1 to 4 corresponded to each of the four plastic side-arms containing odour sources and region 5 was the central region. A filter paper strip containing aliquot of plant odour source was inserted in arm 1 while the rest of the three arms contained control sample (diethyl ether). Time spent in each region was recorded using Olfa software (F. Nazzi, Udine, Italy). If an aphid remained inactive (motionless) for the first 2 minutes of a replicate that replicate was discarded. Ten replicates were performed for each odour source. All bioassays were performed between 10:00 to 13:00.

3.3 Statistical analysis

Aphid Clip Cage Bioassay

Differences in the mean number of live aphids on control and CJ treated plants was compared for each brassica cultivar at two time-points (48 and 96 h) using generalised linear models (GLM) fitted with Poisson probability distributions. Differences in the mean number of aphid nymphs larviposited onto control and CJ treated plants were

compared for each brassica cultivar at two time-points (48 and 96 h) using GLMs fitted with quasi-Poisson probability distributions to account for overdispersion. Plant treatment (i.e., control vs. CJ treated) was a fixed factor.

Aphid Settlement Bioassay

Differences in the mean number of aphids settling on control and CJ treated plants were compared for each brassica cultivar using GLMs with Poisson or quasi-Poisson probability distributions depending on dispersion. Plant treatment (i.e., control vs. CJ treated) was a fixed factor.

Olfactometer Bioassays

The behavioural response of *M. persicae* was tested in two ways. For experiments with one treated arm vs. three solvent control treatments, data were analysed by a paired t-test. In this analysis, the time spent by aphids in treated and solvent arms of the four-arm olfactometer were compared. In experiments where the response in two treatment arms vs. two arms of solvent control was compared, data were first converted into proportions then log-ratio transformed before analysis by one-way analysis of variance and Holm-Sidak mean separation (Mwando et al.,2018). Data were examined for a Gaussian distribution using the Shapiro-Wilk test prior to analysis. All statistical analyses were carried out using R (v 4.0.3) (R Core Development Team, 2021).

3.4 Results

3.4.1 Clip-cage bioassay

After 48 h, there was no significant reduction in adult *M. persicae* survival on five brassica cultivars treated with CJ in clip cage experiments (Fig. 3.3A).The number of aphids surviving was lower on CJ treated brassica cultivars but the difference was not statistically significant. A similar trend was observed after 96 h, with no significant

difference in aphid mortality was recorded on brassica cultivars except ‘Samurai’. Cultivar Samurai had an increase in number of dead aphids (P = 0.04) compared to control plants after 96 h of CJ treatment.

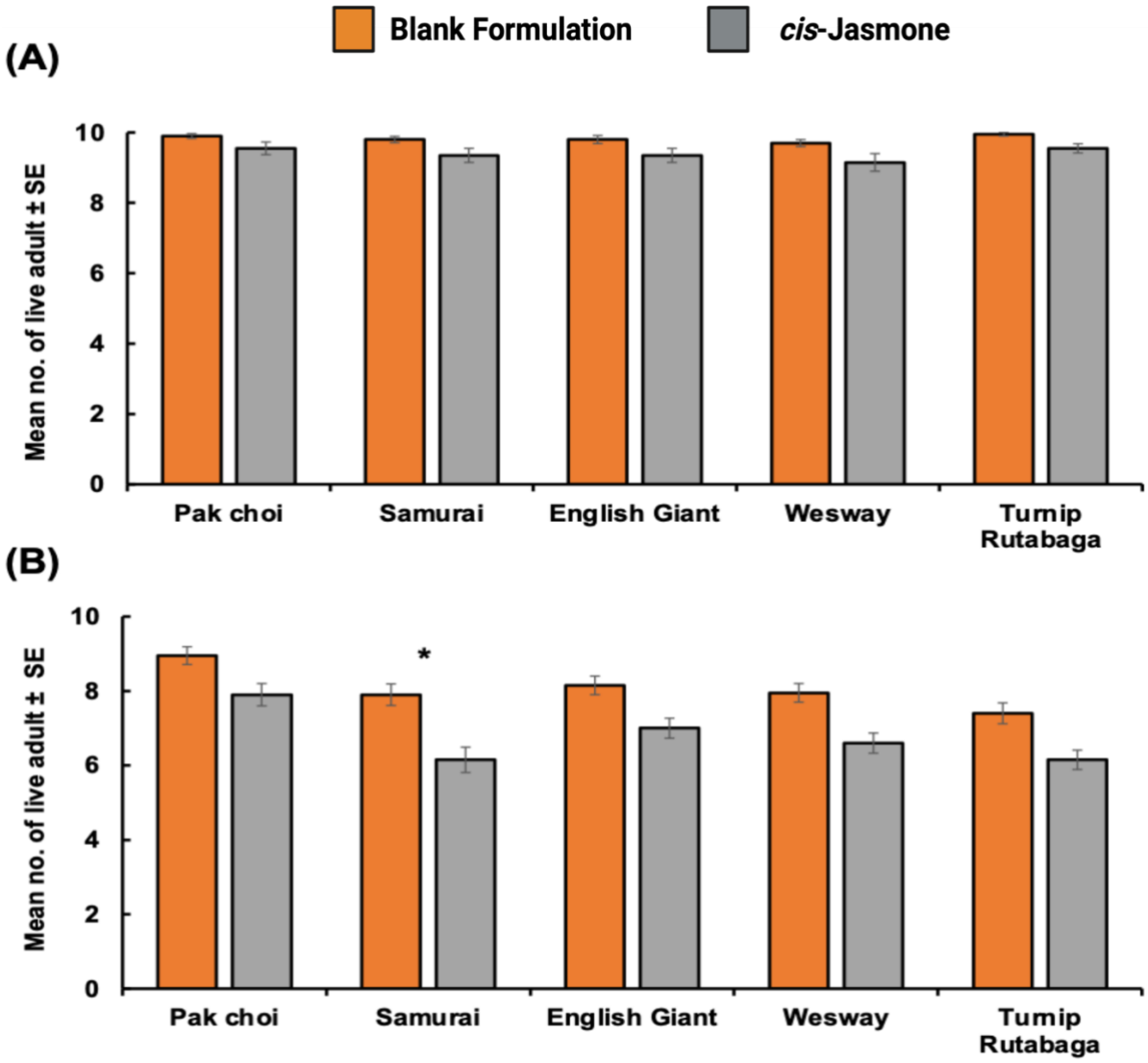

FIGURE 3.3 | *Myzus persicae* adult survival (Mean ± SE) out of the original 10 individuals after (a) 48 h and (B) 96 h in clip cage on five brassica cultivars (n = 10) treated with *cis*-Jasmone or blank formulation (control). Asterisk denotes differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (generalised linear models with Poisson probability distributions).

There was a significant reduction in nymph production on CJ treated plants across both time points (Fig. 3.4A, B). Mean larviposition on CJ treated plants of all brassica cultivars was significantly reduced after 48 h (Fig. 3.4A), decreasing by 35 % from 23.97 on control treated plants to 15.54 on CJ treated plants. Larviposition was reduced most on Wesway ($P < 0.001$; 41 % reduction in larviposition) and the least on Samurai ($P < 0.001$; 21% reduction in larviposition). Similarly, mean larviposition on CJ treated plants of all brassica cultivars was also significantly reduced after 96 h (Fig. 3.4B), decreasing by 39 % from 57.18 on control plants to 34.81 on CJ treated plants. Larviposition was reduced most on 'Samurai' ($P < 0.001$; 59 % reduction in larviposition) and the least on 'Turnip Rutabaga' ($P = 0.004$; 24 % reduction in larviposition).

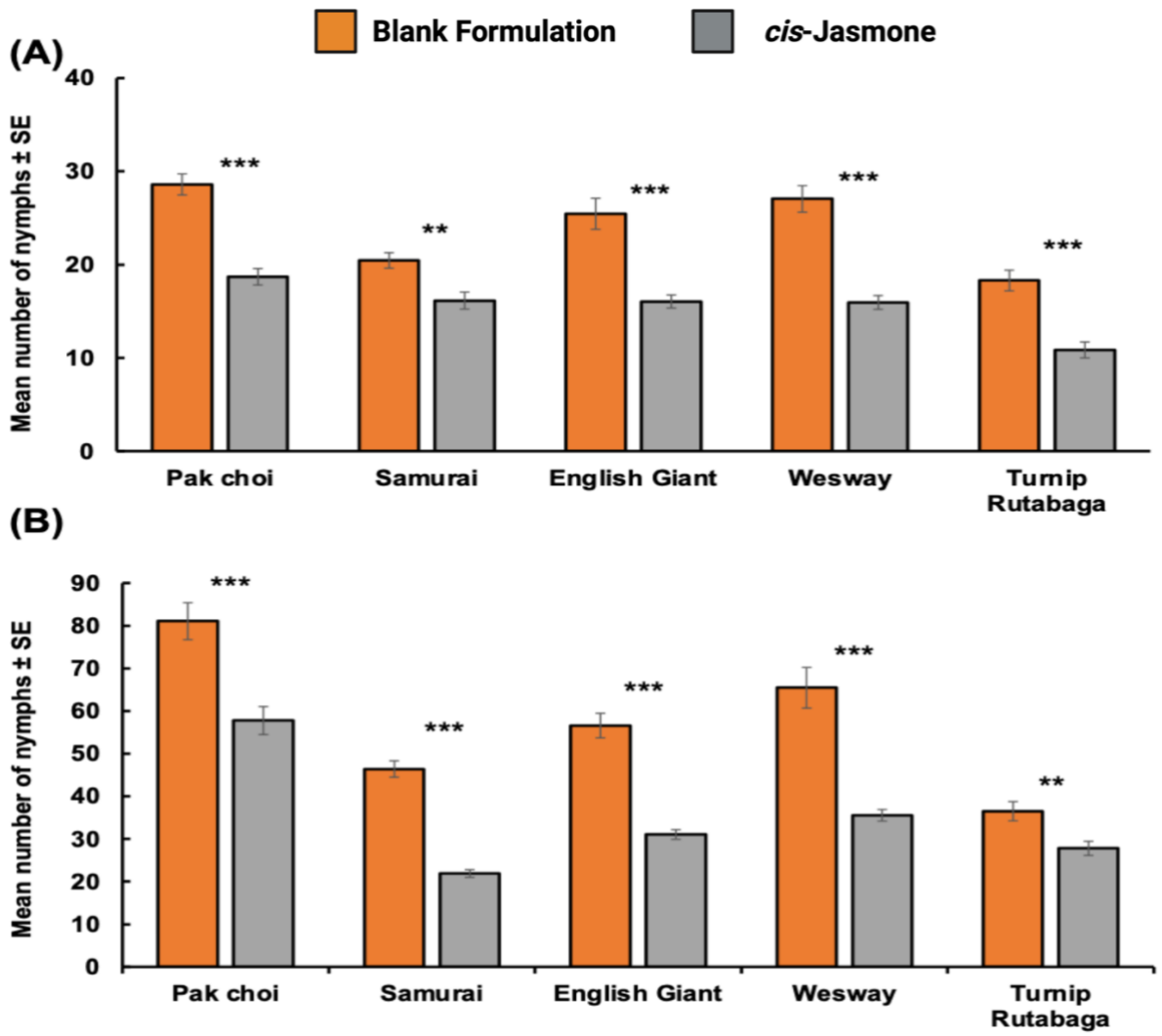

FIGURE 3.4 | *Myzus persicae* larviposition (Mean ± SE) after (A) 48 h and (B) 96 h in clip cages on five brassica cultivars (*n* = 10) treated with *cis*-Jasmone or blank formulation (control). Asterisks denote differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (generalised linear models with Poisson probability distributions).

3.4.2 Settlement bioassay

In settlement bioassay, where aphids were offered a choice between CJ treated and control plants, a clear and statistically significant reduction in aphid settlement was observed on CJ treated plants (Fig. 3.5). This effect was consistent across all brassica cultivars tested. The preference for control over CJ treated plants was strongest for

the *B. napus* cultivar Wesway, with a mean of only 7.05 aphids settling on CJ treated plants compared to 16.2 aphids settling on control treated plants (GLM with quasi-Poisson distribution: F = 32.39; *d.f.* = 1,38; P < 0.001; 30.3% of aphid settlement occurring on CJ treated plants). Similarly, aphid settlement was significantly reduced in Pak choi (GLM with quasi-Poisson distribution: F = 98.81; *d.f.* = 1,38; P < 0.001), English Giant (GLM with Poisson distribution: X^2 = 86.63; *d.f.* = 1,38; P < 0.001), Samurai (generalized linear model with Poisson distribution: X^2 = 70.4: *d.f.* = 1,38; P < 0.001), and Turnip Rutabaga (GLM with Poisson distribution: X^2 = 41.12; *d.f.* = 1,38; P < 0.001). Pooling data across all cultivars tested, mean aphid settlement on CJ treated plants was 1.86 times lower than on control plants.

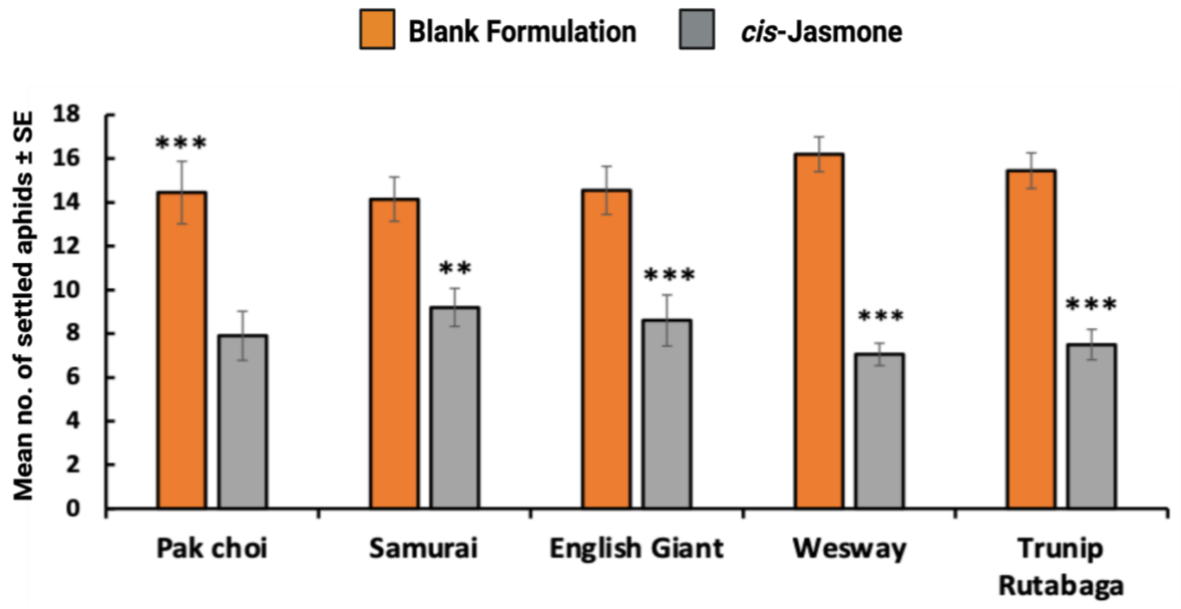

FIGURE 3.5 | Mean settlement of *Myzus persicae* after 24 h on untreated (control) and *cis*-Jasmone (CJ) treated plants in a choice bioassay (50 winged aphids were released in each replicate n = 10). Error bars represent standard error of the mean (± SE). asterisk denote different level of statistical significance * < 0.05, ** < 0.01 and ***

< 0.001 (generalised linear models with either Poisson or quasi-Poisson probability distributions).

3.4.3 Behavioural response of *M. persicae* to odour collected from different brassica cultivars

When presented with a choice between a solvent control (diethyl ether; DEE) and volatiles collected from plants treated with a blank formulation, adult aphids showed no significant preference for either odour source in five of the tested cultivars (Fig. 3.6A). Aphids did, however, spend significantly less time in the treated zone for Wesway (paired *t*-test: $t = 4.69$; *d.f.* = 9; $P = 0.001$) (Fig. 3.6A). However, when plants were CJ treated and aphids were presented with a choice of volatiles from a treated plant versus solvent control (DEE), aphids spent significantly less time in the treated zone of the olfactometer with volatiles from CJ treated plants for 4 out of the 5 cultivars tested (Fig. 3.6B). 'Turnip Rutabaga' was the only cultivar in which volatiles from CJ-treated plants were not repellent. CJ-treated 'Samurai' and 'Pak choi' were the most repellent. When also allowed to choose between volatiles from CJ treated plants and untreated control plants (Fig. 3.6C), aphids spent significant longer in the olfactometer zone with volatiles from blank formulation control plants for 'Pak choi' and 'Samurai' cultivars.

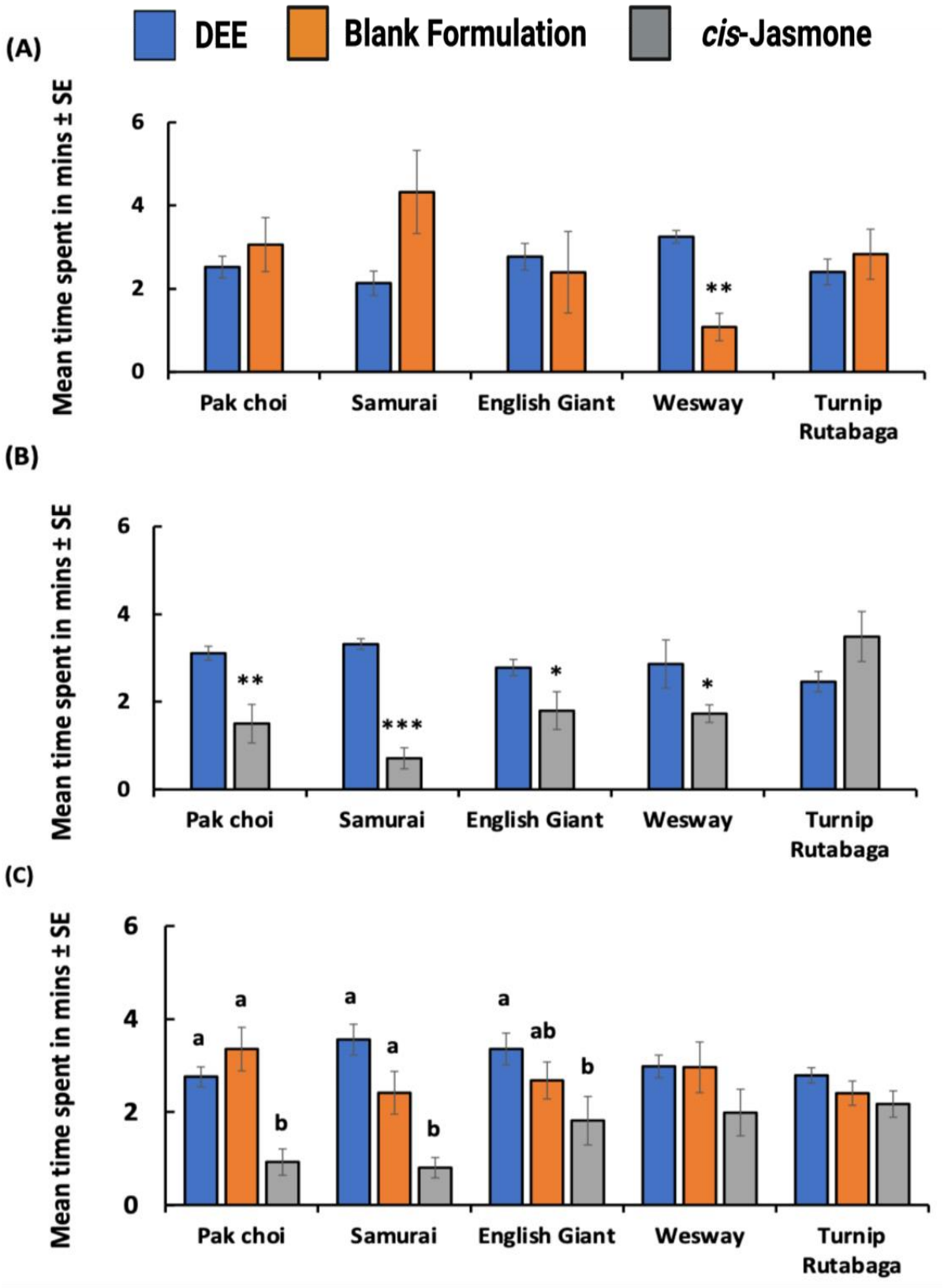

FIGURE 3.6 | Behavioural responses of *Myzus persicae* females to volatile organic compounds (VOCs) from five brassica genotypes in an olfactometer bioassay.

Individual aphids were given 12 minutes to make a choice between (a) one arm of blank formulation treated plants (BF – tween 80 and water) vs. three solvent diethyl ether (DEE) arms, (b) one arm of *cis*-Jasmone treated plants (CJ) vs. three solvent DEE arms and (c) two different treatment arms (BF = blank formulation treated plants and CJ = *cis*-Jasmone treated plants) vs. two solvent DEE arms. The values shown are mean time spent in arm ± standard error (SE) (n = 10). For Fig. 3.6A and 3.6B, Brassica genotypes capped with asterisks show a denote differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (paired t-test). For Fig. 3.6C, different letters above bars indicate statistically significant differences between treatments (P < 0.05), based on post-hock test.

3.5 Discussion

The current study supports previous studies showing that *cis*-Jasmone treatment of plants made them more resistant to insect pests (Bruce *et al.*, 2003; Sobhy *et al.*, 2017; Sobhy *et al.*, 2020). Various crop plants including tomato, maize, wheat potato, soybean treated with CJ were shown to have enhanced levels of resistance (Bruce *et al.*, 2008; Moraes *et al.*, 2009; Oluwafemi *et al.*, 2013; Disi *et al.*, 2017; Sobhy *et al.*, 2017). CJ application activates the plant defense system to produce volatile organic compounds that have a repellent effect on pest (Birkett *et al.*, 2000; Bruce *et al.*, 2008; Oluwafemi *et al.*, 2013). In this study, several bioassays were performed to assess the performance of *M. persicae* on CJ treated and control brassica crop plants that had not been tested previously. We found that the performance of *M. persicae* was reduced on treated plants in terms of survival and fecundity. *M. persicae* also showed less preference for CJ treated plants than untreated control plants.

All five brassica cultivars did not show any significant difference in aphid mortality in clip-cage bioassay after 48 h. However, the number of live adults was lower on CJ

treated plants compared to control plants. Effects of CJ treatment of brassica cultivars on aphids were more apparent after 96 h compared to 48 h since the aphids have been on the plants for longer time and therefore were more affected by any negative effects of the CJ treatment, Samurai was the most responsive cultivar with a significant high increase in the mortality of aphids on CJ plants. Overall number of live aphids was lower on all CJ plants throughout all five cultivars. In contrast to adult aphids, CJ treatment had a strong effect on nymph production, as per results, it can be seen that plants treated with CJ were less suitable for aphid reproduction. Data collected from both series (48 h and 96 h) showed that CJ treatment induces changes in plant physiology that affect the aphid's reproductive capability. If the experiment was continued for longer than 96 h it is likely that even bigger effects would have been observed. The combined effect of increased mortality and reduced larviposition can be considered to 'slow down' aphid population growth. Lower reproductive rate is an important effect of CJ treatment because the aphid's fast reproductive rate is one of the main characteristics that makes it one of the most notorious pests (Leather, 2017). The difference in aphid survival and larviposition is due to the upregulation of plant defence induced by CJ treatment (Dewhirst *et al.*, 2012; Sobhy *et al.*, 2020).

Settlement bioassays showed that aphid colonisation was significantly lower on CJ treated plants for all five cultivars. This experiment shows that low aphid colonisation would further slowdown the aphid infestation, which would be an important step in controlling the aphid population on the plant. Behavioural bioassays revealed that aphids spent less time in the arm of olfactometer treated with CJ treated volatiles i.e. CJ treated plants were repellent to aphids. In olfactometer bioassays, volatiles collected from CJ treated plants were repellent to aphids for most of cultivars. Surprisingly, volatiles collected from Turnip rutabaga showed no repellent effect on

aphids, although volatile analysis showed a 4-fold increase in volatile emission with CJ treatment. This suggests that composition or quality of volatile components is more important than the quantity of the component to repel the pest. Also a repellent effect was observed in another brassica cultivar (Chinese kale) although volatile emission was not increased by CJ treatment (Ali *et al.*, 2021). This again proves the type of volatile chemicals released matters. Based on these results we can say CJ acts as a plant defence elicitor to induce brassica crop plant resistance against aphids.

The current results showed a similar pattern to previously reported results for CJ induced defence with other plant species. For instance; CJ treated plants showed reduced pest growth and reproduction, reduced settlement of the pest population and volatiles collected from CJ plants were avoided. These effects have been observed in several other crop plants such as wheat, potato and soybean (Bruce *et al.*, 2003; da Graça *et al.*, 2016; Bayram and Tonğa, 2018; Sobhy *et al.*, 2020) but had not been tested in brassica crops previously. To test the effect of CJ on aphid natural enemies, we have performed further bioassays in the following chapter.

Chapter 4. EFFECT OF *cis*-Jasmone ON THE PERFORMANCE AND BEHAVIOUR OF *Diaeretiella rapae*

4.1 Introduction

Aphids are severe threat to many economically important crop plants, including Brassica crops. As earlier discussed in chapter 1, aphids cause direct damage to the crop both by their feeding and indirect damage by transmitting plant viruses. For aphids, control measures include the use of insecticides and biological control methods such as use of parasitoids. Both control measures have limitations in their use. Insecticide use is limited against aphids due to resistance development in aphids and other environmental concerns (Devonshire and Moores, 1982; Moores, Devine and Devonshire, 1994; Martinez-Torres *et al.*, 1999). In nature, natural enemies of aphids have the potential to maintain aphid numbers below the economic threshold. They include a range of predators, parasitoids and diseases, but their late visit to the field and slow growth rates often make them less competent to suppress the fast population growth of aphids in the field where crops are available in abundance to the pest.

It has been observed that breeding programs alter the genetic makeup of crop plants that affect the plant-insect interaction (Benrey *et al.*, 1998; Rodriguez-Saona *et al.*, 2011). Selection of desirable traits for crop domestication may compromise other important aspects, such as defence. Domestication of plants alters the nutrient levels and allelochemistry of the plant, for instance domesticated cranberry plant provides higher quality resource to herbivores and their natural enemies compared to their wild crop relatives (Benrey *et al.*, 1998). Thus altered allelochemistry can be utilised to recruit the potential natural enemies to maintain the pest population below threshold level. Hymenopteran wasps are important biological control agents, which are being

used to control aphids. Artificial release of parasitoids and other biocontrol agents has been successful in greenhouse conditions (Van Lenteren, 2000; Bosco, Giacometto and Tavella, 2008; Yang *et al.*, 2014). However, the use of parasitoid wasps in the field is still at an initial stage with varying levels of control (Abram, Mills and Beers, 2020). Use of parasitoids to control the aphid population is also limited due to differences in generation time (aphids have very short generation times while generation time of parasitoids is longer). It is suggested that establishment of parasitoids should be as soon as first aphids appear in the field in order to provide effective biological control (Pijnakker *et al.*, 2020). However, survival of parasitoids on a small number of aphids is difficult. With low aphid numbers, parasitoids may be unable to locate hosts and this may lead increased dispersal to other fields. To enhance the management of aphids, insecticides may be applied but they have detrimental effects on parasitoids.

To increase efficacy of the biological control agents, a potentially effective measure could be use of plant elicitors to enhance natural plant defence. Plants treated with activators have been found to become more attractive to parasitoid wasps. Several plant elicitors have been identified which are being used as an integral part of integrated pest management (Inbar *et al.*, 1998; Stout, Zehnder and Baur, 2002; Holopainen *et al.*, 2009; Thakur and Sohal, 2013; Sobhy *et al.*, 2014; Conboy *et al.*, 2020). Plant elicitor, *cis*-Jasmone is a potential compound that has been tested on various crops (wheat, potato and bean) and insects; aphids: *Nasonovia ribis-nigri* (lettuce aphids), *Sitobion avenae* (grain aphid), and parasitoid; *Aphidius ervi* (Birkett *et al.*, 2000; Bruce *et al.*, 2003; Moraes *et al.*, 2008, 2009; Bruce *et al.*, 2008; Bruce *et al.*, 2008; Delaney *et al.*, 2013; Disi *et al.*, 2017; Sun *et al.*, 2019; Sobhy *et al.*, 2020). So far, no work has investigated the effect of *cis*-Jasmone on crop brassica plants. If

cis-Jasmone induces plant defence in brassica plants then aphid infestation could be reduced and it may open the way for effective use of biological control agents. Previous studies proved that *cis*-Jasmone treated plants show enhanced defence against aphids and also increased recruitment of parasitoids, however effect of CJ treatment was not studied on brassica crops before.

4.1.1 Aims and objectives

The overall aim of this chapter is to assess the effect of *cis*-Jasmone (CJ) treatment of a range of brassica crop plants with regards to parasitoid performance on treated plants. Within this aim there are three objectives:

- To investigate the foraging behaviour of *D. rapae* on CJ treated brassica plants
- To investigate the parasitisation preference of *D. rapae* on CJ treated brassica plants
- To investigate the behavioural response of *D. rapae* to volatiles collected from CJ treated brassica plants

4.2 Materials and methods

4.2.1 Insects

Myzus persicae aphids were collected from the well-established aphid rearing lab, Centre of Applied Entomology and Parasitology (CAEP) at Keele University. The *M. persicae* clone O was reared on Pak choi, commonly known as Chinese cabbage, in Bugdorm cages (46 x 46 x 46 cm; NHBS Ltd, Devon, UK) under controlled conditions (24 °C ± 2 °C , 38 % RH, 16 h L: 8 h D photoperiod).

Diaeretiella rapae parasitoid wasps were collected from the parasitoid rearing lab, Centre of Applied Entomology and Parasitology (CAEP) at Keele University. To rear parasitoids, mummies of *D. rapae*, attached to plant leaves, were introduced to cages

containing fresh Pak choi plants infested with *M. persicae* and kept under controlled condition (20 °C, 40% RH, 16 h:8 h photoperiod; in a growth chamber (MLR-352-PE; Panasonic, The Netherlands). Upon emergence, parasitoid adults were provided with honey solution (1:1 in water) as food. Only female parasitoids were used in experiments and they were 2–3 day old and mated.

4.2.2 Plants

Five *Brassica* lines: *B. napus* cv. 'Samurai'*, B. napus* cv. 'Wesway'*, B. napus* cv. English giant*, B. napus* cv. 'Turnip rutabaga 57' and *Brassica rapa* subsp. *chinensis* cv. Hanakan (Pak choi) were obtained from Warwick University, UK. All plants were grown in a plant growth chamber (22 °C, 16 h L: 8 h D photoperiod). Brassica plants were grown individually in 7.5 cm pots in James magic compost (Westland Horticulture Limited, UK). Plants with five true leaves plants were used for entrainment.

4.2.3 Plant treatments

Plants were either treated with CJ, for treated plants, or blank formulation, for control plants, following the procedure described in section 3.2.3. of chapter 3.

4.2.4 Parasitoid foraging bioassay

All experiments were conducted under the same conditions of temperature and daylength as used for insect rearing. CJ treated and control plants were used in the experiments. Each line was replicated ten times for CJ treated and control. Treated and control replicates were observed alternately. All replicates done in a single day were of the same line. All experiments were conducted in an open-fronted cage. For each replicate the plant was placed on a turntable at the centre of the cage. With the help of a vial a single female parasitoid was collected from the Bugdorm and released onto the leaf of the plant and its foraging behaviour was monitored by direct

observation, until the parasitoid left the plant. Noldus Observer 4.1 software was used to record the behavioural observations. All parasitoids used were between 24 h to 32 h old and each used only once and then discarded. All experiments were done between 9:00 h and 16:00 h. The following data were recorded during observations of parasitoid foraging; (i) type of behaviour (walking, still, cleaning and flying) (ii) duration of each period of walking, still, cleaning and flying (seconds) (iii) total time spent on the plant by each parasitoid (seconds). Time spent walking, still, and cleaning was recorded, as well as total time spent before the parasitoid left the plant. An observation was terminated when a parasitoid flew away from the plant, which was considered as the foraging ‘‘patch.’’ Time spent on treated and control plants was compared by using a paired *t*-test.

4.2.5 Parasitism bioassay

In this choice test bioassay, the effect of CJ on the parasitism rate of *D. rapae* was assessed on CJ treated and control plants in a Bugdorm cage (60 × 60 × 60 cm; NHS Ltd), which was kept in a controlled environment room (25 °C ± 2 °C, 37 % RH, 16 h L: 8 h D photoperiod). Each Bugdorm contained four plants (two CJ treated and two control) treated and control plants were placed at alternate positions (Fig. 4.1). Each plant was inoculated with 50 adult aphids 2 h prior to the release of parasitoids *D. rapae*. Eight female parasitoids were released in the Bugdorm using a plastic vial following the experimental procedure described in Sun *et al.*, (2020). Parasitoids were released to observe the difference in parasitism behaviour of *D. rapae* on CJ and control plants infested with aphids. After 24 h, parasitoid females were collected from the cages. Experiments were conducted in a climate controlled room (25 °C ± 2 °C, 37% RH, 16 h L: 8 h D photoperiod). After 15 days, the number of mummies collected

from treated and control plants was recorded. The assays were repeated ten times on different experimental days.

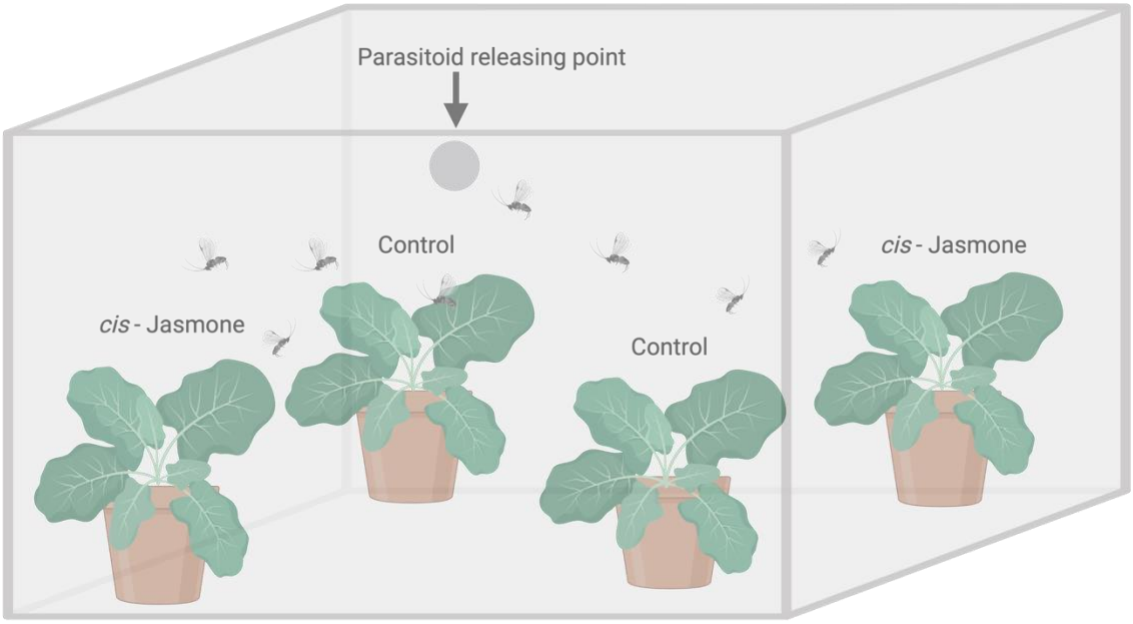

FIGURE 4.1 | Experimental set up for assessing parasitism behaviour of female *D. rapae* on aphid infested (control vs. CJ) plants. Each plant was infested with 50 adult aphids 2 h prior to release of the parasitoid. Plants were removed after 24 h exposure to parasitoids in the Bugdorm cage.

4.2.6 Olfactometer bioassay

In a four-arm olfactometer bioassay, the behavioral response of the parasitoid *D. rapae* was investigated to odours collected from blank formulation (BF) and CJ treated plants. The procedure described in section 3.2.6 of Chapter 3 was followed. Olfactometer bioassays were divided into three series comparing insect responses to different treatments: (i) DEE (solvent blank) and volatile samples collected from BF plants, (ii) DEE and volatile samples collected from CJ treated plants and (iii) DEE and volatile samples collected from BF and CJ treated plants.

4.3 Statistical analysis

Parasitoid Foraging Bioassay

The total time spent by parasitoid wasps foraging on control and CJ treated plants was first analysed for each brassica cultivar using Shapiro-Wilk tests to determine whether the underlying data were Gaussian. As data for this bioassay was non-Gaussian, the response variable (i.e., time) was square root transformed and re-analysed using Shapiro-Wilk tests to confirm that transformed data were Gaussian. After transformation, differences in mean total parasitoid foraging time between control and CJ treated plants was evaluated for each brassica cultivar using two sample t-tests.

Parasitism Bioassay

Differences in the mean number of mummified aphids on control and CJ treated plants were compared for each brassica cultivar using GLMs with Poisson or quasi-Poisson probability distributions depending on dispersion. Plant treatment (i.e., control vs. CJ treated) was a fixed factor.

The behavioural response of *D. rapae* was tested in two ways. For experiments with one treated arm vs. three solvent control treatments, data were analysed by a paired *t*-test. In this analysis, the time spent by aphids in treated and solvent arms of the four-arm olfactometer were compared. In experiments where the response in two treatment arms vs. two arms of solvent control was compared, data were first converted into proportions then log-ratio transformed before analysis by one-way analysis of variance and Holm-Sidak mean separation (Mwando et al.,2018). Data were examined for a Gaussian distribution using the Shapiro-Wilk test prior to analysis.

All statistical analyses were carried out using R (v 4.0.3) (R Core Development Team, 2021).

4.4 Results

4.4.1 Parasitoid Foraging bioassay

In a foraging bioassay, parasitoid wasps spent substantially longer on CJ treated plants than on control plants (Fig. 4.2). There was a 5.1x increase in mean time spent on CJ treated Pak choi plants (two-sample *t*-test, $P = 0.004$), a 4.6x increase on Turnip Rutabaga (two-sample *t*-test, $P = 0.001$), a 4.5x increase on Wesway (two-sample *t*-test, $P = 0.001$), a 3.9x increase on Samurai (two-sample *t*-test, $P = 0.013$), and a 2.8x increase on English Giant (two-sample *t*-test, $P = 0.04$).

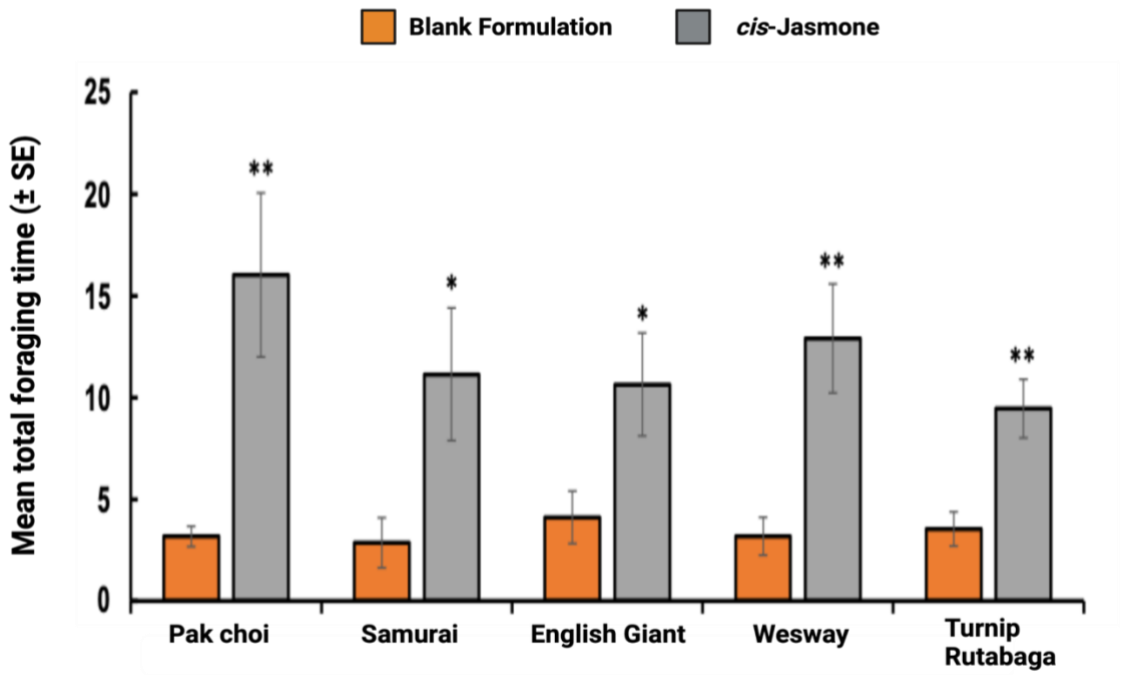

FIGURE 4.2. Mean total time spent foraging (± SE) by *Diaeretiella rapae* on untreated (control) and *cis*-Jasmone (CJ) treated plants ($n = 10$). Error bars represent standard error of the mean (SE). Brassica genotypes capped with asterisks show a significant difference between control and CJ treatment with differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (two-sample *t*-tests).

4.4.2 Parasitism bioassay

In parasitism bioassay, three out of five CJ treated brassica lines showed a significant increase in number of parasitised aphids (mummies) (Fig. 4.3). The largest increase in parasitism was observed on Samurai (P = 0.001; 121 % increase), though Pak choi had the greatest total number of mummified aphids. (Fig. 4.4).

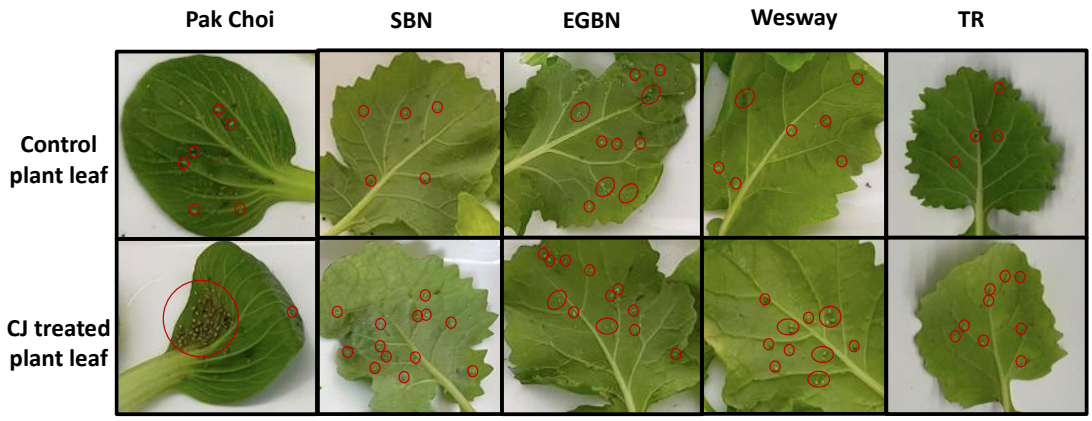

FIGURE 4.3 | Brassica plants leaves (Control and CJ) showing number of mummified aphids (red circle).

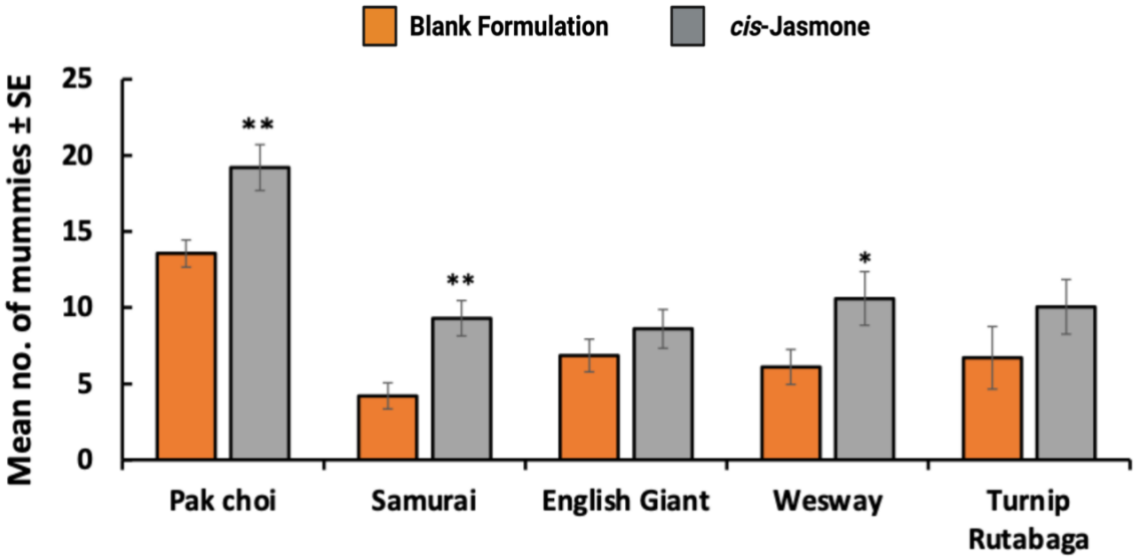

FIGURE 4.4 | Mean Number (± SE) of mummified *M. persicae* on Blank formulation and *cis*-Jasmone treated plants 15 days after exposure to parasitoid *D. rapae* (*n* = 10).

Brassica cultivars capped with asterisks show a differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (two-sample *t*-tests).

4.4.3 Behavioural response of *Diaeretiella rapae* to odour collected from different brassica cultivars

Olfactometer bioassay series 1 was a choice between a solvent control (diethyl ether; DEE) and volatiles collected from plants treated with a blank formulation. Genotypes Wesway and English Giant showed significant deterrence to parasitoids (P = 0.0001 and 0.002 respectively) (Fig. 4.5A). Parasitoids spent more time in the treated zone for the other three genotypes but the difference was not significant (Fig. 4.5A). However, in series 2, when plants were CJ treated and parasitoids were presented with a choice of volatiles from a treated plant versus solvent control (DEE), parasitoids spent significantly more time in the treated zone of the olfactometer with volatiles from CJ treated plants for 3 out of the 5 cultivars tested (Fig. 4.5B). English Giant had the highest parasitoid preference for volatiles from CJ treated plants (P < 0.01) while Samurai had the least preference (P > 0.05). In series 3, when parasitoids were also allowed to choose between volatiles from CJ treated plants and untreated control plants (Fig. 4.5C), parasitoids spent significantly longer in the olfactometer zone treated with volatiles from CJ plants for all brassica cultivars tested: Pak choi (P < 0.04), Samurai (P < 0.01), Turnip rutabaga P < 0.001, Wesway (P < 0.001) and English Giant (P = 0.003). An increase of 291% and 64% in mean time spent by *D. rapae* was recorded when the olfactometer arm treated with CJ treated Turnip rutabaga and Samurai plant volatiles was compared to the arm treated with plant volatiles treated with blank formulation respectively.

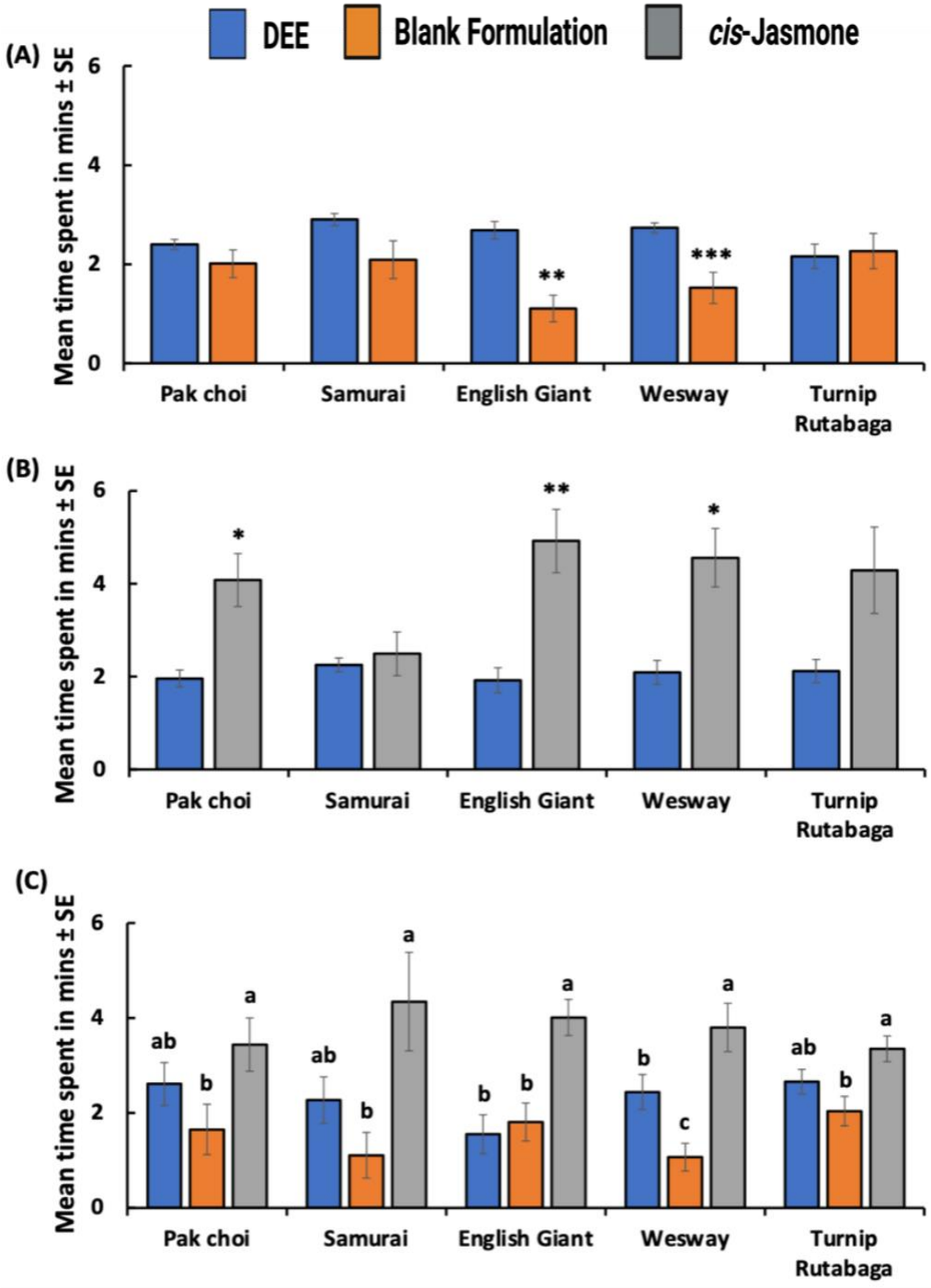

FIGURE 4.5 | Behavioural responses of *Diaeretiella rapae* females to volatile organic compounds (VOCs) from five brassica genotypes in an olfactometer bioassay. Individual parasitoids were given 12 minutes to make a choice between (a) one arm of blank formulation treated plants (BF – tween 80 and water) vs. three solvent diethyl ether (DEE) arms, (b) one arm of *cis*-Jasmone treated plants (CJ) vs. three solvent

DEE arms and (c) two different treatment arms (BF = blank formulation treated plants and CJ = *cis*-Jasmone treated plants) vs. two solvent DEE arms. The values shown are mean time spent in arm ± SE (*n* = 10). For Fig. 4.5A and B, Brassica genotypes capped with asterisks show a denote differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (paired *t*-test). For Fig. 4.5C, different letters above bars indicate statistically significant differences between treatments ($P < 0.05$), based on post-hock test.

4.5 Discussion

Biological control is an important component of Integrated pest management (IPM) (Howarth, 1991; Orr, 2009; Naranjo, Ellsworth and Frisvold, 2015) that plays a vital role in supressing pest populations in the field and leads to reduction in the use of chemical pesticides (Howarth, 1991; Hokkanen and Lynch, 2003). Plants release a plethora of volatile organic compounds which can change when a plant faces an abiotic or biotic stresses (Spinelli *et al.*, 2011; Effah, Holopainen and McCormick, 2019). Plants produce herbivore induced plant volatiles (HIPVs) under herbivore attack and these volatiles are used as a signal cue by biocontrol agents to locate the host plants in search of feeding or ovipositional sites (Dicke and Baldwin, 2010). Previous studies have found that release of HIPVs enhances the efficacy of biological control agents; parasitoid, predators (James, 2003; Hare, 2011; War *et al.*, 2011; Turlings and Erb, 2018).

A number of natural compounds have been used as defence elicitors to activate plant indirect defence and release of induced volatiles (Kessler and Baldwin, 2001; Holopainen *et al.*, 2009; Aranega-Bou *et al.*, 2014). Induced volatile emission enhances biological control by increasing the number of biocontrol agents, their visits and/or foraging time in the crop field (Gols, Posthumus and Dicke, 1999; Bruce *et al.*,

2008; Sobhy *et al.*, 2014; Bruce *et al.*, 2017). Recruitment of biocontrol agents such as predators, parasitoids is one of the key benefits of plant induced defence whether it is caused by herbivory or application of defence elicitors (Cortesero, Stapel and Lewis, 2000; Khan *et al.*, 2008; Peñaflor and Bento, 2013; Sobhy *et al.*, 2014). An extensive literature is available that documents attraction of natural enemies to plants damaged by pests (Room, 1990; Khan *et al.*, 2008; Peñaflor and Bento, 2013; Pirk, 2021). Similar effects have been observed when plants are treated with natural compounds and a significant increase in parasitoid foraging has been recorded (Khan *et al.*, 2008; Sarah Y. Dewhirst *et al.*, 2012; Sobhy *et al.*, 2014; Sobhy, Bruce and Turlings, 2018).

This chapter describes the effect of *cis*-Jasmone treatment of brassica crop plants on the behaviour of aphid parasitoid *D. rapae.* A foraging bioassay was performed to observe the time duration parasitoids spent on blank formulation and CJ treated plants. This bioassay was performed because retention for foraging after arrival is important for a good level of parasitism in the field (Budenberg, Powell and Clark, 1992). Similar studies, performed with the model plant *Arabidopsis*, showed that parasitoids spent double the time on CJ treated plants compared to control plants (Bruce *et al.*, 2008). Longer retention time on plants increases the opportunity for the parasitoid to parasitise the pest. Here, we also found a significant increase in foraging time, with parasitoids spending up to 4.5 fold more time on CJ treated plants than on control plants. The increase in foraging time observed on the brassica cultivars in the current study could be because of changes induced by CJ treatment in host plant as reported in previous studies (Moraes *et al.*, 2008; Bruce *et al.*, 2008; Bayram and Tonğa, 2018). The effect of CJ treatment on the volatile profile of brassica plants will

be discussed in next chapter. The change in volatile emission of treated plants could influence the foraging behaviour of parasitoid.

Besides this, another experiment 'parasitism bioassay' was also performed that supported the findings of the foraging bioassay. An overall increase of 50% in number of mummified aphids was recorded on CJ treated plants when compared with blank formulation treated (BF) plants. Out of five brassica cultivars, three cultivars showed a significantly higher number of mummified aphids. In particular, cultivar Samurai, Wesway and Pak choi showed a significant increase of 2.21 x, 1.53 x and 1.42 fold in number of mummified aphids on CJ plants compared to control plants respectively, while Turnip rutabaga and English Giant showed the least increase. The effect of CJ application at the tritrophic level has been tested with several plants, and most of these previous studies support its role as a stimulator of biological control agents. Behavioral responses of several control agents have been observed with *cis*-Jasmone compound or CJ treated plants; for instance; *Coccinella septempunctata*, showed preference for CJ in a wind tunnel bioassay when exposed to CJ induced bean plant volatiles (Birkett *et al.*, 2000),

To investigate the behavioural response of *D. rapae* to plant volatiles a series of olfactometer bioassays was performed using different combinations of solvent blank control and plant volatile treatments collected from blank formulation and CJ treated plants. Parasitoids spent a longer time in the zone treated with volatiles collected from CJ treated plants. Plant volatiles collected from CJ treated plants had a significant attractant effect on *D. rapae*. All five genotypes, had CJ-induced plant volatiles that acted as an attractant for parasitoid. Cultivars 'Wesway" and 'English giant showed the highest level of induced effect after CJ treatment and were highly attractive to *D. rapae*. As mentioned in the last chapter, olfactometer studies again proved that

changes in the plant volatile blend can play a critical role in inducing insect behavioural changes. The high preference of *D. rape* to CJ plants may be due to the induced effect of CJ in brassica lines. For instance; English Giant and Wesway volatile samples were repellent before CJ treatment but after treatment volatiles samples were highly attractive to *D. rapae*.

Earlier study on Arabidopsis reported that CJ treated Arabidopsis plants also release volatile compounds that act as an attractant to *Aphidius ervi* which spent a longer time foraging on CJ plants compared to control (Bruce *et al.*, 2008). In tobacco, the parasitic efficiency of *Campoletis chloridae* was enhanced when it was exposed to *Helicoverpa armigera* infested plants treated with CJ (Sun *et al.*, 2020).*Telenomus podisi* was highly attracted to soybean plants exposed to CJ application (Moraes *et al.*, 2009). During a field experiment, CJ treatment of wheat plants was responsible for attraction of aphid parasitoids; *Collyria coxator* and predator ladybird beetle *Coccinella septempunctata* (Bayram and Tonğa, 2018). The current findings suggest that CJ treatment could be used to induce plant defence that will improve the biological control of aphids in brassica crops by increasing parasitoid visits and foraging time. To investigate the differences induced by CJ in volatile emission, we have performed volatile analysis in the following chapter.

Chapter 5. IDENTIFICATION AND COMPARISON OF VOLATILE COMPOUNDS RELEASED BY *cis*-Jasmone TREATED AND UNTREATED BRASSICA PLANTS USING COUPLED GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

5.1 Introduction

Plants release a diverse range of volatile compounds, depending on the species and the environmental conditions, these plant volatiles interact with their surroundings (neighbouring plants, herbivores, pests and parasitoids) (Bouwmeester *et al.*, 2019; Erb and Kliebenstein, 2020). The volatile blend emitted by the plants represents the physiological status of the plant and is used as a cue by herbivores to recognise the host and non-host plants (Meiners, 2015; Giron-Calva, Li and Blande, 2017; Webster and Cardé, 2017). There are various factors that affect the uniqueness of the volatile blend that include the plant's health and stress exposure (biotic and abiotic)(Gouinguené and Turlings, 2002; Pedrol, González and Reigosa, 2006). Changes in volatile emission can caused by naturally induced changes (deficiency of nutrients, herbivore attack, allelopathy; plant-plant signalling) or by artificially applying plant defence elicitors (Kessler and Baldwin, 2001; Lou *et al.*, 2005; Pare *et al.*, 2005; Pedrol, González and Reigosa, 2006; Peñaflor and Bento, 2013). Use of defence elicitors to manipulate plant defence (direct and indirect) is an interesting approach that can be used by plant scientists to study plant responses or even to develop new crop protection treatments (Benhamou, 1996; Hegde *et al.*, 2012; Bektas and Eulgem, 2015; Zhang and Gleason, 2020; Mouden *et al.*, 2021).

Any qualitative or quantitative change in plant volatile emission can affect plant-herbivore interactions (Silva *et al.*, 2017; Takabayashi and Shiojiri, 2019). This change can be the consequence of any abiotic (light, drought, salinity, nutrients) and/or biotic stresses (pest and pathogens) the plant is exposed to (Gouinguené and Turlings,

2002; Pedrol, González and Reigosa, 2006). During biotic stress, plant recognises the damage caused and enzymes released into the host body by the invaders, and activates defence pathways that result in the production of antibiotic and defensive organic compounds that repel the stress causing entity (Giron *et al.*, 2016; Wielkopolan and Obrępalska-Stęplowska, 2016; Ighodaro and Akinloye, 2018; Turlings and Erb, 2018). An accumulating body of evidence suggests that herbivore attack leads to the synthesis of organic compounds (Arimura, Kost and Boland, 2005). It has been observed that herbivore attacks is responsible for the induction of herbivore induced plant volatiles (HIPVs) and these HIPVs play an important role in mediating intra- and interspecific interactions (Arimura, Kost and Boland, 2005; D'Alessandro and Turlings, 2006; Rodriguez-Saona and Frost, 2010; Najar-Rodriguez *et al.*, 2015). HIPVs are critically important in conveying the message from the plant (under attack) to neighbouring plants (plant-plant signalling) and also in recruiting biocontrol agents (predators and parasitoids) (D'Alessandro and Turlings, 2006; Giunti *et al.*, 2018). Many parasitoid species use plant volatiles induced by herbivore feeding or oviposition to locate their prey (Giunti *et al.*, 2018; Turlings and Erb, 2018).

A number of studies have revealed that there are plant defence elicitor compounds that can induce a similar pattern of HIPV emission in host plants to that induced by herbivores. This occurs when plants are exposed to synthetic elicitor compounds such as methyl salicylate (Park *et al.*, 2007), methyl jasmonate (Wang and Zheng, 2005; Bi *et al.*, 2007), *cis*-Jasmone (Birkett *et al.*, 2000; Bruce *et al.*, 2008; Sobhy *et al.*, 2020), Prohydrojasmon (Mandour *et al.*, 2013; Uefune, Ozawa and Takabayashi, 2014; Yoshida *et al.*, 2021), benzothiazole (Wendehenne *et al.*, 1998; López-Gresa *et al.*, 2019), ß-aminobutyric acid (Jakab *et al.*, 2001; Cohen, 2002; Balmer *et al.*, 2015), laminarin (Esnault *et al.*, 2005) and chitosan (Vasyukova *et al.*, 2001; Li *et al.*, 2013;

Zhu *et al.*, 2021). These compounds play an important role in inducing the plant defence system when a plant is exposed to them (Holopainen *et al.*, 2009). These compounds are known as plant defense elicitors. Defence elicitor compounds induce direct and indirect defence against pests by activating the appropriate genes and signalling pathways (Bektas and Eulgem, 2015). Activation of defence related genes and pathways induces synthesis of defensive structures and compounds that affect pest population directly and/or indirectly (Mello and Silva-Filho, 2002; War *et al.*, 2012). Most plants treated with defence elicitors showed elevated levels of volatile emission (Obara, Hasegawa and Kodama, 2002; Lou *et al.*, 2005; Ballhorn, Kautz and Schädler, 2013; Sobhy *et al.*, 2017). *cis*-Jasmone is one of these defence elicitors that induces defence responses in a number of plants; Arabidopsis (Bruce *et al.*, 2008), wheat (Bruce *et al.*, 2003), potato (Sobhy *et al.*, 2017), tomato (Disi *et al.*, 2017), tobacco (Sun *et al.*, 2019), soybean (Moraes *et al.*, 2009; Egger and Koschier, 2014; Bayram and Tonğa, 2018), cotton (Hegde *et al.*, 2012), sweet pepper (Sarah Y. Dewhirst *et al.*, 2012), barley (Delaney *et al.*, 2013) and maize (Oluwafemi *et al.*, 2013) against several pests (*Myzus persicae*, *Sitobion avenae*, *Macrosiphum euphorbiae*, *aphis gossypii*). CJ treatment also made the plant more attractive to biocontrol agents of the pests (Bruce *et al.*, 2008; Sobhy *et al.*, 2017).

In chapter 3 and 4, performance and behavioural responses of *M. persicae* and *D. rapae* to the odour of the CJ treated brassica lines were studied. *M. persicae* and *D. rapae* respond to plant odour by spending less and more time in treated zone of odour collected from CJ treated plants respectively indicating repulsion of aphids but attraction of parasitoids. This behavioural response of insects may be due to the presence of single volatile compound or a blend of more than one compound. Plants produce a plethora of volatile compounds and so the first step is to collect and analyse

an entrainment sample for volatile analysis to get information of the compounds present in the sample. Using coupled gas chromatography-mass spectrometry (GC-MS), it is possible to analyse and identify the compounds present in the volatile blend. This chapter provides details of work carried out for analysing the volatile compounds collected from CJ treated and untreated control brassica plants by coupled GC-MS.

5.1.1 Aims and objectives

The aim of the chapter was to identify the chemicals and compare the presence of volatile compounds in the headspace of different lines of brassica treated with CJ and blank formulation (control). To achieve this aim, GC-MS was used, GC stands for Gas Chromatography, which is used to separate the chemicals while MS stands for Mass Spectrometry which is used to identify the compounds.

5.2 Materials and methods

5.2.1 GC-MS sample preparation

Sample preparation is a critical step to analyse the chemical compounds present in the headspace of plants. For each sample, 100 µl of plant headspace sample were transferred into a sample vial (Supelco, 2 ml, PTFE/Silicone). For the safety of GC-syringe and to raise the samples' surface level, an insert was placed in the sample vial. Each sample was properly labelled to avoid any confusion or errors.

5.2.2 Gas chromatography (GC) analysis of headspace samples

Headspace samples were analysed on a 7820A GC coupled to a 5977b single quad mass selective detector (Agilent Technologies, Cheadle, UK). The GC was fitted with a non-polar HP5-MS capillary column (30 mm x 0.25 mm x 0.25 µm film thickness) coated with 5% phenyl-methylpolysiloxane (Agilent Technologies) and used hydrogen carrier gas at a constant flow rate of 1.2 ml/min. Automated injections of 1 µl were

made using a G4513A autosampler (Agilent Technologies) in splitless mode (285°C), with oven temperature programmed from 35°C to 5 min then at 10°C/min to 285°C. compounds were identified according to their mass spectrum, linear retention index relative to retention times of *n*-alkanes, and co-chromatography with authentic compounds. Dr. Islam helped me in interpreting the data.

5.2.3 Mass spectrometry (MS)

The mass spectrum was used as the main approach for the identification of compounds. In Mass spectrometry, molecules are first transformed into charged ions, after which, they are separated out on the basis of their mass and charge. The scale that records the masses of these charged ions according to their abundance is called the mass spectrum. In simple terms, the mass spectrometer has an inlet where molecules enter and are passed to the ionisation chamber where they are bombarded with a high energy electron beam to break the compound into fragment ions. This electron beam generates a positively charged molecule ion which is the consequence of removal of an electron. The positively charged molecular ion then breaks into smaller positively charged particles and each particle bear only a single positive charge. These single positive charged ions are then accelerated by an electric field and then moved to mass analyser. The mass analyser contains strong magnetic field that deflects and separates these charged ions into a curve path according to their charge and mass (Gross, 2006). The fragmentation pattern generated is the mass spectrum and it can be used to identify compounds by comparison with other mass spectra from authentic standards. The mass spectrum is characteristic of the compound.

5.4 Statistical analysis

A univariate analysis (F-test) of variances was performed to investigate whether the concentrations of individual volatile compounds differed between wild and cultivated potato plants. All univariate analyses were performed using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA, USA).

5.5 Results

5.5.1 Volatile analysis

GC-MS analysis of volatile samples collected from five cultivars of brassica found 24 detectable volatile compounds belonging to 8 functional classes (alcohols, aldehydes, aliphatic hydrocarbons, benzenoids, esters, ketones, Nitrogen-containing compounds and terpenes). CJ application induced qualitative and quantitative changes in volatile profile. CJ treated plants showed an increase in volatile emission. All five cultivars had quantitative changes in volatile profile: volatile emission was increased the most in Wesway (up to 14-fold), while the lowest increase was recorded with English Giant (2-fold) (Fig. 5.1). Table 5.1 shows the details of the compounds identified from control and treated plants. CJ treated English giant produced six main compounds; nonanal, (*E*)-3-tetradecene, dihydrojasmone, CJ, methyl isothiocyanate and benzyl nitrile while Pak choi showed a large increase in green leaf volatile (*Z*)-3-hexenyl acetate and 2-ethyl-1-hexanol production together with CJ, limonene and citronellol after treatment with CJ. Samurai was the brassica cultivar that showed a high emission of CJ itself together with a smaller increase in emission of (*Z*)-3-hexenyl acetate and 2-ethyl-1-hexanol and β-elemene. β-Elemene, (*E,E*)-α-Farnesene, 2-ethyl-1-hexanol and p-Cymen-7-ol with notable induction of methyl salicylate (MeSA) and CJ were the main compounds release by turnip rutabaga. A marked increase in emission of about 10 volatiles including CJ, 2-ethyl-1-hexanol, (*Z*)-3-hexenyl acetate, β-elemene and p-

Cymen-7-ol was recorded in Wesway. The quantitative changes in volatile emission are shown in Fig. 5.1.

Table 5.1. Emission (in ng; mean ± SE; *n* = 3) of volatiles released by *cis*-Jasmone treated (CJ) and blank formulation treated (Control) plants [1].

Plant volatile	KI	English Giant		Pak Choi		Samurai		Turnip Rutabaga		Wesway	
		Control	CJ	Control	CJ	Control	CJ	Control	CJ	Control	CJ
Alcohols											
2-ethyl-1-hexanol	1030	0.6±0.1	0.7±0.1	**0.34±0.02**	**2.17±0.3**	**0.51±0.06**	**3.35±0.4**	**0.97±0.5**	**3.61±0.5**	**0.36±0.2**	**5.92±0.9**
1-octanol	1071	ND	ND	ND	ND	**ND**	**0.31±0.08**	**ND**	**0.37±0.1**	**ND**	**0.67±0.2**
2-butyl-1-octanol	1277	0.02±0.01	0.6±0.2	**ND**	**0.59±0.2**	0.06±0.04	0.41±0.2	**0.03±0.02**	**0.52±0.1**	0.05±0.02	1.41±0.5
Aldehydes											
Nonanal	1104	**0.7±0.2**	**3.4±0.4**	ND	0.06±0.04	**0.07±0.06**	**1.15±0.2**	**0.43±0.2**	**1.61±0.3**	**0.08±0.06**	**2.62±0.6**
Decanal	1206	**0.03±0.01**	**0.17±0.01**	ND	0.17±0.06	0.05±0.04	0.34±0.1	0.22±0.1	0.41±0.02	**0.04±0.03**	**0.97±0.2**
Aliphatic hydrocarbons											
Dodecane	1200	0.06±0.05	0.13±0.03	0.03±0.01	0.07±0.05	0.04±0.03	0.17±0.09	0.05±0.04	0.18±0.07	**ND**	**0.62±0.06**
(*E*)-3-tetradecene	1385	0.4±0.2	1.5±0.2	1.16±0.1	1.34±0.6	0.18±0.07	0.92±0.6	0.13±0.1	0.51±0.2	**ND**	**3.48±0.9**
Benzenoids											
MeSA[#]	1192	ND	ND	ND	ND	ND	ND	**0.05±0.04**	1.57±0.4	ND	2.5±0.6
Benzothiazole	1229	ND	ND	ND	0.19±0.1	ND	0.77±0.3	**ND**	**0.63±0.1**	ND	0.39±0.3
Esters											
cis-3-hexenyl acetate	1005	2.3±0.3	2.9±0.5	0.43±0.3	2.81±1.2	**0.91±0.1**	**2.32±0.2**	**1.05±0.2**	**2.11±0.09**	**1.28±0.4**	**5.3±0.8**

2-ethylhexyl acetate	1129	0.17±0.01	0.22±0.01	0.08±0.03	ND	0.14±0.07	0.19±0.08	0.05±0.03	0.19±0.01	ND	ND
Ketones											
Dihydrojasmone	1369	0.10±0.04	1.09±0.6	**ND**	**0.27±0.03**	**ND**	**0.63±0.07**	ND	ND	ND	ND
cis-Jasmone	1394	**0.12±0.06**	**1.53±0.3**	**ND**	**1.76±0.5**	**ND**	**10.9±2.7**	**0.16±0.13**	**1.6±0.4**	**ND**	**11.7±2.9**
N-containing compounds											
Methyl isothiocyanate	992	**0.08±0.06**	**0.5±0.1**	0.05±0.04	0.09±0.07	**0.09±0.07**	**0.42±0.01**	ND	ND	ND	0.23±0.2
Benzyl nitrile	1144	**ND**	**1.7±0.2**	0.04±0.03	0.73±0.24	ND	ND	**ND**	**1.26±0.3**	ND	0.95±0.8
Terpenes											
D-limonene	1030	0.2±0.1	0.5±0.1	ND	1.97±1.1	0.61±0.4	0.56±0.2	0.41±0.2	1.27±0.4	ND	2.17±1.1
Eucalyptol	1032	ND	0.3±0.1	ND	ND	0.07±0.05	ND	ND	ND	ND	ND
Citronellol	1229	0.15±0.09	0.22±0.1	**ND**	**1.03±0.2**	0.11±0.08	ND	ND	ND	ND	ND
DMNT#	1116	ND	ND	ND	ND	ND	ND	**ND**	**0.79±0.2**	ND	ND
p-cymen-7-ol	1289	0.35±0.2	0.54±0.08	**0.24±0.12**	**0.85±0.02**	0.32±0.08	0.67±0.3	**0.32±0.1**	**3.31±0.5**	**0.23±0.1**	**4.67±1.2**
α-cedrene	1411	ND	ND	0.06±0.02	0.24±0.05	ND	ND	ND	ND	ND	ND
β-elemene	1391	**1.41±0.1**	**2.47±0.1**	ND	ND	**0.75±0.1**	**2.79±0.4**	**0.47±0.3**	**7.7±1.7**	**1.23±0.4**	**4.75±0.5**
β-curcumene	1514	0.18±0.02	0.38±0.01	**ND**	**0.31±0.07**	ND	ND	**ND**	**0.36±0.1**	**ND**	**0.38±0.03**
(*E*,*E*)-α-farnesene	1508	**0.05±0.04**	**0.69±0.1**	0.12±0.1	0.31±0.06	**0.03±0.02**	**1.22±0.03**	**0.68±0.1**	**4.58±0.7**	**0.18±0.1**	**0.66±0.06**

[1]Plants were treated 24 h before the start of VOCs air entrainment. Under each chemical class, VOCs are ordered in accordance with their increasing retention time in a gas chromatograph and Kovats index. Bold values indicate significant differences between treatments (*t*-test; $P < 0.05$). # [DMNT: (E)-4,8-dimethyl-1,3,7-nonatriene; MeSA: Methyl salicylate; ND: Not Detected]. VOCs were tentatively identified based on spectra, Kovats retention index and NIST 17 library matches. KI: Kovats index determined on the intermediately non polar HP5-MS column (https://webbook.nist.gov/; http://www.pherobase.com/).

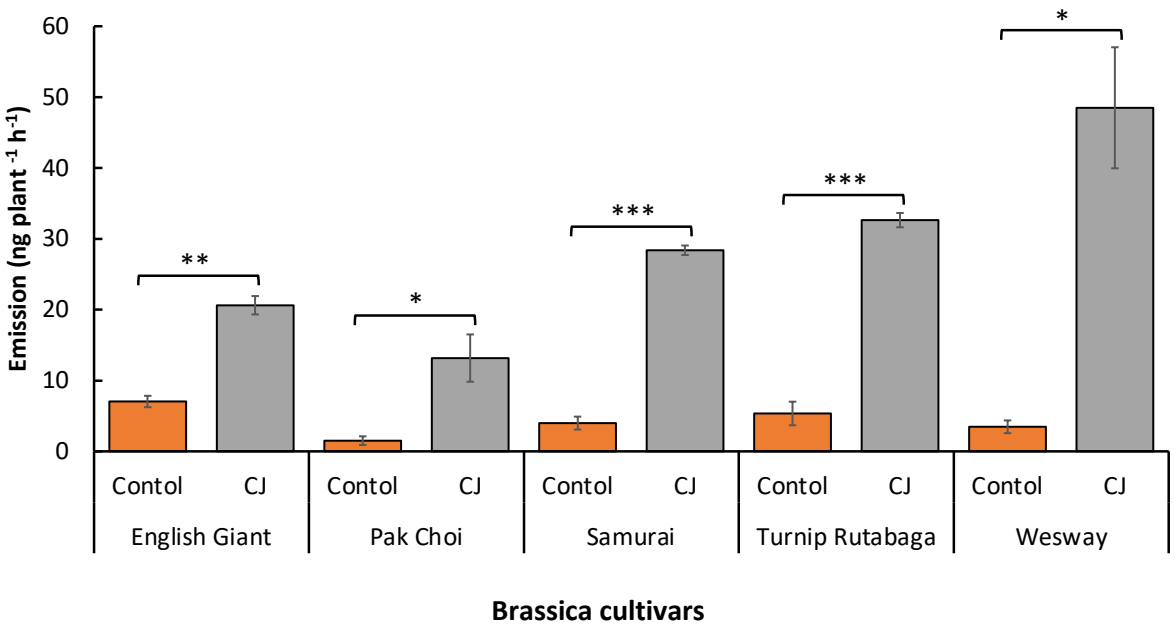

FIGURE 5.1 | Total amount (mean nanogram plant $^{-1}$ h^{-1} ± SE) of identified volatile organic compounds (VOCs) emitted from the five brassica cultivars with and without CJ treatment. Asterisks indicate statistically significant differences: *** < 0.001 and * < 0.005 (paired *t*-test).

5.6 Discussion

Chapter 3 demonstrated that *M. persicae* is repelled by the odour of CJ treated brassica lines and Chapter 4 demonstrated that *D. rapae* was attracted to CJ-induced volatiles, but the semiochemicals responsible for eliciting the insect behavioral responses were not identified. It was reported in earlier chapters that *cis*-Jasmone treatment induces defense in brassica plants that resulted in lower performance and settlement of aphids on treated plants. In contrast, *D. rapae* spent a longer time foraging and increased aphid parasitism on plants treated with CJ compared to control. Behavioral bioassays showed that aphids spent less time in the arm treated with volatiles collected from CJ treated plant. This chapter has provided identification of compounds and quantification of changes in volatile emission induced by *cis*-Jasmone treatment of the five brassica genotypes. To identify the chemicals that might be

associated with the results obtained from behavioural bioassays, we performed chemical analysis of headspace samples collected from CJ treated brassica plants and control plants using GC-MS.

Data obtained from the GC-MS analysis revealed changes in the emitted compounds in blends of treated and control plants. CJ treated plants released a richer blend of volatiles compared to control plants. There was a significant increase in the number of emitted compounds as well as the amount produced. CJ treated plants released a volatile blend rich in volatile compounds such as (*E*)-3-tetradecene, (*Z*)-3-hexenyl acetate, 2-ethyl-1-hexanol, methyl isothiocyanate, dihydrojasmone, limonene, citronellol, methyl salicylate (MeSA), β-elemene, (*E,E*)-α-Farnesene and p-Cymen-7-ol. Besides this, CJ production is also induced by CJ treatment and is coming from the plant. This is not slow release from the treatment itself because there would not be so much difference between the different cultivars if it was.

The effect of compounds identified from CJ treated plant volatiles has already been tested against herbivorous pests. Previous studies revealed that these induced volatile compounds have the potential to negatively affect plant pests, for instance; (*E*)-3-tetradecene acts as a repellent for *Tricholusia ni* and *Epilachna varivestis* (Liu, Norris and Marti, 1988; Liu, Norris and Lyne, 1989; Deepak *et al.*, 2019); methyl isothiocyanate causes mortality of *Otiorhynchus saculatus* (Borek *et al.*, 1997); dihydrojasmone derivatives affect the foraging activity of *M. persicae* (Paprocka *et al.*, 2018); limonene was effective against whiteflies, mealybugs and scale insects (Hollingsworth, 2005; Conboy *et al.*, 2019); citronellol has been found responsible for oviposition reduction of herbivorous pests leafhopper, *Monochamous alternatus* and *Delia radicum* (Saxena and Basit, 1982; Klocke, Darlington and Balandrin, 1987; Lamy *et al.*, 2017); β-elemene as a constituents of essential oil (*Tetradium glabrifolium*,

Evodia rutaecarpa and *Zanthoxylum rhoifolium*) showed strong repellency and larvicidal activity against Asian tiger mosquito (*Aedes albopticus*), *Triboilum castaneum, Lesioderma serricorne Liposcelis bostrychophila and Bemisia tabaci* (Christofoli *et al.*, 2015; Liu *et al.*, 2015; Cao *et al.*, 2018). (*E,E*)-α-Farnesene induces changes in behaviour of codling moth (Sutherland, Hutchins and Wearing, 1974; Yan *et al.*, 2003) and p-Cymen-7-ol was found behaviourally active compounds when tested against tephritid pests and spring aphid *Kaburagia rhusicola* (Aluja *et al.*, 2020; Zhu *et al.*, 2020).

Furthermore, compounds released with CJ treatments of brassica lines were also effective when tested with biocontrol agents i.e., predators and parasitoids. For example, *cis*-3-hexenyl acetate attracted endoparasitoid *Campoletis chloridae* (Sun *et al.*, 2020). Treatment of field plots with MeSA attracted predator insects from families Braconidae, Empididae, Sarcophidae and ladybirds e.g. *Coccinella septempunctata* as well as parasitoids (*Chrysopa nigricornis*) of soybean aphid (James, 2003; James and Price, 2004; Mallinger, Hogg and Gratton, 2011). In behavioural bioassay females *Aphis craccivora*, *Lobesia botrana* showed preference to p-Cymen-7-ol (Katerinopoulos *et al.*, 2005; Mitra *et al.*, 2021). Some of these compounds have been found attractive to several plant pests too that can be useful in developing new management practices based on trap cards that will help in reducing pest burden on crop plants by mass trapping (Weinzierl *et al.*, 2005; Tewari *et al.*, 2014).

The chemical analysis of plant volatiles revealed significant increases in volatile emission from CJ treated plants and provided evidence of both qualitative and quantitative changes. However, these changes in volatile emission varied with genotype. An increased number of plant volatiles emitted was observed in genotypes Wesway and Pak choi when treated with CJ: the number of identified compounds was

eight and ten in the blend released by the plants treated with blank formulation but increased to 18 for both these genotypes after CJ treatment (genotypes Wesway and Pak choi respectively). Turnip rutabaga also showed an increase in the number of emitted compounds from 15 to 19. All five brassica genotypes had enhanced qualitative and quantitative emission of volatiles after exposure to CJ. Interestingly, compounds released by plants treated with CJ were responsible for inducing behavioural changes in insects (*M. persicae* and *D. rapae*) in the four-arm olfactometer, which will be discussed in detail in the next chapter.

Several plants have been studied to test the effect of CJ on plant volatile emission such as, wheat, barley, soybean, bean, tomato. CJ treatment of wheat caused an increase in emission of (*E*)-ocimene, (*E*)-*1R,9S*-caryophyllene and 6-methyl-5-hepten-2-one (Pickett *et al.*, 2007; Moraes *et al.*, 2008). Eight VOCs were induced 100 - to 1000 fold after *cis*-Jasmone exposure of barley and wheat plants, these induced volatiles include (*Z*)-3-hexanol, (*Z*)-3-hexanol, (*Z*)-3-hexanyl acetate, (Z)-ß-ocimene, linalool, ß-caryophyllene, (*E*)- ß-farnesene, and Indole (Delaney *et al.*, 2013). CJ treatment of soybean showed increased levels of flavonoids (Da Graça *et al.*, 2016), while an increase in level of *cis*-3-hexenal, monoterpene (ß-cymene, γ-Terpinene, m-cymene and α-phellandrene) and one sesquiterpenes (germacrene-C) compounds were recorded when tomato plant treated with CJ following *Spodoptera exigua* infestation (Disi *et al.*, 2017). A significant increase in emission of (*E*)-ocimene was also recorded with *Vicia faba* (Moraes *et al.*, 2008). These induced compounds have antibiotic and behavioural effects on insect pests. Antibiotic compounds reduce the growth and development of the pest while behaviourally active compounds repel the pests (Bruce *et al.*, 2003; Pickett *et al.*, 2007; Moraes *et al.*, 2008; Delaney *et al.*, 2013; Disi*et al.*, 2017). These induced compounds have the opposite effect on natural

enemies, and help the plant in recruiting predators and parasitoids of the pests (Birkett *et al.*, 2000; Bruce *et al.*, 2008; Moraes *et al.*, 2009; Hegde *et al.*, 2012; Bayram and Tonğa, 2018). Based on the evidence from chemical analyses, and insect bioassay data, it is clear that CJ treatment induces plant defence against insect pests.

Chapter 6. BEHAVIOURAL RESPONSES OF *M. persicae* AND *D. rapae* TO SYNTHETIC ANALOGUES OF IDENTIFIED COMPOUNDS

6.1 Introduction

The previous chapter described the identification and comparison of volatile compounds released by brassica plants treated with CJ and control (blank formulation). It was observed that volatile blends released by CJ treated plants were enriched in a number of volatile compounds compared to control plants. Five cultivars of brassica, released 18 new compounds when treated with CJ. Volatile samples collected from CJ treated plants were repellent to aphids and attractive to parasitoids. The volatile blends released by plants act as a mediator between the plant and its associated community, such as; neighbouring plants, herbivores including pests and biocontrol agents. Qualitative and quantitative aspects of the volatile blend released from a plant determine the behavioural response of herbivores that can move towards the releasing plant or away from it. Any change in concentration or composition of the volatile blend can affect the plant-herbivore interaction.

Plants release a specific blend of volatile compounds, which has unique characteristics in terms of the combination and concentration of compounds, (Bruce, Wadhams and Woodcock, 2005; Baldwin, 2010; Xu and Turlings, 2018). Any change in chemical composition of the volatile blend can affect how herbivores respond (Vucetic *et al.*, 2014; Ninkovic *et al.*, 2019; Paudel Timilsena, Seidl-Adams and Tumlinson, 2020). It has been seen that whole blend of volatile can elicit different behavioural responses from insects compared to constitutional components when presented as individual components (Bruce and Pickett, 2011). The combination and concentration of compounds present in a volatile blend are vital for the perception of

an odour by the insect olfactory system (McCormick, Unsicker and Gershenzon, 2012). Herbivores use combinations of volatiles to recognise the host/non-host and infested/non-infested plant (De Moraes, Mescher and Tumlinson, 2001; McCormick, Unsicker and Gershenzon, 2012; Kigathi *et al.*, 2019). Previous studies suggested that insects showed a reduced response when exposed to individual compounds of a volatile blend that was behaviourally active when used as a whole sample. However, when applied as mixtures in combination with other compounds a stronger behavioural response was obtained (Bruce and Pickett, 2011; Clavijo Mccormick, Gershenzon and Unsicker, 2014; Dahlin *et al.*, 2018). Similarly, concentration also plays a crucial role: different behavioral responses have been reported with different concentrations of compounds (Clavijo Mccormick, Gershenzon and Unsicker, 2014; Dahlin *et al.*, 2018). Studies were conducted in this chapter to determine the exact behavioural response of aphids to individual compounds present in the volatile blend released by CJ treated brassicas. Synthetic analogues of identified compounds were used individually in the four-arm olfactometer bioassays. Behavioural responses of *M. persicae* and *D. rapae* were tested in olfactometer bioassays using standard concentrations of synthetic analogue as an odour source in one arm vs. hexane (solvent) in rest of the three arms. This chapter describes the behavioural responses of aphids and parasitoids to synthetic analogue of volatile compounds.

6.1.1 Aims and objectives

The aim of the chapter was to determine the effect of individual volatile compounds, identified from CJ-induced brassica plants, on the behaviour of *M. persicae* and *D. rapae*. To achieve this aim, a standard concentration of synthetic analogue of identified volatile compounds was used as an odour source. Olfactometer bioassay was performed to record the behavioural responses of insects to individual compounds.

6.2 Materials and methods

6.2.1 Insects

Myzus persicae aphids were collected from the well-established aphid rearing lab, Centre of Applied Entomology and Parasitology (CAEP) at Keele University. The *M. persicae* clone O was reared on Pak choi, commonly known as Chinese cabbage, in Bugdorm (46 x 46 x 46 cm; NHBS Ltd, Devon, UK) under controlled conditions (30 °C, 38 % RH, 16 h L: 8 h D photoperiod).

Diaeretiella rapae parasitoid wasps were collected from the parasitoid rearing lab, Centre of Applied Entomology and Parasitology (CAEP) at Keele University. To rear parasitoids, mummies of *D. rapae*, attached to plant leaves, were introduced to cages containing fresh Pak choi plants infested with *M. persicae* and kept under controlled condition (20 °C, 40% RH, 16 h:8 h photoperiod). Upon emergence, parasitoid adults were provided with honey solution (1:1 in water) as food. Only female parasitoids were used in experiments and they were 2–3 day old and mated. Both insects were initially obtained from Harper Adams University, UK.

6.2.2 Chemicals

Chemical standard tested individually in olfactometer bioassays were *cis*-3-hexenyl acetate (≥98%), Dodecane (≥99%), 1-octanol (≥99%), trans-ß-farnesene (90%), ß-curcumene (≥96%), 2-ethylhexyl acetate (≥99%), Benzyl nitrile (98%), methyl isothiocyanate (97%), ß-elemene (≥98%), 2-ethyl-1-hexanol, (E)-3-Tetradecene (≥97%), Benzothiazole (≥97%), Eucalyptol (≥99%),Citronellol (≥99%), *cis*-Jasmone (≥97%), 2-butyl-1-octanol, Decanal (≥98%), methyl salicylate (≥99%), p-Cymen-7-ol (>98%), Dihydrojasmone (≥95%), α-cedrene (≥97%), nonanal (≥98%), limonene (96%). Each synthetic compound was tested at concentration of (100ng/µl in hexane).

All synthetic compounds including hexane were purchased from sigma Aldrich (Gillingham, UK).

6.2.3 Test stimuli

Filter paper strips (cut to 5 x 20 mm) were treated with an aliquot (10 µl) of standard compound sample, applied using a micropipette (Drummond “microcaps”; Drummond Scientific Co., USA), and allowed to evaporate for 30 s before placing in odour source inlet.

6.2.4 Behavioural bioassays

A four arm olfactometer was used to determine the behavioural response of *M. persicae* and *D. rapae* to the volatile compounds as described in Chapter 2 (section 2.2.5). An aliquot (10 µl) of each test compound of standard concentration (100 ng/µl) was applied to a filter paper strips. Ten µl hexane was applied on filter paper strips as solvent (control). Insects were exposed to synthetic volatile compounds for 12 minutes and ten replicates were performed for each compound. The behavioural responses of *M. persicae* and *D. rapae* were tested to the synthetic analogues of compounds identified from headspace samples collected from five brassica cultivars.

6.3 Statistical analysis

Olfactometer bioassay

Data on the behavioural response of *M. persicae* and *D. rapae* were analysed by a paired t-test (one tail). In this analysis, the time spent by the tested individuals in treated and the average of three control arms in the four-arm olfactometer were compared (Bruce *et al.*, 2003).

6.4 Results

6.4.1 Behavioural response of aphids to the synthetic analogue of compounds identified in headspace of CJ treated brassica cultivars

Fig. 6.1A Shows time spent in the treated region of olfactometer compared to control when each compound was tested individually at the standard concentration of 100 ng. Out of 23 volatile compounds, six compounds elicited a significant change in aphid behaviour. Out of these six compounds, five were repellent to aphids while one acted as an attractant. The compounds that had a repellent effect were methyl isothiocyanate (p = 2.536E-05), *cis*-Jasmone (p = 0.03) , β-elemene (p = 0.006), (*E*)-3-tetradecene (p = 0.04), and methyl salicylate (p = 0.03). Among these six compounds, methyl isothiocyanate and β-elemene were the most repellent. In contrast, α-cedrene (p = 0.04) was the only compound to which aphids were attracted and spent more time in the arm treated with synthetic compound. The other 17 compounds had no significant effect on aphids.

6.4.2 Behavioural response of parasitoids to the synthetic analogue of compounds identified in headspace of CJ treated brassica cultivars

Fig. 6.1B Shows time spent by parasitoids in the treated region of olfactometer compared to control when each compound was tested individually at the standard concentration of 100 ng. Out of 23 identified volatile compounds, parasitoids spent significantly longer time in the arms treated with the following ten individual compounds; *cis*-Jasmone, citronellol, dihydrojasmone, methyl salicylate, (*E*)-3-tetradecene, β-farnesene, p-cymen-7-ol, β-elemene, methyl isothiocyanate, and benzyl nitrile. Among these ten compounds, *cis*-Jasmone (p = 0.0004), citronellol (p = 0.0007), dihydrojasmone (p = 0.001), were the compounds with highest attractant

effect on parasitoids while methyl isothiocyanate (p = 0.0258), and benzyl nitrile (p = 0.0102) had the least significant effect on *D. rapae.*

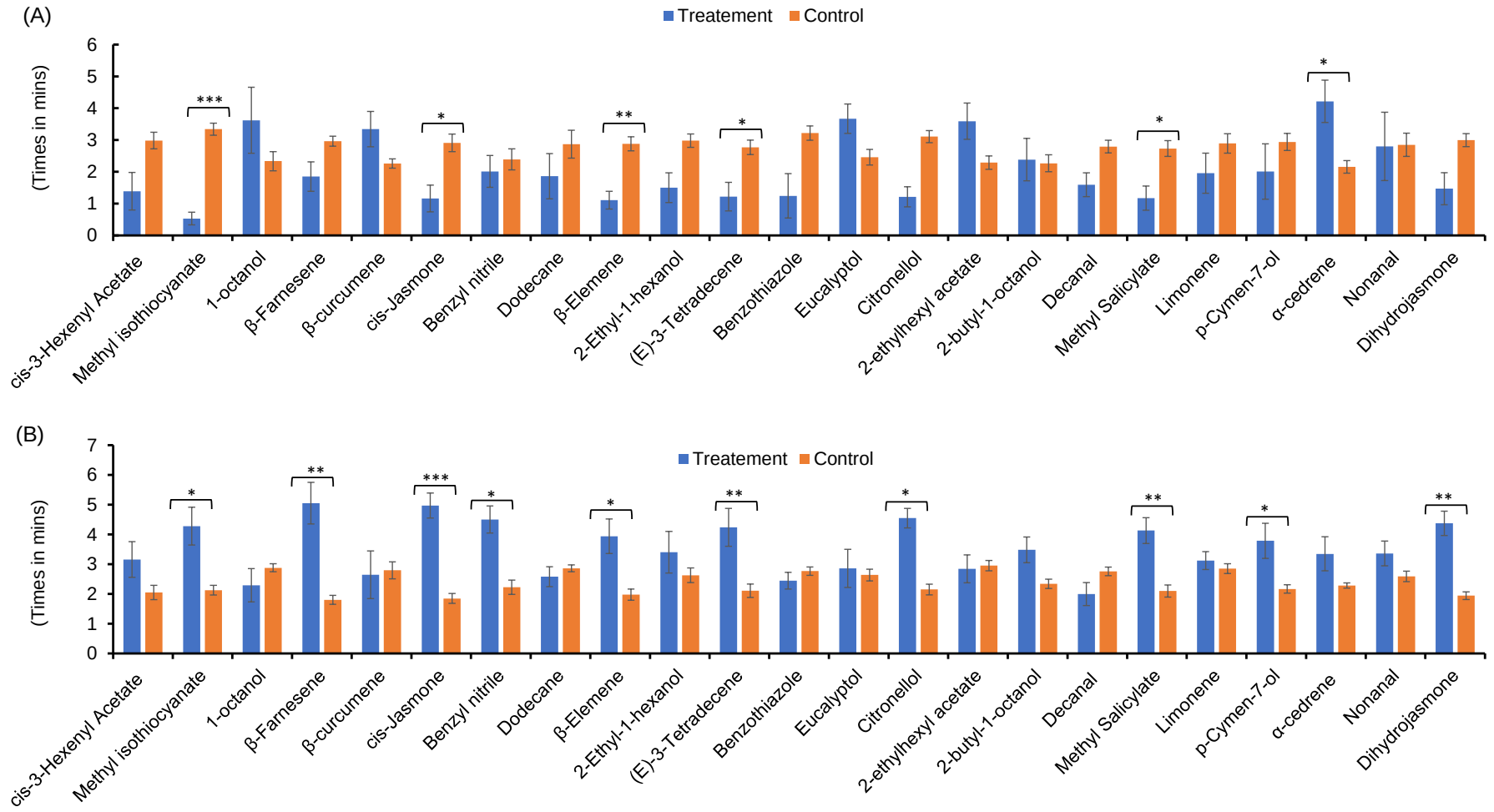

FIGURE 6.1 Behavioural responses of *M. persicae* (A) and *D. rapae* females (B) to standard compounds. Individual aphids were given 12 minutes to make a choice between (a) one arm treated with synthetic compound vs. three solvent (Hexane) arms. The shown values are mean time spent in arm ± standard error (SE) (*n* = 10). Asterisks show a denote differing levels of statistical significance: * < 0.05, ** < 0.01 and *** < 0.001 (paired *t*-test) (Treatment; synthetic compound, control; Hexane).

6.4. Discussion

The previous chapter described the identification of brassica volatiles and changes that occurred after CJ treatment. Previous studies of CJ induced volatile organic compounds (VOCs), in other plant species, showed that the induced blend contains either a higher number of VOCs or elevated levels of volatile compounds (Moraes *et al.*, 2008; Bruce *et al.*, 2008; Delaney *et al.*, 2013; Disi *et al.*, 2017; Sobhy *et al.*, 2017). The induced volatiles have deterrent effects on plant pests and alter their performance by direct and indirect defense responses (Delaney *et al.*, 2013; Oluwafemi *et al.*, 2013). Studies have shown that the change in volatile emission is responsible for the direct and indirect defense of plants to the pests (Dudareva and Pichersky, 2008; Bruce *et al.*, 2008; Holopainen *et al.*, 2009). CJ treatment of the plant induces changes in gene expression levels, that increases defence and reduces the pest population on the host plant (Moraes *et al.*, 2008). This results in the induction of chemical pathways such as those leading to benzoxazinoid and phenolic acid production which are responsible for plant resistance and allelopathic effects to pests (Guenzi and McCalla, 1966; Niemeyer, 1988; Moraes *et al.*, 2008).

The total identified compounds in treated and untreated brassica plant volatiles were 24 in number. In this chapter, we tested synthetic analogues of all identified compound on an individual basis. The compound DMNT was not tested because of unavailability of the compound. To test the effect of these compounds on insect behaviour, behavioural bioassays were performed using a four-arm olfactometer and each compound was tested in a separate bioassay. Behavioural bioassay revealed that out of 23 compounds, six compounds had a significant effect on aphid behaviour where out of six, five compounds were repellent and one was attractive. Plant volatiles benzenoids (methyl salicylate), ketones (*cis*-Jasmone), aliphatic hydrocarbons ((*E*)-3-

tetradecene), and terpenes (β-elemene, α-cedrene) induced significant changes in *M. persicae* behaviour. On the other hand, parasitoid *D. rapae* showed significant attraction to ten out of the 23 compounds. *D. rapae* showed significant preference for terpenes (Citronellol, β-elemene, (*E,E*)-α-farnesene, p-cymen-7-ol), Ketones (*cis*-Jasmone, dihydrojasmone), Nitrogen-containing compounds (methyl isothiocyanate, benzyl nitrile), benzenoids (methyl salicylate) and aliphatic compound ((E)-3-tetradecene).

The compounds identified from CJ treated plant volatiles, in the current study, have been tested against various species of herbivore pests in previous studies. These studies revealed that the induced volatiles compounds have potential to negatively affect plant pests, for instance; (*E*)-3-tetradecene acts as a repellent for *Tricholusia ni* and *Epilachna varivestis* (Liu, Norris and Marti, 1988; Liu, Norris and Lyne, 1989; Deepak *et al.*, 2019); methyl isothiocyanate causes mortality of *Otiorhynchus saculatus* (Borek *et al.*, 1997); dihydrojasmone derivatives affect the foraging activity of *M. persicae* (Paprocka *et al.*, 2018); limonene was effective against whiteflies, mealybugs and scale insects (Hollingsworth, 2005; Conboy *et al.*, 2019); citronellol has been found responsible for oviposition reduction of herbivorous pests leafhopper, *Monochamous alternatus* and *Delia radicum* (Saxena and Basit, 1982; Klocke, Darlington and Balandrin, 1987; Lamy *et al.*, 2017); β-elemene as a constituents of essential oil (*Tetradium glabrifolium*, Evodia rutaecarpa and *Zanthoxylum rhoifolium*) showed strong repellency and larvicidal activity against Asian tiger mosquito (*Aedes albopticus*), *Triboilum castaneum, Lesioderma serricorne Liposcelis bostrychophila and Bemisia tabaci* (Christofoli *et al.*, 2015; Liu *et al.*, 2015; Cao *et al.*, 2018). (*E,E*)-α-Farnesene induces changes in behaviour of codling moth (Sutherland, Hutchins and Wearing, 1974; Yan *et al.*, 2003) and p-Cymen-7-ol was found behaviourally active

compounds when tested against tephritid pests and spring aphid *Kaburagia rhusicola* (Aluja *et al.*, 2020; Zhu *et al.*, 2020).

Furthermore, the compounds released with CJ treatment of plants were also effective when tested with biocontrol agents i.e., predators and parasitoids. For example, CJ treated tobacco plants release *cis*-3-hexenyl acetate was attracted to endoparasitoid *Campoletis chloridae* (Sun *et al.*, 2020), CJ treatment of soybean plants was effective in recruiting the stink bug egg parasitoid *Telenomus podisi* (Moraes *et al.*, 2009); an abundance of predatory thrips *Aeolothrips intermedius*, *Chrysoperla carnea* was observed in the cotton field treated with CJ (Bayram and Tonğa, 2018).

The overall number of compounds that had a significant effect on insect behaviour was low compared to the overall number of identified compounds. While it is not unexpected that only a minority of compounds are bioactive, a further explanation for some compounds not showing a significant effect (repellent or attractant) could be lack of appropriate mixture of compounds as these compounds were tested individually. Interestingly compounds that had a significant effect on insects behaviour were found in the brassica plants treated with CJ only. It has been reported that in addition to quality and quantity of VOCs, the combination and mixture of VOCs is also vital for inducing a change in insect behaviour (Bruce and Pickett, 2011). Insects respond to plant volatiles with the help of a highly sensitive olfactory system that is made up of olfactory receptors neurons (ORNs) (Masson and Mustaparta, 1990; Bruce, Wadhams and Woodcock, 2005; Bruce and Pickett, 2011). Blends of plant volatiles play an important role for insects to recognise host, non-host and in making a behavioural decision that could be avoidance or attraction (Wei *et al.*, 2007; Bruce and Pickett, 2011; Cunningham, 2012). The insect olfactory system can detect a single molecule compound but it sometimes needs whole volatile blend to recognise a plant as a host

or non-host (Webster *et al.*, 2010). Individual compounds could be insufficient to induce a behavioural changes because of lack of the combination of compounds present in the whole volatile blends (Beyaert and Hilker, 2014). That could be the reason for fewer compounds responsible for significant behavioral change in *M. persicae* and *D. rapae,* because the behavioural activity induced by blends of volatiles were higher when insects were exposed to whole blends. However, the key aspect here was to characterise the bioactivity of compounds induced by *cis*-Jasmone. Compounds that repelled aphids and attracted parasitoids were successfully identified. The identification and characterisation of the *cis*-Jasmone induced compounds that repel pests and attract their natural enemies can be used in developing new pest management strategies.

Chapter 7. GENERAL DISCUSSION

Interactions with host plants play a critical role in the fitness of plant feeding insects. Herbivores with the help of their sophisticated olfactory system detect phytochemicals to allow them to recognise host plants for feeding, oviposition and shelter. Any change in the host plant can alter this interaction that may be useful in pest management. The current research has found that aphid performance and interactions with natural enemies can be altered by host plant genetics, in studies with wild potatoes, and by switching on plant defence with an elicitor, in studies with the plant defence activator *cis*-Jasmone in brassicas.

7.1 Potatoes

Performance and behavioural responses of *M. persicae* were tested on cultivated *S. tuberosum* (Desiree) and accessions of a wild potato species *S. stoloniferum* (18333, 22718, 23072).

M. persicae had lower survival and fecundity on wild potato cultivars

The current results showed that wild potato, *S. stoloniferum*, accessions were more resistant to *M. persicae* compared to *S. tuberosum* (Desiree). In a clip-cage bioassay, a significant increase in mortality and decrease in fecundity was observed on all three wild accessions compared to *S. tuberosum* (Desiree). After 48 h, all wild accessions showed a high aphid mortality, in particular 18333, 23072 and 22718 had an increase of 73%, 67% and 55% respectively compared to *S. tuberosum* Desiree. A further increase in aphid mortality was recorded on all wild accessions after 96 h and a similar pattern of resistance in cultivars was observed. In particular, wild accession 18333, 23072 and 22718 had a high mortality with an increase of 89% 82% and 57% compared to *S. tuberosum* Desiree. The level of resistance varied among wild

accessions. Accession 18333 showed the highest number of dead adults throughout both series of experiments (48 h and 96 h) and only 8% of aphids survived after 96 h.

A similar trend was observed in nymph production. Wild accessions were highly resistant and had significantly lower nymph production than cultivated potato, across both time points (48 h and 96 h). Cultivated potato *S. tuberosum* Desiree had 6.5 fold more larviposition compared to wild accessions 18333 after 48 h and this difference was increased to 15 fold after 96 h. In particular, after 48 h the larviposition on wild accessions 18333, 23072 and 22718 was 85%, 75% and 67% respectively less compared to *S. tuberosum* Desiree. A further decrease of 3% in larviposition was recorded on wild accessions after 96 h, this time 18333 had 93% less larviposition compared to *S. tuberosum* Desiree, while the highest mean larviposition was found on 22718 which was three times lower than on *S. tuberosum* Desiree.

***M. persicae* had less preference for volatiles collected from wild potato cultivars.**

In an olfactometer bioassay, *M. persicae* was repelled by volatiles collected from wild accessions 18333 and 23072 and had a significant repellent effect on *M. persicae*. In contrast, *M. persicae* spent longer time in the olfactometer arm treated with volatiles released by *S. tuberosum* Desiree, however there was no significant difference in time.

***D. rapae* showed high preference for volatiles collected from wild potato cultivars**

In contrast to *M. persicae*, the parasitoid *D. rapae* showed a preference for odour collected from wild accessions over the odour collected from *S. tuberosum* Desiree. However, accession 18333 was the only wild potato accession that had a significant attractant effect on *D. rapae (P* = 0.012) while volatile blend of *S tuberosum* desiree acted as a repellent to *D. rapae* (*p* = 0.016). Volatile samples collected from *S.*

tuberosum Desiree were repellent to *D. rapae*. Taken together, the results showed that cultivated crop potato plants are not only more susceptible to the pest than the wild relatives tested but also less attractive to the biological control agents. The volatile blend of the cultivated potato was not capable of recruiting the biological control agents that can provide an ecosystem service for plant protection in the form of natural enemies.

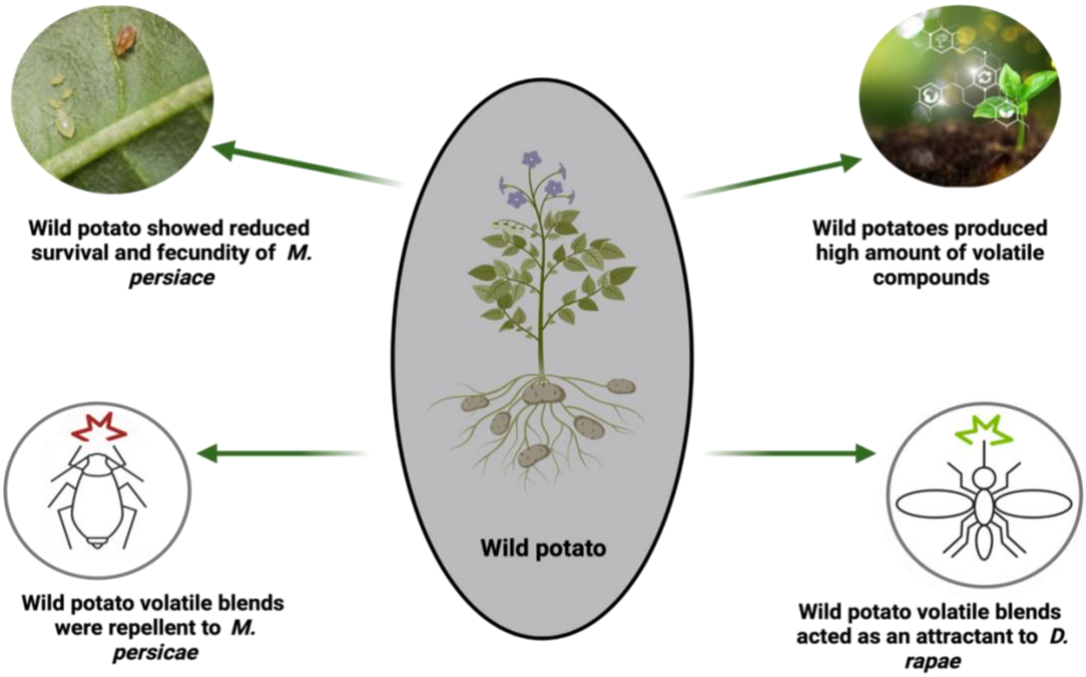

FIGURE 7.1 | A summary of *M. persicae* and parasitoid *D. rapae* performance and behavioural responses on wild accessions of *S. stoloniferum*.

Headspace sampling of wild and cultivated potato lines showed a significant difference in amounts of identified volatiles. The amount and number VOCs recorded from wild potato lines was significantly higher than in cultivated potato. Volatile blends released by wild potato lines showed an increase of volatile compounds that have a deterrent effect on insects. The VOCs identified in the wild potato headspace belong to several classes of VOCs including terpenes, ketones, benzenoids, ketones and alcohol.

Environmentally induced physical, chemical and physiological changes in wild plants may offer an increased level of resistance to pest and pathogens (Xie *et al.*, 2019). Earlier studies reported that wild plants are well adapted to a wide range of habitats, that make them tolerant and genetically more diverse compared to cultivated crop plants (Hails, 2000; HeřmanoVá, Bárta and Čurn, 2007; Fréchette *et al.*, 2010). The wild plants possess high amounts of protective secondary plant metabolites such as alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides (Wink, 1988, 2009; Anderson, Willis and Mitchell-Olds, 2011; Al-Rowaily *et al.*, 2019). The presence of such secondary metabolites in plants affect the overall growth and development of herbivores feed on them, for instance; high amount of saponins in plants reduces growth rates, inhibits enzyme activity and nutrient absorption in digestive tract and also affect the fecundity of the herbivores (Sylwia, Leszczynski and Wieslaw, 2006; Faizal and Geelen, 2013; Badenes-Perez, Gershenzon and Heckel, 2014; Al-Rowaily *et al.*, 2019).

Furthermore, high amount such secondary metabolites affect insects behaviour towards host plant, for instance, plant with high flavonoids content showed high insect mortality and reduce host plant acceptance behaviour of herbivores (Schoonhoven, 1972; Caballero *et al.*, 1986; Dakora, 1995; Kuhlmann and Müller, 2010). The low survival and fecundity of *M. persicae* in clip-cage bioassay may be due to high amount of such antibiotic and defensive secondary metabolites in the tested wild potato lines. In addition, presence of defensive compounds in host plants also affects the interaction between plant and its associated communities including parasitoids and predators (Borek *et al.*, 1997; James, 2003; Yan *et al.*, 2003; James and Price, 2004; Hollingsworth, 2005; Conboy *et al.*, 2019; Aluja *et al.*, 2020; Zhu *et al.*, 2020). The

difference in time spent by *M. persicae* and *D. rapae* is more likely due to the release of defensive volatile compounds by wild potato lines.

The current study shows that there are promising sources of direct aphid resistance in the tested wild potato accessions. Low aphid survival observed suggests that toxic phytochemicals were present in the wild *S. stoloniferum* accessions tested. Although the potential of crop wild relatives as sources of novel resistance to insect pests has been extensively studied. however, exploiting potential resistance traits that are available in wild potato ancestors against insects is understudied. This is attributed to difficulties in identifying the key secondary metabolites that determine resistance and the genes encoding their production. Thus, Identification of the bioactive compounds and genes encoding resistance will be an important topics for future studies. To retain the marketable yield and quality, it will be also be important to test if bioactive compounds are harmful to humans or if they affect the taste of the potatoes. Morphological differences could be seen between the *Solanum* species which were used in experiments; wild accessions had smaller leaves compared to Desiree. Although there could be some relation between aphids and leaf size it is unlikely to explain the high mortality observed in the current study which is more likely due the presence of toxic phytochemicals. The current research findings open up the prospect of breeding for aphid resistance by crossing cultivated and wild potatoes.

7.2 Brassicas

Earlier studies reported that CJ application induces defenses against herbivorous pest in several crop plants, however the effect of CJ on brassica crops was not studied before except in Arabidopsis which is a model plant not the crop plant. In Arabidopsis, CJ treatment induces changes that not only affect the performance of pest but was also responsible for the increase in host plant volatile emission and enhancement in

recruitment of biocontrol agents. Following the research work done on Arabidopsis plant, we decided to investigate the CJ effect on brassica crop plants as brassica crops are of great economic importance and highly affected by several pests including *M. persicae*. A series of performance and behavioural bioassays were performed. To study the effect of CJ on plant volatile emission, entrainment collection was carried out and volatile analysis was also performed.

***M. persicae* had lower survival and fecundity on CJ treated plants**

In clip-cage performance bioassay, fecundity and survival of *M. persicae* was lower on plants treated with CJ. However, the performance of *M. persicae* varied according to the genotype. All five brassica lines responded to CJ treatment and showed lower aphid survival across both series of experiments (48 h and 96 h) however, difference was not significant except 'Samurai', which showed 18% increase in aphid mortality after 96 h. In contrast, all five brassica lines treated with CJ had significantly reduced larviposition across both time points (48 h and 96 h). The number of nymphs produced on control plants was reduced by 35% on CJ plants after 48 h, which showed a potential for CJ application as an integral part of IPM in supressing the aphids population. A reduction in larviposition would slow down aphid population growth rates and this would be valuable in pest management because the high reproduction rate of aphids is one of the main reasons why they are such formidable pests. Cultivar 'Wesway' had the highest reduction of 41% while 'Samurai' had the least decrease (21%) in larviposition after 48 h of CJ. A further decrease in larviposition was observed on CJ plants after 96 h, CJ plants had 39% less nymphs compared to control plants. This change in larviposition on control and CJ treated plants varied among genotypes: This time 'Samurai' showed the highest level of resistance with a 53% reduction in

larviposition while Turnip rutabaga had least reduction of 23% in larviposition after 96 h of CJ treatment.

***M. persicae* showed low preference for CJ treated plants**

In a settlement bioassay, all five brassica lines had significantly lower aphid settlement with a mean reduction of 46% on CJ treated plants. The difference in aphid settlement on CJ treated brassica lines varied from genotype to genotype, for instance; cultivars ‘Wesway’ and ‘Turnip Rutabaga’ had the highest decrease of 56% and 51% in number of settled aphids respectively while ‘Samurai’ had a reduction of 35% in settled aphids on CJ plants. The difference in number of settled aphids on control and CJ plants showed that CJ treatment made the plants less attractive to *M. persicae*.

Olfactometer bioassay results showed a significant change in *M. persicae* behaviour towards the volatiles collected from CJ treated and control brassica lines indicating a repellent effect. In series 1 (diethyl ether vs. blank formulation) ‘Wesway’ was the only cultivar that had negative effect on aphid and *M. persicae* spent significant less time. While in series 2 (diethyl ether vs. CJ), four out of five CJ treated brassica lines were found repellent to *M. persicae.* Cultivars ‘Samurai’, ‘Pak choi’ and ‘English giant’ that had no significant effect on *M. persicae* when treated with blank formulation but after CJ treatment these cultivars showed significant repellent effect on *M. persicae*. In particular, *M. persicae* spent a longer time in the arm treated with volatiles collected from 'Samurai’ treated with blank formulation (Fig 3.6A) however this time was decreased by 84% when *M. persicae* was exposed to volatiles collected from CJ treated ‘Samurai’. Series 3 (diethyl ether vs. blank formulation vs. CJ) also showed a similar pattern of repellent effect of CJ induced volatile samples in olfactometer bioassay (Fig. 3.6C). A mean decrease of 44% in time spent was recorded when *M. persicae* exposed to CJ and blank formulation treated plant volatiles

(Fig 3.6C). In particular, *M. persicae* spent 73%, 69% and 32% less time in the arm treated volatile collected from CJ treated 'Pak choi' 'Samurai' and English Giant plants respectively compared to blank formulation treated plants.

***D. rapae* spent longer time on CJ treated plants**

The results obtained from parasitoid foraging and parasitism bioassays supported the previous literature showing that plants exposed to CJ become attractive to parasitoids. In a parasitoid foraging bioassay, the total foraging time spent by *D. rapae* on control and CJ treated plants was recorded and it was found that parasitoids spent a significant longer time on CJ treated plants for all five brassica lines. In particular, there was an increase of 5.1 fold in time spent on Pak choi, a 4.6 x increase on Turnip rutabaga, a 4.5 x increase on Wesway, a 3.9 x increase on 'Samurai', and a 2.8 x increase on English giant plants when treated with CJ. A similar trend of increase in mean number of parasitised aphids (mummies) on CJ treated plants was observed in a parasitism bioassay, in particular 'Samurai' showed a 2.21 x increase, Wesway 1.53 x increase while a 1.41 x increase was recorded on 'Pak choi'. This shows that CJ treatment can increase aphid parasitism on treated plants compared to control plants.

The series of olfactometer bioassay results showed that CJ treatment of brassica lines significantly increase the time spent by *D. rapae* in olfactometer. In series 1 (diethyl ether vs. blank formulation plant volatiles), 'English Giant' and 'Wesway' plants volatiles had repellent effect on *D. rapae* and parasitoid *D. rapae* spent significant less time in olfactometer arm treated with these volatiles (Fig 4.5A). Interestingly, cultivars 'English Giant' and 'Wesway' which had repellent effect on *D. rapae* before CJ application were found highly attractive after CJ application and time spent to volatiles samples from both cultivars was increased by 4 and 3 fold respectively. In contrast, series 2 (diethyl ether vs. CJ plant volatiles) four out five CJ treated brassica cultivars

had a positive effect (attractant) on *D. rapae* and there was an increase of 2.26 fold in mean time spent when *D. rapae* was exposed to CJ treated plant volatiles compared to solvent diethyl ether (Fig 4.5B). A similar pattern was observed in series 3 (diethyl ether vs. blank formulation vs. CJ plant volatiles), *D. rapae* showed a positive response to all five brassica lines and spent significant longer time in the arm treated with CJ plant volatiles compared to solvent diethyl ether and blank formulation treated plant volatiles, and a mean increase of 2 fold was recorded (Fig. 4.5C).

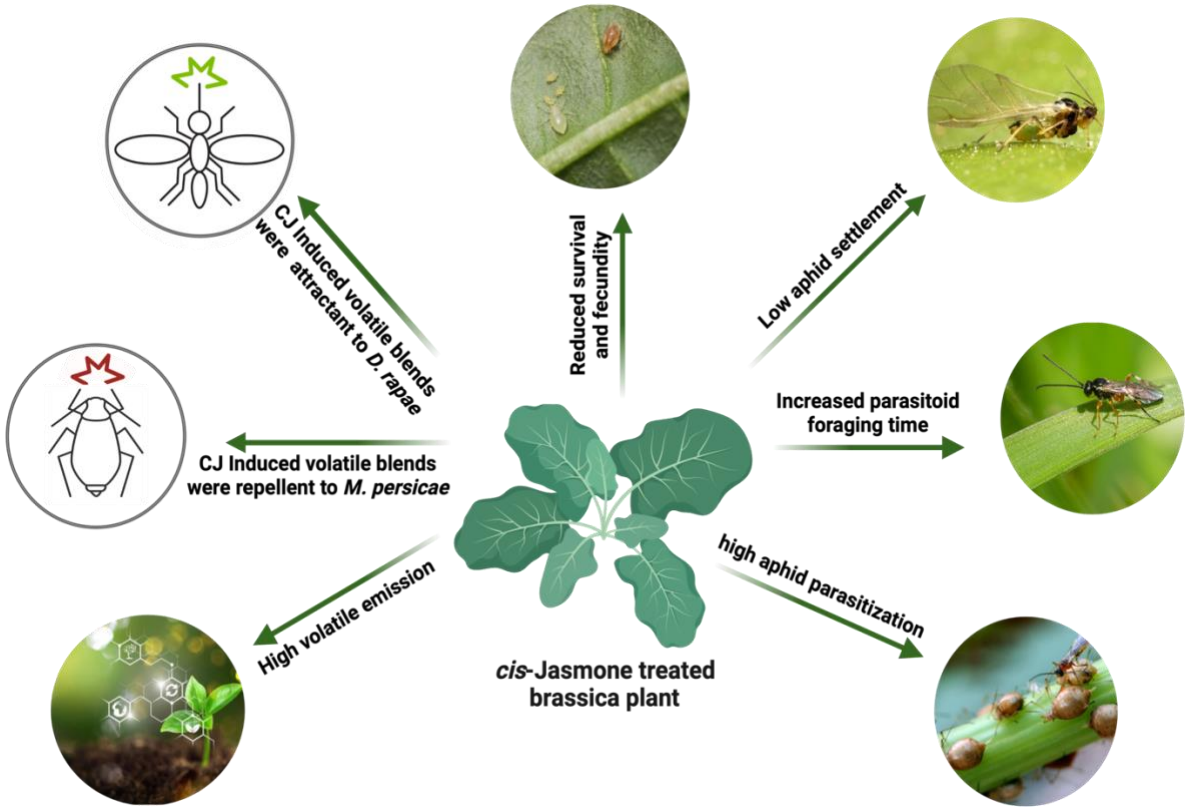

FIGURE 7.2 | A summary of induced resistance and insects (*M. persicae* and *D. rapae*) responses to CJ treated brassicas.

Prospects for exploiting natural and/or chemical plant elicitors as crop protection treatments have been considered for at least two decades (Vallad and Goodman, 2004). The elicitor *cis*-Jasmone was first proposed as a plant defence activator in 2000 (Birkett *et al.*, 2000). *cis*-Jasmone (CJ) was found to be an effective tool in activating

plant defence against sap sucking pests but had not been investigated in the brassica crops before. Previous studies suggested that exogenous application of defence elicitors activates defense pathways in plant that are responsible for synthesis and release of antibiotic and defensive compounds (Bruce *et al.*, 2003; Farag *et al.*, 2005; Lu *et al.*, 2015; Sobhy *et al.*, 2020). The activation of defence related pathways affects the pest's growth, development and preferences towards the host plants (Bruce *et al.*, 2003; Pickett *et al.*, 2007; Moraes *et al.*, 2008; Delaney *et al.*, 2013; Disi *et al.*, 2017; Sobhy *et al.*, 2020). For instance, exogenous application of methyl jasmonate modifies synthesis of terpenoids in treated plants, and an increase level of phenolic compounds has been recorded on plant treatment with salicylic acid, and benzothiadiazole (Miller *et al.*, 2005; Holopainen *et al.*, 2009; Tamaoki *et al.*, 2013). Similarly, CJ treatment of potato, wheat and Arabidopsis plant showed an increase level of volatile compounds that had negative effect on herbivorous pests (Bruce *et al.*, 2003; Bruce *et al.*, 2008; Egger, Spangl and Koschier, 2016; Sobhy *et al.*, 2017). In particular, CJ treated Arabidopsis plants showed activation of distinctive suite of genes, of which a major part evolved in plant metabolism and defense strategies (Bruce *et al.*, 2008; Matthes *et al.*, 2011).

Current study provides strong evidence of induced defence, by decrease in aphid survival and larviposition, reduction in aphids settlement on CJ plants. The reduced performance and settlement of *M. persicae* on CJ could be due to activation of defense pathways responsible for the synthesis of antibiotic and defensive compounds. CJ treated brassica lines had a significant increase in emission of volatile compounds, some of these compounds had repellent effect on *M. persicae* and could be responsible for reduction in time spent by *M. persicae* in olfactometer behavioural bioassay.

Furthermore induced defense also helps the plants to release a specific blend of volatiles that repels the pests and attract natural enemies (Bruce *et al.*, 2003; Bruce *et al.*, 2008; Sobhy, M Erb, *et al.*, 2012; Sobhy *et al.*, 2017; Sobhy, Bruce and Turlings, 2018; Sun *et al.*, 2020). Behavioural bioassay results showed that parasitoid *D. rapae* spent significant longer time when exposed to CJ induced plant volatiles. It is more likely that increase parasitoid foraging time and high number of parasitised aphid (mummies) on CJ treated plants are because of CJ induced plant volatiles. CJ treatment of Arabidopsis also showed reduced performance of pests and upregulation of certain genes.

The above findings enhanced our knowledge about CJ induced defense in brassicas against one of the most serious sap-sucking pests, the peach-potato aphid *M. persicae*. The project outcome provides strong evidences about the potential for using natural compounds to manipulate defensive strategies of the host plants. Earlier studies on other crop plants support our results showing the role of CJ treatment in inducing plant defense. The current study also provides evidences for the attraction of the natural enemy *D. rapae* to CJ treated plants, in which *D. rapae* showed an increase up to 5 fold in foraging time and a significant increase in aphid parasitisation on CJ treated brassicas. Hence, it has been proved that CJ can be used as an effective tool to protect brassica crops and enhance biological control of *M. persicae*. The current study adds to research in this area by elucidating the responses of a major aphid pest and its key parasitoid natural enemy to brassica crop plants induced with the elicitor *cis*-Jasmone. It can be used as an alternative option by farmers struggling for find control measures for this serious sucking pest, especially farmers in the Europe who have been affected by the neonicotinoid ban.

7.3 Future work

The current study opens new ways for the management of the aphid pest *M. persicae* by development of resistant varieties, by transferring resistance genes from wild relatives of crop plants, or inducing plant defense, through application of plant elicitors. These approaches are environmental friendly and perhaps more sustainable than insecticide use. Wild plants possess high genetic diversity that makes them resistant against biotic and abiotic stresses, and utilisation of such genes can help crop plants cope with stresses. On the other hand, exogenous application of plant defence elicitor not only affects the performance of the pest but also is responsible for enhancement of biological control agents. The combined effect of CJ on pest performance and parasitoid recruitment could be useful in developing a sustainable approach for the management of brassica crops against *M. persicae*.

The next step is to test these effects in the field, as the current study was limited to laboratory. The effect of CJ has been tested in the field with wheat but there is a lack of field studies for other crops. It would be interesting to know, how effective this induced defense is in field for brassica crops? Another important thing is to know the genetic mechanism working behind this elicitor induced plant defense, as a little is known about the genetics underpinning these effects. The effect of CJ treatment on plant gene expression was investigated in Arabidopsis where CJ induced genes were identified (Bruce *et al.*, 2008). It would be interesting to know what genes are upregulated on exogenous application of CJ to the crop brassicas studied here. Furthermore, it would also be important to know, how long this induced defence lasts after exposing the plant to the defence elicitor.

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