

**“Screening of Rapeseed-mustard Genotypes and
Eco-friendly Management of Mustard Aphid,
Lipaphis erysimi (Kalt.)”**

By

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**DOCTOR OF PHILOSOPHY IN AGRICULTURE
(Entomology)**

DEPARTMENT OF ENTOMOLOGY

**Dr. Rajendra Prasad Central Agricultural University
Pusa (Samastipur) - 848 125, Bihar, India**

2022

Registration No. - D/ENTO/394/2018-19

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**A THESIS SUBMITTED TO THE
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE AWARD OF THE DEGREE OF**

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(Entomology)**

DEPARTMENT OF ENTOMOLOGY

**Dr. Rajendra Prasad Central Agricultural University,
Pusa (Samastipur) – 848 125, Bihar, India**

2022

Registration No. - D/ENTO/394/2018-19

AFFECTIONATELY DEDICATED TO

My parents

(Sri. Somala Srirama Murthy & Smt. Rajulamma)

and teachers

*“Without whom, I wouldn’t have been able to complete
much of what I have done & become who I am”*

Somala Karthik

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Dated...28 / 01 / 2022

Certificate

This is to certify that the thesis entitled “**Screening of Rapeseed-mustard Genotypes and Eco-friendly Management of Mustard Aphid, *Lipaphis erysimi* (Kalt.)**” submitted in partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN ENTOMOLOGY** of the Post Graduate College of Agriculture, **Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) Bihar**, is a genuine record of **bonafide** research work carried out by **Mr. SOMALA KARTHIK (Regn. No. D/ENTO/394/2018-19)** under my guidance and supervision.

The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and helps received during the course of this investigation and sources of literatures have been duly acknowledged.


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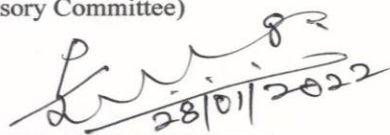
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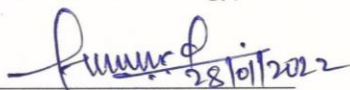
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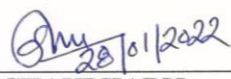
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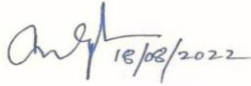
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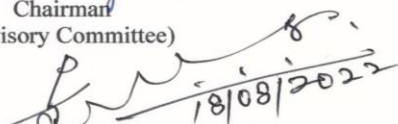
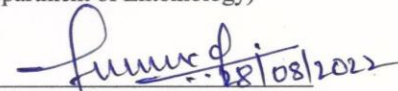
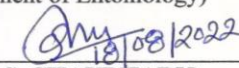
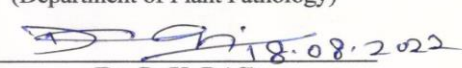
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ABSTRACT

The current investigations were conducted on “Screening of Rapeseed-mustard genotypes and eco-friendly management against mustard aphid, *Lipaphis erysimi* (Kalt.)” during 2019-2020 & 2020-21. The surveys conducted during the year 2020 across India, revealed that *Lipaphis erysimi pseudobrassicae* was present in all the 16 locations surveyed belonging to 13 states and one Union Territory at over altitude ranging from 40.42 m (Dariapur) to 980.80 m (Barapani). The selective RAPD primers viz., OPA-02, OPA-04, F2 and SSR primers viz., ApH04M, Ap-01 and ApH08M were considered as informative for genetic diversity analysis based on polymorphic band percentage (>70), higher PIC, EMR, MI and RP. DNA barcoding of 16 locations with universal primer (LCO1490- Forward and RHCO2- Reverse) employed were amplified at 657 bp of *mtCOI* gene in *Lipaphis erysimi pseudobrassicae*. The phylogenetic tree showed the clustering of all 16 locations with *L. e. pseudobrassicae* reference sequence. Among 16 locations, Jorhat of North-Eastern India was found to be highly diverged with higher branch distance (0.13) in the phylogenetic tree and higher dissimilarity of 30.0 and 31.0 % in RAPD and SSR dendrograms respectively. The association of bacterial endosymbionts with *L. e. pseudobrassicae* showed that *Buchnera* sp, a primary endosymbiont was found in all

the 16 locations. Whereas in secondary endosymbionts, *Wolbachia* sp, *Rickettsia* sp, and *Hamiltonella* sp dominated the most with 81.25 % and *Cardinium* sp liberated with 31.25 %. Among all 16 locations, Jodhpur and Ranchi had a 100 % incidence of endosymbionts and lower incidence was found in Ludhiana, Raipur, Dharwad, Alipur Bihta, Delhi, Jorhat (42.85 %). The inspections on population of alate mustard aphids and natural enemies i.e., adult coccinellids, syrphids and parasitoids in relation to weather parameters revealed their peak on 8th SMW (157.1, 6.48, 5.63, 14.62/ 4 traps) respectively and shown strong positive correlation with maximum temperature (0.445, 0.608*, 0.595*, 0.618*) and sunshine hrs (0.602*, 0.654*, 0.633*, 0.662*) respectively. Different Rapeseed-mustard genotypes were screened against mustard aphid resulting in identification of six promising genotypes among which, genotype RTM 314 had shown low AII (1.3), ADI (1.0) and ARI (1.15). The biochemical analysis also revealed the highest glucosinolates (109.87 μ mole/g) and phenols (6.51 mg/g) content in RTM 314. Further, GC-MS analysis indicated the existence of biologically active compounds like 2-(hydroxymethyl)-2-nitro-1,3-Propanediol; 5-(Methylsulfinyl)pentanenitrile and 1-Isothiocyano-4-(methylsulfinyl)butane only in RTM 314 which could be the reason for resistance against mustard aphid. Among different intercrop combinations, mustard + wheat (3:1) followed by mustard + barley (3:1) have recorded lowest aphid population (41.46, 42.79/ top 10 cm inflorescence of 10 plants) respectively and highest population of coccinellids (8.29, 7.76/ 10 plants), syrphids (6.57, 6.35/ 10 plants), parasitoids (14.01, 13.34/ 10 plants) and spiders (4.39, 4.21/ 10 plants) with superior yield (1213, 1201 kg/ha) respectively. The efficacy of various botanicals was also evaluated against mustard aphid and its natural enemies. Among the botanicals tested on mustard aphid, the % reduction of mustard aphid population over untreated control following the first and the second spray was the highest in Neem Seed Kernel Extract (NSKE) @ 5% (70.69, 87.97 %) followed by Yam Bean Seed Extract (YBSE) @ 5% (66.58, 86.06 %) and Bhang Leaf Extract (BLE) @ 5% (64.25, 84.56 %) respectively besides with higher population of natural enemies. However, based on B: C ratio, BLE @ 5% with 6.86:1 was found to be better followed by NSKE @ 5% (3.61:1) and YBSE @ 5% (3.31:1).

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CHAPTER - I



INTRODUCTION



India is well suited for growing all the major annual oilseed crops. Among them, *Brassica* species are majorly grown in the *Rabi* season that is jointly denoted as Rapeseed-mustard belongs to the family cruciferae (Singh *et al.*, 2018). It is the most essential edible oil crop next to groundnut in India and its cultivation will produce roughly about 30 % of the total oilseeds produced. India reigns over the cultivation of rapeseed-mustard globally with 2nd and 3rd rank in area and production, respectively (Anonymous, 2019). It is grown over 6.23 million hectares area with a production and productivity of 9.34 mt and 1499 kg/ha, respectively in India, while the acreage in Bihar is of 0.08 million ha with production and productivity of 0.11 and 1305 kg/ha, respectively (Anonymous, 2019).

Indian mustard, *Brassica juncea* (L.); Brown sarson, *B. rapa* (L.); Field mustard, *B. campestris* (L.); Gobhi sarson, *B. napus* (L.) and Karan rai, *B. carinata* (A. Braun) for edible oil; Wild mustard, *B. oleracea* (L.) and Black mustard, *B. nigra* (L.) for seed condiments are the important oilseed brassicas cultivated in India. Amongst these, Indian mustard is predominantly grown and contributes a sizable contribution to the production of edible oils (Jat *et al.*, 2019). This group of oilseed crops are grown in both irrigated as well as rainfed conditions and also suitable in mixed cropping.

Rapeseed-mustard crop is of multifaceted uses as edible oil, leafy vegetable, oil cake and also have immense nutritive value. In Northern part of the Indian subcontinent, it is the main source of edible oil and cannot be substituted by any other oil seed crop. The amount of saturated fatty acids is low (8 %) and that of the monounsaturated fatty acids (70 %) and polyunsaturated fatty acids (22 %) are high, rendering mustard oil to be healthy (Satyanshu, 2015). Apart from this, mustard oil has omega-3 in high amounts; it is free from trans fats, cholesterol and has a very little N-6 to N-3 ratio when compared to other edible oils. The green leaves of oilseed brassicas have K, Ca, Mg, Fe, Mn and Zn ions abundantly. *Brassica* oilseed meal has high protein content and rich in phenolic compounds. Plastic, polymers, cosmetics, lubricants, biofumigants, biofuel and pharmaceuticals can also be produced from

rapeseed-mustard. In general, mustard oil is blended with other vegetable oils to improve the profile of fatty acids specifically the omega-3 fatty acid content. Food Safety and Standards Authority of India (FSSAI) has prohibited the blending of mustard oil with any other edible oil in India from October 1, 2020 in order to facilitate manufacture and sale of pure mustard oil for domestic consumption. Therefore, the demand for mustard oil will be spiked further in the coming years. With the aim of replacing the other oils that were used for blending purpose, an additional 5 lakh tonnes of mustard oil will be required, which in turn requires about 12-15 lakh tonnes of additional mustard seed production. Despite of its tremendous advantages and anticipated demand of edible oils, the area under the cultivation of rapeseed-mustard, its production and yield in India are not stable owing to many biotic and abiotic hassles.

Amongst the numerous biotic factors that plummet the Rapeseed-mustard yield, insect pests form the main constraints. So, tackling their problem in these production systems is paramount for drawing productive yields. It has been told that insect-pests of more than 43 species are known to afflict the Indian Rapeseed-mustard, of which nearly a dozen are thought to be most important pests (Purwar *et al.*, 2004). Amongst them, the aphid complex *Lipaphis erysimi erysimi* (Kalt.), *Lipaphis erysimi pseudobrassicae* (Davis), *Brevicoryne brassicae* L., and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) cause catastrophic losses in the production of Rapeseed-mustard worldwide (Müller, 1986; Ronquist and Ahman, 1990; Sæthre *et al.*, 2011; Vidogbéna *et al.*, 2015). Nonetheless, *L. erysimi* group is predominant causing yield losses upto 9-96 % (Singh and Sharma, 2002; Rana, 2005; Parmar *et al.*, 2007).

The *Lipaphis erysimi* group (*Lipaphis erysimi erysimi* and *Lipaphis erysimi pseudobrassicae*) is a specialist aphid group feeding on Brassicas and are morphologically alike but distinctive separated forms or subspecies geographically viz., the European form, *Lipaphis erysimi erysimi* and Indian strain, *Lipaphis erysimi pseudobrassicae* (Müller, 1986; Ronquist and Ahman, 1990). Among which *L. e. pseudobrassicae* is indigenous to Eastern Asia and is considered as the utmost severe pest of oilseed brassica, exclusively in India and other sub-tropical regions of the world (Blackman and Eastop, 2000). This group is known to vector thirteen non-

persistent plant viruses including the important viruses such as cabbage black ring spot and mosaic diseases of turnip, cauliflower, radish etc (Rana, 2005).

The economic losses are caused by the louse shaped pale greenish coloured nymphs as well as the adults. They are seen on the inflorescence, pods, leaves, petioles, tender stems with large number on apical twig and suck the cell sap from these plant parts. Rigorous feeding by these aphids' results in yellowing, curling and consequent drying of leaves, leading to the development of feeble pods and underdeveloped seeds in the pods. Moreover, they secrete honeydew that interferes with photosynthesis (Mandal *et al.*, 2012). They feed on the phloem sap which is a highly instable diet principally composed of sugars and amino acids with high C: N content, poses a problem to their nutritional requirement. Furthermore, the symbiotic bacteria present in their gut are instrumental in producing essential aminoacids from the non-essential ones. *Buchnera aphidicola* is the most commonly transmitted primary symbiont present in most of the aphid species (Munson *et al.*, 1998) while some of the aphid species also bear other species of γ -proteobacteria, *i.e.*, "secondary symbionts" such as *Serratia symbiotica*, *Rickettsia*, *Hamiltonella defensa* and *Regiella insecticola* (Haynes *et al.*, 2003; Moran *et al.*, 2005).

It was found that several insecticides had been effective against mustard aphid in different Rapeseed-mustard growing states along with Bihar (Singh *et al.*, 2009). Nonetheless, the use of various chemical insecticides poses a potential threat to human health besides the environment (Hedlund *et al.*, 2020). Additionally, they are toxic to various beneficial organisms (Coccinellids, syrphids, parasitoids and pollinators such as honey bees) (Singh *et al.*, 2007; Stanley and Preetha, 2016). Therefore, the development of eco-friendly and safe methods for managing mustard aphid is necessary.

The knowledge on the biology of pest is very important because it is influenced by interaction among individuals of the species, their habitats and the surrounding environment including the climatic regime. The study of relationship between insects and its environment provides basic information about the population density and pest management measures to be undertaken for effective management with regard to the population levels (Croft and Hoyt, 1983). Monitoring of the pest population and measuring the richness of natural enemies relatively is important in any pest control programme to regulate the spray schedule of insecticides and to

reduce the problem of pesticide residues (Sarwar, 2009). Abiotic factors like temperature, relative humidity, rainfall and sunshine have a substantial influence on the population of insect pests (Agarwal and Dadheech, 1999).

Development of insect resistant cultivars offers a long-term solution and easier to adopt, which would be effective for the insect pest management programmes. The precise knowledge on resistance sources is likely the primary step towards developing of an insect resistant cultivar (Stoner and Shelton, 1988). Plant resistance results from antixenosis, antibiosis, tolerance, or a combination of these (Painter, 1951; Kogan and Ortman, 1978) also, identification of secondary metabolites which have biocidal properties against aphids could be pivotal factor for Host Plant Resistance (HPR). Therefore, identification of resistant genotypes against mustard aphid will be an economical and environmentally friendly approach to mitigate the yield losses.

Intercropping is another viable option for combating crop insect pests (Baliddawa, 1985; Rao *et al.*, 2012). It entails growing not less than two crop species on the same land at the same time. The mechanism behind pest management through intercropping is that non-host crops are unlikely to be infested with insect pests. It is a latent cultural practise for pest management because it diversifies crops in a given agro-ecosystem, reducing insect population and, as a result, their attack (Pimentel, 2005). In the intercropping systems, non-host crops that are grown alongside the main crop can generally emanate volatile chemicals that are harmful to insect pests, providing some sort of protection (Sulvai *et al.*, 2016), perhaps by attracting the natural enemies by the emanation of volatile organic chemicals or directly fending off the insect pests (Dassou and Tixier, 2016). Therefore, the use of intercropping methods and means provides scope to protect crops through natural pest management.

Botanicals are better alternatives to conventional chemical pesticides in agriculture as they are eco-friendly and safe for humans. Plants can manufacture a potpourri of secondary metabolites that have insecticidal properties (Wink and Schäfer, 2009). Crude extracts from plants, plant parts or whole plants were used for several centuries (Weinzierl, 2000). Several secondary metabolites have insecticidal, repellent, and antifeedant properties. Moreover, they act as a feeding deterrent, an ovipositional deterrent and a growth and development inhibitor for insects (Rice and Coats, 1994; Isman, 2000). As a result, using botanicals as part of an integrated insect management programme can significantly reduce the use of synthetic insecticides.

As a result of climate change and global trade of agricultural products, it is essential to ascertain the continuing changes in the genome of mustard aphid and its speciation. The precise identification of aphids is vital since there are variations amongst species, biotypes, and strains in insecticide susceptibility and the transmission of viruses (Nauen and Elbert, 2003). This provides useful perceptions of control strategies that will be most effective. Therefore, exact identification of mustard aphid species through molecular markers is necessary for their effective management strategies. Estimation of genetic variation within the species also helps to know their evolutionary history and their invasive potential. Identifying the endosymbiont diversity in mustard aphid populations could also help us to determine their association, role in their survival, thereby developing effective management strategies for the pest control.

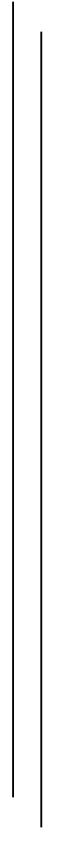
Hence, the current study was meant to comprehend the molecular characterization and genetic diversity of mustard aphid and its endosymbionts in the Indian subcontinent, monitoring of mustard aphid and its natural enemies vis-à-vis abiotic factors, to find out the interactions between different Rapeseed-mustard genotypes with mustard aphids and to develop eco-friendly methods for management of mustard aphid by using different modules such as intercropping and botanicals with the objectives given below:

1. Molecular characterization and phylogenetic diversity of *Lipaphis erysimi* and identification of its endosymbionts in India.
2. Monitoring of alate mustard aphid and natural enemies through yellow sticky traps in relation to weather parameters.
3. Screening of different Rapeseed-mustard genotypes against mustard aphid.
4. Effect of intercrops on the incidence of mustard aphid and natural enemies.
5. To study the efficacy of botanicals on mustard aphid and natural enemies under field conditions.





CHAPTER - II



REVIEW OF LITERATURE



For better understanding of the current study entitled “Screening of Rapeseed-mustard Genotypes and Eco-friendly Management of Mustard Aphid, *Lipaphis erysimi* (Kalt.)” various literatures have been reviewed under the headings listed below.

2.1 The Aphid Complex of Brassicaceae

Aphids are a horde of insect pests that restrain the productivity of Brassicaceae crops around the world. They are specialised phloem sap feeders that cause notable losses in the yield of many crops due to their ability to reproduce rapidly. It is of high economic importance owing to its high fecundity, short generation period, parthenogenetic viviparity, telescoping of generations, and distinctive feeding mechanism (Goggin, 2007). The major aphid species found on Brassica crops worldwide are *Lipaphis erysimi erysimi* (Kalt.), *Lipaphis erysimi pseudobrassicae* (Davis), *Brevicoryne brassicae* L., and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). The distinguishing characters among these aphids were discussed as follows:

Table 2.1 Diagnostic and distinguishing characters of aphid complex in Brassicaceae crops

Characters	<i>Lipaphis erysimi erysimi</i>	<i>Lipaphis erysimi pseudobrassicae</i>	<i>Brevicoryne brassicae</i>	<i>Myzus persicae</i>
Body shape	Small to medium-sized yellowish green, grey green or olive green with a faint white wax bloom and two rows of dark bands on the thorax and abdomen that merge into a single band near the abdomen's tip	Small to medium-sized yellowish green, grey green or olive green with a slight white wax bloom and two longitudinal rows of dark bands on the thorax and abdomen that merge into a single band near the abdomen's tip	Yellowish green covered with white waxy powder	Pear shaped pale yellow to green, appendages transparent
Frontal tubercles	Diverging, not distinctly exceeding vertex	Diverging, not distinctly exceeding vertex	Diverging	Pointed inwards/pronounced converging
Cornicles	Longer and lighter than cauda	Cornicles are light in colour and are noticeably longer than the cauda	Shorter than cauda	Longer than cauda, swollen near the base
Cauda	Tongue shaped	Tongue shaped	Cone shaped or broadly triangular	Pointed
Chromosome no.	2n=10	2n=9	2n=16	2n=12

2.2 Biology

Mustard aphid has been observed to be holocyclic but the species is predominantly anholocyclic in warmer climates (Blackman and Eastop, 2000). It has two modes of reproduction for producing young ones: sexual reproduction and parthenogenesis. Parthenogenesis appears to be the convention as males are very rare, only observed in the cooler months and almost exclusively viviparous females throughout the year (Kawada and Murai, 1979). It has four instars of nymphs. Depending on the season, the reproductive period ranges from 12 to 21 days. During the growing season, it has six complete and overlapping generations, and the life cycle lasts 19-31 days (Kawada and Murai, 1979).

2.3 Host range

Lipaphis erysimi is an oligophagous insect pest group mainly found on host plants of Cruciferae (Brassicaceae). It feeds on many genera and species of Cruciferae, including *Brassica*, *Barbarea*, *Capsella*, *Iberis*, *Lepidium*, *Erysimum* etc. (Blackman and Eastop, 2000). In addition to *Brassica* species, Asteraceae, Tropaeolaceae and Caryophyllaceae were also the certain host plant families in India.

2.4 Distribution

Lipaphis erysimi is globally distributed and can be found in Asia, Africa, Australia, Europe, North America, South America and New Zealand (Blackman and Eastop, 1994). In India it is found in Rajasthan, Haryana, Punjab, Himachal Pradesh, Uttar Pradesh, Bihar, West Bengal, Gujarat, Assam, Meghalaya etc.

2.5 Damage

Large aphid colonies could deform the plants and cause the leaves to curl, shrivel, and yellow by sucking sap from phloem. The *Lipaphis erysimi* is typically found and prefer on the inflorescence (Blackman and Eastop, 1994) than leaves for infestation and produce honeydew, which acts as a medium for the growth of sooty mould.

2.6 Molecular identification and phylogenetic diversity of *Lipaphis erysimi* and its endosymbionts in India

Reliable identification is one of the main constraints faced by ecologists when studying insect communities (Gotelli, 2004). Morphologically identical aphid species

exhibits distinct biological, physiological and genetic variation. Molecular markers like mitochondrial cytochrome oxidase I (*mtCOI*), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Sequence Characterized Amplified Regions (SCAR) can be used for resolving different aphid species. DNA molecular markers are superior to other markers for assessing genetic variation because they are unaffected by environmental factors, less expensive and polymorphic in nature and have high reproducibility rate (Marshall, 1995). According to Avise *et al.* (2012) variation in *mtDNA* sequence provides information on intraspecific evolution and aids in phylogenetics, systematics and genetic diversity studies (Anstead *et al.*, 2002; Ortiz-Rivas and Martinez-Torres, 2010). RAPD markers which produce different fragment lengths do not require precise DNA sequence information and exhibits higher amount of polymorphism (Guo *et al.*, 2017). SSR are tandem repeat sequences with widely distributed loci, high repeatability and used to detect the polymorphism (Balloux and Lugon-Moulin, 2002; Selkoe and Toonen, 2006). Where, 16S/23S *rRNA* genes are used for molecular identification and phylogenetic relationship studies between aphid species and their associated endosymbionts.

Flavie and Pascal (1998) used three RAPD primers (A07, A09, A11) to identify *Aphis gossypii*, collected from various plant hosts in 18 different locations in Southern France, Laos and Portugal.

Clements *et al.* (2000) used nine RAPD primers (OPA-02, OPA-07, OPA-09, OPA-12, OPA-15, OPA-16, OPA-18, OPA-19, OPA-20) for detecting genetic polymorphisms between *Myzus nicotianae* and *Myzus persicae*.

Caillaud *et al.* (2004) used 15 SSR primers (A1A09M, A1A12M, A1B04M, A1B07M, A1B08M, A1B12M, A1C04M, A1C09M, ApF08M, ApG10M, ApH04M, ApH05M, ApH08M, ApH10M, ApH12M) to identify genetic diversity between *Acyrtosiphum pisum* and *Acyrtosiphum loti*.

Hameed *et al.* (2012) studied the genetic diversity of *B. tabaci* with RAPD primers (OPA-04, OPA-09, OPA-11, OPB-13, OPC-01, OPC-04, OPC-10, OPC-15, OPO-06 and OPO-18).

Rebijith *et al.* (2012) used mitochondrial cytochrome oxidase-1 (*COI*) gene to identify both *Aphis gossypii* and *Myzus persicae*. Molecular diversity analyses

revealed that both *A. gossypii* and *M. persicae* didn't have considerable genetic variability as expected.

Kinyanjui *et al.* (2016) also used the mitochondrial cytochrome oxidase-1 (*COI*) gene and identified the morphologically indistinguishable *Aphis craccivora* and *Aphis fabae*.

Fening *et al.* (2020) else recently used the mitochondrial cytochrome oxidase-1 (*COI*) gene to identify the generalist aphid, *Myzus persicae* and specialist aphid, *Lipaphis erysimi pseudobrassicae* on cabbage in Ghana.

2.6.1 Bacterial endosymbionts associated with aphid species

As insects rely on their intracellular bacteria for proper growth and development, microbial endosymbionts are found in around half of all the insects (Kikuchi, 2009; Kuechler *et al.*, 2013). These are classified to Primary (P) and Secondary (S) endosymbionts. P-endosymbionts are obligatory and are transferred vertically (Baumann, 2005). Whereas, S-endosymbionts are facultative and are transmitted both horizontally and vertically (Chiel *et al.*, 2009). Apart from P-endosymbiont, *Buchnera*, aphids additionally have been reported to harbor six S-endosymbionts like *Arsenophonus* sp, *Wolbachia* sp, *Hamiltonella* sp, *Cardinium* sp, *Fristchea* sp and *Rickettsia* sp (Gueguen *et al.*, 2010). S-endosymbiont populations vary between different species of aphids in terms of their infection frequencies, spatial localization and geographical ranges (Gottlieb *et al.*, 2008; Skaljic *et al.*, 2010).

***Buchnera* sp:** These are P-endosymbionts in aphids that live in bacteriocytes cells and provide required nutrients like essential amino acids and carotenoids which are deficient in the host (Douglas, 1998).

***Wolbachia* sp:** These are the most extensively distributed S-endosymbionts. These are maternally transmitted and involved in host reproductive manipulation by feminizing genetic males, parthenogenesis and cytoplasmic incompatibility (Werren *et al.*, 2008). It also benefits the host by providing necessary nutrients for the nymph's entire growth and is associated with increased percentage of female progeny, longer life span and shorter juvenile development period (Xue *et al.*, 2012).

***Arsenophonus* sp:** Similar to *Hamiltonella*, *Arsenophonus* relates with the viral coat protein and aids in virus transmission (Rana *et al.*, 2012).

***Rickettsia* sp:** These are small gram-negative endosymbionts related to surge host fitness, leads to a higher proportion of females and faster developmental stages (Himler *et al.*, 2011). It also confers resistance to heat shock as well as increased insecticide susceptibility (Brumin *et al.*, 2011).

***Cardinium* sp:** These induce cytoplasmic incompatibility mostly. The co-infection of *Cardinium* and *Wolbachia* sp results in a high female to male sex ratio (White *et al.*, 2011).

***Hamiltonella* sp:** These are maternally transmitted endosymbionts residing in the bacteriocytes where they can produce amino acids and cofactors (Rao *et al.*, 2012). It is linked to increased fitness and virus transmission by encoding GroEL proteins with begomovirus coat proteins (Morin *et al.*, 1999).

***Spiroplasma* sp:** *Spiroplasma* sp is mutualistic symbionts found in *Drosophila* and aphids that protect the host from parasitic wasps (Xie *et al.*, 2010).

Haynes *et al.* (2003) have shown the existence of Proteobacterium, *Buchnera* and accessory bacteria like *Spiroplasma*, *Rickettsia* using 16S *rRNA* gene in *Acyrtosiphon pisum* and *Aphis fabae*.

Jousselin *et al.* (2013) identified *Arsenophonus* strains in aphids of genus *Aphis* using 16S *rRNA* gene.

Gauthier *et al.* (2015) revealed the existence of *Buchnera aphidicola*, *Wolbachia*, *Erwinia*, *Pantoea*, *Propionibacterium* and *Ralstonia* in pea aphid using 16S *rRNA* gene.

Jousselin *et al.* (2016) confirmed the presence of *Serratia symbiotica* and *Buchnera aphidicola* in *Cinara* (Aphididae: Lachninae) species, using 16S *rRNA* gene sequencing.

Fortuna (2019) confirmed the incidence of *Buchnera aphidicola* and *Spiroplasma* in *Lipaphis pseudobrassicae* using 16S *rRNA* gene.

Liu *et al.* (2021) identified the presence of *Buchnera*, *Pectobacterium* and *Wolbachia* associated with social aphid, *Pseudoregma bambucicola* using 16S *rRNA* gene.

Xu *et al.* (2021) identified the *Buchnera aphidicola*, *Serratia*, *Arsenophonus* and *Wolbachia* using 16S *rRNA* gene in hormaphidine aphids.

2.7 Monitoring of alate mustard aphids and natural enemies through yellow sticky traps in relation to weather parameters

Several weather factors influence aphid population growth (Rashid *et al.*, 2009), and they seem to be influenced easily by the unfavourable weather conditions and at worst they are likely killed by these unsuitable abiotic phenomena (Jones, 1979). Extreme heat exterminates the symbiotic bacteria up on which some aphids rely on, rendering the aphids infertile (Hughes, 1963). The timing of the spring migration is heavily influenced by winter temperatures and the resulting aphid mortality (Bale *et al.*, 1988). It is clear that temperatures above 30°C prolong development, increase immature stage mortality, diminish adult longevity, and reduce fertility (Kersting *et al.*, 1999). Plant growth stage and temperature exhibits a significant interaction on reproductive life span, total life span, and production of nymphs per female of wheat aphid (Girma *et al.*, 1990). Innate capacity for increase of *M. persicae* is generally higher at temperatures between 15°C and 25°C because of more rapid development and earlier attainment of the maximum fecundity rate. Temperature also influences the innate capacity for increase by modifying developmental time, survival rate, and fecundity rate (Barlow, 1962). As temperature increases fecundity, generation time and life expectancy decreases, nymphs exposed to 35°C failed to complete their development (McCornack *et al.*, 2004).

Kumar *et al.* (2000) observed that mustard aphids positively correlated with temperature and negatively correlated with relative humidity.

Rana *et al.* (2001) found lower temperatures (less than 7.8°C) and high relative humidity (above 92 %) have a negative impact on the mustard aphid population.

Jat *et al.* (2006) observed mustard aphid in 3rd SW (48 aphids/5 plants), which augmented progressively and spiked (295 aphids/ 5 plant) in the 7th SW and disappeared completely after the 11th SW.

Ansari *et al.* (2007) witnessed the arrival of mustard aphid in the 1st fortnight of January with peak population on 10th February. Higher and average temperatures had a positive correlation with the aphid population, while the lower temperatures and relative humidity had a negatively non-significant effect.

Debjani and Akhtar (2008) observed that population of mustard aphids are positively correlated with temperature and negatively correlated with the relative humidity.

Hasan and Singh (2011) observed the appearance of mustard aphid in 2nd SW during 2007-2008 and 2008-2009 crop seasons with peak population (207.22 and 262.89 aphids/10 cm twig) in 5th SW and lowest (2.56, 0.44 aphids/10 cm twig) in 6th SW respectively.

Venkateswarlu *et al.* (2011) observed the highest population of mustard aphid (169.9 aphids /plant) during 10th SW and a significant positive correlation with maximum temperature ($r = 0.582^*$), minimum temperature ($r = 0.567^*$) and sunshine hours ($r = 0.728^*$) while, significant negative correlation ($r = -0.254^*$) with relative humidity.

Achintya and Debjani (2012) observed that population of mustard aphid had a positive significant correlation with temperatures and bright sunshine hours. Whereas, *Diaretiella rapae* had positive correlation with temperatures and bright sunshine hours.

Singh and Lal (2012) observed that the catches of alate aphids were maximum @186.00 /trap and 280.00/trap during the 8th SW in both the years. The catches of alate mustard aphids positively correlated with temperature, rainfall and bright sunshine hours. However, humidity showed negative correlation with alate aphid catches.

Malik and Sachan (2013) revealed that mustard aphid commenced in the 51st SW and peaked in 8th SW and showed significant positive correlation with maximum temperature, non-significant positive correlation with minimum temperature and non-significant negative correlation with relative humidity.

Kumar (2015) has made an attempt to study the association of alate and apterous mustard aphid population on the yellow sticky traps and on host plants during 2008-09 to 2010-11 crop seasons. He observed the alate aphids on yellow sticky traps as early as 43rd SW with two peaks i.e., 34.6 and 94.93 aphids/trap on 46th SW and 9th SW respectively. Whereas, the apterous aphids first surfaced on the crop plants in the 5th SW, and peaked to 48.00 aphids/plant on 8th SW and almost coincided with the peak trap catches of alate aphids on 9th SW.

Sundar *et al.* (2015) observed the maximum activity of winged aphid during February month and significantly positively correlated with temperature and bright sunshine hours but correlated negatively with both relative humidity and rainfall.

Harjindra *et al.* (2017) observed the initial appearance of mustard aphid in 5th SW of 2014 and 2015 and gradually build-up of population reaching to the peak in 9th and 8th SW of 2014 and 2015 respectively. The population was non-significantly negatively correlated with maximum and minimum temperatures and significantly negatively correlated with relative humidity. The activity of coccinellids too began in 5th SW and had a significant positive correlation with aphid population.

Kalasariya and Parmar (2018) observed the aphid incidence in 3rd week of December in both the years 2012-13, 2013-14 and reached first peak in the 3rd week of February and exhibited the significant negative correlation with minimum temperature ($r = -0.611^*$) and relative humidity in the evening ($r = -0.518^*$) during 2012-13. While in 2013-14, the population of aphid attained during last week of February and displayed significant positive correlation ($r = 0.502^*$) with minimum temperature.

Pal *et al.* (2018) observed the population of mustard aphid on yellow sticky traps from 45th to 14th SW, with peak population (712.4 ± 16.4 aphids/trap) in 7th SW.

Sahoo and Saha (2018) observed the first appearance of alate aphids in 50th SW, then progressively increased and declined after 10th SW. Maximum alate mustard aphids (305-457 alate aphids/trap) was trapped in 8th and 9th SW (February). Among weather factors, alate aphids had a significant positive correlation ($r = 0.698^*$) with maximum temperature.

Kashyap *et al.* (2018) found mustard aphid incidence from the 51st SW with peak infestation in 7th SW. While lady bird beetles and syrphids found on 52nd to 10th SW, whereas, parasitoids were noticed on 2nd SW to 10th SW.

Meena *et al.* (2019) observed alate mustard aphid population from 43rd to 12th SW during 2014-15 and 2015-16 with peak (145.7, 344.4 aphids/trap) in 9th SW respectively and disappeared from 12th SW.

Zia and Haseeb (2019) have observed the initial appearance of mustard aphid from 3rd SW and lasted up to 11th SW. They revealed that population reaches its peak

in 7th SW and positively correlated (0.431) with maximum temperature and significantly negatively correlated (-0.718*) with maximum relative humidity.

Soni *et al.* (2021) studies the effect of abiotic factors on the population growth of mustard aphid complex and its associated natural enemies (coccinellids, syrphids, parasitoids). According to the correlation coefficients, the temperature favored the growth of aphid populations and their natural enemies.

2.8 Screening of different Rapeseed-mustard genotypes against mustard aphid

The evaluation of crop germplasm for insect resistance has provided a renewed impetus for the identification and use of Host Plant Resistance (HPR) as an integral component of pest management around the world. The identification of crop cultivars with insect pest resistance/tolerance has enormous prospective for integrated pest management, particularly in developing countries where subsistence farming is the norm (Sharma *et al.*, 1999). From the perspective of the farmer, the usage of resistant genotypes is a simple, inexpensive, most acceptable and quintessential method of addressing pest problems (Ghugal and Shrivastava, 2015).

Brassica resistant variety breeding programme targets mustard aphid resistance to extract and exploit the best source of resistance in this genetically diverse species (Rohilla *et al.*, 1993). However, there is no high-level resistant source that can be used to develop aphid resistant varieties (Sachan and Purwar, 2007; Kumar and Sangha, 2013). Mustard aphid infestation has been so devastating to India's rapeseed-mustard growing regions that no germplasm is free of it (Arunachalam and Katiyar, 1990). Extensive screening of *Brassica* germplasm in India was futile in identifying any effective source of resistance to mustard aphid (Dhillon *et al.*, 1992). In India, breeding for resistance to this dreadly pest is far from accomplished, as aphids exceed the established threshold levels on crops each year (Chattopadhyay *et al.*, 2005). Antibiosis primarily affects mustard aphids by reducing fecundity and increasing mortality (Dogimont *et al.*, 2010). These antibiotic mechanisms involve the production of proteins and secondary metabolites in response to aphid attacks, which can alter aphid physiology, resulting in increased development duration, a lower survival percentage, and a slower growth rate (Goggin, 2007). An in-depth understanding of host-plant interactions, including metabolic fluxes underlying plant resistance and mechanisms used by aphids to escape plant defences,

is important for advancing the breeding resistant variety programme of cultivated *Brassica*.

Raj *et al.* (1996) assessed the resistance of 10 germplasms of three cruciferous oilseed crops (*Brassica juncea*, *B. napus* and *B. campestris*) against aphid complex. The results revealed that germplasms (P-19, P-17, P-24, Varuna, Emma and white glossy) were comparatively resistant, whereas BSH-1 was highly susceptible to aphid infestation.

Malviya and Lal (2000) screened 78 *Brassica* germplasms against mustard aphid. Amongst, 15 germplasms (DIRA-337, DLSC-1, DLSC-2, BIO-464, BIO-902, KM-999, Kranti, PCC-2, RLM-198, RSK-84, RW-2-2, Purple Mutant, PSR-30, RH-7846, TKG-5 and SKM-9320) were observed as promising germplasms against aphid infestation.

Sharma and Bhadouria (2000) studied response of 53 *Brassica* entries against *L. erysimi* and recorded mean Aphid Infestation Index (AII) at 50 % flowering and pod formation stages. Results indicated that, none of the entries was found resistant under heavy infestation of aphid with high range of Aphid Infestation Index (1.5 to 5.0 AII). However, 15 entries (JMM-927, JMM-915, RH 7846, RH-9501, RJ-14, SEJ-2, GSH-1, PSR-22, RAURD-94-02, RAURD-95-01, SKK-92-13, BIO-902, T-27, PCR-9 and ISN-129) with 1.0 to 2.0 AII were showed resistance against mustard aphid.

Rangrez *et al.* (2004) observed the minimum aphid population (30.6/ top 10 cm inflorescence) on KS-104 while maximum (97.3) on yellow sarson (YSK-151) followed by BSH-1 (94.34).

Ali and Rizvi (2011) screened sixty-five genotypes against mustard aphid and observed minimum aphid population in Kranti, Agrani, Pusa Swarnim, Maya, MYSL-203, PCR-7 and Pusa NDYS-2 genotypes and categorized them into highly resistant.

Chauhan *et al.* (2011) revealed that genotypes T 6342, Glossy B 85, RH 7846, RH 7847 have shown resistance to mustard aphid.

Ghadage (2013) tested different mustard genotypes for aphid resistance and found that SKM-0518 showed minimal susceptibility (1.49 aphid index/plant), while Bio-902 and Kranti had 1.54 and 1.59 aphid index/plant, respectively.

Singh *et al.* (2014) screened various wild crucifers against mustard aphid and have observed that *B. fruticulosa*, *B. spinescens* and *Lepidium sativum* were resistant to mustard aphid with aphid infestation index (AII) ≤ 1 .

Bhati *et al.* (2015) reported that genotypes, GSC-6 and T-27 have performed well against aphid infestation in comparison to the BSH-1 and YST-151.

Mukherjee *et al.* (2017) screened 66 Rapeseed-mustard genotypes for their reaction against mustard aphid at Dholi. They have observed minimum AII (1.2) in the genotypes (Bhawani, DRMR 1165-40, CS 700-3-3-6, PDZ 4, DRMRIJ 13-38, RGN 337 and Varuna) and categorized as resistant.

Maurya *et al.* (2018) screened twenty Rapeseed-mustard genotypes against mustard aphid and have observed that RLM-619 and Pusa Jaganath are highly susceptible and resistant to mustard aphid respectively.

Sreedhar *et al.* (2019) conducted an experiment to evaluate the Rapeseed-mustard genotypes against mustard aphid by the means of aphid infestation index (AII). They have observed the lowest AII (1.0) in NPJ-194 and deliberated it as highly resistant genotype.

Sinha *et al.* (2021) screened forty five Rapeseed-mustard genotypes against mustard aphid and have recorded lowest mean AII values in Durgamani (0.23), RP-9 (0.27), Aravali (0.28), RVM-2 (0.29) and RH-406 (0.31) and characterized as resistant. Whereas, genotypes (DRMRIJ-31, RVM-1, JTC-1, GSL-1, Kranti, GSC-7, RH-749, RGN73) were categorized as abstemiously resistant.

2.8.1 Biochemical analysis of Rapeseed-mustard genotypes against mustard aphid

Plants have evolved various biochemical compounds to protect themselves from insect damage over the course of evolution. Constitutive, inducible, induced, direct, and indirect defences are examples of common plant defence mechanisms. These various biochemicals are present in plants as constitutive defences, but they are considered induced defences when their levels rise in the presence of insects (Dicke and Hilker, 2003). Plant secondary metabolites have a wide range of chemical structures that are important for interacting with other organisms but are not required for plant growth or development. Plant volatiles called phytochemicals are primarily involved in plummeting herbivore attacks that attract pollinators and potential for human health (Taveira *et al.*, 2009).

Herbivorous insects can develop strategies to deal with plant allelochemicals, such as simple avoidance of plants with high level of toxic allelochemicals, as well as changes in physiological processes and metabolic defence mechanisms. *Brassica* plants make a number of different defence compounds, some of which are discussed below:

Singh *et al.* (2000) studied the biochemical characters of nine *Brassica juncea* genotypes against resistance to mustard aphid. They have revealed that correlation coefficient of the mustard aphid population and glucosinolate content had a significant antagonistic relationship, whereas total sugar content shown significant association with mustard aphid colony.

Singh *et al.* (2003) evaluated the relationship between the buildup of population of *L. erysimi* and glucosinolates of different *Brassica* spp. and reported negative correlation between the glucosinolates content and aphid population. The highest glucosinolates content was found in seeds (158.24 $\mu\text{moles/g}$) and leaves (47.52 $\mu\text{moles/g}$) of *B. carinata* whereas lowest in seeds of *B. napus* (76.67 $\mu\text{moles/g}$) and leaves of *B. campestris* cv. YST-151 (20.12 $\mu\text{moles/g}$).

Khattab (2007) found that the content of total phenols in infested leaves (8.12 mg/g) was lower than in healthy leaves (7.10 mg/g) which is accompanied by high activities of both phenol oxidases and peroxidases.

Kular *et al.* (2008) witnessed the lowest aphid population in T-27 (*Eruca sativa*), and highest in purple mutant (*B. juncea*). Purple mutant had the lowest phenol concentration when compared to T-27. The aphid population decreased as the phenolic content in the inflorescence increased, and a similar relationship was observed between the aphid population and the glucosinolate content in the inflorescence and aphid populations.

Dinesh and Singh (2012) observed a greater glucosinolate content in genotype T-27 (105.66 $\mu\text{ mole/g}$), lower in BSH-1 (47.04 $\mu\text{ mole/g}$), whereas phenol content ranged from 1.46 and 2.71 % in BSH-1 and T-27, respectively. The coefficient of correlation of glucosinolate, phenol content and aphid infestation was significant and negative. Contrarily, total sugar content varied from 6.87 % (T-27) to 10.50 % (BSH-1) and showed a significant positive correlation with aphid infestation.

Kumar and Singh (2012) reported that the total phenols content varied from 1.46 % in BSH-1 to 2.71 % in T-27 with significantly negative correlation between phenolic content and aphid infestation.

Kumar and Sangha (2013) revealed that higher levels of glucosinolates and total phenols had a converse relationship with aphid infestation.

Kumar *et al.* (2017) studied the resistance to infestation by mustard aphids and its relationship to some biochemical traits in ten selected varieties (RH-9501, RK-9501, Purple mutant (P-Mutant), RH-7846, JMM-927, Hyola-401, DLSC2, T-27, BSH-1 and YST-151. They have observed that T-27 and DLSC-2 genotypes were less susceptible to aphid infestation and negatively correlated with total glucosinolate and phenol contents, whereas positively correlated with total soluble sugar content.

Kumar *et al.* (2020) ascertained the biochemical basis of resistance to mustard aphid in ten *Brassica* genotypes during 2016-17 at Hisar. They have observed the genotypes (BSH-1, YSH-401) with very low phenol content (1.51 and 1.60 %, respectively). The AII is significantly negatively correlated ($r = -0.920^{**}$) with phenol content and significantly positively correlated with total sugar content ($r = 0.980^{**}$).

Table 2.2: Properties of bioactive compounds identified in *Brassica* and other plants through GC-MS

Sl.No	Compound Name	Properties	References
1	9-Octadecenoic acid (Z)-	Oviposition deterring and repellent	Anderson <i>et al.</i> (1993); Guoqing <i>et al.</i> (2001)
2	9-Octadecenoic acid (Z)-, methyl ester	Antiinflammatory, antiandrogenic, insectifuge	Dr. Duke's Phytochemical and Ethnobotanical Databases
3	9,12-Octadecadienoic acid, methyl ester	Hepatoprotective, antiinflammatory, nematocide, insectifuge, alpha reductase inhibitor, antiandrogenic	Arora and Kumar (2017)
4	9,12-Octadecadienoyl chloride, (Z,Z)	Natural antagonist to AT1 receptors	Jananie <i>et al.</i> (2011)
5	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Anti-arthritic, antiandrogenic, insectifuge	Jananie <i>et al.</i> (2011)

Cont....

6	γ -Sitosterol	Repellent	Gaddagutti <i>et al.</i> (2016)
7	α -Tocopherol	Anti-oxidant, anti-inflammatory	Arora and Kumar (2017)
8	Erucic acid	Anti-nutritional	Rogers (1978)
9	Guanosine	Cytotoxic against T cells lines, antiviral against vero cells infected with HSV-1	Arora and Kumar (2017)
10	Hexadecanoic acid	Antioxidant and anti-inflammatory and oviposition deterring	Guoqing (2001); Patel <i>et al.</i> (2017)
11	Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl) ethyl ester	Hemolytic, pesticide, antioxidant	Tyagi and Agrawal (2017)
12	Hexadecanoic acid, ethyl ester	Antioxidant, pesticide and antimicrobial	Rukshana <i>et al.</i> (2017)
13	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic, pesticide	Zayed <i>et al.</i> (2014)
14	Octanoic acid, 2-dimethylaminoethyl ester	Candidicide, fungicide and pesticide	Dr. Duke's Phytochemical and Ethnobotanical Databases
15	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Hypocholesterolemic, antiarthritic	Dr. Duke's Phytochemical and Ethnobotanical Databases
16	Phytol	Antioxidant, antinociceptive, insecticidal, repellent antimicrobial	Adjalian <i>et al.</i> (2015); Patel <i>et al.</i> (2017)
17	Stigmast-5-en-3-ol, (3.beta., 24S)-	Anti-inflammatory, antipyretic	Arora and Kumar (2017)
18	Tetradecanoic acid	Larvicidal and repellent activity	Siva kumar <i>et al.</i> (2011)
19	Allyl Isothiocyanate	Defense against herbivores	Sharma and Rai (2018)
20	Oxime-, methoxy-phenyl-	Antidotes for nerve agents, reactivate acetylcholinesterase	Sharma and Rai (2018)
21	Methyl .beta.-d-galactopyranoside	Antioxidant, antibacterial	Sharma and Rai (2018)
22	Methallyl cyanide	Biopesticide	Sharma and Rai (2018)

Cont....

23	Stigmasterol	Antimicrobial, anti-inflammatory	Dr. Duke's Phytochemical and Ethnobotanical Database
24	Pentadecanoic acid	Antimicrobial	Dr. Duke's Phytochemical and Ethnobotanical Database
25	9,12-octadecadienoic acid	Antibacterial, fungicidal activity, anticancer, anti-inflammatory, hypocholesterolemia	Kumar and Tanwar (2011)
26	11-cis-octadecenoic acid	Antimicrobial, dietary precursor of C9, T11 conjugated linoleic acid	Mujeeb <i>et al.</i> (2014)
27	(Z)-9-octadecanoic acid	Antimicrobial, fungicidal activity, α -reductase inhibitor, anti-inflammatory	Turgis <i>et al.</i> (2009)
28	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Antimicrobial, antioxidant, antitumor	Tangjitjaroenkun (2018)

2.9 Effect of intercrops on the incidence of mustard aphid and natural enemies

Most insect pests are suppressed by intercropping due to the altered crop canopies and resulting in changed microclimate (Jayaraj, 2002; Degri *et al.*, 2014). One insecticide modification is the use of an intercropping system. It is a non-chemical cultural practise that has the potential to reduce pest infestation by increasing crop diversity (Degri *et al.*, 2012). To maximise the survival and reproduction of beneficial arthropods, pollen and nectar resources, which are often scarce in modern agricultural landscapes, must be provided (Isaacs *et al.*, 2008). Plant diversity can help in providing abundant resources of pollen and nectar besides the pest control by enhancing natural enemy populations from the top down, as well as by other 'bottom-up' effects that act directly on pests (Gurr *et al.*, 2003).

Intercropping is a traditional agricultural practice used to prevent crop yield loss due to pest infestation in various geographical areas around the world. Also, increases biodiversity in the field to encourage environmentally sustainable agriculture production with low inputs of pesticide (Ghaley *et al.*, 2005) as they

provide natural pest control. The straight impact of natural enemies on agriculture herbivores (Symondson *et al.*, 2002; Tschardtke *et al.*, 2005) has received better attention over recent years as a strategy for crop protection (Fiedler *et al.*, 2008; Khan *et al.*, 2008). Ali (2002) informed higher incidence of pea aphid in pea sole crop than intercropping with wheat, Ethiopian mustard. Bedoussac *et al.* (2010) and Lopes *et al.* (2015) also perceived higher densities of pea aphid in pea sole crop than in intercropping with durum wheat.

Singh and Kothari (1997) studied the consequences of intercropping on the dynamics of the mustard aphid population. They compared aphid infestation on monocrop mustard to aphid infestation on mustard intercropped with artemisia, coriander, chamomile, fennel, and dill. It has been discovered that intercropping mustard with fennel results in significantly lower aphid infestation.

Lasker *et al.* (2004) studied the effect of intercrops (barley, wheat, pea, fennel, radish, fenugreek, spinach and coriander) on the prevalence of mustard aphid in mustard and observed the minimum aphid population incidence in mustard+ wheat followed by mustard+barley intercrop combinations.

Monika *et al.* (2005) observed the minimum aphid population in mustard intercropped with coriander, while the highest population was found in mustard grown alone.

Ali and Ansari (2008) observed the lowest incidence of aphid population in mustard intercropped with fennel, followed by mustard intercropped with coriander, wheat and chickpea.

Singh and Shankar (2010) conveyed that mustard + barley had the lowest aphid population and the highest mustard yield, followed by mustard + wheat. In terms of aphid population, lentil and radish were comparable to the sole mustard crop.

Sarwar (2011) reported that mustard intercropped with cereals supported lower aphid population and higher seed yield than monoculture.

Nassab *et al.* (2013) determined the effects of wheat-rapeseed intercropping on the population density of wheat aphid and its natural enemies. They have observed the lowest wheat aphid population density with increase of its natural enemies.

Lopes *et al.* (2016) found lowest pest populations in wheat-based intercropping systems while increasing the natural enemy diversity when compared to pure stands.

Akbar *et al.* (2017) studied the effect of different companion crops (wheat, barley, fennel, onion, garlic and maize) on aphid population in canola. They have observed the minimum aphid infestation in canola grown along with garlic, onion and fennel.

Nisar and Rizvi (2018) determined the effect of intercropping cabbage, cauliflower and mustard with combinations of fennel, onion and garlic on the incidence of aphid population. They observed the minimum aphid population in mustard + garlic followed by mustard + onion and mustard + fennel over sole mustard crop.

Reddy *et al.* (2018) intercropped mustard with wheat, onion, garlic, chickpea, cowpea and coriander. They found that mustard intercropped with coriander had the lowest aphid population.

2.10 To study the efficacy of botanicals on mustard aphid and natural enemies under field conditions

Botanical insecticides are organic and natural insecticides derived from plants, and they have naturally occurring defensive properties. Plants can synthesise a number of secondary metabolites that aren't required for growth and development but are important in pest defence (Rosenthal and Berenbaum, 1991; Wink and Schäfer, 2009). The use of secondarily produced metabolites for crop protection against insect species most likely began thousands of years ago (Thacker, 2002). For centuries, people from different cultures around the world have used crude plant extracts (Weinzierl, 2000). Because of their favourable profile for human health and the environment, they are regarded as desirable alternatives to conventional chemical pesticides in agriculture. Many secondary metabolites have antifeedant, repellent and insecticidal properties and also act as insect growth regulators and inhibit reproduction (Rice and Coats, 1994; Isman, 2000).

Several factors appear to be limiting botanicals' success at the farmer level, most remarkably regulatory barriers and the handiness of competing products (newer synthetics). Botanical insecticides are paramount suited for organic food production in developed countries, but in developing countries, they play a much larger role in food

production and postharvest protection. As a result, they quickly became a hot topic of scientific research for safe food production through environmentally friendly pest control. The crude aqueous extracts of *Azadirachta indica*, *Cannabis sativa*, *Calotropis gigantean*, *Cassia angustifolia*, *Chrysanthemum cinerariifolium*, *Derris elliptica*, *Nicotiana tabacum*, *Ageratum conyzoides*, *Parthenium hysterophorus*, *Lantana camera* etc. were reported to have insecticidal as well as repellent properties against aphids, thrips, mites and other sucking insect pests and the concentration of plant extract had an inverse relationship with repellent activity (Pluke *et al.*, 1999; Weinzierl, 2000).

Turner *et al.* (1980) reported that leaf extract of *Cannabis* works very well as pesticides, despite the fact that they contain very little cannabinoid. *Cannabis* contains over 400 different chemical compounds majorly terpenes, ketones and esters.

Kashyap *et al.* (1991); Bajpai and Sharma (1992); Ross and ElSohly (1996) reported that spraying leaf extracts of bhang on crop plants can act as an oviposition deterrents and insect repellents.

Srivastava and Guleria (2003) ascertained 34 plant extracts against mustard aphid and revealed that *Allium sativum*, *Ageratum conyzoides*, *Lantana camera*, *Cannabis sativa*, *Cymbopogon citrates*, *Pinus roxburghii* and *Ricinus communis* exhibited insecticidal activity against aphid.

Singh (2007) assessed the efficacy of different indigenous plants and their derivatives against mustard aphid. It was observed that among all the treatments oxydemeton-methyl @ 0.25 % and NSKE @ 5% were the most effective and reduced the population significantly by 64.2 and 57.9 %, respectively.

Chand and Tiwari (2010) have observed that after 24 hours of feeding with neem extract, bhang and parthenium, the larvae consumed significantly less leaf area.

Chandel *et al.* (2012) observed the highest mortality of mustard aphid (70.82 %) with NSKE followed by lagundi (69.40 %), congress grass (65.38 %), arusa (55.81 %), lantana (51.70 %) leaf extracts and untreated control (1.5 %).

Benelli *et al.* (2018) studied the activity of *Cannabis* extract against *Myzus persicae*, *Spodoptera littoralis* and *Musca domestica*. They have observed the higher toxicity of *Cannabis* extract against *M. persicae* and *M. domestica*.

Bhatta *et al.* (2019) studied the bio-efficacy of different botanicals against mustard aphid, under field conditions. They have observed the highest percentage of aphid reduction with cypermethrin (93.1 %) followed by NSKE @ 5% (88.46 %).

Singh *et al.* (2019) studied the bio-efficacy of different botanicals against mustard aphid and natural enemies. They have observed the maximum reduction of aphid population (91.6 %) over control with Imidacloprid @ 40 g a.i/ha followed by 80.9 % in NSKE @ 5%.

Singh *et al.* (2019) assessed the effect of various tuber-based bio-pesticides on mustard aphid. The results of four years revealed that among various tuber-based bio-pesticides, yam bean seed extract @ 2 and 5% evidenced most effective in reducing the aphid population and at par with chemical dimethoate after first and second spraying.

Kumar *et al.* (2020) studied the bio-efficacy of different botanicals against mustard aphid and found maximum % reduction (93.2 %) of aphid population maximum in dimethoate 30 EC @ 625 ml/ha followed by neem seed kernel extract @ 5% (71.4 %).

2.10.1 Insecticidal compounds of Yam bean seed and Bhang leaves

Li *et al.* (2009) studied the insecticidal compounds from methanol extract seeds of yam bean by Thin Layer Chromatography (TLC) to determine their toxicity against *Aphis gossypii*, *Plutella xylostella*, *Aedes albopictus* and *Herse convolvuli*. They have identified a total of fourteen compounds and their insecticidal activity against different tested insects was different. However, they have noted that the main insecticidal compound was not rotenone but 12a-hydroxyrotenone.

Yongkhamcha and Indrapichate (2012) studied the insecticidal activity of both ethanolic and water seed extracts of yam bean, mint weed and celery on *Aedes aegypti*. They have observed that, among all the extracts yam bean seed extract with ethanol was most toxic to both larvae and adults.

McPartland and Sheikh (2018) reviewed that *Cannabis* can biosynthesize about 140 terpenoids, which have insecticidal and repellent activity. They collectively constitute the plant's essential oil and are responsible for arthropod deterrence.

Ahmed *et al.* (2020) evaluated the methanol leaf extracts of *Artemisia argyi*, *Citrullus colocynthis* and *Cannabis indica* to *Brevicoryne brassicae* at laboratory

conditions and identified various insecticidal compounds through GC-MS. Following field testing, they concluded that all of the plant extracts comprises significant insecticidal properties and could be used as botanical insecticides.

Jin *et al.* (2020) profiled secondary metabolites from flowers, leaves, stem barks and roots of *Cannabis* through GC-MS, LC-MS and LC-UV-MS. They have identified a total of 47 terpenoids, 14 cannabinoids 7 flavonoids and 3 sterols.

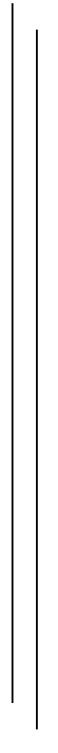
Table 2.3 Properties of bioactive compounds identified in Yam bean seed and Bhang leaf crude extract through GC-MS

Sl.No	Compound Name	Properties	References
1.	Phenylfuranocoumarin	Oviposition deterrents	Phrutivorapongkul <i>et al.</i> (2002)
2.	Pachyrrhizid	Oviposition deterrents	Phrutivorapongkul <i>et al.</i> (2002)
3.	Rotenone	Insecticidal	Yongkhamcha and Indrapichate (2012)
4.	myrcene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
5.	(E)- β -caryophyllene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
6.	α -Terpinolene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
7.	β -Sesquiphellandrene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
8.	β -Selinene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
9.	Limonene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
10.	β -Bisabolene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)
11.	α -farnesene	Insecticidal, repellent activity	McPartland and Sheikh (2018); Ahmed <i>et al.</i> (2020); Jin <i>et al.</i> (2020)





CHAPTER - III



MATERIALS AND METHODS



MATERIALS AND METHODS

To achieve the goals set forth in the current investigation entitled “Screening of Rapeseed-mustard Genotypes and Eco-friendly Management of Mustard Aphid, *Lipaphis erysimi* (Kalt.)”, systematic procedures and protocols were used. This chapter contains information on the materials and the methods used throughout the investigation.

3.1 Physiographic situations of the experimental site

The research farm at Dholi is situated at 25°59'10.7"N latitude and 85°40'51.5"E longitude in Eastern India with an altitude of 50.86 m above Mean Sea Level (MSL) in the Gandak command area. The condition of the soil varies from loamy to sandy loam. Dholi falls under agroclimatic zone of Middle Gangetic Plains. The maximum temperatures recorded in the month of April-May (hottest months) with the maximum average temperature around 38°C and in the month of January (coldest month) the minimum average temperature of 7°C.

3.2 Survey and collection of mustard aphid across India

Surveys were conducted to collect the mustard aphid samples from 14 states/Union territories *i.e.*, Bihar (Dholi, Dariapur, Alipur Bihta), Uttarakhand (Pantnagar), Punjab (Ludhiana), Rajasthan (Jodhpur), Uttar Pradesh (Jhansi), Jharkhand (Ranchi), West Bengal (Mohanpur), Chattisgarh (Raipur), Karnataka (Dharwad), Andhra Pradesh (Chintapalli), Manipur (Imphal), Meghalaya (Barapani), Delhi (IARI), Assam (Jorhat). The collected samples were preserved in 99.9 % ethanol and stored at -20°C until further studies.

3.3 Morphological, molecular characterization and phylogenetic divergence of *Lipaphis erysimi*

The mustard aphid samples collected from 16 locations were subjected to morphological identification and molecular characterization.

Table 3.1: Details of interlocation survey conducted during the year 2020

Sl.No.	Location	District	State/ UT	Agro-climatic zone	Date of Collection	Latitude	Longitude	Elevation
1.	Pantnagar	Udham Singh Nagar	Uttarakhand	Western Himalayan	31-01-2020	29°01'19.9"N	79°29'26.9"E	227.21 m
2.	Ludhiana	Ludhiana	Punjab	Trans-Gangetic Plains	25-01-2020	30°54'03.6"N	75°51'26.3"E	254.49 m
3.	Jodhpur	Jodhpur	Rajasthan	Western Dry Region	18-01-2020	26°21'07.8"N	73°02'56.5"E	236.9 m
4.	Jhansi	Jhansi	Uttar Pradesh	Central Plateau and Hills	29-01-2020	25°30'53.7"N	78°32'38.6"E	216.41 m
5.	Ranchi	Ranchi	Jharkhand	Eastern Plateau and Hills	02-02-2020	23°26'36.2"N	85°19'10.2"E	628.9 m
6.	Mohanpur	Paschim Medinipur	West Bengal	Lower Gangetic Plains	23-01-2020	22°59'26.9"N	88°27'00.6"E	40.66 m
7.	Raipur	Raipur	Chhattisgarh	Eastern Plateau and Hills	05-02-2020	21°13'58.1"N	81°42'39.8"E	291.63 m
8.	Dharwad	Dharwad	Karnataka	Southern Plateau and Hills	15-01-2020	15°29'43.8"N	74°59'13.1"E	698.41 m
9.	Chintapalli	Visakhapatnam	Andhra Pradesh	East Coast Plains and Hills	06-02-2020	17°52'40.4"N	82°20'26.4"E	839.13 m
10.	Barapani	Ri-Bhoi	Meghalaya	Eastern Himalayan	28-01-2020	25°41'07.8"N	91°54'57.7"E	980.80 m
11.	Imphal	Imphal West	Manipur	Eastern Himalayan	30-01-2020	24°48'54.3"N	93°53'24.7"E	782.14 m
12.	Dholi	Muzaffarpur	Bihar	Middle Gangetic Plains	25-01-2020	25°59'10.7"N	85°40'51.5"E	50.86 m
13.	Dariapur	Lakhisarai	Bihar	Middle Gangetic Plains	01-02-2020	25°13'01.3"N	86°04'19.7"E	40.42 m
14.	Alipur Bihta	Patna	Bihar	Middle Gangetic Plains	01-02-2020	25°27'19.1"N	85°27'43.4"E	41.11 m
15.	IARI	Delhi	Delhi	Trans Gangetic Plains	22-02-2020	28°37'56.3"N	77°09'44.9"E	232.33 m
16.	Jorhat	Jorhat	Assam	Eastern Himalayan	02-02-2020	26°43'28.2"N	94°11'51.3"E	97.58 m

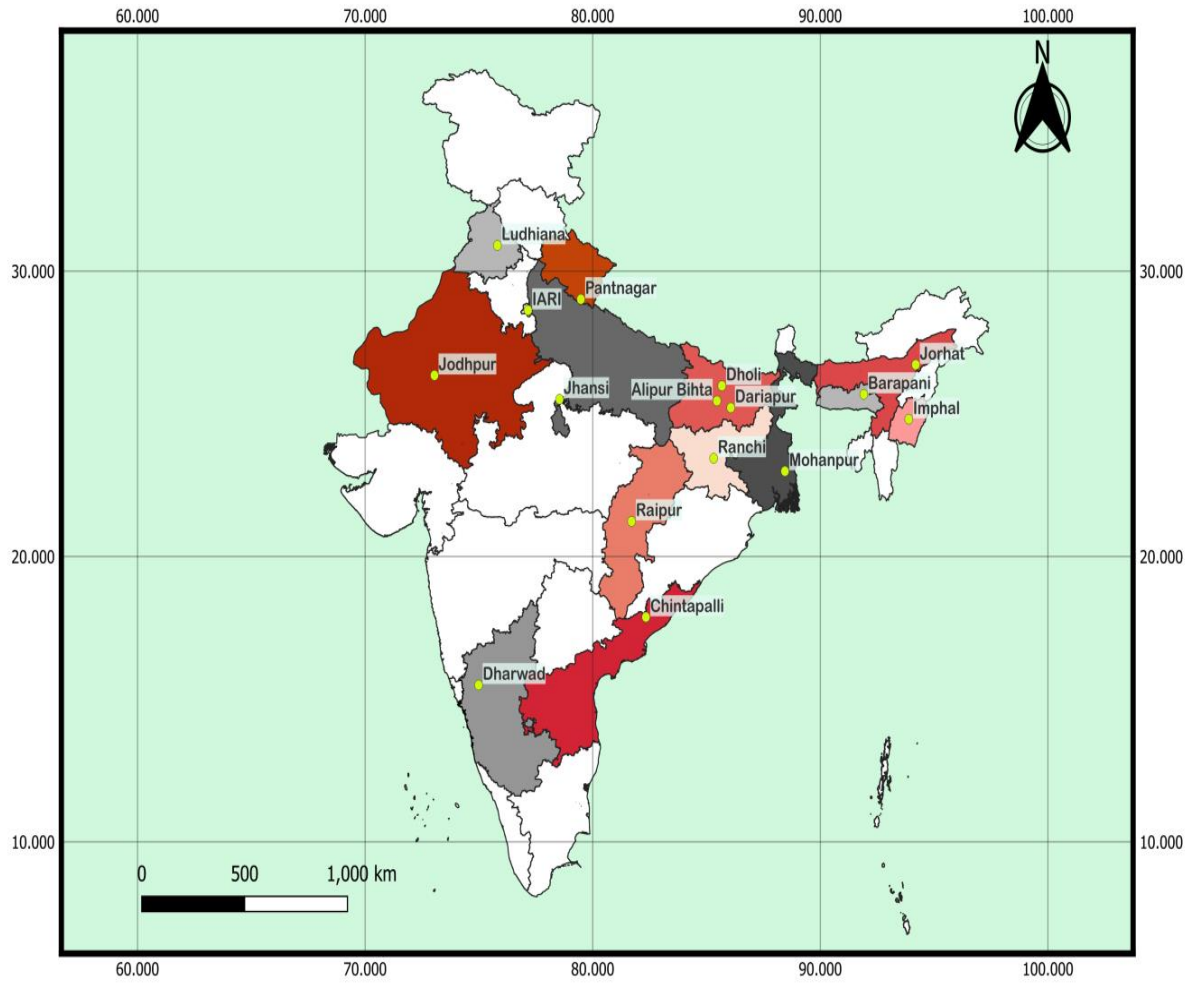


Fig 3.1: Interlocation survey during the year 2020

3.3.1 Morphological identification of *Lipaphis erysimi*

To confirm the species identity of *L. erysimi*, small to medium sized yellowish green, grey green or olive green, with two longitudinal rows of dark bands on the thorax and abdomen which unite into a single band near the tip of the abdomen; frontal tubercles- diverging but not distinctly exceeding vertex; cornicles- not dark and distinctly longer than the cauda; cauda- tongue shaped, were examined under a compound microscope as described by Blackman and Eastop (2000).

3.3.2 Molecular characterization of *Lipaphis erysimi*

After morphological confirmation of *L. erysimi* samples collected from different locations across India with varying altitudes (Table 3.1), they were further characterized at molecular level.

3.3.2.1 Extraction of DNA by CTAB (Cetyl Trimethyl Ammonium Bromide) method

i) Stock solutions

Table 3.2: List of reagents and composition of stock solution for DNA extraction

Sl. No	Reagents	Composition
1	1 M Tris-HCl (pH 8.0)	121.1 g of Tris hydroxyl methane aminomethane was dissolved in 800 ml double distilled H ₂ O and final volume made up to 1 liter and pH was adjusted to 8.0 with NaOH.
2	0.5 M EDTA (pH 8.0)	186.1 g of sodium ethylene diamine tetra-acetate was added to 500 ml of double distilled H ₂ O and final volume made up to 1 liter and pH was adjusted to 8.0 with NaOH.
3	5M NaCl	292.2 g of NaCl was added to 500 ml of H ₂ O and final volume made up to 1 liter.
4	3M Sodium acetate (pH 5.2)	408.1 g of sodium acetate was dissolved in 800 ml of distilled H ₂ O and final volume made to 1 liter and pH was adjusted to 5.2 with glacial acetic acid.

Cont....

5	2 % CTAB	2 g of CTAB was dissolved in 100 ml of distilled H ₂ O
6	CTAB Buffer (100 mL)	Following chemicals were mixed to prepare 100 mL of CTAB buffer: 5M NaCl (28 ml); 1M Tris HCl (10 ml); 0.5M EDTA (4 ml); 2 % CTAB (2 g); β-mercaptoethanol (200 µl) Finally volume was maintained to 100 ml by adding 55.8 ml of double distilled water.
7	70 % Ethanol	70 ml of 99.9 % ethanol was mixed with 30 ml of double distilled water and kept in refrigerator for cooling.
8	TE buffer	1 M Tris HCl (pH 8.0)-10 ml; 0.5 M EDTA (pH 8.0)- 2 ml and final volume made up to 1 liter with double distilled H ₂ O.
9	Phenol: Chloroform: Isoamyl alcohol (25:24:1)	25 ml of equilibrated phenol and 24 ml of Chloroform was mixed with 1 ml of Isoamyl alcohol to make 100 ml solution of 25:24:1 (P:C:I).
10	Chloroform : Isoamyl alcohol (24:1)	96 ml of Chloroform was mixed with 4 ml of Isoamyl alcohol to make 100 ml solution of 24:1 (C:I).

3.3.2.2 Isolation of genomic DNA

Protocol for genomic DNA isolation of *L. erysimi* adults was standardized in the Molecular laboratory, Department of Entomology and Molecular laboratory II, Department of Plant Breeding and Genetics, PG College of Agriculture, RPCAU, Pusa. CTAB method was modified to isolate the genomic DNA (Cubero *et al.*, 1999).

- Five adults from each location were taken and placed on a heating block to allow the ethanol to evaporate.
- The samples were crushed with micro-pestles in 500 µl of 2 % of CTAB buffer: 100 mM Tris HCl- 1.576 gm, 1.4 M NaCl- 8.1816 gm, 20 mM EDTA - 0.744 gm, 2 % CTAB- 2 gm (pH 8.0)) that had been preheated at 65°C for 10 minutes and was added with 10 µl of 20 % Sodium Dodecyl Sulphate (SDS), 10 µl of 0.1 % of 2-mercaptoethanol and 10 µl of Proteinase-K.
- The suspension was incubated for 45 minutes at 65°C with the tubes being inverted for every 15 minutes.

- Equal volume of PCI (25:24:1) was added after being cooled at room temperature. The suspension was then centrifuged at 14000 rpm for 10 minutes at 4°C.
- The upper aqueous layer was separated in a new micro centrifuge tubes by adding an equal volume of ice-cold isopropanol and stored at -20°C for overnight and the precipitated pellet was cleaned with 70 % chilled ethanol with centrifugation at 10000 rpm for 10 minutes at 4°C.
- The ethanol was then removed and the genetic material was allowed for air dry for few minutes before being dissolved in 50 µl 1X TE buffer.

3.3.2.3 Qualitative and quantitative analysis of DNA

Quality and quantity of DNA was analysed by two methods.

1. Agarose Gel Electrophoresis (AGE)

Agarose gel (0.8 %) was formulated by dissolving 0.8 gm agarose in 100 mL TAE buffer (Tris-acetate-EDTA). After cooling, 5.0 µL EtBr (Ethidium Bromide) was added. Gel was casted in gel casting tray. Wells were formed by inserting comb in the gel casting tray. DNA samples together with tracking dye (6x Methanol blue) were loaded in the well. Gel was run in the electrophoretic unit for 45 min at 100 V. After 45 min, gel was taken out carefully from gel casting tray and placed into Syngene Gel Documentation system. Gel picture was taken using InGenius3 software and saved in jpg format.

2. Spectrophotometer

A spectrophotometer model Biophotometer was used to check the purity of DNA (Eppendorf TM). Absorbance has been recorded at both the wavelengths (A260/A280; A260/A230) and the purity was determined by the ratio of A260/A280. A260/A280 value between 1.8 and 2.0 indicates that the DNA quality is pure. If the concentration of DNA was found more, then it was diluted to get a working solution of 100 ng/ µl (Sambrook *et al.*, 1989).

$$\text{Dilution of DNA} = \frac{\text{Concentration of DNA required (ng/}\mu\text{l)} \times \text{Total volume required (}\mu\text{l)}}{\text{Available concentration of DNA (ng/}\mu\text{l)}}$$

3.3.2.4 PCR amplification of Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) primers

The genomic DNA isolated from adult *L. e. pseudobrassicae* was amplified with 11 RAPD and 8 SSR primers (Table 3.4) to assess the molecular differences between *L. pseudobrassicae* populations collected from 16 interlocation. PCR was performed with a 20 μ l reaction mixture comprising of 2 μ l template DNA (100 ng), 1.5 μ l of forward and reverse primers, 8 μ l of *Taq* mixture and 8.5 μ l of nuclease free water. Thermo cycler was performed under the following conditions: initial denaturation at 95°C for 2.30 sec, 35 cycles of denaturation at 94°C for 45 sec, annealing at 94°C for 30 sec and extension at 72°C for 5 min followed by final extension of 72°C for 10 min. PCR products were visualized by 2.2 % agarose gel stained with 5 μ l ethidium bromide and observed by Syngene gel documentation system.

Table 3.3: Components of PCR reaction

Sl. No.	Contents	Volume (μ l)
1	Genomic DNA	2 μ l
2	PCR <i>Taq</i> mixture	8 μ l
3	Forward and Reverse primer	1.5 μ l
4	Nuclease free water	8.5 μ l
	Total volume	20 μl

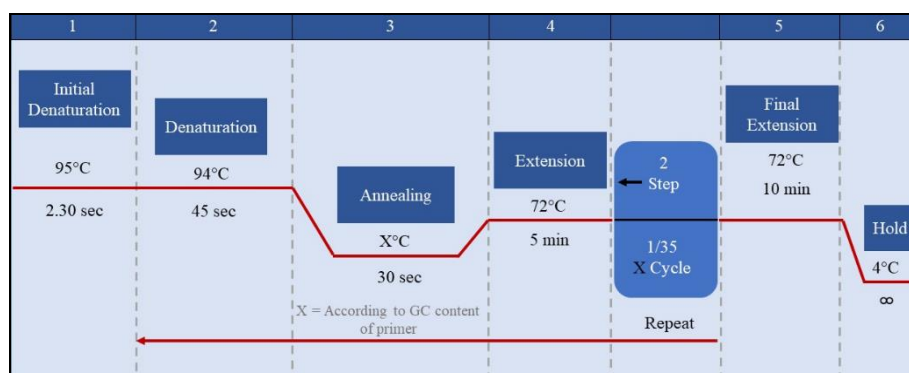


Figure 3.2: Thermal cycling programme

Data interpretation

The presence and absence of amplicons were manually scored as one and zero, respectively. The genetic distance or dissimilarity was calculated using the Jaccard coefficient (Jaccard, 1908) and dendrogram was constructed by NTsys software, the scored marker data matrix and Principal Component Analysis (PCA) was analyzed using the standard procedure in DARwin 6 software (Perrier *et al.*, 2003) based on dissimilarity coefficients and UPGMA (Unweighted Pair-Group Method with Arithmetical averages). The total amplified bands, number of polymorphic bands, number of monomorphic bands, Percentage of Polymorphic Bands (PPB) were calculated for each RAPD and SSR markers.

Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Resolving Power (RP) and Marker Index (MI) were calculated to determine the informativeness of RAPD and SSR markers.

$$\text{PIC} = 1 - \sum p_i^2 \text{ (Anderson } et al., 1993)$$

Where,

p_i is the frequency of the i^{th} allele of the locus

$$\text{MI} = \text{EMR} \times \text{PIC} \text{ (Varshney } et al., 2007)$$

Effective Multiplex Ratio (EMR) is the product of the fraction of polymorphic bands and the number of polymorphic bands (Kumar *et al.*, 2009). The Resolving Power (RP) of each primer was calculated according to the Prevost and Wilkinson (1999).

$$\text{RP} = \sum \text{Ib}, \text{ Where Ib represents the informative fragments}$$

Ib can be represented on a scale of 0/1 by the following formula

$$\text{Ib} = 1 - (2 \times |0.5 - p_i|)$$

Where,

p_i is the proportion of accessions containing the i^{th} band

Table 3.4: Details of primers used in the study

Sl. No	Primer	Nucleotide Sequence	T _m (°C)	Expected Size (bp)	Reference
RAPD primers					
1	OPA-02	5'-TGCCGAGCTG-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
2	OPA-04	5'-AATCGGGCTG-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
3	OPA-10	5'-GTGATCGCAG-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
4	OPA-11	5'-CAATCGCCGT-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
5	OPA-13	5'-CAGCACCCAC-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
6	OPA-15	5'-TTCCGAACCC-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
7	OPA-20	5'-GTTGCGATCC-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
8	OPR-07	5'-ACTGGCCTGA-3' (10)	37.3	300-600 bp	Lima <i>et al.</i> (2000)
9	F2	5'-GAGGATCCCT-3' (10)	37.3	300-600 bp	De Barro and Driver (1997)
10	F12	5'-ACGGTACCAG-3' (10)	37.3	300-600 bp	De Barro and Driver (1997)
11	H9	5'-TGTAGCTGGG-3' (10)	37.3	300-600 bp	Serçe <i>et al.</i> (2007)
SSR primers					
1	ApF08M	F: 5'-TAATCCGTCGTAATTGCGTT-3' (20)	50.0	299-355 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-TAAGCCCTCACTCACCCCTC-3' (20)			
2	ApG10M	F: 5'-CAACGACGGCGGCTATACTA-3' (20)	50.0	219-251 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-ACGAGAGCTTCCGGCGTAT-3' (20)			
3	ApH05M	F: 5'-ACGAGAGCTTCCGGCGTAT-3' (20)	51.0	224 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-CAACGACGGCGGCTATACTA-3' (20)			
4	ApH08M	F: 5'-GCGCACAGTGCGTATACATT-3' (20)	51.0	175 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-TATTACAACGCACGTCATCG-3' (20)			
5	ApH12M	F: 5'-CTTCCACAAGAACTCCGGT-3' (20)	56.0	225-228 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-CTCGGTAACCACCTTGGTAG-3'(20)			

Cont...

6	ApH04M	F: 5'-CGCATCGAGTGTCGTATTAT-3' (20)	56.0	256-260 bp	Caillaud <i>et al.</i> (2004)
		R: 5'-GTTCCAAGGTCCTCTCTTCC-3' (20)			
7	Ap-01	F: 5'-AACCGGAGGGCCAAGACT-3' (18)	50.0	155 bp	Kurokawa <i>et al.</i> (2004)
		R: 5'-ACGACGTCTAGGTCGAGCAC-3' (20)			
8	Ap-03	F: 5'-GCAGCAAACAGCAGGTGTA-3' (21)	51.0	166 bp	Kurokawa <i>et al.</i> (2004)
		R: 5'-ACAATTGCTCGATGGTCCTC-3' (20)			
9	Ap-05	F: 5'-TCACCAAGGCATCCCTCTAC-3' (20)	53.0	100-304 bp	Kurokawa <i>et al.</i> (2004)
		R: 5'-GTAGCGAATTTTCCGGTTG-3' (20)			
Endosymbiont specific 16s rRNA gene					
1	<i>Buchnera</i> sp	F: 5'-GAGCTTGCTCTCTTTGTCGGCAA-3' (23)	54.0	1000 bp	Raina <i>et al.</i> (2015)
		R: 5'-CTTCTGCGGGTAACGTCACGAA-3' (22)			
2	<i>Wolbachia</i> sp	F: 5'-CGGGGAAAATTTATTGCT-3' (19)	54.0	650 bp	Raina <i>et al.</i> (2015)
		R: 5'-AGCTGTAATACAGAAAGGAAA-3' (21)			
3	<i>Arsenophonus</i> sp	F: 5'-CGTTTGATGAATTCATAGTCAA-3' (23)	54.0	630 bp	Raina <i>et al.</i> (2015)
		R: 5'-GGTCCTCCAGTTAGTGTACCCAAC-3' (25)			
4	<i>Rickettsia</i> sp	F: 5'-GCTCAGAACGAACGCTGG-3' (18)	54.0	900 bp	Gottlieb <i>et al.</i> (2006)
		R: 5'-GAAGGAAAGCATCTCTGC-3' (18)			
5	<i>Cardinium</i> sp	F: 5'-GCGGTGTA-3' (19)	54.0	450 bp	Weeks <i>et al.</i> (2003)
		R: 5'-ACCTCTTCTTTAACTCAAGCCT-3' (22)			
6	<i>Hamiltonella</i> sp	F: 5'-AGCACAGTTTACTGAGTTCA-3' (20)	54.0	960 bp	Tsuchida <i>et al.</i> (2006)
		R: 5'-TACGGYTACCTTGTTACGACTT-3' (22)			
7	<i>Spiroplasma</i> sp	F: 5'-AGAGTTTGATCMTGGCTCAG-3' (20)	54.0	450 bp	Tsuchida <i>et al.</i> (2006)
		R: 5'-TAGCCGTGGCTTTCTGGTAA-3' (20)			
Universal primer					
1	<i>mtCOI</i>	F: 5'-GGTCAACAAATCATAAAGATATTGG-3' (25)	52.0	920 bp	Simon <i>et al.</i> (1994)
		R: 5'-TAAACTTCAGGCTGACCAAAAAATCA-3' (26)			

3.3.3 Phylogenetic analysis of mustard aphid using partial mitochondrial cytochrome oxidase I (*mtCOI*) gene

In polymorphism studies, isolates from each cluster were selected and subjected to partial *mtCOI* sequencing to confirm the identity of mustard aphid species.

3.3.3.1 Polymerase Chain Reaction (PCR) protocol and DNA sequencing

The desired gene, partial mitochondrial cytochrome oxidase I (*mtCOI*) was amplified using primer, *mtCOI* and the amplified product of *mtCOI* was sequenced.

. The Components of PCR reaction mixture and PCR cycling conditions were mentioned in the (Table 3.3 and Figure 3.1) respectively. The amplicons were visualized in 2.0 % Agarose gel electrophoresis through staining with 6 µl EtBr at 100 V for 30 min. The PCR product was sent to Eurofins Genomics Bangalore, Karnataka for sequencing. The PCR products were purified according to manufacturer's protocol using a QIA quick PCR purification kit. Dideoxy Cycle Sequencing was performed using the chain-termination method and ABI Prism Big Dye reaction kit (ver. 3.1). On an ABI 3130XL genetic analyzer, the PCR products were sequenced from both ends and the termination products were analyzed. Sequencher 4.10 was used to edit the sequences and assemble the contigs.

The sequences obtained were analyzed for sequence homology using the nucleotide BLAST, submitted via BankIt in NCBI (National Center for Biotechnology Information) and eventually GenBank accession numbers were received.

3.3.3.2 Phylogenetic analyses

Sequences for comparisons were retrieved from NCBI and aligned through Clustal Omega online software (Sievers *et al.*, 2011); Phylogenetic and molecular evolutionary analyses were performed by using the software MEGA X (Molecular Evolutionary Genetic Analysis Version X) (Kumar *et al.*, 2018). Maximum Likelihood fits of 24 different nucleotide substitution models was performed to choose the best model for phylogenetic tree construction (Felsenstein, 1981). Estimates of Evolutionary divergence between sequences and Maximum Composite Likelihood Estimate of the pattern of nucleotide substitution were calculated.

Neutrality tests like Fisher's test (Fisher, 1935) and Tajima test (Tajima, 1989) were conducted to verify the sequences that do not fit neutral theory.

The 16 sequences of the partial *mtCOI* gene of interlocation were compared to the following sequences of *L. erysimi pseudobrassicae* and six out groups (*Myzus persicae*, *Brevicoryne brassicae*, *Cinara longipennis*, *Melanaphis japonica*, *Schizaphis graminum*, *Rhopalosiphum padi*) available in the NCBI database (Table 3.5). Using invertebrate mitochondrial genetic code, the nucleotide sequences were converted into amino acid sequences and aligned using Expasy-translate (Gasteiger *et al.*, 2003).

Table 3.5: Details of reference sequences (retrieved from NCBI, GenBank)

Sl. No	Aphid species	Accession number
1	<i>Lipaphis erysimi pseudobrassicae</i>	GU668619
2	<i>Myzus persicae</i>	JF883917
3	<i>Brevicoryne brassicae</i>	KR033127
4	<i>Cinara longipennis</i>	GU457805
5	<i>Melanaphis japonica</i>	MH820489
6	<i>Schizaphis graminum</i>	MF828645
7	<i>Rhopalosiphum padi</i>	KR346127

3.3.4 Molecular identification and diversity of bacterial endosymbionts associated with *Lipaphis erysimi*

Extracted DNA of *L. erysimi* was used for PCR amplification and detection of endosymbionts. Using specific gene targets such as 16S *rRNA*, *Buchnera* sp, *Wolbachia* sp, *Arsenophonus* sp, *Rickettsia* sp, *Cardinium* sp, *Hamiltonella* sp and *Spiroplasma* sp were identified among 16 locations.

Gene specific 16S *rRNA* of bacterial endosymbionts were screened through PCR. Components of PCR reaction mixture and PCR cycling conditions were mentioned in the (Table 3.3 and Figure 3.1) respectively. The PCR reaction was carried out in Gradient Thermal Cycler (Agilent technologies). The amplified products were run on a 2.5 % Agarose gel with a 1X TAE buffer and EtBr (6 µl) in a gel electrophoresis system at 100 V for 45 minutes with a 100 bp DNA ladder (G-Biosciences). The presence of bands in the image captured by the gel documentation

system was carefully examined and the diversity of associated bacterial endosymbionts was studied.

3.4 Monitoring of alate aphids and natural enemies through yellow sticky traps in relation to weather parameters

Yellow sticky traps (14.9 × 21 cm) with dry adhesive were used in this study for monitoring the population of alate aphids and natural enemies. Four replicates of each trap were deployed along the four perimeters (one each in North, South, East, West sides) in the experimental farm, TCA, Dholi following recommended package of practices except crop protection measures during *Rabi* 2019-20 and 2020-21. Traps in each replication were tied and randomly assigned to four fixed bamboo sticks. Yellow sticky traps were deployed for weekly interval from the field, recovered, brought to the laboratory and evaluated for the alate aphids and natural enemies under a microscope. Data were recorded from each trap on the population build-up of alate aphids and associated natural enemies.

3.4.1 Impact of weather factors on the population of alate aphids and natural enemies

To investigate the effects of abiotic factors on the population growth of alate aphids and their natural enemies, the meteorological data (weekly mean) for temperature (maximum and minimum), morning (7 00 hrs) and evening (14 00 hrs) relative humidity (%), rainfall (mm) and sunshine (hrs) during 2019-2020 to 2020-2021 were obtained from Pusa observatory, RPCAU, Pusa.

Statistical analysis

The mean alate aphid counts and natural enemies were statistically analysed for correlation and regression with meteorological observations.

3.5 Screening of different Rapeseed-mustard genotypes against mustard aphid

Thirty rapeseed-mustard genotypes (Table 3.8), along with Yellow sarson (66-197-3) (Susceptible Check), Kranti (National Resistant Check), BSH-1 (National Susceptible Check) were acquired from All India Coordinated Research Project (AICRP) on Rapeseed and Mustard, Sewar, Bharatpur (Rajasthan), India. The experiment was conducted in Randomized Block Design (RBD) and replicated three times in a plot size (3 × 1.5m) of paired rows with 30 x 15 cm row to row and plant to

plant spacing. Except for the use of insecticides, the genotypes were grown in accordance with the recommended agronomic package of practises. The sowing was done on 30th November and 1st December, during *Rabi*, 2019-20 and 2020-21 respectively. The observations on population of mustard aphid, Aphid Population Index (API), Aphid Damage Index (ADI) and Aphid Resistance Index (ARI) were recorded at flowering and pod stage. For this, the aphid population count was taken from ten randomly selected plants of top 10 cm inflorescence in each replication. The ARI were determined based on API and ADI values and ultimately derived the resistance category of each genotype (Table 3.6).

Table 3.6 Categorization of Rapeseed-mustard genotypes for resistance to mustard aphid based on the scale given by Dhillon *et al.* (2018)

Sl.No	Aphid Population Index (API)	Aphid Damage Index (ADI)	Aphid Resistance Index (ARI)	Resistance category
1.	1 = No or less than 20 aphids on the inflorescences of test plant.	1 = Normal plant growth, no signs of injury, no leaf curling or yellowing.	(API+ADI/2)	0.0-1.0 = Highly Resistant
2.	2 = upto 25 % inflorescences have 21- 100 aphids on the test plant.	2 =Plant growth is average, with some curling and yellowing of the leaves, as well as flowering and fruiting.	(API+ADI/2)	1.1-2.0 = Resistant
3.	3 = upto 50 percent of inflorescences have 101- 250 aphids on the test plant.	3 = Plant growth is poor, with curling and yellowing leaves on some branches, drying of a few flowers, and poor pod setting.	(API+ADI/2)	2.1-2.5 = Moderately Resistant
4.	4 = upto 75 % inflorescences have 251- 500 aphids on the test plant.	4 = Plant growth is slowed, with heavy curling and yellowing of leaves throughout the plant, as well as drying and curling of nearly half of the inflorescence, poor flowering, and rare pod setting.	(API+ADI/2)	2.6-3.5 = Susceptible
5.	5 = 100 % of inflorescences have more than 500 aphids on the test plant.	5 = Severe stunting and ragged plant appearance, yellowing and curling of nearly all leaves, complete drying of inflorescence without flower, and immature drying of pods if any.	(API+ADI/2)	3.6-5.0 = Highly Susceptible

	R₁ 3m	R₂	R₃
1.5m	G1	G8	G7
	G2	G9	G25
	G3	G10	G26
	G4	G11	G27
	G5	G12	G28
	G6	G13	G15
	G7	G14	G16
	G28-SC	G1	G17
	G8	G29-NRC	G18
	G9	G2	G28-SC
	G10	G3	G19
	G11	G4	G20
	G12	G5	G21
	G13	G6	G22
	G14	G30-NSC	G23
	G29-NRC	G7	G8
	G15	G25	G9
	G16	G26	G10
	G17	G27	G11
	G18	G28	G12
	G19	G15	G13
	G20	G16	G14
	G21	G17	G1
	G22	G18	G29-NRC
	G23	G28-SC	G2
	G30-NSC	G19	G3
	G25	G20	G4
	G26	G21	G5
	G27	G22	G6
	G28	G23	G30-NSC

Table 3.7: List of Rapeseed-mustard genotypes screened against mustard aphid

Genotype.No.	Genotypes
G1	RCH1
G2	RMM 10-1-1
G3	DRMRCI 96
G4	DRMR HJ 2403 (Hybrid)
G5	RGN 229
G6	RH 1676
G7	SKM 1626
G8	RGN 443
G9	PBR 385
G10	DRMRIJ 31
G11	RH 7846
G12	PR 2016-4
G13	DRMR 2017-16
G14	NPJ 229
G15	RH 1424
G16	PDZ 11
G17	PDZ 12
G18	RH (OE) 1706
G19	PDZ 1
G20	GSH 2196
G21	AKGS 19-8
G22	GSH 1699
G23	CS 2005-143
G24	GSH 2180
G25	HNS 0702
G26	RTM 1624
G27	RTM 314
G28	Yellow sarson (66-197-3)
G29	Kranti
G30	BSH-1

Fig 3.3 Field layout of screening of Rapeseed-mustard genotypes against mustard aphid

3.5.2 Biochemical analysis

The biochemical constituents *viz.*, Glucosinolates, total phenols and total soluble sugars of promising Rapeseed-mustard genotypes were estimated in Department of Entomology and Department of Biochemistry, RPCAU, Pusa by the following procedures:

3.5.2.1 Glucosinolates estimation:

The procedure for estimating glucosinolates in rapeseed-mustard genotypes was followed according to the Kumar *et al.* (2004).

Principle: Sodium tetrachloropalladate (Na_2PdCl_4) forms a complex with the sulphur of glucosinolates, yielding a red compound that can be measured at 405nm.

Reagents:

- i. 60 % Methanol
- ii. 0.2 mM Na_2PdCl_4
- iii. Double distilled water (dd H_2O)

Procedure:

The inflorescence of promising genotypes was collected and heated in an oven at 100°C to deactivate the myrosinase enzyme. The dried inflorescence was ground in a mortar and pestle, and 0.3 g was transferred to the test tubes. Then 300 μl (0.3 ml) of 60 % methanol was added into the tubes and heated in a water bath at 80°C for 10 min to make sure that methanol was evaporated. 4 ml of dd H_2O was added, and the tubes were heated again at 80°C for 15 min before being centrifuged at 3000 rpm for 10 min. For sample analysis, 40 μl of supernatant was taken into each tube separately in duplicates. Each tube was then filled with 4 ml of 0.2 mM Na_2PdCl_4 reagent and left at room temperature for one hour. Optical Density (OD) reading was recorded at 405 nm along with standard and unknown samples in the spectrophotometer. As a standard, Sinigrin (16.6 to 83.0 μg) was used.

3.5.2.2 Estimation of total phenols:

Total phenol content in Rapeseed-mustard genotypes was estimated by the procedure given by Swain and Hillis (1959).

Principle: When phosphomolybdic acid reacts with phenols in alkaline medium in the presence of Folin-Ciocalteu reagent, a blue-colored complex is formed which can be measured at 760 nm.

Reagents

- i. 80 % Methanol
- ii. Folin-Ciocalteu reagent
- iii. 20 % Sodium carbonate (Na_2CO_3)

Procedure: 0.25 g inflorescence from promising Rapeseed-mustard genotypes was homogenised in 10 ml of 80 % methanol and placed in a water bath for two hours. The mixture was then filtered through Whatman No. 1 filter paper, and the total volume was reduced to 10 ml. A 0.5 ml extract was taken from the supernatant layer and placed in test tubes, which were then evaporated by placing the test tubes in a boiling water bath. To dissolve the residue, 6.5 ml of distilled water was added to the test tubes, followed by 0.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 1ml of saturated Na_2CO_3 solution was added to this and incubated for one hour in the dark before being read at 760 nm. As a standard Gallic acid (10 μg to 100 μg) was used and phenol content was expressed as mg/g gallic acid equivalent.

3.5.2.3 Estimation of Total soluble sugars:

Total soluble sugars were determined using anthrone reagent according to the method described by Yemm and Willis (1954).

Reagents:

- i. Anthrone reagent: 0.4 g anthrone was dissolved in 100 ml concentrated H_2SO_4
- ii. 80 % methanol

Procedure: 300 mg inflorescence of each promising Rapeseed-mustard genotypes were homogenised in 80 % methanol. The filter was collected and refluxed three times with 80 % methanol in hot water bath for 15 min. The supernatant from each extraction was cooled and decanted into another test tube and diluted to 5 ml with 80 % methanol. The resulting extract was used to calculate total soluble sugars.

Estimation: An aliquot of 0.2 ml was taken in test tubes and evaporated to dryness in a boiling water bath. The residue in the tube was dissolved in 1 ml of distilled water

and mixed with 4.0 ml of the anthrone reagent after cooling and heated in water bath for 5 min, cooled and absorbance at 620 nm was measured with a Spectrophotometer. The concentration of the total sugars was calculated from the standard curve of glucose (10-100 µg).

3.5.3 Gas Chromatography- Mass Spectrometry (GC-MS) for bioactive compounds in promising Rapeseed-mustard genotypes

GC-MS analysis of promising Rapeseed-mustard genotypes for bio active compounds was executed in Advanced Instrumental Research Facility (AIRF), Jawaharlal Nehru University (JNU) New Delhi.

Extraction

The collected 10 gram inflorescences of selected promising Rapeseed-mustard genotypes were allowed to dry in the shade at room temperature being rinsed in tap water to remove impurities. Dried inflorescence of each genotype was ground using mortar and pestle in the presence of 80 % HPLC-grade methanol as a solvent for extraction. After each extraction, the material was transferred to the separate conical flasks and kept for 48 hours at room temperature with timely interval shaking. Finally, the extraction was filtered through filter paper (Whatman No. 1) and subjected to drying at room temperature for 48 hours. The solvent-free extract was precisely weighed to 0.5 g, dissolved in 10 ml of HPLC-grade methanol, and stored at 4°C in glass-stoppered vials until use.

Procedure

The prepared extracts were subjected to Gas Chromatography-Mass Spectrometry analysis in order to determine the number of compounds. Secondary metabolites and oleo/aromatic compounds were characterised using an Agilent 6890 gas chromatograph and 5975B mass spectrometer in trace ion detection mode with a Programmable Temperature Vaporizer injector (PTV). The chromatographic separation was carried out on a fused silica HP-5 m (0.25 mm × 30 m × 0.25 µm film thickness) capillary column. The modified oven programming was set according to (Medini *et al.*, 2009): oven kept isothermally at 80°C for 2 min, increased from 80 °C to 200°C at the rate of 4°C /min for two min and from 180°C to 230°C at the rate of 25°C /min for 13 min. One microliter of the extract was injected in the split mode at 260°C. Helium was used as the carrier gas, at a flow rate of 3.0 ml/min and a split

ratio of 10:0. Elements were detected using Electron Ionization (EI), with scan times and mass ranges of 0.05 sec and 40-600 m/z, respectively, and a run time of 45 minutes.

Identification of bioactive compounds

The bioactive compounds were identified by computing their retention time in comparison to (C9-C18) n-alkenes and data for genuine compounds available in NIST, Willey and Perfumery libraries, as well as matching their mass spectrum fragmentation configurations with corresponding data stored in the GC-MS data system's mass spectra library and their published mass spectra. The electronic integration of the FID peak area of each recognised compound yielded the relative fraction amount of that compound.

3.6. Effect of intercrops on the incidence of mustard aphid and natural enemies

3.6.1 Experimental design

The experiment was set up in a Randomized Block Design (RBD) with nine treatments and three replications. The plots (3.3 x 2 m size) were separated by 1 m and 1.5 m between treatments and replicates respectively with inter-row spacing, 30 cm and intra-row spacing of 15 cm. This gives a total of 17 rows per experimental plot with 20 plants per row. Sowing was done on 29th November and 30th November in *Rabi*, 2019-20 and 2020-21 respectively. Mustard variety yellow sarson (66-197-3) was sown as a sole crop and eight intercrops *viz.*, wheat, barley, chickpea, lentil, pea, linseed, coriander and fennel were sown in one row between three rows of mustard (3:1) as a replacement of particular row of mustard. The details of treatments and intercrop varieties were presented in Table 3.8. Throughout the crop growth period, standard agronomic practises were followed without any pest control measures were used.

Table 3.8: Details of the intercrop combinations used in the experiment

Treatments	Name of Treatments	Ratio
T ₁	Mustard + Wheat (HD 2967)	3:1
T ₂	Mustard + Barley (Ratna)	3:1
T ₃	Mustard + Chickpea (Pusa-256)	3:1
T ₄	Mustard + Lentil (HUL-57)	3:1
T ₅	Mustard + Peas (HUDP-15)	3:1
T ₆	Mustard + Linseed (TL99)	3:1
T ₇	Mustard + Coriander (RD-44)	3:1
T ₈	Mustard + Fennel (Rajendra Saurabh)	3:1
T ₉	Mustard sole crop (Control)	-

3.6.2 Data collection

The population of mustard aphids was observed on the top 10 cm of the inflorescence of selected ten randomly selected plants and whereas, natural enemies (coccinellids, syrphids, parasitoids, spiders) count was taken from whole plant basis of ten randomly selected plants with direct visual observation for at least 5 to 10 minutes at fortnightly interval beginning from 35 days after sowing till the harvest of the crop. Predators were grouped individually into coccinellids, syrphids and spiders and their count in both immature and mature stages was taken. However, since aphid parasitoids were difficult to count in the field, we documented the number of mummified aphids found on selected plants and randomly collected on each sample date, brought to the laboratory for adult emergence and identified under a microscope. Identification of adult coccinellids, syrphids and spiders was made based on familiarity with species commonly encountered and also through photographs captured in the field. The effect of intercrop combinations on mustard yield (kg/ha) was also being recorded from each plot separately to determine the effects of treatment on yield

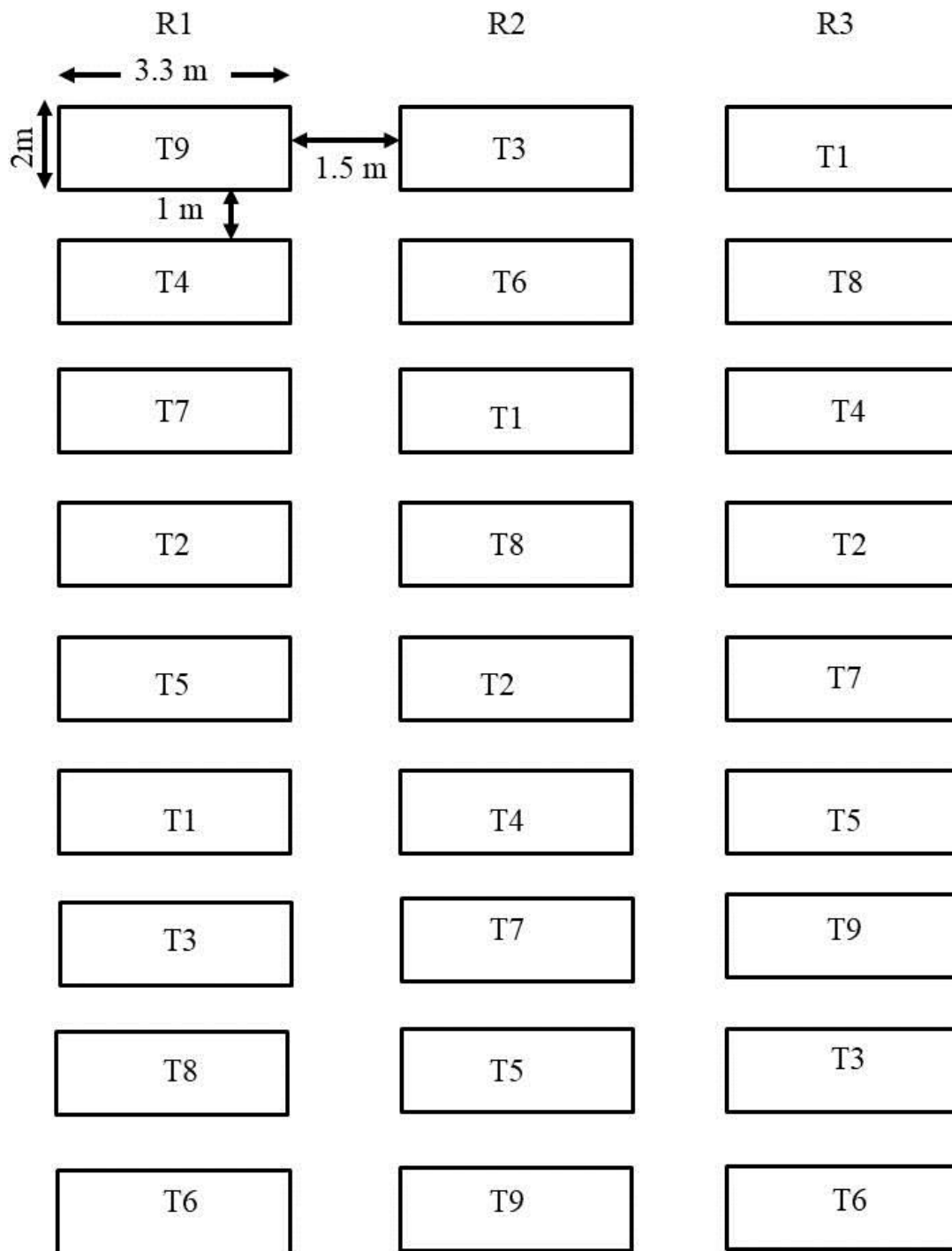


Figure 3.4: Field layout of effect of intercrops on the incidence of mustard aphid and natural enemies

3.6.3 Statistical analysis

The data was subjected to analysis of variance (ANOVA) and Tukey's HSD test (SPSS 19) software for comparing means significantly at the 5% level.

3.7. Effect of botanicals on mustard aphid and its natural enemies under field conditions

The study was conducted at the research farm, T.C.A. Dholi, during 2019-2020 and 2020-2021 cropping season. The experiment was laid out in a Randomized Block Design (RBD) with seven treatments and three replications, yellow sarson (66-197-3) used as a test variety. The plots (3×3.5m size) were separated by a 0.5 m and 1 m between treatments and replications respectively. The details of treatments used in the experiment are presented in Table 3.9.

3.7.1 Neem Seed Kernel Extract (NSKE) and Yam Bean Seed Extract (YBSE)

The collected seeds of neem and yam bean were dried under shade and crushed into powder with mortar and pestle. The 5 % w/v NSKE and YBSE were prepared by dissolving 500 g neem seed powder and yam bean seed powder into 10 L of water for 24 hours with regular stirring (Nboyine *et al.*, 2013). The resulting solutions were filtered through muslin cloth and applied on the field.

3.7.2 Preparation of leaf extracts

The leaves of the different test plants *i.e.*, Lamb's quarter (*Chenopodium album*), Field bindweed (*Convolvulus arvensis*), Bhang (*Cannabis sativa*) were washed with distilled water and spread on blotting paper to dry them at room temperature, later they were crushed into a paste using mortar and pestle. The 5 % w/v leaf extracts were prepared by dissolving 500 g paste into 10 L of water for 24 hours with regular stirring (Degri *et al.*, 2013). The resulting solutions were filtered through muslin cloth before spraying. All the treatments were sprayed twice at fortnightly interval starting from 50% flowering stage.

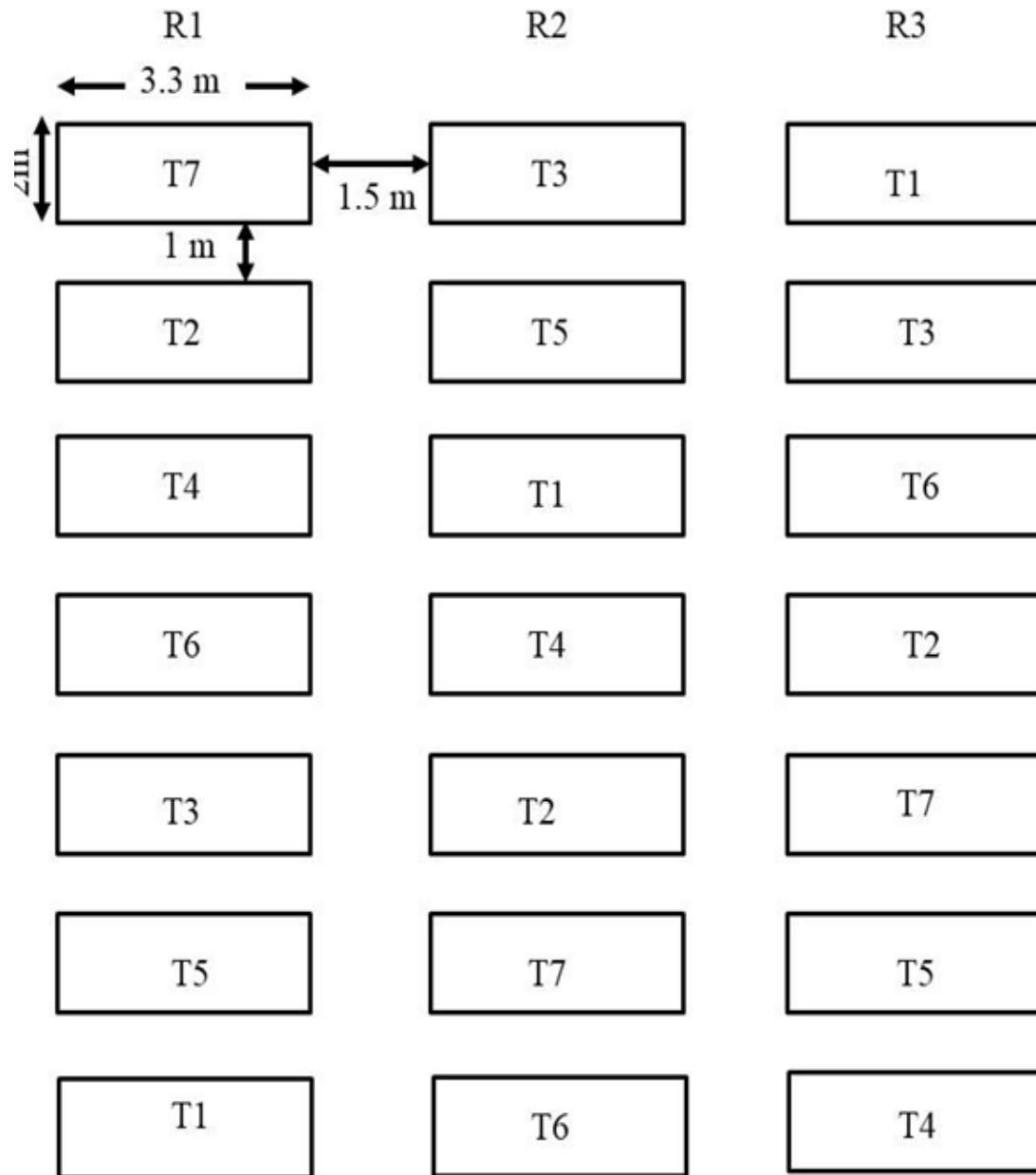


Figure 3.5: Field layout of effect of botanicals against mustard aphid and its natural enemies

3.7.3 Gas Chromatography- Mass Spectrometry (GC-MS) for bioactive compounds in selected botanicals

GC-MS analysis of selected botanicals *viz.*, yam bean seed and bhang leaf for bio active compounds was performed in Advanced Instrumental Research Facility (AIRF), Jawaharlal Nehru University (JNU) New Delhi.

Extraction

After removing impurities by rinsing in tap water, the 10 gm YBS and Bhang leaves were allowed to dry in the shade at room temperature. The dried seeds and leaves were grounded using mortar and pestle in the presence of 80 % HPLC-grade methanol as a solvent for extraction. After each extraction, the material was transferred to the separate conical flasks and kept for 48 hours at room temperature with timely interval shaking. Finally, the extraction was filtered through filter paper (Whatman No. 1) and subjected to drying at room temperature for 48 hours. The solvent-free extract was precisely weighed to 0.5 g and dissolved in 10 ml HPLC-grade methanol before being stored in glass-stoppered vials at 4°C until use. The procedure of GC-MS analysis and identification of compounds is described earlier (3.5.3).

Table 3.9: Details of treatments used in the experiment

Treatments	Name of Treatments	Scientific/trade name	Conc./dosage
T ₁	Neem Seed Kernel Extract (NSKE)	<i>Azadirachta indica</i>	5 %
T ₂	Lamb's Quarter Leaf Extract (LQLE)	<i>Chenopodium album</i>	5 %
T ₃	Field Bindweed Leaf Extract (FBLE)	<i>Convolvulus arvensis</i>	5 %
T ₄	Bhang Leaf Extract (BLE)	<i>Cannabis sativa</i>	5 %
T ₅	Yam Bean Seed Extract (YBSE)	<i>Pachyrhizus erosus</i>	5 %
T ₆	Dimethoate 30 EC (Check)	Rogor	0.03%
T ₇	Untreated control	-	-

3.7.4 Data collection

The mustard aphid population was recorded from top 10 cm inflorescence of ten randomly selected plants at one day before and 3, 5, 7 and 10 days after each spray. Whereas, natural enemies (coccinellids, syrphids, parasitoids, spiders) count was taken from whole plant basis of ten randomly selected plants with direct visual observation for at least 5 to 10 minutes. The yield (kg/ha) was also being recorded from each plot separately to determine the effects of treatment on yield.

Statistical analysis

The data was subjected to an analysis of variance (ANOVA) as well as the Tukey's HSD test for significantly comparing means at the 5% level (SPSS 19 software). The percent reduction in aphid population in each treatment compared to the control was calculated using Henderson and Tilton (1955) formula.





CHAPTER - IV



RESULTS AND DISCUSSION



The results of investigation on “Screening of Rapeseed-mustard Genotypes and Eco friendly Management of Mustard Aphid, *Lipaphis erysimi* (Kalt.)” are presented under the following titles:

- 4.1 Molecular characterization and phylogenetic diversity of *Lipaphis erysimi* and its endosymbionts in India.
- 4.2 Monitoring of alate mustard aphid and natural enemies through yellow sticky traps in relation to weather parameters.
- 4.3 Screening of different Rapeseed-mustard genotypes against mustard aphid.
- 4.4 Effect of intercrops on the incidence of mustard aphid and natural enemies.
- 4.5 To study the efficacy of botanicals on mustard aphid and natural enemies under field conditions.

4.1 Molecular characterization and phylogenetic divergence of *Lipaphis erysimi* and its associated endosymbionts in India

The molecular characterization and phylogenetic divergence of *Lipaphis erysimi* from different locations were investigated. For this a survey was conducted during, 2020 and collected mustard aphid samples from 16 locations of India. Further, the collected samples were morphologically confirmed and subjected for molecular studies.

4.1.1 Morphological confirmation of *Lipaphis erysimi*

To confirm the species identity of *Lipaphis erysimi*, shape of frontal tubercles, cauda, length and colour of cornicles were examined under a compound microscope (Plate 1) as described by Blackman and Eastop (2000).



Plate 4.1: Key morphological characters of *Lipaphis erysimi*

Note: a. two longitudinal rows of dark bands on the thorax and abdomen b. Frontal tubercles are diverging, not distinctly exceeding vertex c. Cornicles are not dark and distinctly longer than the cauda d. Cauda- tongue shaped

4.5.2 Evaluation of RAPD and SSR primers for understanding the genetic diversity of *Lipaphis erysimi*

Genomic DNA extracted from one adult each of 16 locations were amplified with 10 RAPD and 9 SSR primers by Gradient Thermal Cycler. The amplified products were separated on a 2.5 % Agarose in Gel electrophoresis and visualized with Syngene Gel documentation system. Further, the amplicon patterns obtained were analyzed for polymorphism.

4.5.2.1 Details on amplification of RAPD region in genomic DNA of *Lipaphis erysimi* collected from different locations

Genetic diversity analysis of *Lipaphis erysimi* was carried out using 10 RAPD primers and informativeness of markers was determined by calculating Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Marker Index (MI) and Resolving Power (RP).

RAPD polymorphism

All the ten RAPD primers were amplified with polymorphic percentage ranging from 42.86 (OPA-13) to 100.00 (OPR-07, F2, H9) yielding a total of 60 bands which varied from three (H9) to ten (OPA-02) from all the locations. Whereas, the number of monomorphic bands varied from zero (OPR-07, F2, H9) to four (OPA-13). The mean number of bands and polymorphic bands per primer were 5.54 and 4.81 respectively (Table 4.1). Similarly, Vanlerberghe-Masutti and Chavigny (1998) identified a total of 37 polymorphic bands in *Aphis gossypii*. According to Chen *et al.* (2009), the number of RAPD bands generated per primer varied from 2 to 12, with an average of six bands per primer and 72 monomorphic bands out of 178 total bands.

The PIC of 10 primers varied from zero to 0.69 with an average of 0.44. The minimum PIC value was observed in OPA-10 (0.29) and maximum of 0.69 in OPA-04, specifies high allelic variations.

Table 4.1: Details on amplification of RAPD region in genomic DNA of *Lipaphis erysimi*

Sl. No	Primers	T(°C)	TB	PB	MB	PPB (%)	PIC	EMR	MI	RP
1	OPA-02	37.2	10	09	01	90.00	0.67	8.10	5.42	2.74
2	OPA-04	37.2	08	07	01	87.50	0.69	6.13	4.26	2.86
3	OPA-10	37.2	06	03	03	50.00	0.29	1.50	0.43	1.13
4	OPA-11	37.2	05	04	01	80.00	0.51	4.00	2.05	0.86
5	OPA-13	37.2	07	03	04	42.86	0.31	1.29	0.39	0.87
6	OPA-20	37.2	07	05	02	71.43	0.41	3.57	1.47	2.49
7	OPR-07	37.2	06	06	00	100.00	0.56	6.00	3.38	2.25
8	F2	37.2	08	08	00	100.00	0.66	8.00	5.31	4.12
9	F12	37.2	06	05	01	83.33	0.31	4.17	1.31	2.25
10	H9	37.2	03	03	00	100.00	0.41	3.00	1.23	0.37
	Total	-	60	53	-	-	-	-	-	-
	Mean	-	5.54	4.81	-	-	0.44	4.16	2.29	1.81

Note: T (°C): Annealing temperature, TB: Total Band, PB: Polymorphic Band, MB: Monomorphic Band, PPB (%): Percentage of Polymorphic Band, PIC: Polymorphism Information Content, EMR: Effective Multiplex Ratio, MI: Marker Index, RP: Resolving Power.

The EMR values of RAPD primers ranged from 1.29 to 8.10 with a mean value of 4.16. The maximum EMR was observed in OPA-02 (8.10), followed by F2 (8.00) and minimum in OPA-13 (1.29) followed by 1.50 in OPA-10. The MI values ranged from 0.39 to 5.42 with a mean of 2.29. The maximum MI was observed in OPA-02 (5.42), followed by F2 (5.31) and minimum in OPA-13 (0.39), followed by 0.43 in OPA-10. The RP values ranged from 0.37 to 4.12 with a mean of 1.81. The maximum RP was observed in F2 (4.12) and minimum in H9 (0.37) followed by 0.86 in OPA-11.

The above primers *viz.*, OPA-02, OPA-13, OPA-20 were also used to study the genetic relationship of various aphid species by Clements *et al.* (2000). Among 10 primers evaluated OPA-02, OPA-04, OPA-11, OPA-20, OPR-07, F2, F12 and H9 were informative, with more than 70.00 polymorphic band percentage. Similarly, Quieroz *et al.* (2017) found more than 70 % polymorphism in OPA-11 (73.8) and considered it as informative. Based on higher PIC, EMR, MI and RP; OPA-02, OPA-04 and F2 were shown to have greater informativeness than other primers (OPA-11, OPA-20, OPR-07, F12 and H9).

Genetic relationship of *Lipaphis erysimi* in different locations using RAPD markers

A genetic dissimilarity dendrogram and Principal Component Analysis (PCA) were created using DARwin 6 software to compute comparative amplification data of ten RAPD primers (Figure 4.1). The dendrogram yielded six clusters *i.e.*, C1Aia α , C1Aia β , C1Aib, C1Aii, C1B, C2. The major clusters, C1 and C2 were found to be 30.0 % dissimilar to each other. Whereas, cluster C1 was subdivided into C1A and C1B with 29.0 % dissimilarity. Further, cluster C1A was subdivided into C1Ai and C1Aii with 27.0 % dissimilarity and cluster C1Ai was subdivided into C1Aia and C1Aib with 26.0 % dissimilarity. Whereas, cluster C1Aia was subdivided into C1Aia α and C1Aia β with 23.0 % dissimilarity.

The first cluster C1Aia α contained six locations *i.e.*, Dariapur, Mohanpur, Jhansi, Imphal, Ludhiana, Alipur Bihta. Among which Alipur Bihta shared 21.0 % dissimilarity with Dariapur, Mohanpur, Jhansi, Imphal and Ludhiana. Whereas, Ludhiana shared 19.0 % dissimilarity with Dariapur, Mohanpur, Jhansi, Imphal. Further, Imphal shared 18.0 % dissimilarity with Dariapur, Mohanpur and Jhansi. However, Dariapur, Mohanpur and Jhansi shared lesser dissimilarity percentage of 17.0. The second cluster C1Aia β contained only one location, Dholi which shared 23.0 % dissimilarity with cluster C1Aia α . The third cluster C1Aib contained two locations *i.e.*, Chintapalli, and Pantnagar with 22.0 % dissimilarity. The fourth cluster, C1Aii contained Ranchi, Jodhpur, Barapani. Of these Barapani shared 25.0 % dissimilarity with Ranchi and Jodhpur which shared 20.0 % dissimilarity. The fifth cluster C1B contained Delhi, Raipur and Dharwad. Among which, Dharwad shared 28.0 % dissimilarity with Delhi and Raipur. Whereas, Delhi and Raipur shared 24.0 % dissimilarity. The sixth cluster C2 of Jorhat location sharing 30.0 % dissimilarity with C1.

The above results showed that Dariapur and Mohanpur which belong to Eastern region of India were found to share 17.0 % (lower) dissimilarity (Figure 4.1). Chintapalli and Pantnagar from Southern and Northern India were grouped within the same cluster with 22.0 percent dissimilarity. Interestingly, Ranchi of Eastern and Jodhpur of Western India formed in the same cluster sharing 20.0 percent dissimilarity (Figure 4.1). Further, Jorhat of North Eastern India was found to be highly diverged (30.0 % dissimilarity) from other locations and observed with higher branch distance (0.13) in the phylogenetic tree (Figure 4.1).

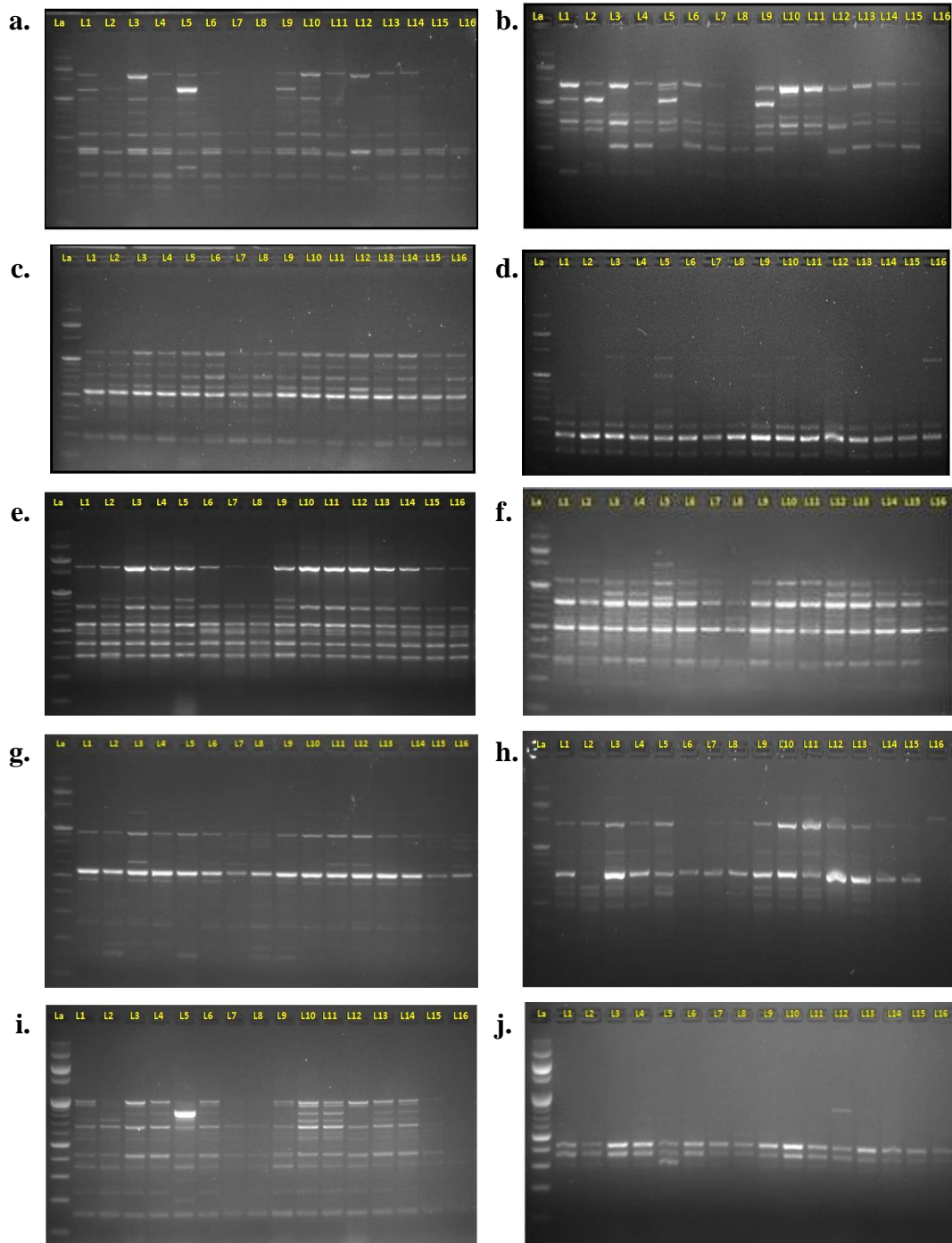
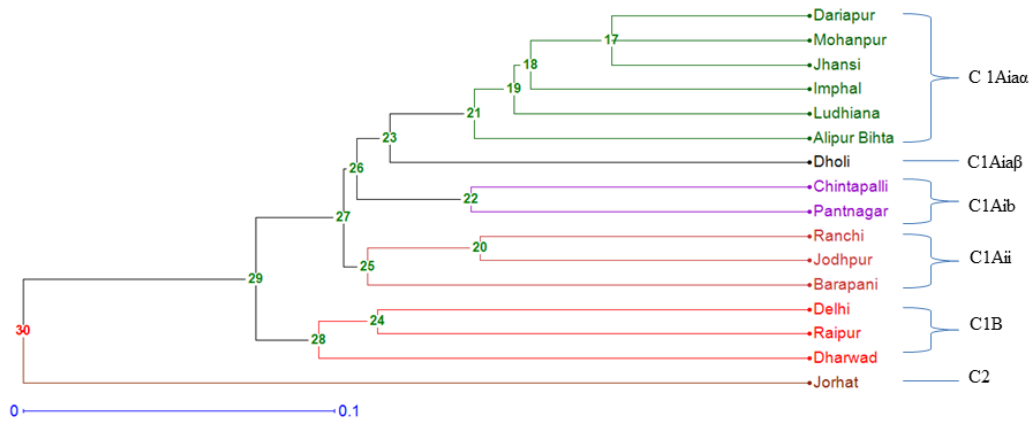


Plate 4.2: Random Amplified Polymorphic DNA fragments generated from *Lipaphis erysimi* collected from 16 locations (L1-L16)
 a. OPA-02 b. OPA-04 c. OPA-10 d. OPA-11 e. OPA-13
 f. OPA-20 g. OPR-07 h. F2 i. F12 j. H9

a)



b)

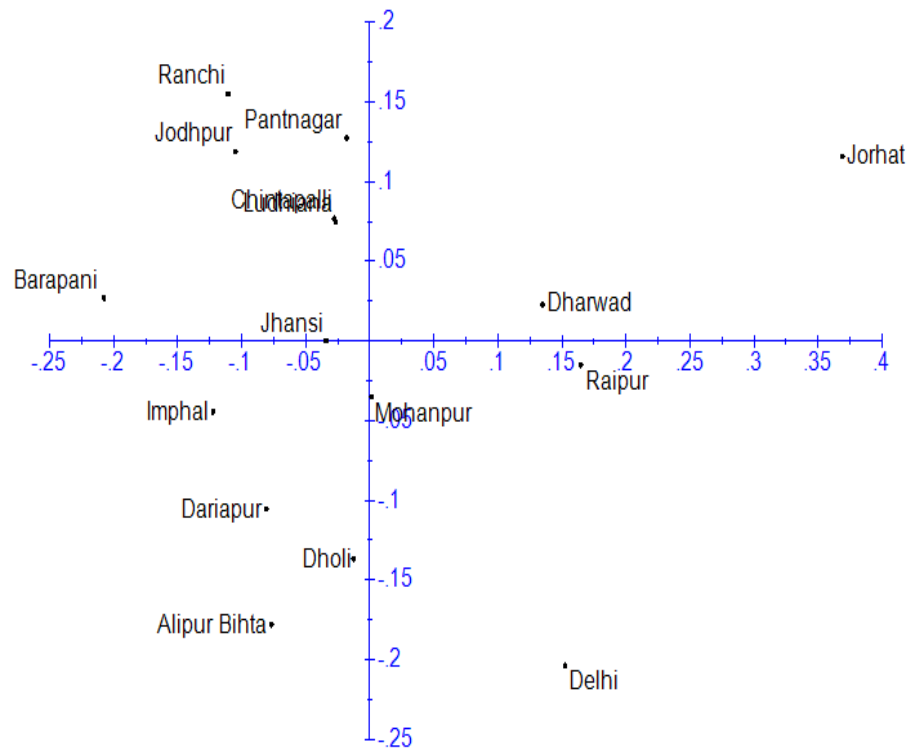


Figure 4.1: Dendrogram inferred from matrix of pair wise distances in RAPD analysis of *Lipaphis erysimi* from different locations using UPGMA a) Dendrogram b) PCA

Principal Component Analysis

The dendrogram was well supported by Principal Component Analysis (PCA), in which Ranchi, Jodhpur (C1Aii) formed together in first quadrant and Jorhat, Dharwad, Raipur, Delhi are situated at very distance in the second and fourth quadrant. The locations present on the left side of the perpendicular line are sharing a lower dissimilarity percentage whereas on the right side of perpendicular line are sharing a higher dissimilarity percentage which are reliable with the clusters formed in the dendrogram.

4.5.3 Details on amplification of SSR region in genomic DNA of *Lipaphis erysimi* from different locations

Genetic diversity analysis of *Lipaphis erysimi* was carried out using nine SSR primers and informativeness of markers was determined by calculating Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Marker Index (MI) and Resolving Power (RP).

SSR polymorphism

All the SSR primers were amplified with polymorphic percentage ranging from zero (ApH12M) to 100.00 (ApH04M, ApH08M, Ap-01 and Ap-05) yielding a total of 32 bands which varied from one (ApH12M, Ap-03) to seven (Ap-01). Whereas, the number of monomorphic bands varied from zero (ApH04M, ApH08M, Ap-01, Ap-05) to one (ApF08M, ApG10M, ApH05M, ApH12M, Ap-03). The mean number of total bands and polymorphic bands per primer were 3.55 and 3.00 respectively (Table 4.2). Similarly, Jun *et al.* (2012) used SSR markers and observed 26 polymorphic bands in *Aphis glycines*.

The PIC determines informativeness of primer. The PIC of nine primers varied from 0.00 to 0.87 with a mean of 0.39. The minimum PIC was observed in ApH12M (0.00) and maximum value of 0.87 in ApH04M, representing high allelic variations. The EMR determines the fraction of polymorphic fragments. The EMR values of primers ranged from 0.00 to 7.00 with a mean value 2.32. The maximum EMR was observed in Ap-01 (7.00), followed by 4.00 in ApH04M, Ap-05 and minimum (0.00) in ApH12M, Ap-03 followed by 1.00 in ApH08M. The MI characterizes the capacity of primer to detect polymorphic loci. The MI values ranged from 0.00 to 5.05 with an mean of 1.41. The maximum MI was observed in Ap-01 (5.05), followed by

APH04M (3.46) and minimum in ApH12M, Ap-03 (0.00), followed by ApH05M (0.41).

Table 4.2: Details on amplification of SSR region in genomic DNA of *Lipaphis erysimi*

Sl. No	Primers	T(°C)	TB	PB	MB	PPB (%)	PIC	EMR	MI	RP
1	ApF08M	56.0	03	02	01	66.66	0.35	1.33	0.46	1.25
2	ApG10M	56.0	04	03	01	75.00	0.27	2.25	0.60	1.25
3	ApH04M	56.0	04	04	00	100.00	0.87	4.00	3.46	1.25
4	ApH05M	56.0	03	02	01	66.66	0.31	1.33	0.41	1.13
5	ApH08M	52.0	05	05	00	100.00	0.54	1.00	0.54	2.88
6	ApH12M	56.0	01	00	01	00.00	0.00	0.00	0.00	0.00
7	Ap-01	56.0	07	07	00	100.00	0.72	7.00	5.05	2.62
8	Ap-03	56.0	01	00	01	00.00	0.00	0.00	0.00	0.00
9	Ap-05	56.0	04	04	00	100.00	0.53	4.00	2.10	1.50
	Total	-	32	27	-	-	-	-	-	-
	Mean	-	3.55	3.00	-	-	0.39	2.32	1.41	1.32

Note: T (°C): Annealing temperature, TB: Total Band, PB: Polymorphic Band, MB: Monomorphic Band, PPB (%): Percentage of Polymorphic Band, PIC: Polymorphism Information Content, EMR: Effective Multiplex Ratio, MI: Marker Index, RP: Resolving Power

The RP indicates the discriminatory potential of the primers. RP values ranged from 0.00 to 2.88 with a mean of 1.32. The maximum RP values were observed in ApH08M (2.88) and minimum in Ap-03 and ApH12M (0.00).

The above primers *viz.*, ApF08M, ApG10M, ApH04M, ApH05M, ApH08M, ApH12M, Ap-01, Ap-03, Ap-05 were also been used by Caillaud *et al.* (2004) and Kurokawa *et al.* (2004) to examine the genetic relationship in *Acyrtosiphon pisum*. SSR primers (ApG10M, ApH04M, ApH08M, Ap-01, Ap-05) recorded more than 70 % polymorphism were found to be informative and having a potential use in the generation of molecular profiles of *Lipaphis erysimi*. ApH04M, Ap-01, ApH08M were revealed to have greater informativeness than other primers (ApF08M, ApG10M, ApH05M, ApH12M, Ap-03, Ap-05) based on higher PIC, EMR, MI and RP.

Genetic relationship of *Lipaphis erysimi* in different locations using SSR markers

A genetic dissimilarity dendrogram and Principal Component Analysis (PCA) were created using DARwin 6 software to compute comparative amplification data of nine SSR primers (Figure 4.2). The dendrogram yielded seven clusters *i.e.*, C1Aia α , C1Aia β , C1Aib α , C1Aib β , C1Aii, C1B, C2. The major clusters, C1 and C2 were found to be 31.0 % dissimilar to each other. Whereas, cluster C1 was subdivided into C1A and C1B with 30.0 % dissimilarity. Further, cluster C1A was subdivided into C1Ai and C1Aii with 29.0 % dissimilarity and cluster C1Ai was subdivided into C1Aia and C1Aib with 28.0 % dissimilarity. Cluster C1Aia was subdivided into C1Aia α and C1Aia β with 27.0 % dissimilarity. Whereas, cluster C1Aib was subdivided into C1Aib α and C1Aib β with 24.0 % dissimilarity.

The first cluster C1Aia α contained Imphal, Barapani, Chintapalli and Jhansi sharing 25.0 % dissimilarity. Among which Jhansi and Chintapalli; Imphal and Barapani shared 19.0 and 20.0 % dissimilarity respectively. The second cluster, C1Aia β contained Mohanpur and Ludhiana with 23.0 % dissimilarity and shared 27.0 % dissimilarity with C1Aia. The third cluster, C1Aib α contained Dariapur, Ranchi, Dholi. Among which Dariapur, Ranchi shared 17.0 % dissimilarity with each other and 22.0 % with Dholi. The fourth cluster, C1Aib β contained Jodhpur and Pantnagar with 21.0 % dissimilarity. The fifth cluster, C1Aii contained Delhi, Alipur Bihta and Raipur. Among these Delhi and Alipur Bihta shared 18.0 % dissimilarity with each other and 26.0 % with Raipur. The sixth cluster, C1B with Dharwad has shared 30.0 % dissimilarity with C1A. Further, the seventh cluster, C2 with Jorhat has shared 31.0 % dissimilarity with C1 cluster.

The above results showed that, Imphal and Barapani, which belongs to North Eastern region of India were found to share 20.0 % dissimilarity and were also clustered in the phylogenetic tree (Figure 4.5). Whereas, Dariapur, Dholi and Ranchi belongs to eastern India were grouped within the same cluster with 22.0 percent dissimilarity. Interestingly, Chintapalli of Southern India and Jhansi of Northern India formed in the same cluster sharing 19.0 percent dissimilarity (Figure 4.2). Among all the locations surveyed, Jorhat of North-Eastern India was found to be highly diverged (31.0 % dissimilarity) from other locations and noted with higher branch distance (0.13) in the phylogenetic tree (Figure 4.2).

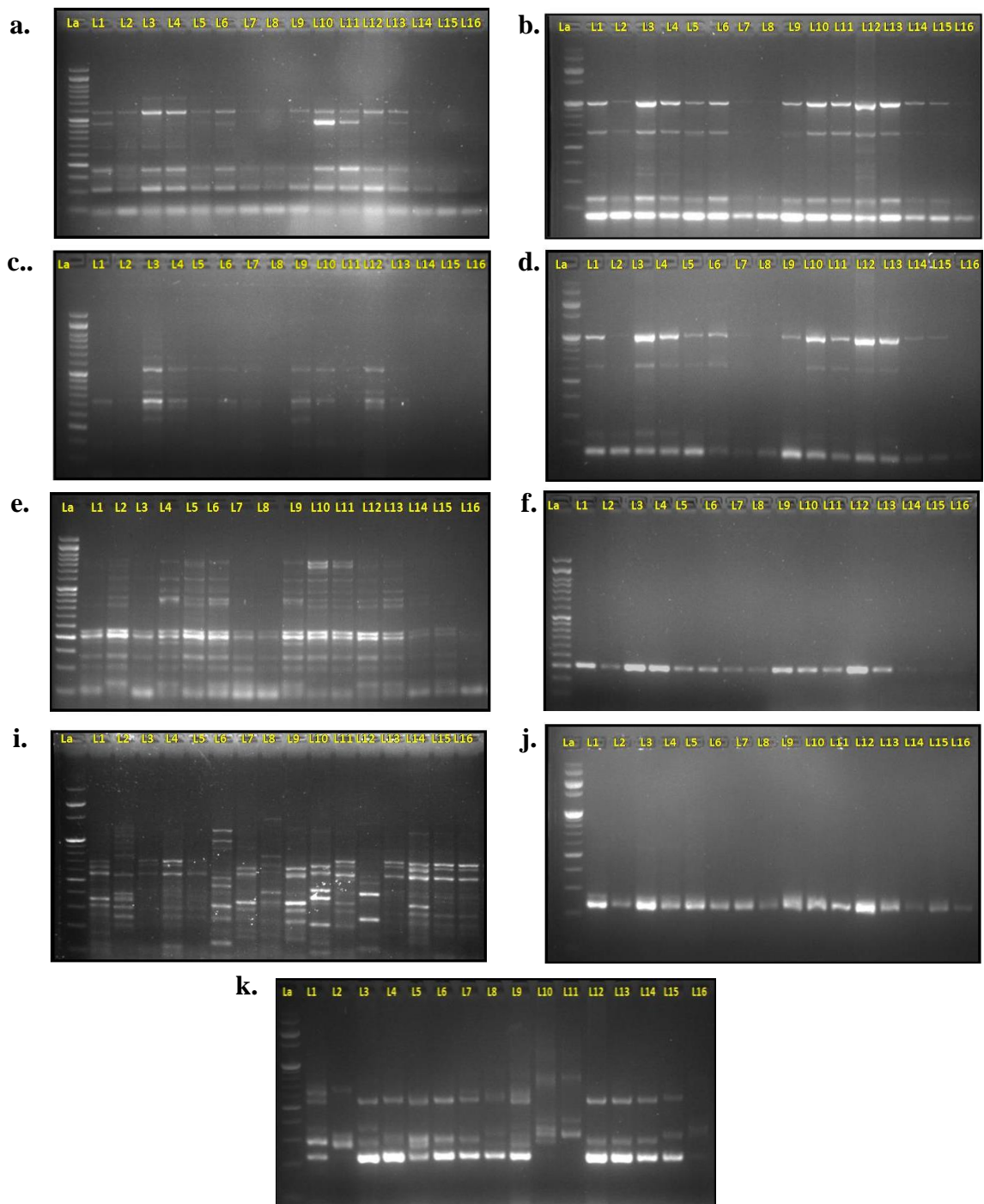
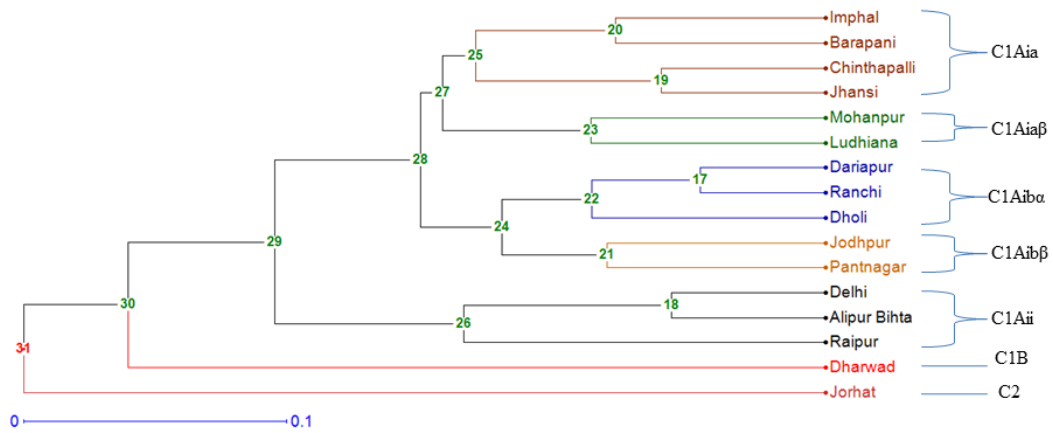


Plate 4.3 : Simple Sequence Repeats generated from genomic DNA of *Lipaphis erysimi* collected from 16 locations (L1-L16)

a. ApF08M **b.** ApG10M **c.** ApH04M **d.** ApH05M **e.** ApH08M
f. ApH12M **g.** Ap-01 **h.** Ap-03 **i.** Ap-05

a)



b)

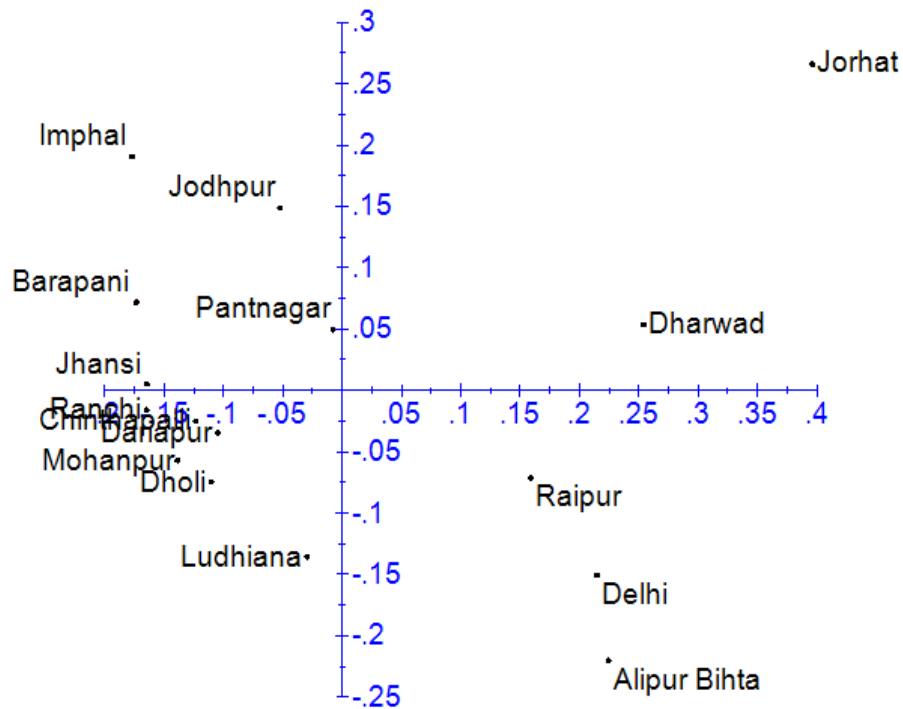


Figure 4.2: Dendrogram inferred from matrix of pair wise distances in SSR analysis of *Lipaphis erysimi* from 16 locations using UPGMA a) Dendrogram b) PCA

In the present study the genetic variations observed through RAPD and SSR analysis between geographical populations separated by longitudinal and elevational range may be due to the local thermal regimes, insecticide usage, migration and some abiotic and biotic factors. Similarly, Wang *et al.* (2017) observed a genetic variation among the cotton aphid populations from different locations of China and anticipated that host plant specialization, insecticide usage and climate change could be the reason for the genetic variation among the populations. Recently Reddy *et al.* (2022) observed the genetic variation among the *H. armigera* populations of different locations in Uttarakhand and assumed that these variations could be due to adaptation to local thermal regimes besides the latitudinal and altitudinal range of species.

Principal Component Analysis

The dendrogram was well supported by Principal Component Analysis (PCA), in which Ranchi, Dariapur, Dholi (C1Aib α) formed together in third quadrant and Jorhat, Dharwad, Raipur, Delhi, Alipur bihta are situated at very distance in the second and fourth quadrant. The locations present on the left side of the perpendicular line are sharing a lower dissimilarity percentage whereas on the right side of perpendicular line are sharing a higher dissimilarity percentage which are reliable with the clusters formed in the dendrogram.

4.5.4 Molecular identification of *Lipaphis erysimi* through DNA barcoding of mitochondrial cytochrome oxidase I (*mtCOI*) gene

The molecular identification of collected samples from 16 locations was investigated using *mtCOI* (LCO1490- Forward and RHC02- Reverse) primer. The primer amplified 625 bp (Plate 4.7) fragment of the mitochondrial cytochrome oxidase I (*mtCOI*) gene. BLASTN search results of the sequences of 16 locations obtained from Dideoxy Cycle sequencing confirmed the sub-species identity of *Lipaphis erysimi pseudobrassicae* with 99 % similarity. Further, the sequences were submitted to NCBI GenBank nucleotide sequence databases and received the accession numbers (Table 4.4). The DNA barcodes of *L. e. pseudobrassicae* from 16 locations were given in the table 4.3. Similar primer was used by Hebert *et al.* (2003) and amplified the *mtCOI* gene of several insects.

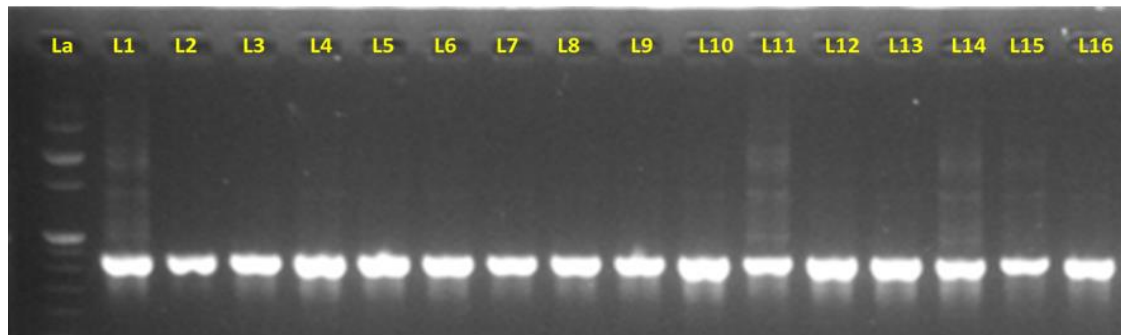


Plate 4.4: Gel picture of *Lipaphis erysimi* mitochondrial cytochrome oxidase I (*mtCOI*) gene from 16 locations (L1-L16)

L1:Pantnagar L2: Ludhiana L3: Jodhpur L4: Jhansi L5: Ranchi L6: Mohanpur
 L7: Raipur L8: Dharwad L9: Chintapalli L10: Barapani L11: Imphal L12: Dholi
 L13: Dariapur L14: Alipur Bihta L15: Delhi L16: Jorhat

Table 4.3: DNA barcodes of *Lipaphis erysimi pseudobrassicae* from 16 locations

Sl. No	Location	DNA Barcode
1.	Pantnagar	
2.	Ludhiana	
3.	Jodhpur	
4.	Jhansi	
5.	Ranchi	
6.	Mohanpur	
7.	Raipur	
8.	Dharwad	
9.	Chintapalli	
10.	Barapani	
11.	Imphal	
12.	Dholi	
13.	Dariapur	
14.	Alipur Bihta	
15.	Delhi	
16.	Jorhat	



Table 4.4 Accession numbers received from NCBI, GenBank for *L. e. pseudobrassicae* of 16 locations

Sl. No.	Locations	Accession numbers
1	Pantnagar	OK001958
2	Ludhiana	OK087344
3	Jodhpur	OK087345
4	Jhansi	OK087346
5	Ranchi	OK087347
6	Mohanpur	OK087348
7	Raipur	OK087349
8	Dharwad	OK087350
9	Chintapalli	OK087351
10	Barapani	OK087352
11	Imphal	OK087353
12	Dholi	OK087354
13	Dariapur	OK087355
14	Alipur Bihta	OK087356
15	Delhi	OK087357
16	Jorhat	OK087358

4.5.4.1 Multiple sequence alignments of partial nucleotide sequence of *Lipaphis erysimi pseudobrassicae*

Multiple sequence alignment of nucleotides was performed using Clustal Omega software. The nucleotide sequences aligned were used to study the evolutionary relationship of *L. e. pseudobrassicae* from 16 locations by constructing a phylogenetic tree.

Multiple alignments of nucleotide sequences obtained in this study identified 438 identical nucleotides fully conserved residues (*) and 141 Single Nucleotide Polymorphisms (SNPs) (Figure 4.3). Whereas in the multiple sequence alignment of amino acid sequences, we found 114 fully conserved residues (*), 52 conserved amino acid residues (.) and 23 semi-conserved amino acid residues (:.) (Figure 4.4). This higher number of nucleotide similarity indicates that they evolved from a common origin and also SNPs can be used for differentiating the haplotypes. Similarly, Wang *et al.* (2016) obtained five single-nucleotide polymorphisms with the ability to distinguish two apterous biotypes of *Aphis gossypii* collected from cotton and cucumber in China.

Lipaphis_erysimi_pseudobrassicaceae_Barapani_OK087352	-----CACAAATCATAAAGATATAGGAACCTTATACTCTTATTTGGAAATTTGGTTAG	53
Lipaphis_erysimi_pseudobrassicaceae_Imphal_OK087353	-----TACAAATCATAATGATATGGAGGAATATACATATATTTGGAAAGTAGATCAT	53
Lipaphis_erysimi_pseudobrassicaceae_Jorhat_OK087358	-----AACAAAATATAAAGATATAGGAACCTTATACTCTTATTTGGAAATTTGGTTAG	53
Lipaphis_erysimi_pseudobrassicaceae_Mohanpur_OK087348	-----AACAAATCATAAAAATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Ranchi_	-----AACAAATCATAAAGATATTTGGAACTTTATACATCTTATTTGGATCAACAAA	53
Lipaphis_erysimi_pseudobrassicaceae_Jhansi_OK087346	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Alipur_Bihta_OK087356	-----AACAAATCATAAAGATATTTGGAACTTTATACATCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Ludhiana_OK087344	-----ACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	52
Lipaphis_erysimi_pseudobrassicaceae_Jodhpur_OK087345	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Pantnagar_OK001958	TTTGGTCAACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	60
Lipaphis_erysimi_pseudobrassicaceae_Raipur_OK087349	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Delhi_OK087357	-----AACAAATATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Dharwad_OK087350	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Chintapalli_OK087351	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Dholi_OK087354	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
Lipaphis_erysimi_pseudobrassicaceae_Dariapur_OK087355	-----AACAAATCATAAAGATATTTGGAACTTTATACTCTTATTTGGAAATTTGATCAG	53
	*** * **** * * * * * * * * * * * * * * * * *	
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Lipaphis_erysimi_pseudobrassicaceae_Imphal_OK087353	GTAGAATCGGATCATCTCCAAGAAATTTAATTCGTTGAGAAATTAAGACAAATTAAGGCCAA	113
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Lipaphis_erysimi_pseudobrassicaceae_Ranchi_	GTATAATCGGATCATCTCTAGAATTTAATTCGTTTAGAATTAAGACAAATTAATCCAA	113
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Lipaphis_erysimi_pseudobrassicaceae_Chintapalli_OK087351	--TNHKDGTLYLFLFGIWSGMIGSSLSILIRLELSQNSININNNQLYNVDITPAFIMIF	58
Lipaphis_erysimi_pseudobrassicaceae_Dholi_OK087354	--TNHKDGTLYLFLFGIWSGMIGSSLSILIRLELSQNSININNNQLYNVDITPAFIMIF	58
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Lipaphis_erysimi_pseudobrassicaceae_Imphal_OK087353	--TNHNDIGMNYMLFGSSSCSISGSSPSILIRNVEVSRKASINDNHQYNDVATHPAIVMI	58
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	**.*:*:*.* ** **.* **	
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Lipaphis_erysimi_pseudobrassicaceae_Jorhat_OK087358	IYPLSNNFHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Ranchi_	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
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Lipaphis_erysimi_pseudobrassicaceae_Raipur_OK087349	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Delhi_OK087357	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Jhansi_OK087346	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Pantnagar_OK001958	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	180
Lipaphis_erysimi_pseudobrassicaceae_Ludhiana_OK087344	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Daripour_OK087355	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Jodhpur_OK087345	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Dharwad_OK087350	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Chintapalli_OK087351	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Dholi_OK087354	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Barapani_OK087352	MYPLLSHNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
Lipaphis_erysimi_pseudobrassicaceae_Imphal_OK087353	IYPLSNNIAHNNISVDLIFSLHLAGISSILGAINFICTILNMMPNMKLNQIPLFPWS	178
	:** **.*:*:*:* ** **.* **	
Lipaphis_erysimi_pseudobrassicaceae_Mohanpur_OK087348	ILITISILLNLFPLLAGAITMLNLRKLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Jorhat_OK087358	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Ranchi_	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Alipur_Bihta_OK087356	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Raipur_OK087349	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Delhi_OK087357	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Jhansi_OK087346	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Pantnagar_OK001958	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	227
Lipaphis_erysimi_pseudobrassicaceae_Ludhiana_OK087344	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Daripour_OK087355	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Jodhpur_OK087345	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Dharwad_OK087350	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Chintapalli_OK087351	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Dholi_OK087354	ILITAIILILSLPVLGAGAITMLLTDRLNLTSTFFPAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Barapani_OK087352	ILITGFFLILFLPVLGAGNTMLMDRKLNTSFLFELAGGGDPYLFQP-	224
Lipaphis_erysimi_pseudobrassicaceae_Imphal_OK087353	ILNTGILLILFLPVLGAGNTMLMDRKLNTSFLFELAGGGDPYLFQP-	224
	** *..* * *.* **.* **.* **.* **.* **.* **.* **.* **.* **.* **.* **.*	

Figure 4.4: Multiple sequence alignment of partial amino acid sequence of *L. e. pseudobrassicaceae* from 16 locations

[*] Fully conserved residue, [:] Conserved residue, [.] Semi conserved residue.

4.5.5 Phylogenetic analysis of *L. e. pseudobrassicae* from 16 locations using partial mitochondrial cytochrome oxidase I (*mtCOI*) gene

Phylogenetic analysis of *L. e. pseudobrassicae* was done using partial mitochondrial cytochrome oxidase I (*mtCOI*) gene in MEGA X software. For the best nucleotide substitution pattern, the model with lowest Bayesian Information Criterion (BIC) *i.e.*, Tamura 3-parameter with Gamma distribution (T92+G) of BIC (7774.347) was chosen (Table 4.5) for constructing the phylogenetic tree. Discrete Gamma distributions (+G) were used to model non-uniformity of evolutionary rates across invariable evolutionarily sites (+I). The study included 24 nucleotide sequences, for a totality of 675 positions in the ultimate dataset. Similar to our studies, Rakauskas *et al.* (2014) chosen Tamura 3-parameter model with Gamma distribution (T92+G) for *Rhopalosiphum padi* and *Rhopalosiphum nymphaeae* mitochondrial (*COI*) variability studies in Lithuania.

4.5.5.1 Phylogenetic analysis among *L. e. pseudobrassicae* of 16 locations

Phylogenetic analysis of *L. e. pseudobrassicae* was performed using 24 sequences of *mtCOI* *viz.*, final sequences of *L. e. pseudobrassicae* from 16 locations along with one reference sequence of *L. e. pseudobrassicae* and seven out groups *i.e.*, *Schizaphis graminum*; *Rhopalosiphum maidis*; *Rhopalosiphum padi*; *Melanaphis japonica*; *Cinara longipennis*; *Brevicoryne brassicae*; *Myzus persicae* with 675 positions in the ultimate dataset (Figure 4.5). Phylogenetic tree was constructed using Tamura 3-parameter with Gamma distribution (T92+G) and the evolutionary distances were estimated using the Maximum Composite Likelihood approach.

The phylogenetic tree showed two major clusters of *mtCOI* sequences of 16 locations and 7 out groups. First cluster comprised of the sequences of all the 16 locations *i.e.*, Pantnagar, Ludhiana, Jodhpur, Jhansi, Ranchi, Mohanpur, Raipur, Dharwad, Chintapalli, Barapani, Imphal, Dholi, Dariapur, Alipur Bihta, Delhi and Jorhat along with reference sequence of *L. e. pseudobrassicae* GU668619. Out of these, Dariapur and Delhi; Jodhpur and Jhansi formed together in one sub cluster. Barapani, Imphal, Jorhat of North-eastern India and Mohanpur of Eastern

Table 4.5: Maximum Composite Likelihood fits of 24 different nucleotide substitution models of *L. e. pseudobrassicae* of 16 locations

Model	Parameters	BIC	AICc	<i>lnL</i>	(+I)	(+G)	<i>R</i>	<i>f</i> (A)	<i>f</i> (T)	<i>f</i> (C)	<i>f</i> (G)	<i>r</i> (AT)	<i>r</i> (AC)	<i>r</i> (AG)	<i>r</i> (TA)	<i>r</i> (TC)	<i>r</i> (TG)	<i>r</i> (CA)	<i>r</i> (CT)	<i>r</i> (CG)	<i>r</i> (GA)	<i>r</i> (GT)	<i>r</i> (GC)
T92+G	48	7774.347	7406.767	-3655.234	n/a	0.85	0.96	0.375	0.375	0.125	0.125	0.082	0.027	0.070	0.082	0.070	0.027	0.082	0.211	0.027	0.211	0.082	0.027
T92+G+I	49	7778.660	7403.429	-3652.558	0.00	0.85	0.96	0.375	0.375	0.125	0.125	0.082	0.027	0.070	0.082	0.070	0.027	0.082	0.211	0.027	0.211	0.082	0.027
HKY+G	50	7782.367	7399.484	-3649.580	n/a	0.85	0.95	0.349	0.401	0.140	0.110	0.089	0.031	0.061	0.078	0.077	0.025	0.078	0.223	0.025	0.194	0.089	0.031
TN93+G	51	7785.032	7394.498	-3646.080	n/a	0.88	0.95	0.349	0.401	0.140	0.110	0.090	0.031	0.047	0.078	0.091	0.025	0.078	0.263	0.025	0.149	0.090	0.031
HKY+G+I	51	7786.420	7395.887	-3646.774	0.00	0.85	0.95	0.349	0.401	0.140	0.110	0.089	0.031	0.061	0.078	0.077	0.025	0.078	0.222	0.025	0.194	0.089	0.031
TN93+G+I	52	7789.048	7390.863	-3643.256	0.00	0.88	0.95	0.349	0.401	0.140	0.110	0.090	0.031	0.047	0.078	0.091	0.025	0.078	0.263	0.025	0.149	0.090	0.031
T92+I	48	7790.289	7422.710	-3663.205	0.35	n/a	0.95	0.375	0.375	0.125	0.125	0.083	0.028	0.070	0.083	0.070	0.028	0.083	0.209	0.028	0.209	0.083	0.028
HKY+I	50	7798.176	7415.293	-3657.484	0.35	n/a	0.94	0.349	0.401	0.140	0.110	0.090	0.031	0.061	0.078	0.077	0.025	0.078	0.221	0.025	0.193	0.090	0.031
TN93+I	51	7799.218	7408.684	-3653.173	0.34	n/a	0.94	0.349	0.401	0.140	0.110	0.091	0.032	0.046	0.079	0.092	0.025	0.079	0.263	0.025	0.146	0.091	0.032
GTR+G	54	7804.742	7391.256	-3641.439	n/a	0.91	0.95	0.349	0.401	0.140	0.110	0.107	0.020	0.048	0.093	0.094	0.024	0.050	0.269	0.025	0.153	0.086	0.031
GTR+G+I	55	7808.733	7387.598	-3638.602	0.00	0.91	0.95	0.349	0.401	0.140	0.110	0.107	0.020	0.048	0.093	0.094	0.024	0.051	0.269	0.024	0.153	0.086	0.031
GTR+I	54	7819.053	7405.567	-3648.594	0.34	n/a	0.95	0.349	0.401	0.140	0.110	0.108	0.021	0.047	0.094	0.094	0.023	0.053	0.271	0.023	0.150	0.085	0.030
T92	47	7877.959	7518.032	-3711.872	n/a	n/a	0.84	0.375	0.375	0.125	0.125	0.088	0.029	0.066	0.088	0.066	0.029	0.088	0.198	0.029	0.198	0.088	0.029
TN93	50	7882.731	7499.848	-3699.762	n/a	n/a	0.85	0.349	0.401	0.140	0.110	0.096	0.033	0.043	0.084	0.088	0.026	0.084	0.254	0.026	0.136	0.096	0.033
HKY	49	7891.691	7516.459	-3709.074	n/a	n/a	0.84	0.349	0.401	0.140	0.110	0.095	0.033	0.058	0.083	0.073	0.026	0.083	0.211	0.026	0.183	0.095	0.033
GTR	53	7893.858	7488.023	-3690.829	n/a	n/a	0.85	0.349	0.401	0.140	0.110	0.121	0.021	0.045	0.105	0.092	0.023	0.054	0.265	0.020	0.142	0.085	0.025
K2+G	47	8043.815	7683.888	-3794.800	n/a	1.05	1.20	0.250	0.250	0.250	0.250	0.057	0.057	0.136	0.057	0.136	0.057	0.057	0.136	0.057	0.136	0.057	0.057
JC+G	46	8051.797	7699.521	-3803.623	n/a	1.07	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K2+I	47	8055.454	7695.526	-3800.619	0.32	n/a	1.12	0.250	0.250	0.250	0.250	0.059	0.059	0.132	0.059	0.132	0.059	0.059	0.132	0.059	0.132	0.059	0.059
JC+G+I	47	8055.843	7695.915	-3800.814	0.00	1.07	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K2+G+I	48	8058.419	7690.840	-3797.270	0.00	1.05	1.29	0.250	0.250	0.250	0.250	0.055	0.055	0.141	0.055	0.141	0.055	0.055	0.141	0.055	0.141	0.055	0.055
JC+I	46	8066.977	7714.701	-3811.213	0.32	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K2	46	8107.061	7754.786	-3831.255	n/a	n/a	1.08	0.250	0.250	0.250	0.250	0.060	0.060	0.130	0.060	0.130	0.060	0.060	0.130	0.060	0.130	0.060	0.060
JC	45	8119.695	7775.072	-3842.404	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083

Note: TR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor; *lnL*: Maximum Likelihood value; BIC: Bayesian Information Criterion; AICc: Akaike Information Criterion, corrected.

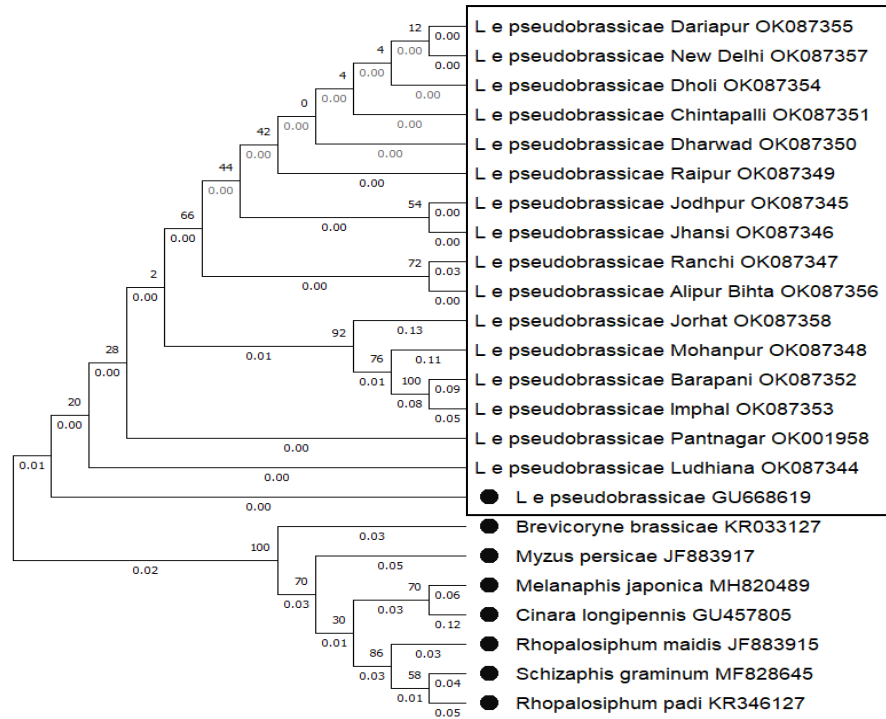
India are formed together in another sub cluster. Ranchi and Alipur Bihta formed in one sub cluster belongs to Jharkhand and Bihar respectively. Whereas all the out groups *i.e.*, *Rhopalosiphum padi*, *Schizaphis graminum*, *Rhopalosiphum maidis*, *Melanaphis japonica*, *Cinara longipennis*, *Myzus persicae* and *Brevicoryne brassicae* are formed into second cluster. These genetic variations between the populations of different geographical locations could be due to latitudinal and altitudinal range of species along with insecticide usage, migration, abiotic and biotic factors. Similarly, Wang *et al.* (2017) in cotton aphid and Reddy *et al.* (2021) in *H. armigera* observed the genetic variations through partail *mtCOI* gene analysis and also assumed that these genetic variations could be due to latitudinal, altitudinal range of species, insecticide usage, and adaptations to local abiotic conditions.

Patterns of nucleotide substitution in *L. e. pseudobrassicae* of 16 locations

The Maximum Composite Likelihood estimate of nucleotide substitution patterns for sequences was presented using MEGA X (Kumar *et al.*, 2018). This analysis involved 24 nucleotide sequences with 675 positions in the ultimate dataset. Transitional substitution rates are bolded, while transversional substitution rates are in italics (Table 4.6).

The nucleotide frequencies are A= 34.98 %, T/U= 40.16 %, C= 13.96 % and G= 10.90 %. The base composition of the *mtCOI* gene fragment was skewed towards Adenine (A) and Thymine (T) with an overall 75.14 %. According to Lynch (2008), a biased transition/transversion ratio was a universal feature of nucleotide diversity, which is consistent with our findings. The transition/transversion rate ratios are $k1=1.545$ (purines) and $k2= 2.554$ (pyrimidines). The overall transition/transversion bias (R) is 0.814, where $R= [A*G*k1 + T*C*k2]/ [(A+G)*(T+C)]$. Wondji *et al.* (2007) reported that conservation of genomic evolution is positively correlated with the ratio of transitions to transversions.

a)



b)

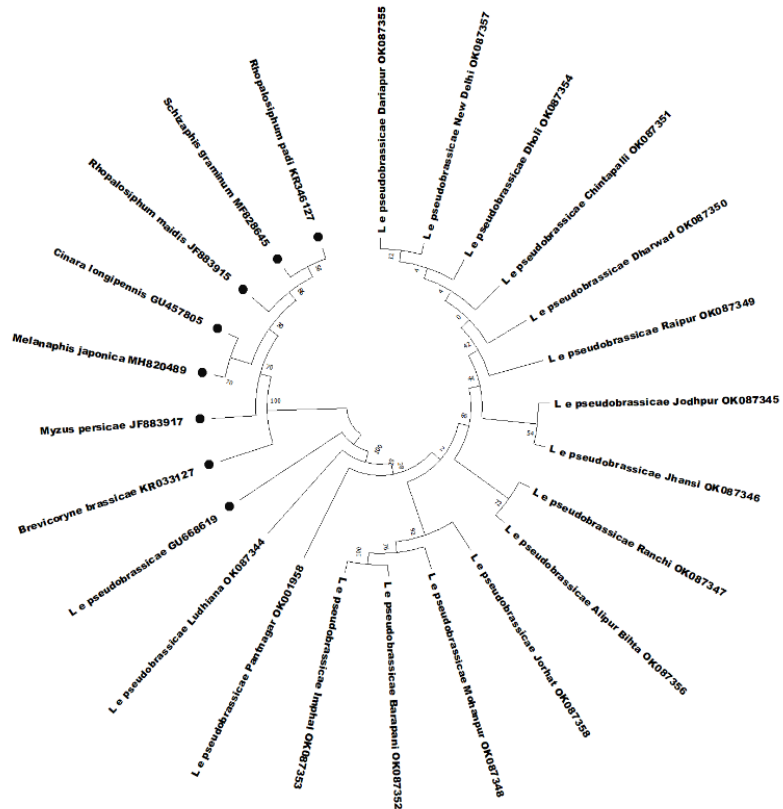


Figure 4.5: Phylogenetic tree for partial *mtCOI* gene sequence of *L. e. pseudobrassicae* of 16 locations based on Maximum Likelihood method and HKY+G model a) Rectangular tree with branch length b) Circular tree without branch length

Table 4.6: Maximum Composite Likelihood estimate of the pattern of nucleotide substitution in *L. e. pseudobrassicae* of 16 locations

	A	T	C	G
A	-	9.82	<i>3.41</i>	4.12
T	8.55	-	8.72	<i>2.66</i>
C	8.55	25.07	-	<i>2.66</i>
G	13.21	9.82	<i>3.41</i>	-

Note: The rates of transitional substitutions are bolded, while those of transversional substitutions are in italics

Tests for neutrality

Two different tests of selective neutrality *i.e.*, Tajima's and Fisher's exact test were performed for 24 sequence pairs. These tests were conducted using MEGA X software to determine the nature of selection/departure from neutrality.

Fisher's exact test

The Nei-Gojobori method was used to calculate the number of synonymous and non-synonymous differences between sequences with 195 positions in the ultimate dataset. The locations having statistically significant P value ($P < 0.05$) showed deviation from other locations and supported neutral theory of evolution. In this study among all the locations, Jorhat and Barapani; Imphal and Mohanpur are having $P < 0.05$ (Table 4.7). The locations with $P > 0.05$ were found to be non-significant indicating that they were deviated from neutral theory of evolution. Similarly, Zhang and Hewitt *et al.* (1997) reported P values lower than 0.05 were considered significant at the 5 % level neutral theory of evolution. Wang *et al.* (2017) found that 111 of the 330 locus population combinations deviated considerably from Hardy-Weinberg equilibrium, as determined by Fisher's exact test in cotton aphid, *Aphis gossypii*.

Table 4.7: Fisher's exact test of *L. e. pseudobrassicae* of 16 locations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1																									
2	0.50																								
3	0.50	0.40																							
4	0.26	0.20	0.51																						
5	1.00	1.00	1.00	0.52																					
6	0.15	0.06	0.08	0.06	0.19																				
7	0.40	0.32	0.50	0.26	1.00	0.07																			
8	0.63	0.50	0.80	0.41	1.00	0.09	0.63																		
9	0.63	0.50	0.80	0.41	1.00	0.09	0.63	1.00																	
10	0.31	0.41	0.39	0.34	0.34	0.11	0.35	0.41	0.41																
11	0.11	0.18	0.15	0.18	0.43	0.00	0.14	0.17	0.17	0.52															
12	0.63	0.50	0.80	0.41	1.00	0.09	0.63	1.00	1.00	0.41	0.17														
13	0.50	0.63	0.63	0.32	1.00	0.08	0.50	0.80	0.80	0.43	0.18	0.80													
14	0.65	0.57	0.73	0.50	1.00	0.12	0.65	1.00	1.00	0.34	0.23	1.00	0.73												
15	0.50	0.40	0.63	0.32	1.00	0.08	0.80	0.80	0.80	0.39	0.15	0.80	0.63	0.73											
16	1.00	1.00	1.00	0.51	1.00	0.32	1.00	1.00	1.00	0.04	0.09	1.00	1.00	1.00	1.00										
17	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00	0.25									
18	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.05	0.00								
19	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00							
20	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.03	0.00	0.00	0.00						
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00					
22	0.41	0.64	0.33	0.33	1.00	0.08	0.41	0.41	0.41	0.46	0.10	0.41	0.41	0.33	0.41	1.00	0.00	0.00	0.00	0.00	0.00				
23	0.01	0.02	0.01	0.01	0.09	0.00	0.01	0.01	0.01	0.24	0.02	0.01	0.02	0.03	0.01	0.39	0.00	0.00	0.00	0.00	0.00	0.00			
24	0.01	0.01	0.01	0.00	0.03	0.00	0.01	0.01	0.01	0.09	0.02	0.01	0.01	0.02	0.01	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	

Note: 1. *L. e. pseudobrassicae* Pantnagar; 2. *L. e. pseudobrassicae* Ludhiana; 3. *L. e. pseudobrassicae* Jodhpur; 4. *L. e. pseudobrassicae* Jhansi; 5. *L. e. pseudobrassicae* Ranchi; 6. *L. e. pseudobrassicae* Mohanpur; 7. *L. e. pseudobrassicae* Raipur; 8. *L. e. pseudobrassicae* Dharwad; 9. *L. e. pseudobrassicae* Chintapalli; 10. *L. e. pseudobrassicae* Barapani; 11. *L. e. pseudobrassicae* Imphal; 12. *L. e. pseudobrassicae* Dholi; 13. *L. e. pseudobrassicae* Dariapur; 14. *L. e. pseudobrassicae* Alipur Bihta; 15. *L. e. pseudobrassicae* Delhi; 16. *L. e. pseudobrassicae* Jorhat; 17. *Schizaphis graminum*; 18. *Rhopalosiphum maidis*; 19. *Rhopalosiphum padi*; 20. *Melanaphis japonica*; 21. *Cinara longipennis*; 22. *L. e. pseudobrassicae*; 23. *Brevicoryne brassicae*; 24. *Myzus persicae*.

Tajima's neutrality test

After removing ambiguous positions for each sequence pair, a total of 675 positions in the final dataset were evaluated. An excess of low frequency polymorphisms is indicated by a negative Tajima's D value (-1.450944) signifying population size expansion and is consistent with neutral theory (Table 4.8). These results are in parallel with Wang *et al.* (2016) who found significant dataset in *Aphis gossypii* with Tajima's $D = -2.218$.

Table 4.8: Tajima's neutrality test of *L. e. pseudobrassicae* of 16 locations

m	S	p_s	Θ	π	D
24	339	0.502222	0.134489	0.086114	-1.450944

Note: m = number of sequences; S = Number of segregating sites; $p_s = S/n$; $\Theta = p_s/a_1$; π = nucleotide diversity; D is Tajima test statistic

4.5.6 Molecular identification and diversity of bacterial endosymbionts associated with *L. e. pseudobrassicae* of 16 locations

In the current study, seven bacterial endosymbionts were investigated *viz.*, one primary endosymbiont, *Buchnera* sp and six secondary endosymbionts namely *Wolbachia* sp, *Arsenophonus* sp, *Rickettsia* sp, *Cardinium* sp, *Hamiltonella* sp and *Spiroplasma* sp by amplifying specific 16s *rRNA* gene.

4.5.6.1 Identification of bacterial endosymbionts associated with *L. e. pseudobrassicae* of 16 locations

The presence of various bacterial endosymbionts from 16 locations were confirmed by the amplification of 16s *rRNA* gene (Table 4.9, Plate 4.8). The occurrence of primary endosymbiont, *Buchnera* sp was 100.0 %. Whereas, secondary endosymbionts *Wolbachia* sp, *Rickettsia* sp, and *Hamiltonella* sp accounted for 81.25 % followed by *Arsenophonus* sp (50.0 %), *Spiroplasma* sp (43.75 %), and *Cardinium* sp (31.25 %). The 16S *rRNA* gene has also been used by Gauthier *et al.* (2015); Jousselin *et al.* (2016) to investigate the variety of bacterial communities associated with aphid species. Buchner (1965); Douglas (1993); Sandström and Moran (1999) reported that *Buchnera* sp, exhibits mutualistic relationship with the aphid species, which provides vital elements that the aphid diet lacks. All secondary endosymbionts

were amplified unevenly in 16 locations, among which *Wolbachia* sp was amplified in all the locations except Mohanpur, Alipur Bihta and Delhi. Similarly, *Rickettsia* sp was amplified in all the locations except Pantnagar, Ludhiana, Barapani. Whereas, *Arsenophonus* sp was amplified in eight locations viz., Ludhiana, Raipur, Dharwad, Chintapalli, Dholi, Alipur Bihta, Delhi, Jorhat. However, *Cardinium* sp amplified in only Pantnagar, Jodhpur, Ranchi, Barapani, Dholi. On the other hand *Hamiltonella* sp was amplified in all except Raipur, Dharwad, Jorhat and *Spiroplasma* sp amplified only in Pantnagar, Jodhpur, Jhansi, Ranchi, Chintapalli, Barapani, Dholi.

4.5.6.2 Diversity of bacterial endosymbionts associated with *L. e.pseudobrassicae* of 16 locations

The diversity of seven endosymbionts in all samples varied from 42.85 to 100.00 % with maximum (100.00 %) was observed in Jodhpur and Ranchi. Whereas, 85.71 % in Pantnagar, Jhansi, Barapani, Dholi and 71.42 % in Chintapalli, Imphal, Dariapur. While, minimal diversity (42.85) was observed in Ludhiana, Raipur, Dharwad, Alipur Bihta, Delhi and Jorhat followed by 57.14 % in Mohanpur.

Multiple bacterial endosymbiont associations were more common in all the 16 locations. Similarly, Haynes *et al.* (2003) found multiple bacterial endosymbionts in pea and black bean aphids. According to Vautrin *et al.* (2008), multiple bacterial associations may have been caused by a positive interaction between the symbionts. Whereas, both primary and secondary endosymbionts prevalence could be influenced by a range of factors, including environmental conditions, geographic location etc. (Morag *et al.*, 2012; Karut and Tok, 2014; Goretty *et al.*, 2019).

Table 4.9: Diversity of bacterial endosymbionts in *L. e. pseudobrassicae* of 16 locations

Sl.No.	Location	<i>Buch</i>	<i>Wsp</i>	<i>Ars</i>	<i>Rickt</i>	<i>Card</i>	<i>Ham</i>	<i>Spr</i>	Total amplified	Percentage (%)
1.	Pantnagar								6	85.71
2.	Ludhiana								3	42.85
3.	Jodhpur								7	100.0
4.	Jhansi								6	85.71
5.	Ranchi								7	100.0
6.	Mohanpur								4	57.14
7.	Raipur								3	42.85
8.	Dharwad								3	42.85
9.	Chintapalli								5	71.42
10.	Barapani								6	85.71
11.	Imphal								5	71.42
12.	Dholi								6	85.71
13.	Dariapur								5	71.42
14.	Alipur Bihta								3	42.85
15.	Delhi								3	42.85
16.	Jorhat								3	42.85
	Total amplified	16	13	8	13	5	13	7		
	Percentage (%)	100.0	81.25	50.00	81.25	31.25	81.25	43.75		

Note: Empty and filled cells indicates the absence and presence of bacterial endosymbionts respectively in *L. e. pseudobrassicae* of 16 locations *Buch*- *Buchnera*, *Wsp*- *Wolbachia*, *Ars*- *Arsenophonus*, *Rickt*- *Rickettsia*, *Card*- *Cardinium*, *Ham*- *Hamiltonella*, *Spr*- *Spiroplasma*

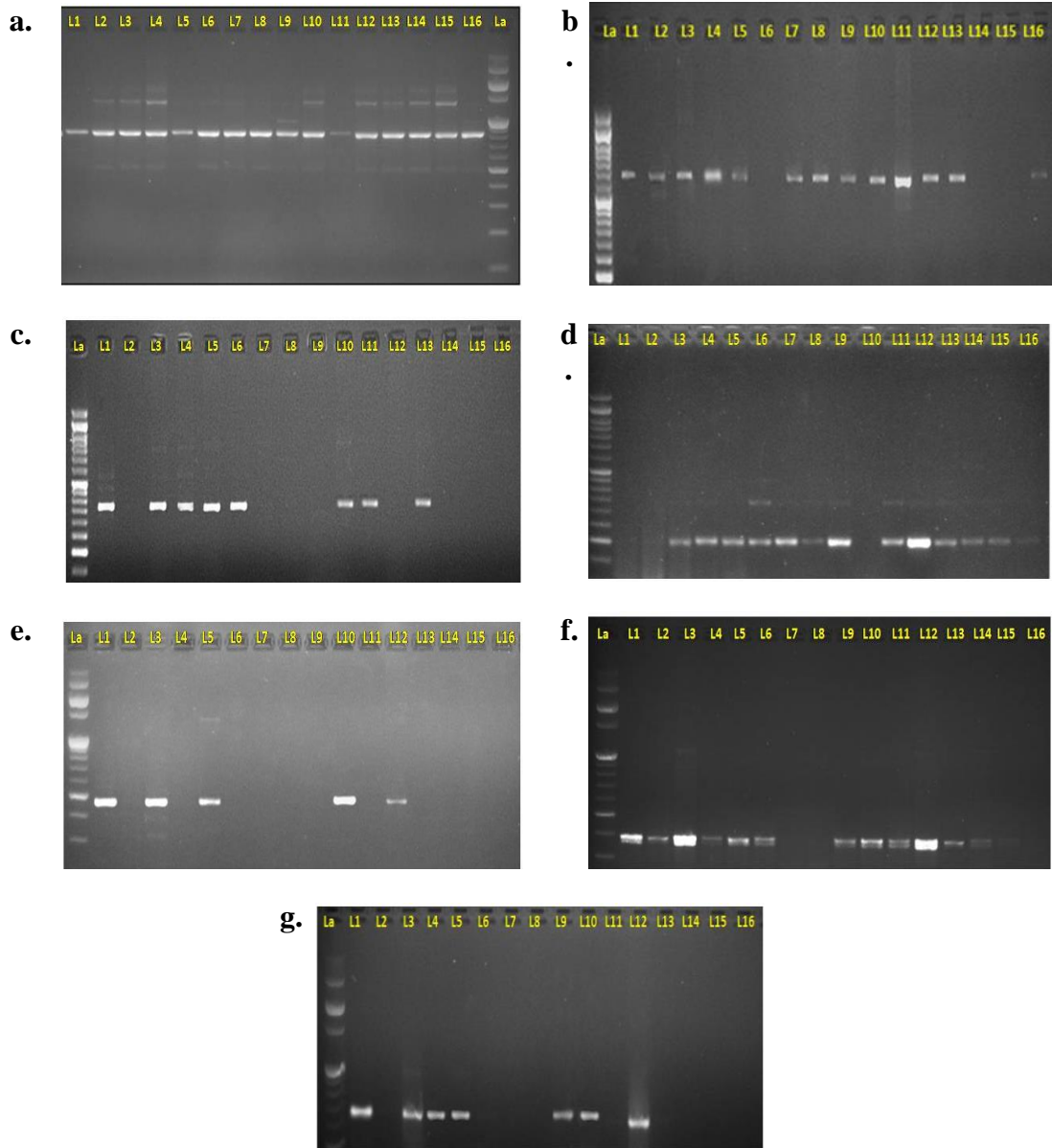


Plate 4.5: Amplification of Endosymbiont specific 16s rRNA gene of *Lipaphis erysimi* of 16 locations (L1-L16)

a. *Buchnera* sp b. *Wolbachia* sp c. *Arsenophonus* sp d. *Rickettsia* sp e. *Cardinium* sp
 f. *Hamiltonella* sp g. *Spiroplasma* sp

4.2 Monitoring of alate aphids and natural enemies on yellow sticky traps in relation to weather parameters

Adhesive yellow traps were installed on four cardinal directions *viz.*, North, South, East, West in the field and aphid counts and natural enemies which trapped were recorded at weekly intervals from date of germination till the harvest of the crop for the two consecutive years *Rabi*, 2019-20 & 2020-21.

4.2.1 Population of alate aphids and natural enemies on yellow sticky traps in relation to weather parameters during *Rabi*, 2019-20

The data pertaining to mean alate aphids and natural enemies population per four traps at weekly intervals during *Rabi*, 2019-20 (Table 4.10, Figure 4.6) showed that, the population of alate aphids commenced first on the 52nd SW (8.32/4 traps) and reached maximum on 8th SW (168.66/4 traps) and remains active upto 12th SW (14.35/4 traps). The alate aphids caught in traps exhibited a substantial increase on 8th SW, after this the catches steadily decreased. The population of natural enemies *i.e.*, adult coccinellids, syrphids and parasitoids were commenced first on the 1st SW (0.42, 0.31, 0.68/ 4 traps respectively) and reached their peak on 8th SW (6.22, 5.63, 15.44/ 4 traps respectively). Further, the correlation coefficient between prevailing weather factors and population of alate aphids, adult coccinellids, syrphids and parasitoids (Table 4.13) clearly indicated that population of alate aphids are positively correlated with maximum temperature ($r=0.508$), minimum temperature ($r=0.183$), morning relative humidity ($r=0.084$), rainfall ($r=0.063$), significantly positively correlated with sunshine hrs (0.637*) and negative correlation with evening relative humidity ($r=-0.354$). The multiple regression equation ($Y=-1061.05+23.612X_1-21.122X_2+4.853X_3+4.951X_4-2.241X_5+9.739X_6$) presented in table 4.13 explains that all the weather variables influenced the alate aphid population to a range of 84.10 % ($R^2=84.10$). Unlike alate aphids the population of adult coccinellids exhibited a significant positive correlation with maximum temperature ($r=0.634^*$) and sunshine hrs ($r=0.646^*$) and positive correlation with minimum temperature (0.416), morning relative humidity (0.226), rainfall (0.209) and negative correlation with evening relative humidity ($r=-0.347$). The multiple regression equation ($Y=-62.372+1.245X_1-0.865X_2+0.399X_3+0.143X_4-0.090X_5+0.021X_6$) explains that all the weather variables impacted the adult coccinellid population to a range of 83.85 % ($R^2=83.85$). Similarly, the population of adult syrphids also had a significant positive correlation

with maximum temperature ($r= 0.620^*$) and sunshine hrs ($r= 0.638^*$) and positively correlated with minimum temperature ($r= 0.401$), morning relative humidity (0.199), rainfall (0.187) and negatively correlated with evening relative humidity ($r=-0.335$). The multiple regression equation ($Y=-55.654+1.085X_1-0.791X_2+0.388X_3+0.097X_4-0.056X_5-0.067X_6$) explains that all the weather parameters influenced the adult syrphid population to a range of 84.00 % ($R^2= 84.00$). The population of adult parasitoids also significantly positively correlated with maximum temperature ($r=0.625^*$) and sunshine hrs ($r=0.630^*$) and positively correlated with minimum temperature ($r=0.412$), morning relative humidity ($r=0.198$), rainfall ($r=0.218$) and negatively correlated with evening relative humidity ($r=-0.335$). The multiple regression equation ($Y=-158.85+3.214X_1-2.292X_2+1.074X_3+0.296X_4-0.169X_5-0.211X_6$) explains that all the weather variables influenced the adult parasitoids population to a range of 80.00 % ($R^2= 80.00$).

4.2.2 Population of alate aphids and natural enemies on yellow sticky traps in relation to weather parameters during *Rabi*, 2020-21

It is obvious from Table 4.11 and Figure 4.7 that during *Rabi*, 2020-21 the population of alate aphids commenced first on the 51st SW (3.98/4 traps) a week earlier than the before season and reached maximum on 8th SW (145.62/4 traps) and remains active up to 12th SW (4.25/4 traps). The alate aphids caught in traps showed a substantial rise on 8th SW, after this the catches steadily decreased. The population of natural enemies i.e., adult coccinellids, syrphids and parasitoids were also commenced first on the 52nd SW (0.45, 0.32, 1.29/ 4 traps respectively) a week earlier than the previous years and reached their peak on 8th SW (6.74, 5.63, 13.79/ 4 traps respectively). Further, the correlation coefficient between prevailing abiotic factors and population of alate aphids, adult coccinellids, syrphids and parasitoids (Table 4.13) clearly indicated that population of alate aphids have positively correlated with maximum temperature ($r=0.491$), minimum temperature ($r=0.295$), sunshine hrs (0.540) and negatively correlated with morning relative humidity ($r=-0.497$) and evening relative humidity ($r=-0.427$). According to multiple regression equation ($Y=605.217+16.380X_1-17.716X_2-11.217X_3+4.165X_4+0X_5+1.929X_6$) of alate aphids presented in table 4.13 the value of R^2 explains that all the weather variables accounted for 64 % ($R^2= 64.00$) variation in the incidence of alate aphid populations. However, the adult coccinellids bared a significant positive correlation with

maximum temperature ($r=0.560^*$) and sunshine hrs ($r=0.579^*$) and positive correlation with minimum temperature (0.395) and negative correlation with morning relative humidity (-0.546) and evening relative humidity (-0.434). The multiple regression equation ($Y=27.397+0.757X_1-0.714X_2-0.565X_3+0.241X_4+0X_5+0.121X_6$) explains that all the weather variables influenced the adult coccinellid population to a range of 66.89 % ($R^2= 66.89$). Similarly, the population of adult syrphids are significantly positively correlated with maximum temperature ($r=0.552^*$) and sunshine hrs ($r=0.575^*$), positively correlated with minimum temperature (0.390) and negatively correlated with relative humidity of both morning (-0.536) and evening (-0.431). The multiple regression equation ($Y=22.135+0.528X_1-0.526X_2-0.434X_3+0.179X_4+0X_5+0.167X_6$) explains that all the weather variables influenced the adult syrphid population to a range of 63.80 % ($R^2= 63.80$). The population of adult parasitoids also significantly positively correlated with maximum temperature ($r=0.596^*$), sunshine hrs ($r=0.619^*$), positively correlated with minimum temperature (0.460) and negatively correlated with relative humidity of both morning (-0.541) and evening (-0.453). The multiple regression equation ($Y=46.537+1.036X_1-0.978X_2-0.964X_3+0.436X_4+0X_5+0.683X_6$) explains that all the weather variables impacted the adult parasitoids population to a range of 61.28 % ($R^2= 61.28$).

Based on the pooled mean of *Rabi*, 2019-20 & 2020-21 (Table 4.12, Figure 4.8) it is clearly revealed that the alate aphid populations were first commenced on the 51st SW (2.00/4 traps) and reached maximum on 8th SW (157.1/4 traps) and remains active up to 12th SW (9.3/4 traps). Whereas, the adult coccinellids, syrphids and parasitoids commenced first on the 52nd SW (0.23, 0.16, 0.65/ 4 traps respectively) with peak population on 8th SW (6.48, 5.63, 14.62/ 4 traps respectively). The correlation analysis (Table 4.14) revealed that population of alate aphids are positively correlated with maximum temperature ($r=0.445$), minimum temperature ($r=0.180$), significantly positively correlated with sunshine hrs (0.602*) and negatively correlated with relative humidity of both morning ($r=-0.252$) and evening ($r=-0.367$). The multiple regression analysis ($Y=-170.47+22.656X_1-17.641X_2-7.260X_3+7.688X_4-5.454X_5 + 10.486X_6$) explains that all the weather variables accounted for 71.50 % ($R^2= 71.50$) variation on the incidence of alate aphid populations. However, adult coccinellids are significantly positively correlated with maximum temperature ($r=0.608^*$) and sunshine hrs ($r=0.654^*$), positively correlated

with minimum temperature (0.382) and negatively correlated with morning (-0.321) and evening relative humidity (-0.460). The multiple regression equation ($Y = -13.682 + 0.925X_1 - 0.569X_2 - 0.263X_3 + 0.334X_4 - 0.241X_5 + 0.515X_6$) explains that all the weather variables influenced the adult coccinellid population to a range of 75.96 % ($R^2 = 75.96$). Similarly, the population of adult syrphids are significantly positively correlated with maximum temperature ($r = 0.595^*$) and sunshine hrs ($r = 0.633^*$), positively correlated with minimum temperature (0.371) and negatively correlated with morning (-0.305) and evening relative humidity (-0.446). The multiple regression analysis revealed that ($Y = -14.386 + 0.797X_1 - 0.501X_2 - 0.172X_3 + 0.257X_4 - 0.185X_5 + 0.362X_6$) all the weather variables contributed to a range of 72.25 % ($R^2 = 72.25$) adult syrphid population. The population of adult parasitoids also significantly positively correlated with maximum temperature ($r = 0.618^*$), sunshine hrs ($r = 0.662^*$) positively correlated with minimum temperature (0.413) and negatively correlated with morning (-0.292) and evening relative humidity (-0.442). The multiple regression analysis ($Y = -44.135 + 2.301X_1 - 1.232X_2 - 0.524X_3 + 0.793X_4 - 0.591X_5 + 0.919X_6$) revealed that all the weather variables contributed to an extent of 75.54 % ($R^2 = 75.54$) adult parasitoids population.

The present investigation corroborates the findings of Malik and Sachan (2013) who observed the occurrence of mustard aphid in the third week of December (51st SMW) with peak population in February second week (7th SMW). The period of activity of mustard aphid is in conformity with the results of Bhati *et al.* (2015) who reported the incidence of mustard aphid from December last week (52nd SMW) to March second week (10th SMW) with peak population on second week of February (6th SMW). Kalasariya and Parmar (2018) also reported that mustard aphid commenced during December third week (51st SMW), increased with fast multiplication and reached peak during February third week (8th SMW), after which aphid population gradually declined with the rise in temperature and maturity of the crop. Our findings are also consistent with those of Kulkarni and Patel (2001), who found that the population of coccinellids began to appear in the final week of January (5th SMW), peaked in the third week of February (8th SMW), and then began to fall from February last week (9th SMW). While, Singh *et al.* (2011) observed that population of coccinellids started from the January last week (4th SMW) and progressively increased with peak population on 9th SMW. However, Bhaskar and

Virakatanath (2002), Badjena and Mandal (2005) observed the peak population on 11th SMW. Contrarily, Mishra and Kanwat (2003) and Kumar and Paul (2017) observed the first arrival of coccinellids in the 1st and 2nd SMW with peak population in 6th and 7th SMW respectively. Kulkarni and Patel (2001) and (Mishra and Kanwat 2018) observed the initial appearance of syrphids in the 2nd SMW and its population reached peak during 5th and 6th SMW. Similarly, Devi *et al.* (2011) also observed the maximum syrphid population on 5th and 6th SMW. However, Varshney *et al.* (2017) observed the first appearance of coccinellids, syrphids and parasitoids during 4th SMW and gradually the populations attained peak during 9th SMW. The findings of Raja *et al.* (2014) are in support to the current study, who reported that population of mustard aphid, coccinellids and parasitoids are positively correlated with maximum, minimum temperature, bright sunshine hours and negatively correlated with relative humidity. Similarly, Akthar *et al.* (2010); Yadav and Agrawal (2018) stated that the population of parasitoids and coccinellids respectively are positively correlated with maximum, minimum temperature, bright sunshine hours and negatively correlated with relative humidity.

Table 4.10: Monitoring of alate mustard aphids and natural enemies population through yellow sticky traps during Rabi 2019-20 in relation to weather parameters

SMW	Mean number per 4 traps				Temperature (°C)		RH (%)		Rainfall (mm)	Sunshine (hrs)
	Alate Aphids	Adult Coccinellids	Adult Syrphids	Adult Parasitoids	Max.	Min.	07 00 hrs	14 00 hrs		
50	0	0	0	0	23.9	11.3	92	75	22	1.3
51	0	0	0	0	18	9.1	91	74	0	0.9
52	8.32	0	0	0	15.7	5.9	92	74	0	1.4
1	26.42	0.42	0.31	0.68	19.1	9.3	90	77	0	0.8
2	19.11	0.23	0.17	0.44	17.1	8.7	94	83	1.8	0.8
3	48.08	0.98	0.72	1.86	19.9	10.9	93	76	4.4	2.9
4	59.28	1.34	0.97	2.56	20.8	7.6	90	62	0	6.8
5	95.32	2.06	1.67	3.95	22.7	9.5	93	67	0	7.4
6	102.44	2.59	2.22	6.12	23.3	8.8	87	56	0	8.4
7	146.34	5.35	4.12	13.67	25.0	10.9	90	64	0	7
8	168.66	6.22	5.63	15.44	26.5	13.5	94	71	24	8.3
9	125.33	5.86	4.95	13.66	26.1	13.9	95	64	0	8
10	92.66	4.23	3.12	10.04	27	15.7	91	67	8.2	8.1
11	35.25	2.12	1.75	5.31	26.5	15.3	93	57	4.4	6.5
12	14.35	0.68	0.46	1.16	30.3	16.9	87	59	17	8.3
13	0	0	0	0	33.1	18.3	69	40	0	10.7

SMW- Standard Meteorological Week

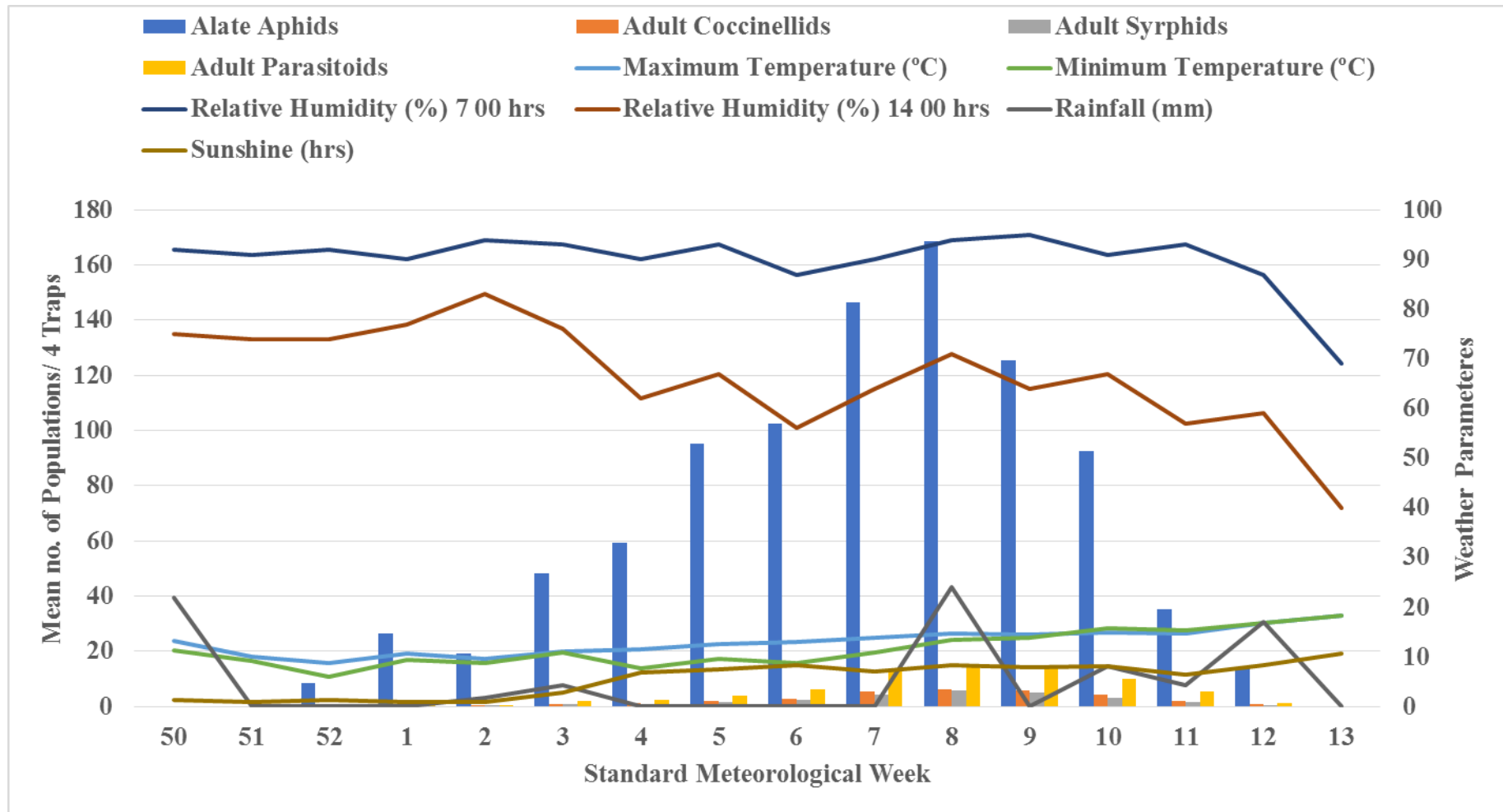


Figure 4.6: Population of alate mustard aphids and natural enemies in relation to weather parameters during *Rabi* 2019-20

Table 4.11: Monitoring of alate mustard aphid and natural enemies through yellow sticky traps during *Rabi* 2020-21 in relation to weather parameters

SMW	Mean number per 4 traps				Temperature (°C)		RH (%)		Rainfall (mm)	Sunshine (hrs)
	Alate Aphids	Adult Coccinellids	Adult Syrphids	Adult Parasitoids	Max.	Min.	07 00 hrs	14 00 hrs		
50	0	0	0	0	22.7	12	95	72	0	0.9
51	3.98	0	0	0	19.8	8.4	96	71	0	3.8
52	18.54	0.45	0.32	1.29	22.9	8	94	65	0	6.8
1	29.22	0.83	0.71	2.04	24.45	11.24	94	65	0	6.4
2	20.37	0.66	0.49	1.51	21.67	10.95	95	68	0	4.92
3	8.41	0.39	0.21	0.78	15.6	8.22	93	77	0	0.17
4	13.02	0.48	0.33	1.08	16.54	8.27	94	82	0	2.02
5	22.87	1.24	1.06	2.75	20.44	7.2	94	74	0	3.2
6	94.32	2.85	2.08	4.62	24.81	10.68	90	62	0	6.14
7	128.21	4.69	3.38	7.22	26.94	12.24	90	58	0	7.52
8	145.62	6.74	5.63	13.79	28.08	13.98	93	71	0	8.02
9	121.34	5.67	4.62	11.24	29.24	15.52	84	55	0	9.55
10	99.55	4.92	3.83	10.05	30.34	16.91	92	64	0	8.82
11	38.63	2.56	1.67	6.43	30.14	17.6	88	56	0	7.16
12	4.25	0.22	0.15	0.55	33.91	18.97	82	42	0	7.72
13	0	0	0	0	34.27	17.18	87	45	0	8.3

SMW- Standard Meteorological Week

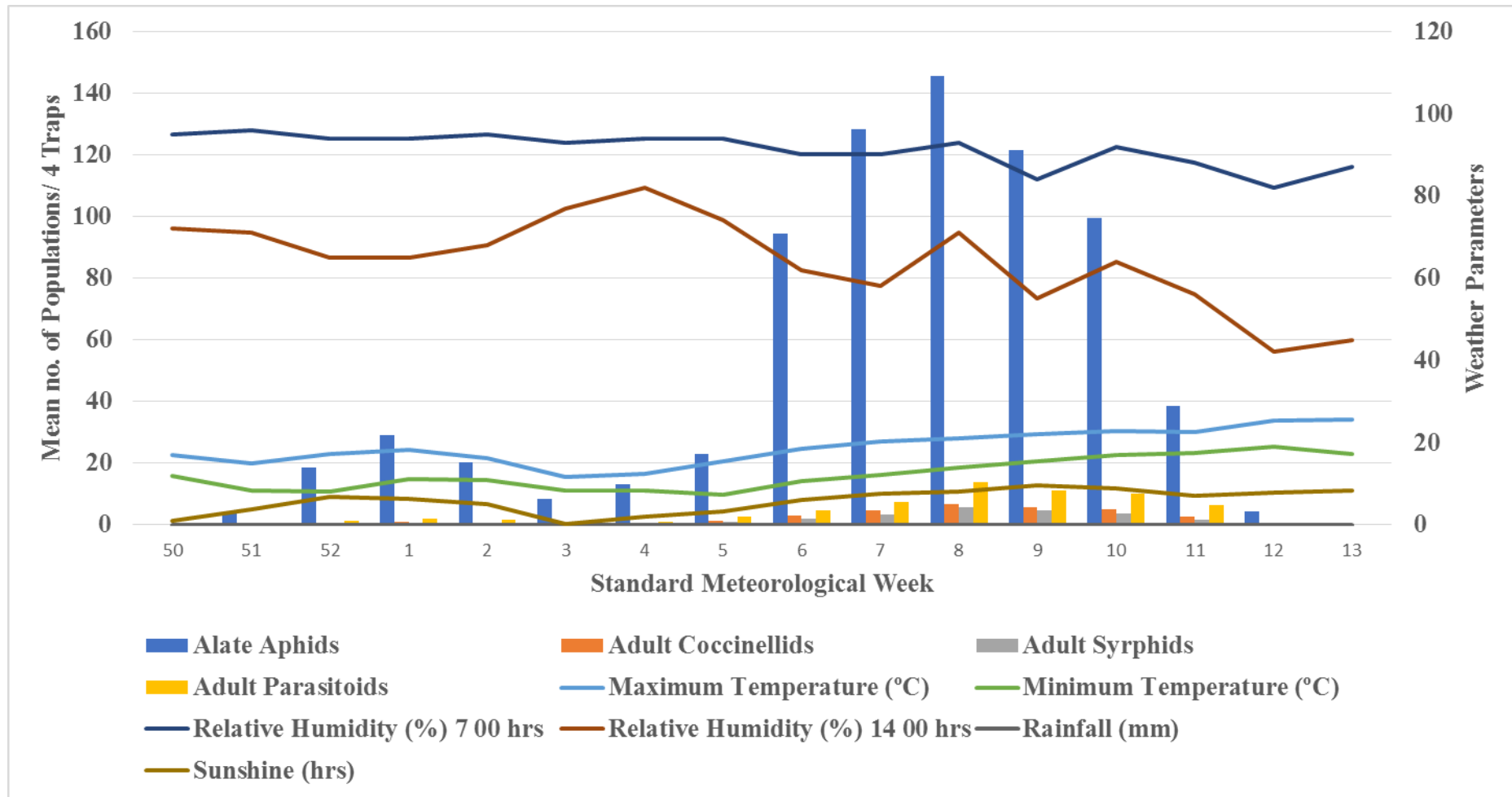


Figure 4.7: Population of alate mustard aphids and natural enemies in relation to weather parameters during *Rabi* 2020-21

Table 4.12: Monitoring of alate mustard aphid and natural enemies through yellow sticky traps in relation to weather parameters (based on pooled mean of Rabi, 2019-20 & 2020-21)

SMW	Mean number per 4 traps				Temperature (⁰ C)		RH (%)		Rainfall (mm)	Sunshine (hrs)
	Alate aphids	Adult Coccinellids	Adult Syrphids	Adult Parasitoids	Max.	Min.	07 00 hrs	14 00 hrs		
50	0	0	0	0	23.3	11.65	94	74	11	1.1
51	2.0	0	0	0	18.90	8.75	94	73	0	2.4
52	13.4	0.23	0.16	0.65	19.30	6.95	93	70	0	4.1
1	27.8	0.63	0.51	1.36	21.78	10.27	92	71	0	3.6
2	19.74	0.45	0.33	0.98	19.39	9.83	95	76	0.9	2.9
3	28.2	0.69	0.47	1.32	17.75	9.56	93	77	2.2	1.5
4	36.2	0.91	0.65	1.82	18.67	7.94	92	72	0	4.4
5	59.1	1.65	1.37	3.35	21.27	8.35	94	71	0	5.3
6	98.4	2.72	2.15	5.37	23.96	9.74	89	59	0	7.3
7	137.3	5.02	3.75	10.45	26.42	11.57	90	61	0	7.3
8	157.1	6.48	5.63	14.62	27.29	13.74	94	71	12	8.2
9	123.3	5.77	4.79	12.45	27.97	14.71	90	60	0	8.8
10	96.1	4.58	3.48	10.05	28.67	16.31	92	66	4.1	8.5
11	36.9	2.34	1.71	5.87	28.07	16.45	91	57	2.2	6.8
12	9.3	0.45	0.31	0.86	32.01	17.935	85	51	8.5	8.0
13	0	0	0	0	33.69	17.74	78	43	0	9.5

SMW- Standard Meteorological Week

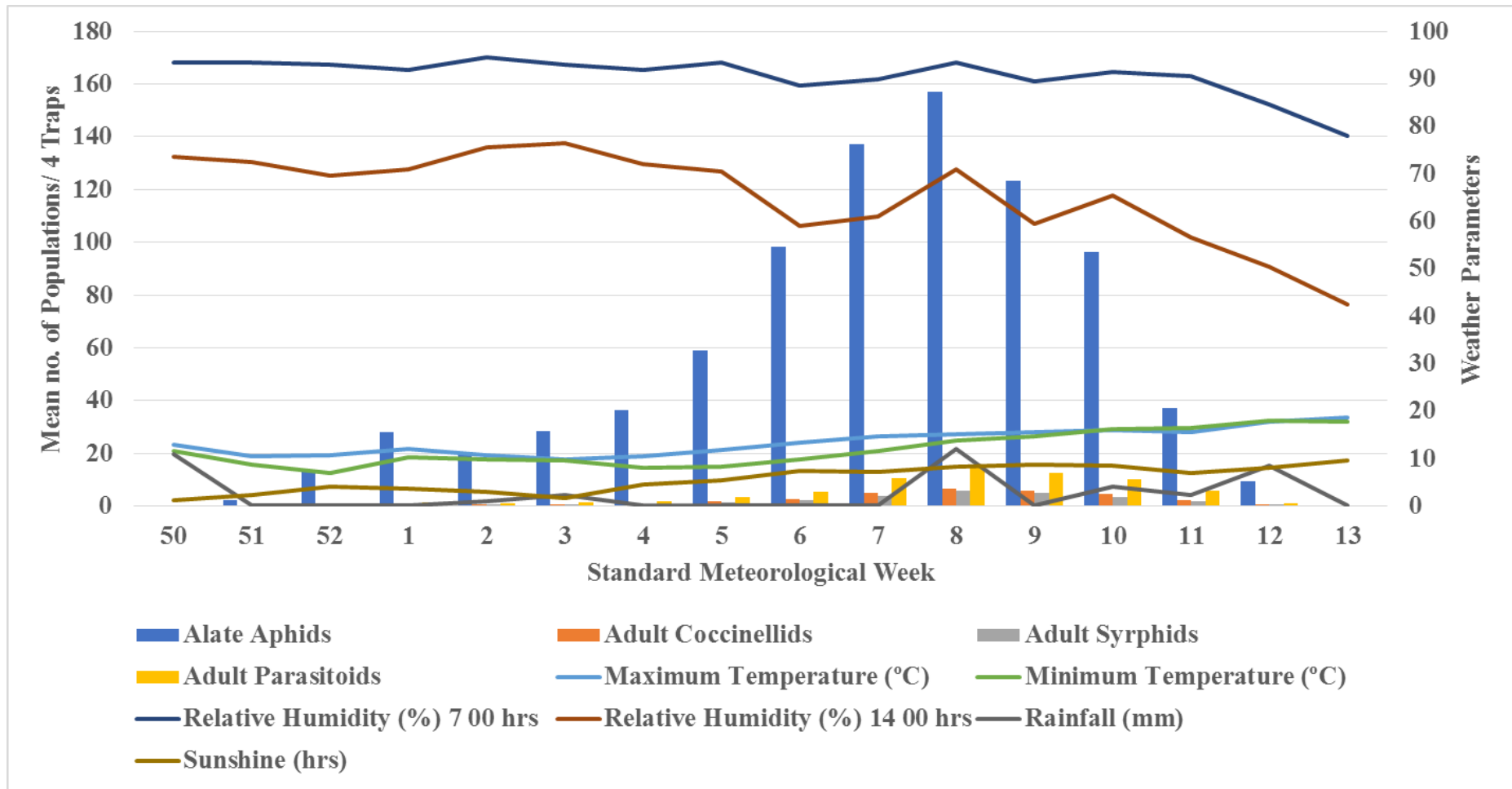


Figure 4.8: Population of alate mustard aphids and natural enemies in relation to weather parameters (based on pooled mean of Rabi 2019-20 and 2020-21)

Table 4.13: Correlation coefficient and regression analysis between weather parameters and population of alate aphids and natural enemies during Rabi, 2019-20 & 2020-21

Year	Dependent Variables	Correlation coefficient						Multiple regression equation	R ² (%)
		Temperature ° (C)		RH (%)		Rainfall (mm)	Sunshine (hrs)		
		Max.	Min.	7 00 hr	14 00 hr				
2019-20	Alate aphids	0.508	0.183	0.084	-0.354	0.063	0.637*	$Y = -1061.05 + 23.612X_1 - 21.122X_2 + 4.853X_3 + 4.951X_4 - 2.241X_5 + 9.739X_6$	84.10
	Adult Coccinellids	0.634*	0.416	0.226	-0.347	0.209	0.646*	$Y = -62.372 + 1.245X_1 - 0.865X_2 + 0.399X_3 + 0.143X_4 - 0.090X_5 + 0.021X_6$	83.85
	Adult Syrphids	0.620*	0.401	0.199	-0.335	0.187	0.638*	$Y = -55.654 + 1.085X_1 - 0.791X_2 + 0.388X_3 + 0.097X_4 - 0.056X_5 - 0.067X_6$	84.00
	Adult Parasitoids	0.625*	0.412	0.198	-0.335	0.218	0.630*	$Y = -158.85 + 3.214X_1 - 2.292X_2 + 1.074X_3 + 0.296X_4 - 0.169X_5 - 0.211X_6$	80.00
2020-21	Alate aphids	0.491	0.295	-0.497	-0.427	-	0.540	$Y = 605.217 + 16.380X_1 - 17.716X_2 - 11.217X_3 + 4.165X_4 + 0X_5 + 1.929X_6$	64.00
	Adult Coccinellids	0.560*	0.395	-0.546	-0.434	-	0.579*	$Y = 27.397 + 0.757X_1 - 0.714X_2 - 0.565X_3 + 0.241X_4 + 0X_5 + 0.121X_6$	66.89
	Adult Syrphids	0.552*	0.390	-0.536	-0.431	-	0.575*	$Y = 22.135 + 0.528X_1 - 0.526X_2 - 0.434X_3 + 0.179X_4 + 0X_5 + 0.167X_6$	63.80
	Adult Parasitoids	0.596*	0.460	-0.541	-0.453	-	0.619*	$Y = 46.537 + 1.036X_1 - 0.978X_2 - 0.964X_3 + 0.436X_4 + 0X_5 + 0.683X_6$	61.28

Weather parameters: X₁ -Maximum temperature (°C), X₂ -Minimum temperature (°C), X₃ -Relative humidity at 7 hrs (%), X₄ -Relative humidity at 14 hrs (%), X₅ -Rainfall (mm), X₆ - Sunshine (hrs)

Coefficient of determination (R²)

*Significant at P = 0.05

Table 4.14: Correlation coefficient and regression analysis between weather parameters and population of alate aphids and natural enemies (based on pooled mean of two years *i.e.*, 2019-20 & 2020-21)

Dependent Variables	Correlation coefficient						Multiple regression equation	R ² (%)
	Temperature (°C)		RH (%)		Rainfall (mm)	Sunshine (hrs)		
	Max.	Min.	7 00 hr	14 00 hr				
Alate aphids	0.445	0.180	-0.252	-0.367	-0.038	0.602*	$Y = -170.47 + 22.656X_1 - 17.641X_2 - 7.260X_3 + 7.688X_4 - 5.454X_5 + 10.486X_6$	71.50
Adult Coccinellids	0.608*	0.382	-0.321	-0.460	0.091	0.654*	$Y = -13.682 + 0.925X_1 - 0.569X_2 - 0.263X_3 + 0.334X_4 - 0.241X_5 + 0.515X_6$	75.96
Adult Syrphids	0.595*	0.371	-0.305	-0.446	0.084	0.633*	$Y = -14.386 + 0.797X_1 - 0.501X_2 - 0.172X_3 + 0.257X_4 - 0.185X_5 + 0.362X_6$	72.25
Adult Parasitoids	0.618*	0.413	-0.292	-0.442	0.088	0.662*	$Y = -44.135 + 2.301X_1 - 1.232X_2 - 0.524X_3 + 0.793X_4 - 0.591X_5 + 0.919X_6$	75.54

Weather parameters: X₁ -Maximum temperature (°C), X₂ -Minimum temperature (°C), X₃ -Relative humidity at 7 hrs (%), X₄-Relative humidity at 14 hrs (%), X₅-Rainfall (mm), X₆- Sunshine (hrs)

Coefficient of determination (R²)

*Significant at P = 0.05

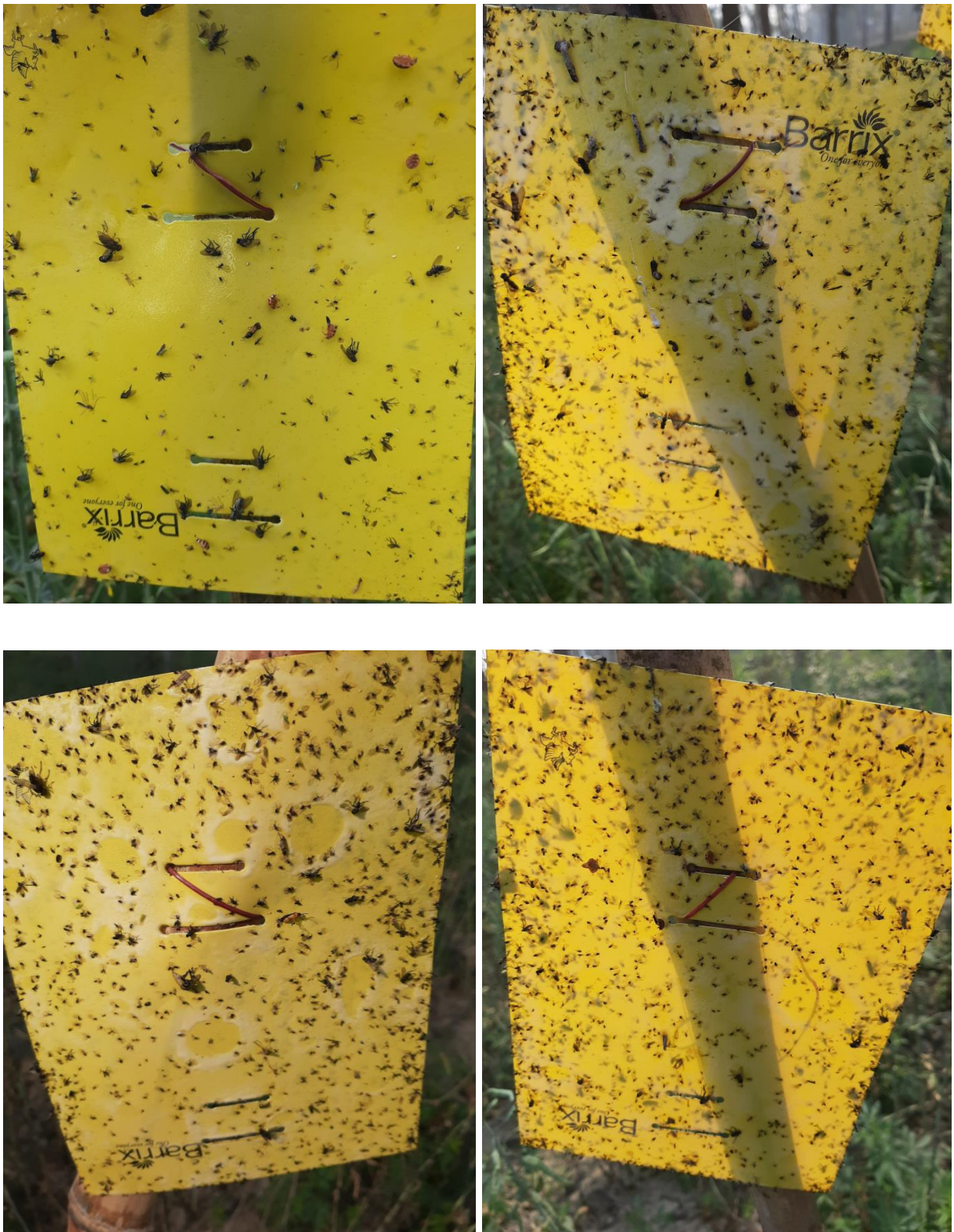


Plate: 4.6 Monitoring of alate aphids and its natural enemies on yellow sticky traps

4.2.1 Screening of Rapeseed-mustard genotypes against mustard aphid during Rabi, 2019-20

Thirty Rapeseed-mustard genotypes were screened under field conditions to examine their reaction against mustard aphid infestation along with three checks Yellow sarson (66-197-3) (Susceptible check), Kranti (National resistant check) and BSH-1 (National susceptible check). The data on number of aphids on top 10 cm inflorescence per 10 randomly selected plants were recorded at 50% flowering and pod stage; Aphid Population Index (API), Aphid Damage Index (ADI) and Aphid Resistance Index (ARI) have been presented in Table 4.15. During the year, 2019-2020 mean aphid population at 50% flowering and pod stage varied from 28.89 to 83.76 and 44.8 to 165.9 in RTM 314 and RCH 1 respectively as compared to checks Yellow sarson (66-197-3) (65.52 to 170.7), Kranti (32.55 to 50.2) and BSH-1 (85.78 to 192.6) respectively. Among all the genotypes only six genotypes (RTM 314, RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699) have harboured lowest aphid population and ranged from 28.89-42.65 and 44.8-79.3 at 50% flowering and pod stage respectively. Similarly, on the basis of total mean aphid population lowest aphid population was recorded in RTM 314 (36.8) followed by RTM 1624 (53.2), GSH 1699 (56.4), RH 7846 (57), AKGS 19-8 (59.3), GSH 2180 (59.5) and highest in RCH 1 (124.8) as compared to checks Yellow sarson (66-197-3) (118.1), Kranti (41.4) and BSH-1 (139.2). Mean API and ADI of both at 50% flowering and pod stage ranged from 1.4 to 4.7 and 1 to 4.9 in RTM 314 and RCH 1 respectively. Based on ARI among all the genotypes RTM 314 recorded lowest (1.2) followed by RTM 1624 (2.1), GSH 1699 (2.2), RH 7846 (2.2), AKGS 19-8 (2.2), GSH 2180 (2.25) and highest in RCH 1 (4.8) as compared to checks Yellow sarson (66-197-3) (3.85), Kranti (2) and BSH-1 (4.25).

4.2.2 Screening of Rapeseed-mustard genotypes against mustard aphid during Rabi, 2020-21

Almost similar trend was recorded during 2020-21 crop season which indicated consistency in the resistance response of Rapeseed-mustard genotypes against mustard aphid over both the years. The mean aphid population at 50% flowering and pod stage varied from 22.23 to 74.33 and 39.67 to 151.24 in RTM 314 and RCH 1 respectively as compared to checks Yellow sarson (66-197-3) (60.88 to

143.98), Kranti (29.89 to 45.33) and BSH-1 (79.56 to 151.66) respectively (Table 4.16). Among all the genotypes only six genotypes (RTM 314, RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699) have harboured lowest aphid population and ranged from 22.23-39.34 and 39.67-69.55 at 50% flowering and pod stage respectively. Similarly, on the basis of total mean aphid population lowest aphid population was recorded in RTM 314 (28.95) followed by RTM 1624 (45.65), GSH 1699 (53.88), RH 7846 (47.02), AKGS 19-8 (52.64), GSH 2180 (50.55) and highest in RCH 1 (112.78) as compared to checks Yellow sarson (66-197-3) (104.75), Kranti (38.94) and BSH-1 (118.72). Mean API and ADI of both at 50% flowering and pod stage ranged from 1.2 to 4.4 and 1 to 4.7 in RTM 314 and RCH 1 respectively. Based on ARI among all the genotypes RTM 314 recorded lowest (1.1) followed by RTM 1624 (2.15), GSH 1699 (2.25), RH 7846 (2.1), AKGS 19-8 (2.15), GSH 2180 (2.12) and highest in RCH 1 (4.55) as compared to checks Yellow sarson (66-197-3) (3.7), Kranti (1.85) and BSH-1 (4).

Based on pooled mean of *Rabi*, 2019-20 & 2020-21 (Table 4.17) mean aphid population at 50% flowering and pod stage varied from 25.56 to 79.05 and 42.22 to 158.56 in RTM 314 and RCH 1 respectively as compared to checks Yellow sarson (66-197-3) (63.30 to 157.32), Kranti (31.22 to 47.78) and BSH-1 (82.67 to 172.11) respectively. Among all the genotypes only six genotypes (RTM 314, RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699) have harboured the lowest aphid population and ranged from 25.56 to 40.44 and 42.22 to 74.40 at 50% flowering and pod stage respectively. Similarly, on the basis of cumulative mean aphid population lowest aphid population was recorded in RTM 314 (32.89) followed by RTM 1624 (49.43), GSH 1699 (55.16), RH 7846 (52), AKGS 19-8 (55.97), GSH 2180 (55.04) and highest in RCH 1 (118.80) as compared to checks Yellow sarson (66-197-3) (104.75), Kranti (38.94) and BSH-1 (118.72). Mean API and ADI of both at 50% flowering and pod stage ranged from 1.3 to 4.6 and 1 to 4.8 in RTM 314 and RCH 1 respectively. Based on ARI among all the genotypes RTM 314 recorded lowest (1.15) followed by RTM 1624 (2.08), GSH 1699 (2.23), RH 7846 (2.15), AKGS 19-8 (2.18), GSH 2180 (2.19) and highest in RCH 1 (4.8) as compared to checks Yellow sarson (66-197-3) (3.78), Kranti (1.93) and BSH-1 (4.13).

During 2019-20 & 2020-21 out of the thirty genotypes, six namely RTM 314 (resistant) and RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699

(moderately resistant) exhibited consistent resistant reaction (Table 2). Thus, two years of field screening resulted in identification of six promising genotypes viz., RTM 314, RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699 against mustard aphid which were further evaluated for mechanism of resistance through biochemical and GC-MS analysis under laboratory conditions.

Similarly, Singh *et al.* (2014) screened wild and cultivated crucifers for their resistance against mustard aphid and observed a lowest AII (0.7 and 1.0) in *Brassica fruticulosa* and *B. spinescens* (wild crucifers) respectively and categorized as resistant and highest AII (4.0 and 5.0) in BSH-1 and Rohini (cultivated crucifers) respectively and categorized as susceptible. Mukherjee *et al.* (2017) screened sixty six advanced rapeseed-mustard breeding genotypes for their reaction against mustard aphid and observed minimum AII (1.2) in the genotypes viz., Bhawani, YST 151, DRMR 1165-40, CS 700-3-3-6, PDZ 4, DRMRIJ 13-38, RGN 337, Varuna and categorized as resistant. Based on the level of infestation Maurya *et al.* (2018) observed lowest infestation on GSC-6, NRCDR 601, Vardhan and Pusa Jagnath. Sreedhar *et al.* (2019) recorded lowest mean aphid index (1.0) in NPJ-194 genotype and categorized as highly resistant.

Biochemical analysis

All the promising genotypes (RTM 314, RTM 1624, GSH 2180, RH 7846, AKGS 19-8 and GSH 1699) obtained after preliminary screening during 2019-2020 were analysed for various biochemical constituents viz., glucosinolates, phenols and total sugars as per the standard procedures and the results are presented as below:

Glucosinolate content

The glucosinolate content of the promising genotypes ranged from 83.66 to 109.87 $\mu\text{mole/g}$ and differs significantly from the susceptible checks (Table 4.19). Highest glucosinolate content was observed in RTM 314 (109.87 $\mu\text{mole/g}$) and lowest was observed in GSH 1699 (86.34 $\mu\text{mole/g}$) in comparison to two susceptible checks viz., BSH-1 (44.21 $\mu\text{mole/g}$) and Yellow sarson (66-197-3) (51.62 $\mu\text{mole/g}$) and one resistant check Kranti (92.45 $\mu\text{mole/g}$). Glucosinolate content in RTM 314 was also found significantly higher (109.87 $\mu\text{mole/g}$) in comparison to other promising genotypes viz., RTM 1624 (95.76 $\mu\text{mole/g}$), RH 7846 (91.21 $\mu\text{mole/g}$), AKGS 19-8 (89.68 $\mu\text{mole/g}$), GSH 1699 (86.34 $\mu\text{mole/g}$) and GSH 2180 (83.66 $\mu\text{mole/g}$). When

glucosinolate content was compared to aphid infestation, genotypes with higher glucosinolate content had fewer aphids per plant (Table 4.20). In all of the promising genotypes, the correlation coefficient between glucosinolate content and mustard aphid population was found to be highly significantly negative ($r=-0.971^{**}$).

Present findings are in close conformity with Ramdhari *et al.* (1994); Chander *et al.* (1997); Singh *et al.* (2000) who found that the genotypes rich in glucosinolate content harboured low mustard aphid population. Biodegradable biocides with antifungal, nematicidal, and insecticidal activities that are catalysed by myrosinase have gotten a lot of attention because of their distinctive biological and chemical properties (Yu *et al.*, 2005). The defence system of Brassicaceae is mainly a chemical nature, comprises glucosinolates and their breakdown products (Broekgaarden *et al.*, 2008; Björkman *et al.*, 2011). Degradation products of glucosinolates, such as isothiocyanates and nitriles, act as feeding deterrents and are toxic to herbivores (Ratzka *et al.*, 2002). Glucosinolates have various effects on different types of pests, such as generalists and specialists (Lankau, 2007). This study discovered a substantial negative association between glucosinolate concentration and aphid population per plant. Previous researchers have also found a link between indole glucosinolate concentrations and the performance of *B. brassicae* and other aphid species (Cole, 1997; Mewis *et al.*, 2005; Santolamazza-Carbone *et al.*, 2014). Simailar to our studies, Yadav and Rana (2018) assessed 30 mustard genotypes for aphid infestation using biochemical bases at flowering stage and found a highly significant negative correlation between glucosinolate content and mustard aphid population. Palial *et al.* (2018) also stated that higher content of glucosinolates served as biochemical bases of resistance in the three introgression lines (I₈, I₇₉ and I₈₂), whereas BSH-1 and PBR-210 showed the reverse trend. Kumar and Sangha (2013) as well looked into the biochemical bases of mustard cultivar resistance to mustard aphid infestation and revealed that glucosinolate levels were inversely related to aphid infestation.

Phenol Content

The phenol content of the promising genotypes ranged from 4.18 to 6.51 mg/g and differs significantly from the susceptible checks (Table 4.19). Highest phenol content was observed in RTM 314 (6.51 mg/g) and lowest was observed in GSH 2180 (4.18 mg/g) in comparison to two susceptible checks *viz.*, BSH-1 (2.56 mg/g) and

Yellow sarson (66-197-3) (3.01 mg/g) and one resistant check Kranti (4.62 mg/g). Phenol content in RTM 314 was also found significantly higher in comparison to other promising genotypes viz., RTM 1624 (5.42 mg/g), RH 7846 (5.01 mg/g), AKGS 19-8 (4.52 mg/g), GSH 1699 (4.39 mg/g) and GSH 2180 (4.180 mg/g). When phenol content was compared to aphid infestation, it was discovered that genotypes with higher phenol content had lower aphid population/plant (Table 4.20). In all of the promising genotypes, the correlation coefficient between phenol content and mustard aphid population was found to be highly significantly negative ($r=-0.891^{**}$).

The findings are in reliable with the following findings from various researchers: Kumar *et al.* (2017); Kumar *et al.* (2020) investigated the biochemical basis of mustard aphid resistance in different rapeseed-mustard genotypes and found that BSH-1 and YSH-401 had the lowest phenol content. Increased levels of total phenols served as a biochemical basis of resistance in the three *Brassica* introgression lines (I₈, I₇₉ and I₈₂), according to Palial *et al.* (2018), whereas BSH-1 and PBR-210 showed the opposite trend. Kumar and Sangha (2013) investigated the biochemical mechanisms behind mustard cultivar resistance to mustard aphid infestation and discovered that total phenols exhibited an inverse association with aphid infestation. Moran and Thompson (2001) also observed a downregulation of phenylalanine ammonia lyase activity in response to *Myzus persicae* in *Arabidopsis* cultivars which are susceptible to aphid infestation and concluded that phenol content has a significant negative correlation with aphid population, indicating its role in plant defence.

Total sugar content

In comparison to the other biochemical parameters studied, total sugar content showed the opposite trend. The sugar content of all promising genotypes was significantly lower than that of susceptible controls (Table 4.19). The total sugar content ranged from 6.42 to 10.84 mg/g in RTM 314 and GSH 2180, respectively. Lowest sugar content was observed in RTM 314 (6.42 mg/g) followed by RTM 1624 (7.08 mg/g), RH 7846 (8.15 mg/g), AKGS 19-8 (9.12 mg/g), GSH 1699 (9.56 mg/g) and GSH 2180 (10.04 mg/g). In all of the promising genotypes, the correlation coefficient between sugar content and aphid population was found to be non-significantly positive ($r=0.643$) (Table 4.20). Similarly, Kumar *et al.* (2017) observed

the higher sugar content in susceptible variety of mustard (BSH-1). Similarly, Joseph and Peter (2007) observed the low amount of total sugars in resistant lines of cowpea against aphid infestation. According to Xu *et al.* (2016), susceptible wheat cultivars to grain aphid infestation had significantly lower sugar levels than resistant cultivars. Similarly, Zhang and Liu (2011) found that susceptible wheat cultivars to *Macrosiphum avenae* had low sugar content.

Activity of phytochemicals identified in the methanol inflorescence extract of promising Rapeseed-Mustard genotypes against mustard aphid

Initially preliminary field screening of thirty genotypes along with susceptible, national susceptible and national resistant check were conducted during 2019-20 & 2020-21. Based on API, ADI and ARI the promising genotypes have been identified viz., RTM 314 (resistant), RTM 1624, GSH 2180, GSH 1699, RH 7846, AKGS 19-8 (moderately resistant) and were subjected to GC-MS analysis along with Yellow sarson (66-197-3) (susceptible check), Kranti (National resistant check) and BSH1 (National susceptible check) for identifying the metabolic compounds which are responsible for resistance/susceptibility of the genotypes to mustard aphid.

A total of 38 (RTM 314), 33 (RTM 1624), 34 (GSH 2180), 33 (RH 7846), 35 (AKGS 19-8), 34 (GSH 1699), 28 (Yellow sarson 66-197-3), 31 (Kranti) and 18 (BSH 1) bioactive compounds have been identified through the GC-MS analysis based on the % area covered and retention time. The bioactive compounds along with their % area, retention time, molecular formulae and molecular weight are given in Table 4.21 to 4.29 and Figure 4.9 to 4.17. More number of compounds was identified in all the promising genotypes in comparison to BSH 1 and Yellow sarson 66-197-3 (Table 4.30). Also the % area covered by the compounds viz., 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, n-Hexadecanoic acid and Hexadecanoic acid methyl ester are higher in all the promising genotypes in comparison to BSH 1 (susceptible check). Furthermore, the compounds viz., α -Tocopherol, γ -Sitosterol which have repellent properties are present in all the promising genotypes unlike BSH 1 (susceptible check). Interestingly, in RTM 314 (resistant genotype) three compounds viz., 2-(hydroxymethyl)-2-nitro-1,3-Propanediol; 5-(Methylsulfinyl) pentanenitrile and 1-Isothiocyano-4-(methylsulfinyl) butane have been identified unlike in other genotypes which may be

the reason for resistance against mustard aphid. During attack by a mustard aphid, glucosinolates which are naturally present in mustard plants are activated by myrosinase enzyme and produce toxic metabolites including isothiocyanates (ITCs), thiocyanates, simple nitriles (CNs) and epithionitriles (Kazana *et al.*, 2007; Sun *et al.*, 2021) which act against aphid damage. Among the products of glucosinolate hydrolysis, the toxicity of glucosinolate-derived ITCs is conveyed by the electrophilic $-N=C=S$ functional group which reacts with intracellular nucleophiles and acts against aphid interaction with rapeseed-mustard (Hansch *et al.* 2012; Jeschke *et al.* 2016). Compounds like 9, 12-Octadecadienoic acid, methyl ester reportedly known to act as insectifuge (Arora and Kumar, 2017). Reddy *et al.* (2021); Reddy *et al.* (2022) also anticipated that 9-Octadecenoic acid (Z) and γ -Sitosterol compounds could have oviposition deterring and repellent activity respectively, against pod borer and pulse beetle in chickpea genotypes. Pesticidal properties of Hexadecanoic acid, methyl ester had been reported by (Chandrasekaran *et al.*, 2011) whereas, n-Hexadecanoic acid by Zayed *et al.* (2014). Sivakumar *et al.* (2011) reported the larvicidal and repellent activity of tetradecanoic acid.

Table 4.15: Evaluation of Rapeseed-mustard genotypes against mustard aphid during Rabi, 2019-20

	Genotypes	Mean aphid population at 50% Flowering stage*	Mean aphid population at Pod stage*	Total mean aphid population*	Mean Aphid Population Index (API)*	Mean Aphid Damage Index (ADI)**	Mean Aphid Resistance Index (ARI)
1	RCH1	83.76	165.9	124.8	4.7	4.9	4.8
2	RMM 10-1-1	62.13	101.7	81.9	3.4	3.6	3.5
3	DRMRCI 96	74.98	158.5	116.7	4.2	4.8	4.5
4	DRMR HJ 2403	72.09	154.3	113.2	3.5	3.7	3.6
5	RGN 229	69.32	138.5	103.9	3.1	3.8	3.45
6	RH 1676	65.44	133.4	99.4	3	3.2	3.1
7	SKM 1626	77.54	149.6	113.5	3	3.3	3.15
8	RGN 443	71.09	106.3	88.7	2.7	3.1	2.9
9	PBR 385	67.56	102.5	85.0	2.7	3.4	3.05
10	DRMRIJ 31	78.45	152.7	115.6	2.9	3.2	3.05
11	RH 7846	42.65	71.3	57.0	2.3	2.1	2.2
12	PR 2016-4	81.56	162.8	122.2	3.1	3.6	3.35
13	DRMR 2017-16	73.65	145.0	109.3	3.3	3	3.15
14	NPI 229	81.56	171.4	126.5	3.55	3.05	3.3
15	RH 1424	79.95	154.3	117.1	3.4	3.4	3.4
16	PDZ 11	74.23	136.6	105.4	3.3	3	3.15
17	PDZ 12	78.98	141.5	110.3	3.5	3.5	3.5
18	RH (OE) 1706	69.05	121.9	95.5	3.15	3.2	3.17
19	PDZ 1	80.65	143.6	112.1	3.5	3	3.25
20	GSH 2196	65.98	114.0	90.0	2.85	2.9	2.87
21	AKGS 19-8	41.99	76.6	59.3	2.3	2.1	2.2
22	GSH 1699	40.76	72.1	56.4	2.4	2	2.2
23	CS 2005-143	78.11	158.6	118.4	3.2	3	3.1
24	GSH 2180	39.78	79.3	59.5	2.4	2.1	2.25
25	HNS 0702	75.97	138.8	107.4	2.95	3	2.97
26	RTM 1624	38.76	67.6	53.2	2	2.2	2.1
27	RTM 314	28.89	44.8	36.8	1.4	1	1.2
28	Yellow sarson (66-197-3) (SC)	65.52	170.7	118.1	3.7	4	3.85
29	Kranti (NRC)	32.55	50.2	41.4	2	2	2
30	BSH-1 (NSC)	85.78	192.6	139.2	4	4.5	4.25
	CD (P=0.05)	8.505	16.613	12.317	0.403	0.407	0.403
	SEm(±)	2.997	5.853	4.863	0.142	0.143	0.142
	CV (%)	7.869	8.053	7.935	8.068	7.948	7.972

* Top 10 cm inflorescence, ** whole plant; Susceptible Check (SC), National Resistant Check (NRC), National susceptible Check (NSC)
API- Aphid Population Index; ADI- Aphid Damage Index; ARI- Aphid Resistance Index

Table 4.16: Evaluation of Rapeseed-mustard genotypes against mustard aphid during Rabi, 2020-21

	Genotypes	Mean aphid population at 50% Flowering stage*	Mean aphid population at Pod stage*	Total mean aphid population*	Mean API*	Mean ADI**	Mean ARI
1	RCH1	74.33	151.24	112.78	4.4	4.7	4.55
2	RMM 10-1-1	55.76	90.07	72.91	3.2	3.6	3.4
3	DRMRC196	66.45	145.58	106.01	4	4.2	4.1
4	DRMR HJ 2403	68.12	141.25	104.68	3.9	3.6	3.75
5	RGN 229	61.55	125.41	93.48	3.2	3.6	3.4
6	RH 1676	58.99	117.58	88.28	3	3.3	3.15
7	SKM 1626	69.41	133.02	101.21	3.15	3.4	3.27
8	RGN 443	65.87	94.05	79.96	2.6	2.8	2.7
9	PBR 385	60.55	99.08	79.81	2.5	2.75	2.62
10	DRMRIJ 31	69.77	134.25	102.01	3.05	3	3.02
11	RH 7846	38.23	55.82	47.02	2.2	2	2.1
12	PR 2016-4	72.76	129.47	101.11	3.7	3.3	3.5
13	DRMR 2017-16	66.89	117.97	92.43	3.15	3.4	3.27
14	NPJ 229	74.32	122.03	98.17	3.3	3.7	3.5
15	RH 1424	71.87	131.73	101.8	3.6	3.5	3.55
16	PDZ 11	65.09	124.61	94.85	3.5	3.3	3.4
17	PDZ 12	69.06	115.81	92.43	3.6	3.35	3.47
18	RH (OE) 1706	61.76	106.97	84.36	3.17	3.4	3.28
19	PDZ 1	72.56	121.55	97.05	3.4	3.6	3.5
20	GSH 2196	59.87	95.77	77.82	2.87	3	2.93
21	AKGS 19-8	36.22	69.07	52.64	2.2	2.1	2.15
22	GSH 1699	39.34	68.43	53.88	2.3	2.2	2.25
23	CS 2005-143	71.98	132.88	102.43	3.1	3.05	3.07
24	GSH 2180	31.56	69.55	50.55	2.25	2	2.125
25	HNS 0702	68.11	125.74	96.92	2.97	3	2.98
26	RTM 1624	31.55	59.76	45.65	2	2.3	2.15
27	RTM 314	22.23	39.67	28.95	1.2	1	1.1
28	Yellow sarson 66-197-3 (SC)	60.88	143.98	104.75	3.5	3.9	3.7
29	Kranti (NRC)	29.89	45.33	38.94	2	1.7	1.85
30	BSH-1 (NSC)	79.56	151.66	118.72	3.9	4.1	4
	CD (P=0.05)	7.699	13.964	11.813	0.392	0.407	0.399
	SEm(±)	2.35	4.92	3.529	0.138	0.143	0.141
	CV (%)	7.858	7.998	7.902	7.910	8.023	7.952

* Top 10 cm inflorescence, ** whole plant; Susceptible Check (SC), National Resistant Check (NRC), National susceptible Check (NSC)
API- Aphid Population Index; ADI- Aphid Damage Index; ARI- Aphid Resistance Index

Table 4.17: Evaluation of Rapeseed-mustard genotypes against mustard aphid (data given are the pooled value of Rabi, 2019-20 & 2020-21)

	Genotypes	Mean aphid population at 50% Flowering stage*	Mean aphid population at Pod stage*	Cumulative mean aphid population*	Mean API*	Mean ADI**	Mean ARI
1	RCH1	79.05	158.56	118.80	4.6	4.8	4.68
2	RMM 10-1-1	58.95	95.88	77.41	3.3	3.6	3.45
3	DRMRCI 96	70.72	152.03	111.37	4.1	4.5	4.30
4	DRMR HJ 2403	70.11	147.78	108.94	3.7	3.7	3.68
5	RGN 229	65.44	131.94	98.69	3.2	3.7	3.43
6	RH 1676	62.22	125.48	93.85	3.0	3.3	3.13
7	SKM 1626	73.48	141.29	107.38	3.1	3.4	3.21
8	RGN 443	68.48	100.18	84.33	2.7	3.0	2.80
9	PBR 385	64.06	100.78	82.42	2.6	3.1	2.84
10	DRMRIJ 31	74.11	143.50	108.80	3.0	3.1	3.04
11	RH 7846	40.44	63.56	52.00	2.3	2.1	2.15
12	PR 2016-4	77.16	146.13	111.64	3.4	3.5	3.43
13	DRMR 2017-16	70.27	131.47	100.87	3.2	3.2	3.21
14	NPJ 229	77.94	146.73	112.33	3.4	3.4	3.40
15	RH 1424	75.91	143.04	109.47	3.5	3.5	3.48
16	PDZ 11	69.66	130.60	100.13	3.4	3.2	3.28
17	PDZ 12	74.02	128.67	101.34	3.6	3.4	3.49
18	RH (OE) 1706	65.41	114.41	89.91	3.2	3.3	3.23
19	PDZ 1	76.61	132.55	104.58	3.5	3.3	3.38
20	GSH 2196	62.93	104.88	83.90	2.9	3.0	2.91
21	AKGS 19-8	39.11	72.83	55.97	2.3	2.1	2.18
22	GSH 1699	40.05	70.26	55.16	2.4	2.1	2.23
23	CS 2005-143	75.05	145.75	110.40	3.2	3.0	3.09
24	GSH 2180	35.67	74.40	55.04	2.3	2.1	2.19
25	HNS 0702	72.04	132.26	102.15	3.0	3.0	2.98
26	RTM 1624	35.16	63.70	49.43	2.0	2.2	2.08
27	RTM 314	25.56	42.22	32.89	1.3	1.0	1.15
28	Yellow sarson 66-197-3 (SC)	63.20	157.32	104.75	3.6	4.0	3.78
29	Kranti (NRC)	31.22	47.78	38.94	2.0	1.9	1.93
30	BSH-1 (NSC)	82.67	172.11	118.72	4.0	4.3	4.13
	CD (P=0.05)	8.063	15.346	11.636	0.398	0.407	0.401
	SEm(±)	2.841	5.407	4.100	0.140	0.143	0.141
	CV (%)	7.866	7.986	7.945	7.995	7.985	7.970

* Top 10 cm inflorescence, ** whole plant; Susceptible Check (SC), National Resistant Check (NRC), National susceptible Check (NSC)
API- Aphid Population Index; ADI- Aphid Damage Index; ARI- Aphid Resistance Index

Table 4.18: Categorization of Rapeseed-mustard genotypes against mustard aphid based on mean Aphid Resistance Indices (ARI) of 2019-20 & 2020-21

Sl.No	Genotypes (with ARI)	Resistance category
1.	NIL	0.0-1.0= Highly Resistant
2.	RTM 314 (1.15)	1.1-2.0= Resistant
3.	RTM 1624 (2.1), GSH 2180 (2.19), RH 7846 (2.15), AKGS 19-8 (2.18), GSH 1699 (2.23), Kranti (1.93)	2.1-2.5= Moderately resistant
4.	RMM 10-1-1 (3.45), GSH 2196 (2.91), RGN 443 (2.8), PBR 385 (2.84), RGN 229 (3.43), PR 2016-4 (3.43), DRMR 2017-16 (3.21), NPJ 229 (3.40), RH 1424 (3.48), PDZ 11 (3.28), PDZ 12 (3.49), RH (OE) 1706 (3.23), PDZ 1 (3.38), DRMRIJ 31 (3.04), RH 1676 (3.13), SKM 1626 (3.21), DRMR HJ 2403 (3.68), HNS 0702 (2.98), CS 2005-14 (3.09)	2.6- 3.5= Susceptible
5.	RCH1 (4.68), DRMRCI 96 (4.30), Yellow sarson 66-197-3 (3.78), BSH1 (4.13)	3.6-5.0= Highly susceptible

Table 4.19: Biochemical constituents of promising Rapeseed-mustard genotypes in relation to aphid infestation

Genotypes	Glucosinolates ($\mu\text{mole/g}$)	Phenols (mg/g)	Total Sugars (mg/g)
RH 7846	91.21 \pm 6.84 ^b	5.01 \pm 0.37 ^{bc}	8.15 \pm 0.61 ^{bc}
AKGS 19-8	89.68 \pm 4.51 ^b	4.52 \pm 0.22 ^{cd}	9.12 \pm 0.45 ^{cd}
GSH 1699	86.34 \pm 3.11 ^b	4.39 \pm 0.15 ^{cd}	9.56 \pm 0.34 ^d
GSH 2180	83.66 \pm 2.10 ^b	4.18 \pm 0.10 ^d	10.04 \pm 0.25 ^d
RTM 1624	95.76 \pm 1.46 ^b	5.42 \pm 0.08 ^b	7.08 \pm 0.10 ^{ab}
RTM 314	109.87 \pm 3.96 ^a	6.51 \pm 0.23 ^a	6.42 \pm 0.23 ^a
Yellow sarson (66-197-3) (SC)	51.62 \pm 2.58 ^c	3.01 \pm 0.15 ^e	12.82 \pm 0.64 ^e
Kranti (NRC)	92.45 \pm 3.50 ^b	4.62 \pm 0.17 ^{cd}	7.84 \pm 0.29 ^{bc}
BSH-1 (NSC)	44.21 \pm 1.35 ^c	2.56 \pm 0.07 ^e	14.23 \pm 0.43 ^f

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.20: Correlation between biochemical constituents of promising Rapeseed-mustard genotypes and Aphid Infestation Index (AII)

Variable	Glucosinolates	Phenols	Total sugars
Aphid Infestation Index (AII)	-0.971**	-0.891**	0.643 ^{NS}

** Significant at P= 0.01

NS= Non-significant

Table 4.21: Bioactive compounds identified from inflorescence of RTM 314 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. Wt
1	8.740	3328700	6.08	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	C ₄ H ₉ NO ₅	151.12
2	9.677	2191772	4.00	5-(Methylsulfinyl)pentanenitrile	C ₆ H ₁₁ NOS	145.23
3	11.493	182260	0.33	Tetradecanoic acid, methyl ester	C ₁₅ H ₃₀ O ₂	242.4
4	12.042	650631	1.19	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37
5	12.581	1073542	1.96	1-Isothiocyano-4-(methylsulfinyl)butane	C ₆ H ₁₁ NOS ₂	177.3
6	13.614	1062343	1.94	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
7	14.081	2267165	4.14	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
8	14.749	104352	0.19	Palmitic acid	C ₁₉ H ₄₀ O ₂ Si	328.6
9	15.252	2326292	4.25	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
10	15.310	2333649	4.26	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
11	15.437	937804	1.71	Phytol	C ₂₀ H ₄₀ O	296.5
12	15.546	276130	0.50	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
13	15.804	14495743	26.47	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
14	15.991	787076	1.44	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282.5
15	16.610	352915	0.64	1,E-8,Z-10-Tridecatriene	C ₁₃ H ₂₂	178.31
16	16.660	321969	0.59	Pentadec-7-ene, 7-bromomethyl-	C ₁₆ H ₃₁ Br	303.32
17	16.954	361048	0.66	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
18	17.075	1527224	2.79	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
19	17.305	240573	0.44	Cyclohexanone, 2-butyl-	C ₁₀ H ₁₈ O	154.25
20	18.227	590955	1.08	Pyrrrolidine-1-dithiocarboxylic acid, 2-oxocyclopentyl ester	C ₁₀ H ₁₅ NOS ₂	229.4
21	18.368	868927	1.59	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
22	18.408	768745	1.40	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
23	18.521	2358896	4.31	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
24	18.557	1969438	3.60	cis-9-Octadecenal	C ₁₈ H ₃₄ O	266.5
25	18.756	635440	1.16	Pentadecanoic acid, 15-hydroxy-	C ₁₅ H ₃₀ O ₃	258.5
26	19.907	1379310	2.52	3-n-Butylthiophene-1,1-dioxide	C ₈ H ₁₂ O ₂ S	242.43
27	20.104	1342979	2.45	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₄₀ O ₄	356.5
28	20.288	389314	0.71	Cyclopentadecanone, 2-methyl-	C ₁₆ H ₃₀ O	238.41
29	22.047	840124	1.53	Phytyl decanoate	C ₃₀ H ₅₈ O ₂	450.8
30	23.243	253563	0.46	Stigmasteryl methyl ether	C ₃₀ H ₅₂ O	428.5
31	23.452	360844	0.66	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
32	23.531	237523	0.43	erythro-9,10-Dibromopentacosane	C ₂₅ H ₅₀ Br ₂	510.5
33	24.563	735154	1.34	Ergost-5-en-3-ol	C ₂₈ H ₄₈ O	400.7
34	25.518	1342373	2.45	γ-Sitosterol	C ₂₉ H ₅₂ O ₂	432.7
35	27.092	373565	0.68	2-Heptadecyloxirane	C ₁₉ H ₃₈ O	282.5
36	27.770	3200010	5.84	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
37	30.303	1773403	3.24	14-Methyl-8-hexadecyn-1-o	C ₁₇ H ₃₂ O	352.2
38	35.890	518873	0.95	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		54760624	100.00			

Table 4.22: Bioactive compounds identified from inflorescence of RTM 1624 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. Wt
1	8.829	1140648	2.50	Xanthosine	C ₁₀ H ₁₂ N ₄ O ₆	284.23
2	12.071	236814	0.52	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37
3	13.615	779681	1.71	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
4	14.082	2470164	5.41	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
5	14.746	151186	0.33	Palmitic acid	C ₁₉ H ₄₀ O ₂ Si	328.6
6	15.250	2408315	5.27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
7	15.308	2152596	4.71	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
8	15.440	155191	0.34	Phytol	C ₂₀ H ₄₀ O	296.5
9	15.544	333461	0.73	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
10	15.800	12803222	31.78	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
11	15.981	309426	0.68	Undec-10-ynoic acid, undecyl ester	C ₂₂ H ₄₀ O ₂	336.6
12	16.610	375410	0.82	1,E-11,Z-13-Octadecatriene	C ₁₈ H ₃₂	248.4
13	16.660	342718	0.75	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280.5
14	16.951	269026	0.59	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
15	17.074	1784725	4.97	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
16	18.132	513953	1.13	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354.5
17	18.368	527653	1.16	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
18	18.404	333700	0.73	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
19	18.519	2798329	6.13	1,8,11-Heptadecatriene, (Z,Z,Z)-	C ₁₇ H ₃₀	234.4
20	18.753	709057	1.55	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
21	19.285	203647	0.45	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.4
22	19.912	1755606	3.84	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
23	20.127	1449201	3.17	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
24	20.265	1491581	3.27	Pyrrolidine-1-dithiocarboxylic acid, 2-oxocyclopentyl ester	C ₁₀ H ₁₅ NOS ₂	229.4
25	22.045	201703	0.44	Phetyl decanoate	C ₃₀ H ₅₈ O ₂	450.8
26	22.113	197989	0.43	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.4
27	23.241	344604	0.75	Stigmasteryl methyl ether	C ₃₀ H ₅₂ O	428.5
28	23.529	337242	0.74	Cholestan-7-one, cyclic 1,2-ethanediyl acetal, (5.alpha.)-	C ₂₉ H ₅₀ O ₂	430.7
29	25.516	570181	1.25	γ -Sitosterol	C ₂₉ H ₅₂ O ₂	432.7
30	27.095	360217	0.79	E-11-Hexadecenal	C ₁₆ H ₃₀ O	238.41
31	27.730	3822162	8.37	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
32	30.292	1552666	3.40	Neronine, 4.beta.,5-dihydro-	C ₁₈ H ₂₁ NO ₆	347.4
33	35.884	592040	1.30	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		45663793	100.00			

Table 4.23: Bioactive compounds identified from inflorescence of GSH 2180 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical formulae	Mol. wt
1	8.830	1201711	1.93	Xanthosine	C ₁₀ H ₁₂ N ₄ O ₆	284.23
2	9.889	262148	0.42	3-tert-butyl-4-hydroxyanisole	C ₁₁ H ₁₆ O ₂	180.24
3	10.035	97820	0.16	Dichloroacetic acid, tridec-2-ynyl ester	C ₁₅ H ₂₄ Cl ₂ O ₂	306.2
4	13.615	1334304	2.29	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
5	14.079	2953276	4.75	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
6	14.746	158594	0.26	Palmitic acid	C ₁₉ H ₄₀ O ₂ Si	328.6
7	15.038	254572	0.41	13-Hexyloxacyclotridec-10-en-2-one	C ₁₈ H ₃₂ O ₂	280.4
8	15.249	2997701	4.83	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
9	15.308	2978419	4.80	(9E,12E)-9,12-Octadecadienoyl chloride	C ₁₈ H ₃₁ ClO	298.9
10	15.364	149357	0.24	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
11	15.449	712014	1.15	Phytol	C ₂₀ H ₄₀ O	296.5
12	15.544	495404	0.80	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
13	15.802	16589872	26.71	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
14	15.982	427225	0.69	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282.5
15	16.610	414323	0.67	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
16	16.660	372343	0.60	7-(Bromomethyl)-7-pentadecene	C ₁₆ H ₃₁ Br	303.32
17	16.952	429647	0.69	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
18	17.074	1676894	3.75	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
19	17.309	191904	0.31	Eicosanoic acid, methyl ester	C ₂₁ H ₄₂ O ₂	326.6
20	17.731	398564	0.64	9-Octadecenamamide, (Z)-	C ₁₈ H ₃₅ NO	281.5
21	18.131	481222	0.77	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
22	18.366	841247	1.35	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
23	18.405	614815	0.99	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
24	18.519	5977944	9.62	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
25	18.557	8113643	13.06	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
26	19.282	159100	0.26	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
27	19.905	1813010	2.92	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
28	20.103	1629263	2.62	14-methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252.4
29	23.236	393701	0.63	Stigmasteryl methyl ether	C ₃₀ H ₅₂ O	428.5
30	23.447	262633	0.42	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
31	25.523	692773	1.12	γ-Sitosterol	C ₂₉ H ₅₀ O	414.7
32	27.719	3277937	5.98	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
33	30.284	1873247	3.02	Neronine, 4.beta.,5-dihydro-	C ₁₈ H ₂₁ NO ₆	347.4
34	35.861	710509	1.14	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		62113517	100.00			

Table 4.24: Bioactive compounds identified from inflorescence of RH 7846 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. Wt
1	8.694	2964677	6.02	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	283.24
2	13.618	931733	1.89	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
3	14.084	1511856	3.07	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
4	15.252	2248323	4.57	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
5	15.312	2512530	5.10	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
6	15.373	173466	0.35	6-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296.5
7	15.441	782919	1.59	Phytol	C ₂₀ H ₄₀ O	296.5
8	15.548	395382	0.80	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
9	15.798	7394933	15.74	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
10	16.139	464119	0.94	Linoelaidic acid	C ₁₈ H ₃₂ O ₂	280.4
11	16.613	322530	0.65	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
12	16.956	361645	0.73	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
13	17.077	1483760	4.39	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
14	17.741	522165	1.06	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	281.5
15	18.136	429367	0.87	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354.5
16	18.372	618819	1.26	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
17	18.411	525409	1.07	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
18	18.562	12489731	25.36	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
19	18.953	184441	0.37	Docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂	354.6
20	19.757	242790	0.49	cis-13-Docosenoyl chloride	C ₂₂ H ₄₁ ClO	357
21	19.917	1448908	2.94	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
22	20.123	1923293	4.40	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
23	20.950	166061	0.34	Chloromethyl 2-chlorododecanoate	C ₁₃ H ₂₄ Cl ₂ O ₂	283.2
24	21.641	273915	0.56	Pentatriacontane	C ₃₅ H ₇₂	492.9
25	22.123	177489	0.36	4-Trifluoromethylbenzoic acid, 10-undecenyl ester	C ₁₉ H ₂₅ F ₃ O ₂	342.4
26	22.984	494162	1.00	Octacosan-14-one	C ₂₈ H ₅₆ O	408.7
27	23.244	301256	0.61	Stigmasteryl methyl ether	C ₃₀ H ₅₂ O	428.5
28	23.458	186810	0.38	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
29	25.526	722329	1.47	γ-Sitosterol	C ₂₉ H ₅₂ O ₂	432.7
30	27.107	362406	0.74	3-Cyclohexylnonadecane	C ₂₅ H ₅₀	350.7
31	27.781	3448007	7.00	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
32	30.304	1328019	2.70	Neronine, 4.beta.,5-dihydro-	C ₁₈ H ₂₁ NO ₆	347.4
33	35.916	578198	1.17	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		49251011	100.00			

Table 4.25: Bioactive compounds identified from inflorescence of AKGS 19-8 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. wt
1	8.814	704694	0.63	Xanthosine	C ₁₀ H ₁₂ N ₄ O ₆	284.23
2	9.875	137771	0.12	1,2,4-cyclopentanetrione, 3-(2-pentenyl)-	C ₁₀ H ₁₂ O ₃	180.2
3	11.486	419634	0.38	Tetradecanoic acid, methyl ester	C ₁₅ H ₃₀ O ₂	242.4
4	12.067	171331	0.15	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37
5	13.608	2286499	2.05	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
6	14.081	8409394	7.93	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
7	15.019	423004	0.38	13-Hexyloxacyclotridec-10-en-2-one	C ₁₈ H ₃₂ O ₂	280.4
8	15.245	4605322	4.14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
9	15.305	4027229	3.62	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
10	15.359	246273	0.22	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296.5
11	15.458	840801	0.76	Phytol	C ₂₀ H ₄₀ O	296.5
12	15.538	858231	0.77	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
13	15.826	41133999	36.96	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
14	15.993	1368260	1.23	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282.5
15	16.601	667620	0.60	1,E-8,Z-10-Tetradecatriene	C ₁₄ H ₂₄	192.34
16	16.652	485950	0.44	1,3-Cyclohexadecanedione, 6-nitro-	C ₁₆ H ₂₇ NO ₄	297.0
17	16.953	268237	0.24	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
18	17.067	4016244	5.34	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
19	17.309	288513	0.26	Methyl 18-methylnonadecanoate	C ₂₁ H ₄₂ O ₂	326.6
20	18.123	349478	0.31	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₃₈ O ₄	354.5
21	18.365	779970	0.70	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
22	18.517	9250608	8.31	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
23	18.553	5267833	4.81	Glycidyl oleate	C ₂₁ H ₃₈ O ₃	338
24	19.123	194787	0.18	10-Undecen-1-al, 2-methyl-	C ₁₂ H ₂₂ O	182.3
25	19.274	361702	0.33	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
26	20.133	1748139	1.57	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
27	20.798	1120920	1.01	Pyrrolidine, 1-(1-oxo-9-octadecenyl)-, (Z)-	C ₂₂ H ₄₁ NO	335.6
28	22.102	477829	0.43	Z,E-3,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266.5
29	23.230	748658	0.67	Stigmast-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	678.0
30	23.446	303553	0.27	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
31	25.536	1004536	0.90	γ-Sitosterol	C ₂₉ H ₅₀ O	414.7
32	27.080	1152912	1.04	(14Z)-14-Tricosenyl	C ₂₄ H ₄₆ O ₂	366.6
33	30.259	4826856	4.34	(R)-(-)-(Z)- 14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₄ O	254.5
34	31.122	3964993	11.12	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
35	35.859	738782	1.36	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		111282319	100.00			

Table 4.26: Bioactive compounds identified from inflorescence of GSH 1699 through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. wt
1	8.732	1730000	1.89	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	283.24
2	9.870	413110	0.45	3',5'-Dimethoxyacetophenone	C ₁₀ H ₁₂ O ₃	180.2
3	13.610	1942268	2.12	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
4	14.081	6466390	7.07	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
5	15.029	233537	0.26	13-Hexyloxacyclotridec-10-en-2-one	C ₁₈ H ₃₂ O ₂	280.4
6	15.247	4240701	4.64	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
7	15.306	3899292	4.27	(9E,12E)-9,12-Octadecadienoyl chloride	C ₁₈ H ₃₁ ClO	298.9
8	15.362	215081	0.24	11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
9	15.451	1005189	1.10	Phytol	C ₂₀ H ₄₀ O	296.5
10	15.542	685191	0.75	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
11	15.819	26005129	30.45	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
12	15.992	771145	1.71	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282.5
13	16.955	598695	0.65	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
14	17.072	2777436	4.31	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
15	17.241	180825	0.20	2-Pyrrolidinone, 1-[2-(4-piperidinyl)ethyl]-	C ₁₁ H ₂₀ N ₂ O	196.29
16	17.305	318095	0.35	Tridecanedioic acid, dimethyl ester	C ₁₅ H ₂₈ O ₄	272.38
17	18.128	229548	0.25	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₃₈ O ₄	354.0
18	18.368	2225455	2.43	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
19	18.407	2234048	2.44	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
20	18.517	6097983	6.67	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
21	18.553	3581305	3.92	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
22	19.137	285562	0.60	Hexadecanal, 2-methyl-	C ₁₇ H ₃₄ O	254.0
23	19.278	330050	0.36	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
24	19.906	2459417	2.69	Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl-	C ₇ H ₁₂ Cl ₂	167.07
25	20.803	452364	0.49	Pyrrolidine, 1-(1-oxo-9,15-octadecadienyl)-	C ₂₂ H ₃₉ NO	333.6
26	22.040	116170	0.13	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	C ₅₇ H ₁₀₄ O ₆	885.4
27	22.109	336567	0.37	Z,E-2,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266.5
28	23.447	507393	0.56	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
29	25.527	1038791	1.14	γ-Sitosterol	C ₂₉ H ₅₀ O	414.7
30	29.673	4276483	7.79	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
31	30.273	3688095	4.03	N-[2-(tetradecyloxy)phenyl]acetamide	C ₂₂ H ₃₇ NO ₂	347.4
32	30.693	462582	0.51	cis-15-Tetracosenoic acid, propyl ester	C ₂₇ H ₅₂ O ₂	408.7
33	35.843	759699	0.83	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
34	37.341	680134	0.74	Octadecanoic acid, 2,3-bis(acetyloxy)propyl ester	C ₂₅ H ₄₆ O ₆	442.6
		91407655	100.00			

Table 4.27: Bioactive compounds identified from inflorescence of Yellow sarson (66-197-3) (Susceptible Check) through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formulae	Mol. Wt
1	8.815	1119069	2.46	Xanthosine	C ₁₀ H ₁₂ N ₄ O ₆	284.23
2	9.933	244491	0.54	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-	C ₁₀ H ₁₂ O ₃	180.2
3	13.617	1051125	2.31	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
4	14.083	2381771	5.24	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
5	14.746	135401	0.30	Palmitic acid	C ₁₉ H ₄₀ O ₂ Si	328.6
6	15.252	2729466	6.01	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
7	15.310	2675739	5.89	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
8	15.368	154203	0.34	6-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296.5
9	15.547	445841	0.98	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
10	15.800	13203355	33.01	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
11	15.981	316991	0.70	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.4
12	16.610	398832	0.88	1,E-8,Z-10-Hexadecatriene	C ₁₆ H ₂₈	220.39
13	16.953	351808	0.77	Fumaric acid, 2-dimethylaminoethyl octadecyl ester	C ₂₆ H ₄₉ NO ₄	439.7
14	17.075	1747057	5.84	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
15	17.731	562544	1.24	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	281.5
16	18.133	475678	1.05	trans,trans-9,12-Octadecadienoic acid, propyl ester	C ₂₁ H ₃₈ O ₂	322.5
17	18.367	701044	1.54	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
18	18.404	468871	1.03	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
19	18.518	2920845	6.43	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
20	19.284	197731	0.44	(6Z,9Z)-6,9-Pentadecadien-1-ol	C ₁₅ H ₂₈ O	224.38
21	19.908	1803040	3.97	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
22	20.110	1755148	3.86	14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252.4
23	22.115	145218	0.32	9-Octadecyne	C ₁₈ H ₃₄	250.5
24	25.526	353918	0.78	Stigmast-5-en-3-ol, (3.beta.)-	C ₂₉ H ₅₀ O	414.7
25	27.092	394718	0.87	3-Cyclohexylnonadecane	C ₂₅ H ₅₀	350.7
26	27.734	2988878	6.58	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
27	30.280	1631563	3.59	Neronine, 4.beta.,5-dihydro-	C ₁₈ H ₂₁ NO ₆	347.4
28	35.862	845789	1.86	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		45442402	100.00			

Table 4.28: Bioactive compounds identified from inflorescence of Kranti (National Resistant Check) through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formula	Mol. Wt
1	8.863	1093766	3.39	(Z)-Caryophyllene	C ₁₅ H ₂₄	204.2
2	13.625	515707	1.60	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
3	14.092	532525	1.65	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
4	15.256	1511136	4.69	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
5	15.315	1619516	5.02	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
6	15.377	120548	0.37	14,17-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
7	15.550	292431	0.91	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
8	16.139	111470	0.35	Methyl 10-trans,12-cis-octadecadienoate	C ₁₉ H ₃₄ O ₂	294.5
9	16.613	270850	0.84	1,E-11,Z-13-Heptadecatriene	C ₁₇ H ₃₀	234.4
10	16.883	9635	0.03	Octadecanoic acid, 2-propenyl ester	C ₂₁ H ₄₀ O ₂	324.5
11	16.953	84823	0.26	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
12	17.077	1364958	4.66	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
13	18.130	99411	0.31	1,3-Cyclododecadiene	C ₁₂ H ₂₀	164.5
14	18.172	87746	0.27	Oleoyl chloride	C ₁₈ H ₃₃ ClO	300.9
15	18.370	275245	0.85	N,6-Dimethyl-5-hepten-2-amine	C ₉ H ₁₉ N	414.25
16	18.409	238992	0.74	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
17	18.524	3913009	12.14	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
18	18.558	5016216	29.46	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	328.41
19	18.705	166272	0.52	6,11-Hexadecadien-1-OL	C ₁₆ H ₃₀ O	328.41
20	19.285	263643	0.82	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
21	19.916	1266894	3.93	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
22	20.120	1583245	4.91	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354.2
23	20.226	331524	1.03	2-Methyl-n-hexacosane	C ₂₇ H ₅₆	380.5
24	21.384	76023	0.24	Heneicosane	C ₂₁ H ₄₄	296.6
25	21.640	479704	1.49	Hexatriacontane	C ₃₆ H ₇₄	507
26	22.984	576815	1.79	Octacosan-14-one	C ₂₈ H ₅₆ O	408.7
27	23.245	271382	0.84	Stigmast-5-en-3-ol, (3.beta.)-	C ₂₉ H ₅₀ O	414.7
28	25.529	840322	2.61	γ-Sitosterol	C ₂₉ H ₅₂ O ₂	432.7
29	27.790	2573577	7.98	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
30	30.307	977059	3.03	Z,Z-3,13-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266.5
31	35.883	442420	1.37	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.6
		32241099	100.00			

Table 4.29: Bioactive compounds identified from inflorescence of BSH-1 (National Susceptible Check) through GC-MS

Peak#	R.Time	Area	Area%	Name	Chemical Formula	Mol. Wt
1	13.625	376782	1.20	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
2	14.093	410974	1.31	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
3	15.257	1165722	4.07	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
4	15.315	1267482	4.03	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296.4
5	15.550	228440	0.73	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
6	16.613	266385	0.85	1,E-8,Z-10-Hexadecatriene	C ₁₆ H ₂₈	220.39
7	17.079	1395898	7.08	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
8	18.174	111239	0.35	Oleoyl chloride	C ₁₈ H ₃₃ ClO	300.9
9	18.372	63030	0.20	Undecanal, 2-methyl-	C ₁₂ H ₂₄ O	184.32
10	18.525	4214965	13.41	1,8,11-Heptadecatriene, (Z,Z)-	C ₁₇ H ₃₀	234.4
11	18.562	8602751	41.65	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238.41
12	19.289	236053	0.75	9,12-Octadecadienoyl chloride, (Z,Z)-	C ₁₈ H ₃₁ ClO	298.9
13	19.917	1148640	3.65	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
14	22.126	154410	0.49	(Z)6-Pentadecen-1-ol	C ₁₅ H ₃₀ O	226.4
15	23.249	279823	0.89	Stigmast-5-en-3-ol, (3.beta.)-	C ₂₉ H ₅₀ O	414.7
16	27.786	2649044	8.43	Sebacic acid, di(dec-4-enyl) ester	C ₃₀ H ₅₄ O ₄	478.7
17	35.887	388055	1.23	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		31436949	100.00			

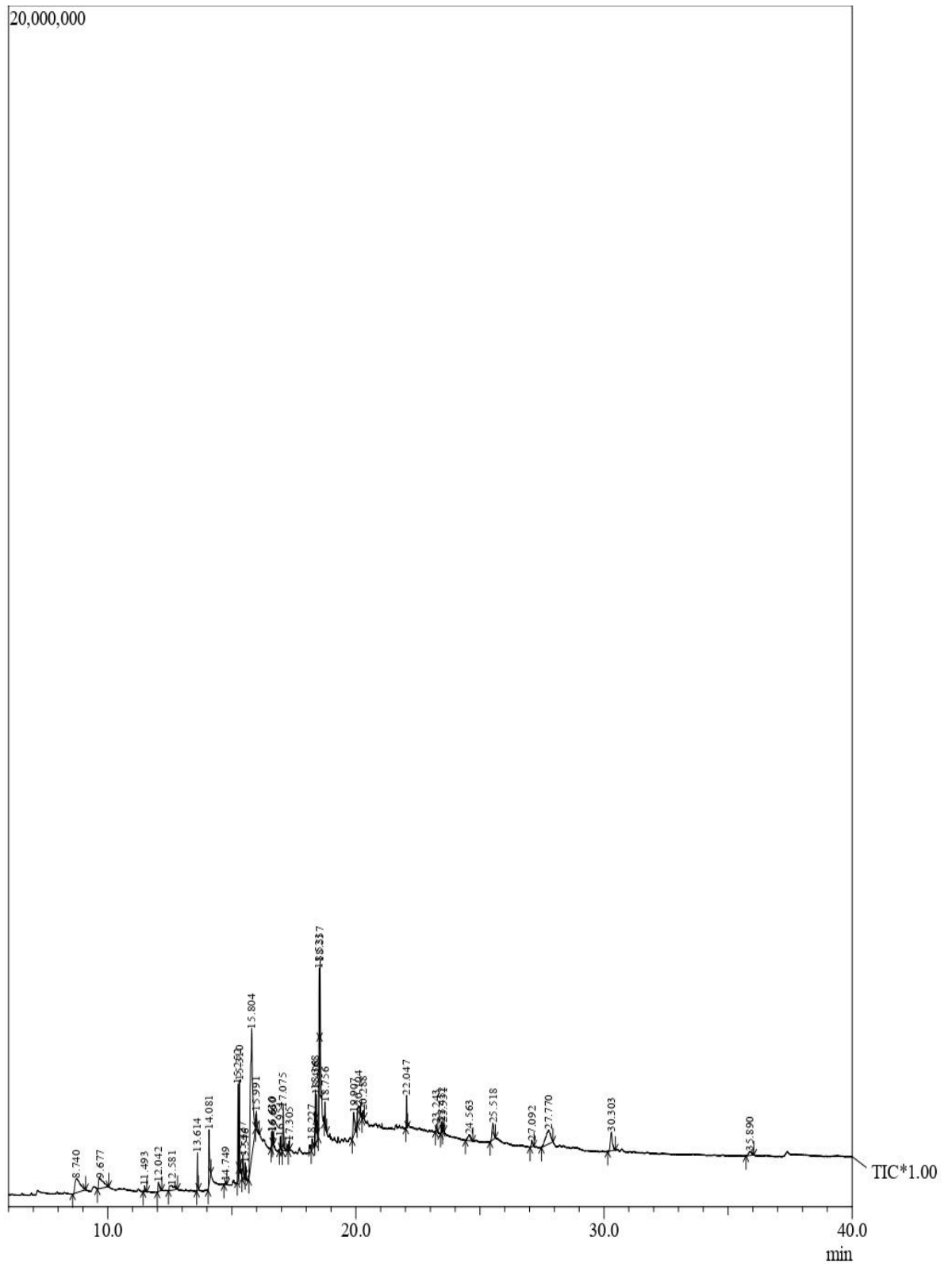


Figure 4.9: GC-MS chromatogram of genotype RTM 314

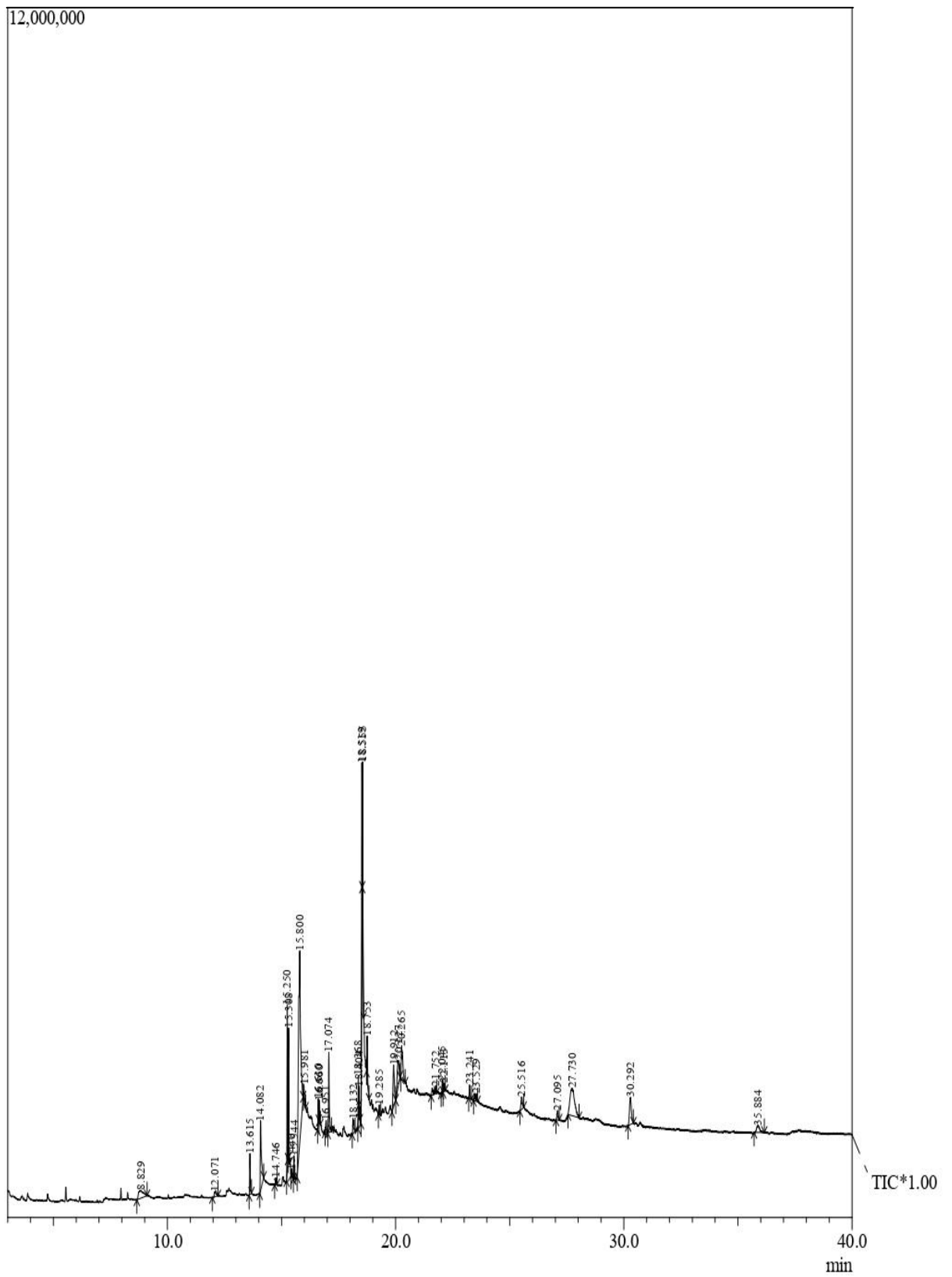


Figure 4.10: GC-MS chromatogram of genotype RTM 1624

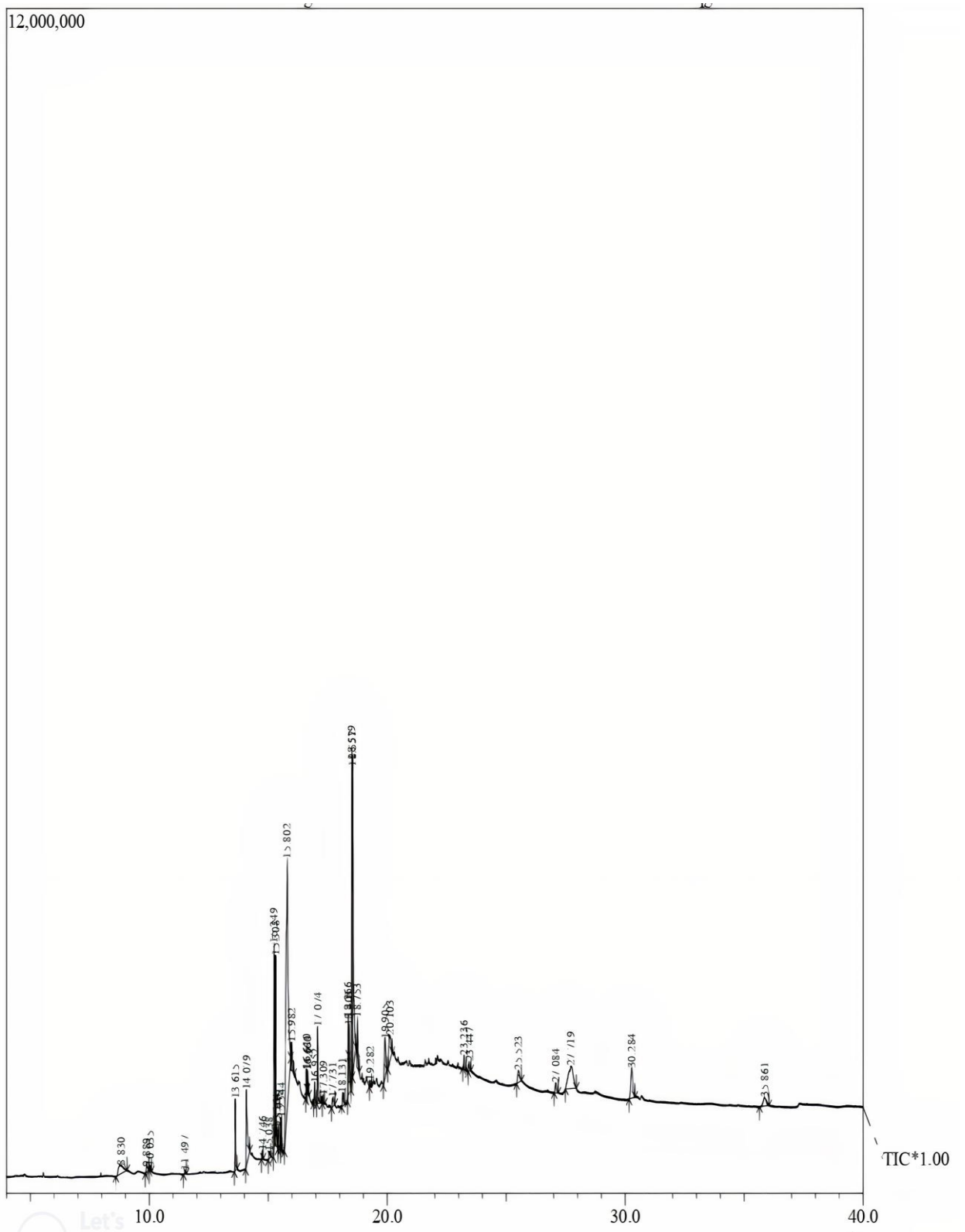


Figure 4.11: GC-MS chromatogram of genotype GSH 2180

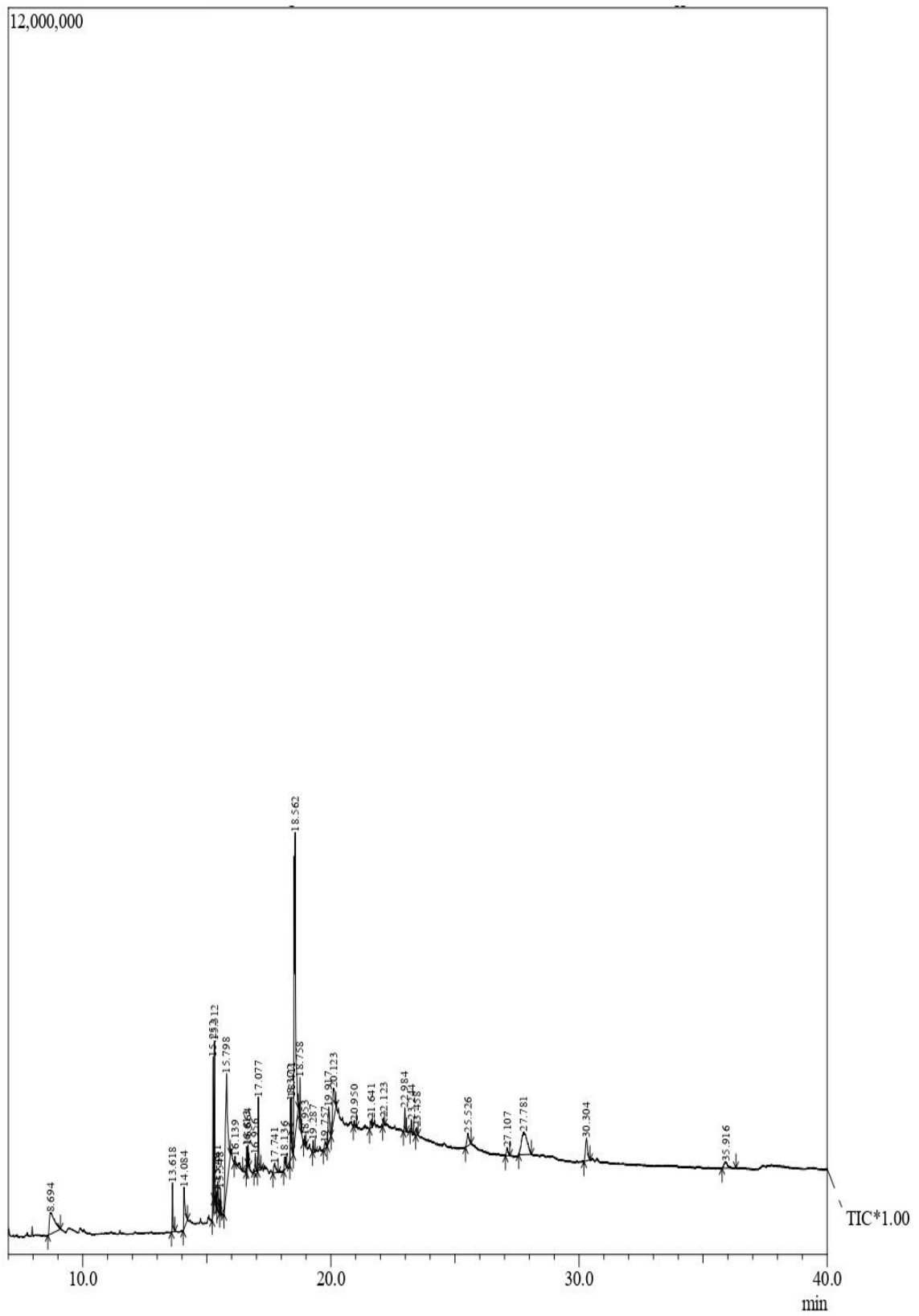


Figure 4.12: GC-MS chromatogram of genotype RH 7846

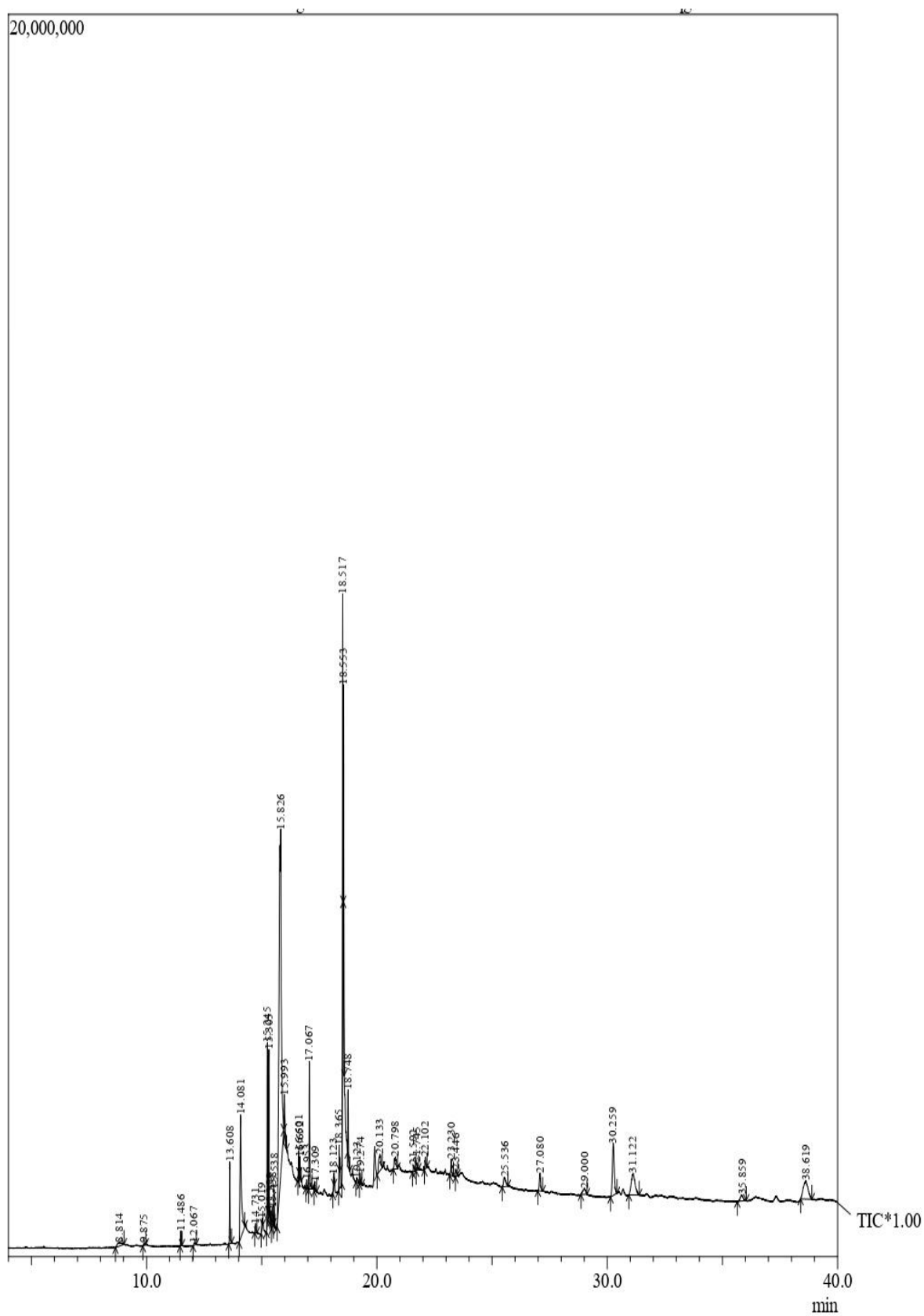


Figure 4.13: GC-MS chromatogram of genotype AKGS 19-8

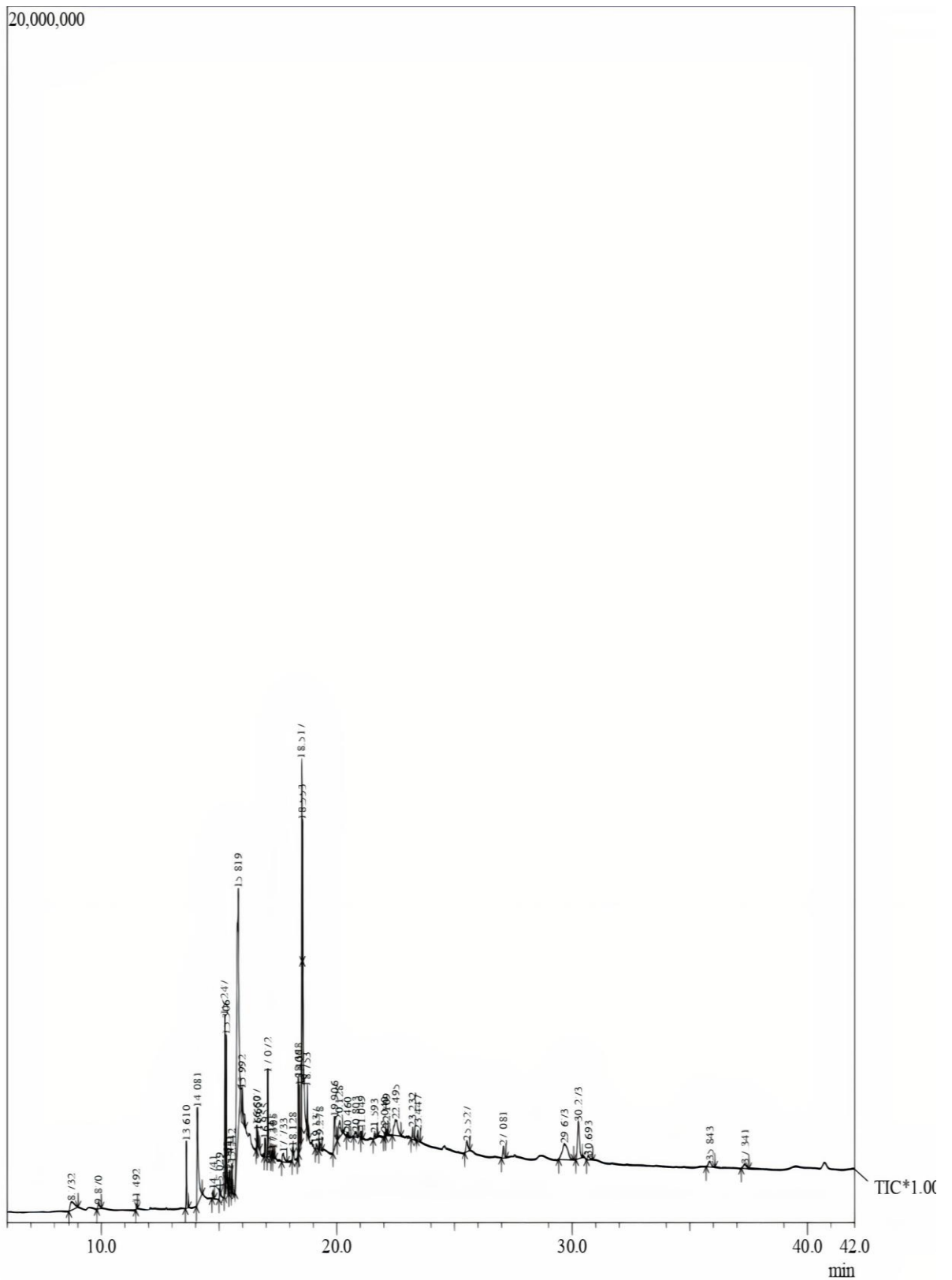


Figure 4.14: GC-MS chromatogram of genotype GSH 1699

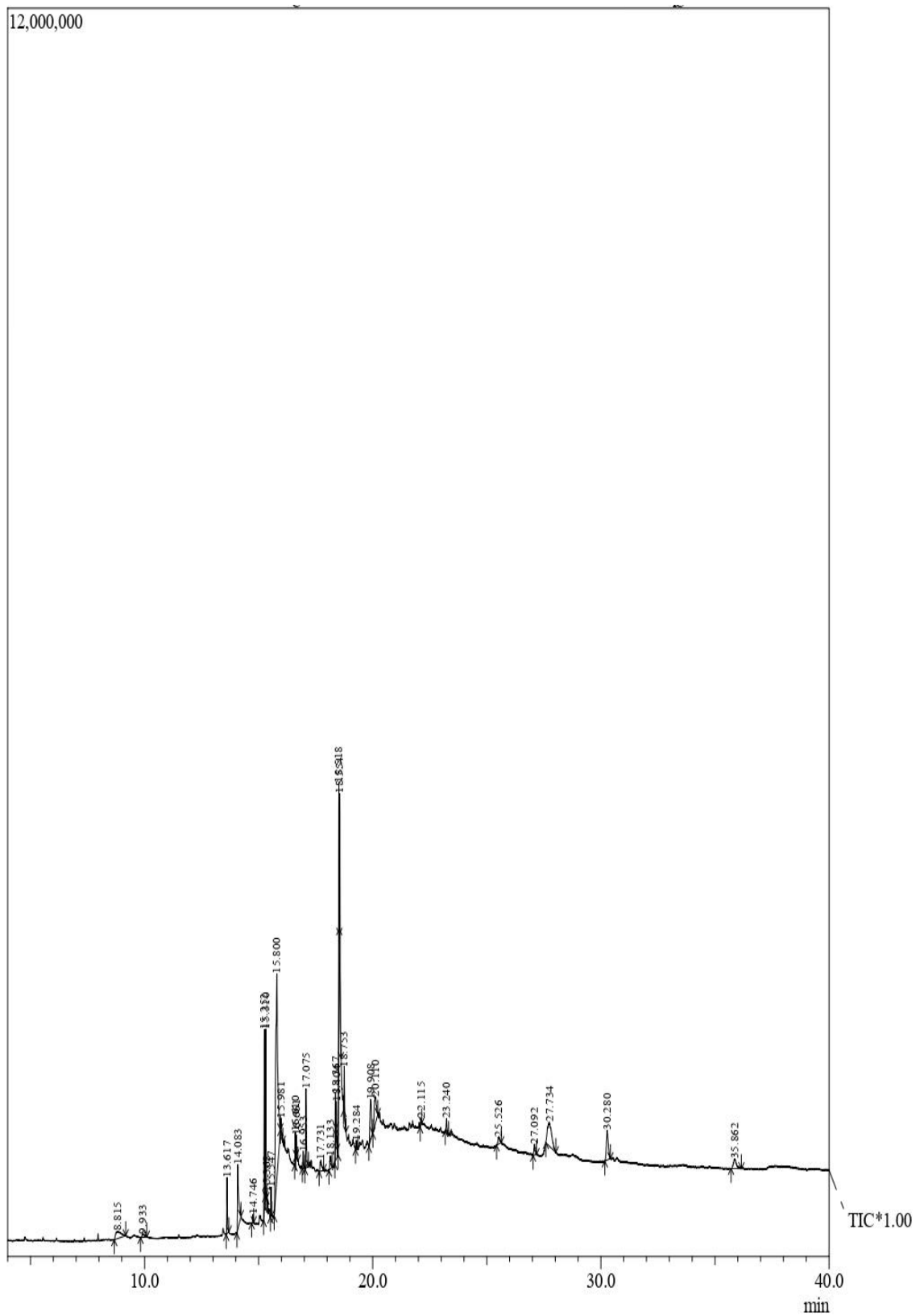


Figure 4.15: GC-MS chromatogram of genotype Yellow sarson (66-197-3)

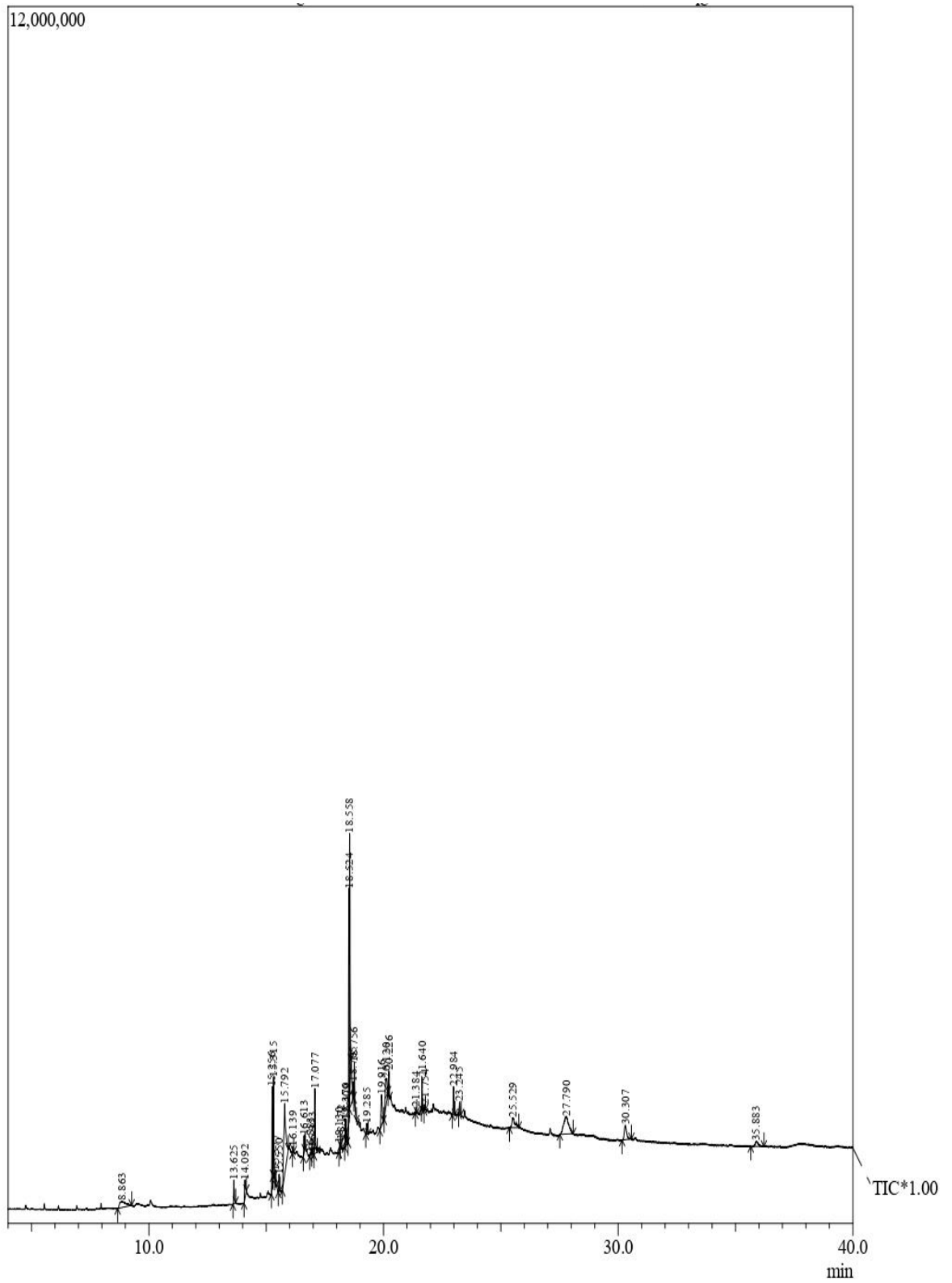


Figure 4.16: GC-MS chromatogram of genotype Kranti

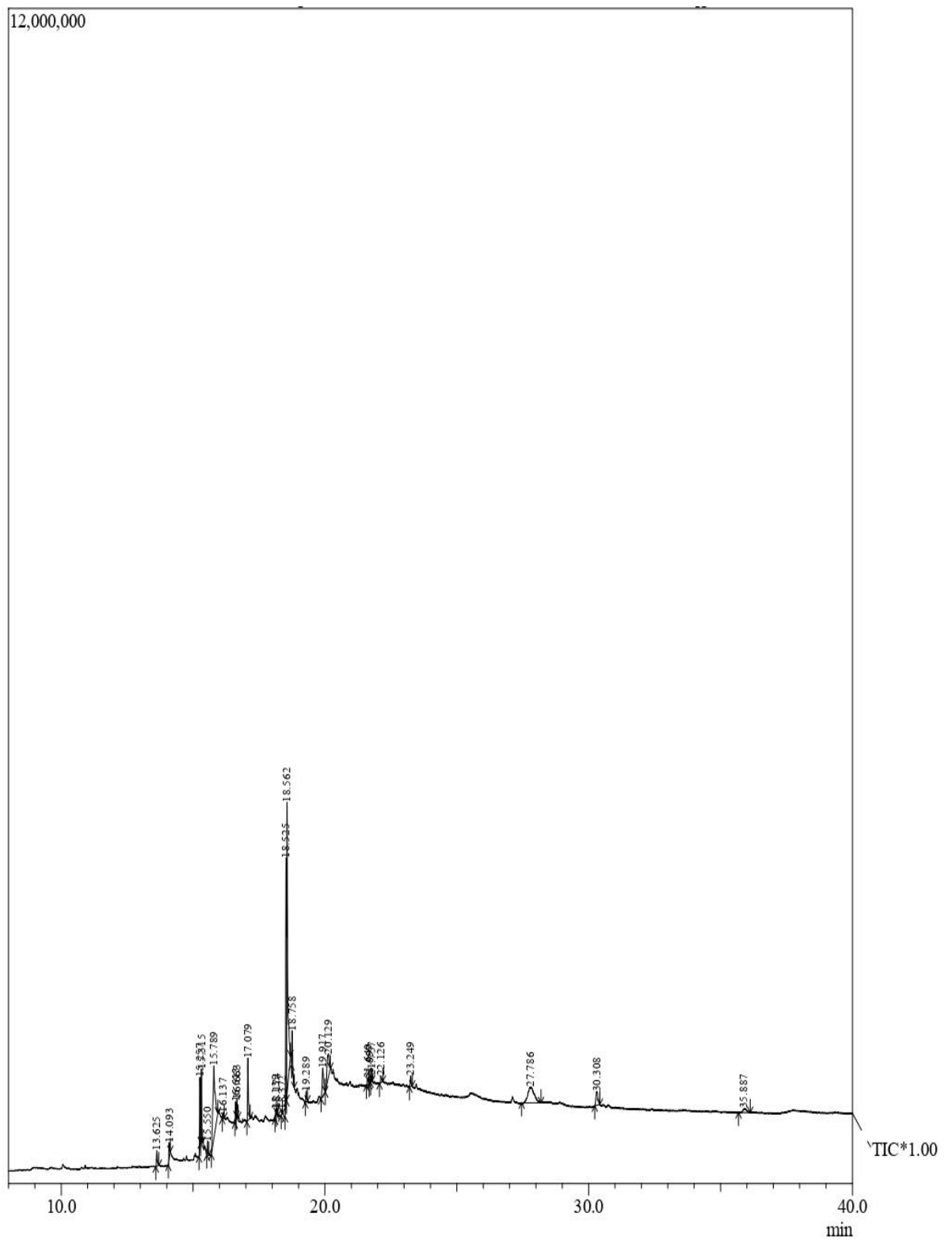


Figure 4.17: GC-MS chromatogram of genotype BSH-1

Table 4.30 Comparison of bio-active compounds of promising Rapeseed-mustard promising genotypes

Retention time	Compound name	Properties	Genotypes									
			RH7846	AKGS 19-8	GSH 1699	GSH 2180	RTM 1624	RTM 314	Yellow Sarson (66-197-3) (SC)	Kranti (NRC)	BSH-1 (NSC)	
8.74	2-(hydroxymethyl)-2-nitro-1,3-Propanediol	Insecticidal										
8.863	(E)-Caryophyllene	Insecticidal, repellent										
9.677	5-(Methylsulfinyl) pentanenitrile	Insecticidal,										
11.486	Tetradecanoic acid, methyl ester	Oviposition deterring and repellent										
12.067	Tetradecanoic acid	Oviposition deterring and repellent										
12.581	1-Isothiocyanato-4-(methylsulfinyl)butane	Insecticidal										
13.614	Hexadecanoic acid, methyl ester	Insecticidal, repellent										
14.081	n-Hexadecanoic acid	Insecticidal, repellent										
15.252	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Oviposition deterring and repellent										
15.451	(E)-Phytol	Insecticidal, repellent										
23.452	α -Tocopherol	Repellent										
25.518	γ -Sitosterol	Repellent										

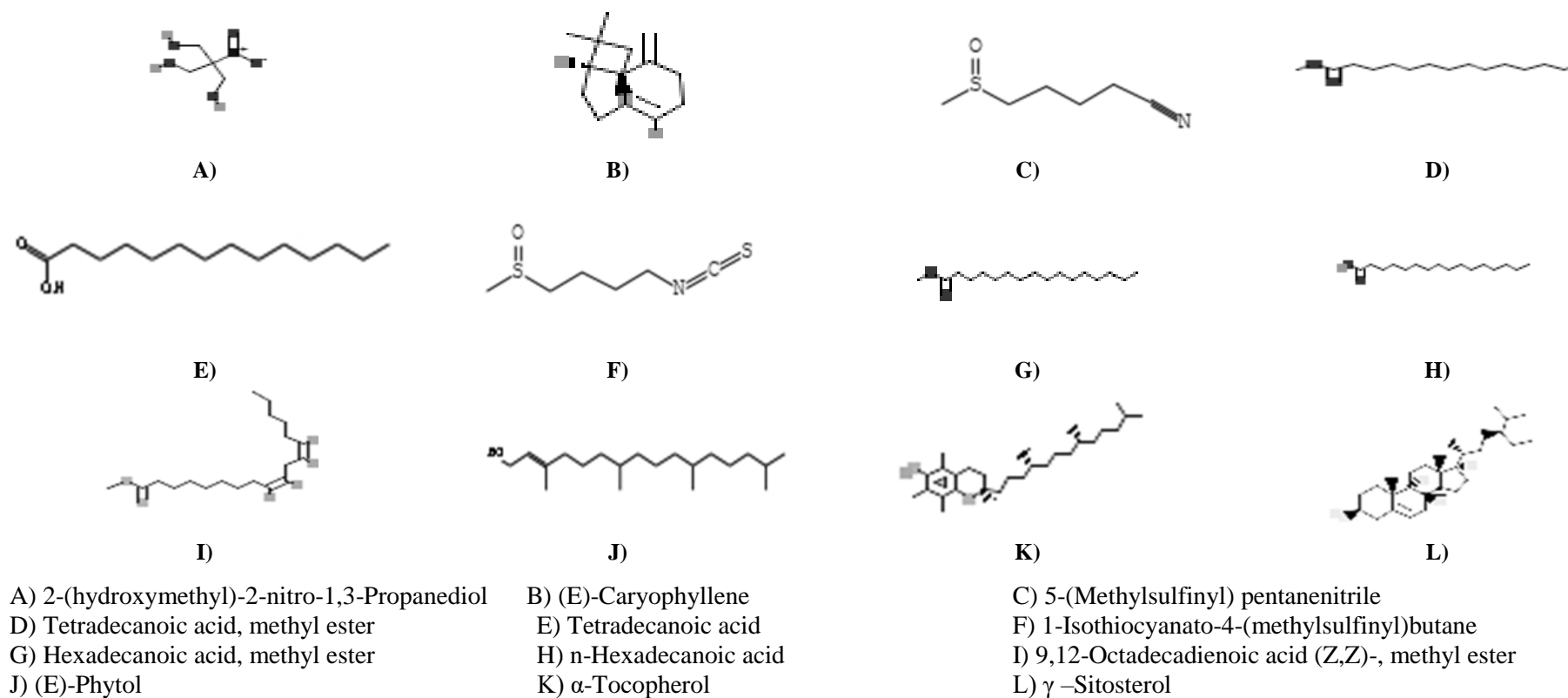


Figure 4.18: Chemical structures of specific compounds with insecticidal properties

4.4 Effect of intercrops on the incidence of mustard aphid and its natural enemies

The current study was carried out during *Rabi*, 2019-20 & 2020-21, to evaluate the effect of different intercrops (wheat, barley, chickpea, lentil, pea, linseed, coriander and fennel) combinations in mustard crop grown at 3:1 ratio on the incidence of mustard aphid and its natural enemies. The results found during both the years are summarized below.

4.4.1 Effect of different intercrops on the incidence of mustard aphid during *Rabi*, 2019-2020 & 2020-2021

The data pertaining to mean aphid population on mustard in different intercrop combinations with 3:1 ratio during *Rabi*, 2019-2020 have been presented in Table 4.31. It was observed that mustard aphid population in all the intercrop combination started from 35 DAS and continued till 95 DAS and attained its peak between 60 to 75 DAS. It revealed that at 35 and 50 DAS, minimum aphid population was observed with mustard + coriander (22.62, 38.48/ top 10 cm inflorescence of 10 plants) and mustard + fennel (24.04, 42.77/ top 10 cm inflorescence of 10 plants) respectively, and shown a significant difference with other intercrop combinations and mustard sole crop (control). However, at 65, 80 and 95 DAS the minimum aphid population was observed in mustard + wheat (71.94, 44.54, 27.85/ top 10 cm inflorescence of 10 plants), which is at par with mustard + barley (73.84, 46.35, 28.09/ top 10 cm inflorescence of 10 plants), mustard + coriander (83.54, 54.95, 37.11/ top 10 cm inflorescence of 10 plants) and mustard + fennel (87.68, 53.93, 35.88/ top 10 cm inflorescence of 10 plants) respectively and shown a significant difference with other intercrops and mustard sole crop (control). Further, on total mean basis, the lowest aphid population was found in mustard + wheat (45.52/ top 10 cm inflorescence of 10 plants) followed by mustard + barley (46.89/ top 10 cm inflorescence of 10 plants), mustard + coriander (47.34/ top 10 cm inflorescence of 10 plants) and mustard + fennel (49.02/ top 10 cm inflorescence of 10 plants) which are statistically at a par with each other and shown a significant difference with other treatments along with mustard sole crop (102.24/ top 10 cm inflorescence of 10 plants).

During the next crop season *Rabi*, 2020-21 (Table 4.31) the trend of population build-up of aphid in different intercrop combinations was more or less similar to the previous crop season but comparatively lower. At 35 and 50 DAS, minimum aphid population was observed with mustard + coriander (16.75, 30.32/ top

10 cm inflorescence of 10 plants) and mustard + fennel (18.15, 34.87/ top 10 cm inflorescence of 10 plants) respectively, and shown a significant difference with other intercrops and mustard sole crop (control). However, at 65, 80 and 95 DAS the minimum aphid population was observed in mustard + wheat (62.24, 36.02, 23.26) followed by mustard + barley (64.72, 37.52, 25.09/ top 10 cm inflorescence of 10 plants), mustard + coriander (73.56, 45.04, 28.32/ top 10 cm inflorescence of 10 plants) and mustard + fennel (74.11, 42.21, 26.54/ top 10 cm inflorescence of 10 plants) respectively which are statistically at par. Further, based on total mean the lowest aphid population was recorded in mustard + wheat (37.40) followed by mustard + barley (38.70), mustard + coriander (38.79), and mustard + fennel (39.18) which are statistically at a par with each other and showed a significant difference with other treatments along with mustard sole crop (85.57/ top 10 cm inflorescence of 10 plants).

On the basis of pooled mean data of two years *i.e.*, 2019-2020 and 2020-2021 (Table 4.32 and depicted in Figure 4.19), it is quite obvious that the intercrops have significant effect on mustard aphid population in comparison to the mustard sole crop (control). Among all the tested intercrop combinations, lowest cumulative mean aphid population was recorded in mustard + wheat (41.46/ top 10 cm inflorescence of 10 plants) followed by mustard + barley (42.79/ top 10 cm inflorescence of 10 plants), mustard + coriander (43.06/ top 10 cm inflorescence of 10 plants) and mustard + fennel (44.10/ top 10 cm inflorescence of 10 plants) which are statistically at par and have showed a significant difference with other intercrop combinations along with mustard sole crop (93.91/ top 10 cm inflorescence of 10 plants).

4.4.2 Effect of different intercrops on the incidence of natural enemies during *Rabi*, 2019-2020 & 2020-2021

It is clear from this experiment that different intercrop combinations had a significant impact on the population of natural enemies *i.e.*, coccinellids (grubs and adults), syrphids (grubs and adults), parasitoids (mummified aphids and adults) and spiders. The results found during both the years *Rabi*, 2019-20 and 2020-21 are summarized below.

Coccinellids

The perusal of data given in Table 4.33 revealed that during *Rabi*, 2019-20 the coccinellid population were influenced by different intercrop combinations. At 35 and 50 DAS the maximum population of coccinellids (3.42, 6.12/ 10 plants) was observed

in mustard + coriander followed by mustard + fennel (3.21, 6.01) respectively and showed a significant difference with other treatments. However at 65, 80 and 95 DAS maximum population of coccinellids were recorded in mustard + wheat (9.88, 16.14, 8.72/ 10 plants) followed by mustard + barley (9.59, 14.97, 8.39/ 10 plants), mustard + coriander (8.68, 13.84, 8.02/ 10 plants) and mustard + fennel (8.89, 13.55, 7.86/ 10 plants) respectively. On a total mean basis the highest coccinellids/ 10 plants were recorded in mustard + wheat (8.54) and barley (8.06) which are at par with coriander (7.92) and fennel (7.88) and showed significant difference with other intercrop combinations along with untreated control (3.35).

During *Rabi* 2020-21 (Table 4.33), more or less similar results were obtained, however the population of coccinellids were comparatively lower than the previous season. Among different intercrop combinations, at 35 and 50 DAS mustard intercropped with coriander and fennel have supported the higher abundance of coccinellids *i.e.*, 3.11 to 5.65 and 2.86 to 5.49/ 10 plants respectively. Remarkably at 65, 80 and 95 DAS maximum population of coccinellids were recorded on mustard + wheat (9.87, 14.88, 8.84/ 10 plants) followed by mustard + barley (8.94, 13.96, 7.98/ 10 plants), mustard + coriander (7.96, 12.68, 6.85/ 10 plants) and mustard + fennel (7.81, 12.56, 6.69/ 10 plants) respectively. Based on total mean maximum coccinellid population was recorded in mustard + wheat (8.04/10 plants) followed by mustard + barley (7.46/10 plants), mustard + coriander (7.25/ 10 plants) and mustard + fennel (7.08/10 plants) which are statistically at par and showed significant difference with other intercrop combinations along with untreated control (3.01/10 plants).

Based on the pooled mean data of *Rabi* 2019-20 and 2020-21 (Table 4.35 and Figure 4.20), intercrop combinations in mustard have shown a significant effect on abundance of coccinellids. Among all the tested intercrop combinations, highest cumulative mean coccinellid population/ 10 plants was greater in mustard + wheat (8.29), followed by mustard + barley (7.76), mustard + coriander (7.59) and mustard + fennel (7.45) which are statistically at par and showed significant difference with other intercrop combinations along with untreated control (3.18).

Syrphids

It is apparent from table 4.34 that mustard intercropped with different crops recorded comparatively higher syrphid population with mustard sole crop. During *Rabi*, 2019-20 at 35 and 50 DAS mustard + coriander and mustard + fennel have housed a higher number of syrphid population *i.e.*, 2.51 to 4.96 and 2.38 to 4.41/ 10

plants, respectively and showed a significant difference with other intercrops combinations and mustard sole crop. However, at 65, 80 and 95 DAS maximum population of syrphids were recorded on mustard + wheat (8.92, 13.25, 7.98/ 10 plants) followed by mustard + barley (8.76, 12.89, 7.66/ 10 plants) respectively. Further, on a total mean basis the highest syrphids/ 10 plants were recorded in mustard + wheat (7.01) and mustard + barley (6.82) which are at par with mustard + coriander (6.71) and mustard + fennel (6.56) and showed significant a difference with other intercrop combinations along with mustard sole crop (2.37).

During *Rabi*, 2020-21 (table 4.34) at 35 and 50 DAS the maximum mean number of syrphid population was recorded in mustard + coriander (2.24 to 4.15/ 10 plants) followed by mustard + fennel (2.02 to 3.85/ 10 plants) respectively. Contrarily at 65, 80 and 95 DAS maximum population of syrphids were recorded in mustard + wheat (7.69, 12.56, 5.81/ 10 plants) followed by mustard + barley (7.52, 11.98, 5.58/ 10 plants), respectively. On a total mean basis maximum syrphid population/ 10 plants was recorded in mustard + wheat (6.12) followed by mustard + barley (5.89), mustard + coriander (5.67) and mustard + fennel (5.58) which are statistically at par and showed significant difference with other intercrop combinations along with mustard sole crop (2.08).

Based on the pooled mean data of *Rabi* 2019-20 and 2020-21, presented in Table 4.35 and depicted in Figure 4.21, it is comprehend that on cumulative mean basis mustard intercropped with wheat supported a greater number of syrphids (6.57/ 10 plants) followed by mustard + barley (6.35/ 10 plants), mustard + coriander (6.19/ 10 plants) and mustard + fennel (5.98/ 10 plants) which are statistically at par and showed significant difference with other intercrop combinations along with untreated control (2.22/ 10 plants).

Parasitoids

The perusal of data given in Table 4.36 noticeably revealed that during *Rabi*, 2019-20 & 2020-21 the population of parasitoids significantly varied among different intercrop combinations. During 2019-20 at 35 and 50 DAS the population of parasitoids were found maximum (4.66 to 9.43/ 10 plants) in mustard + coriander followed by mustard + fennel (4.41 to 9.02) respectively and showed a significant difference with other treatments. Whereas at 65, 80 and 95 DAS maximum population of parasitoids were recorded in mustard + wheat (19.41, 25.66, 13.82/ 10 plants) followed by mustard + barley (18.58, 23.98, 13.18/ 10 plants) respectively. On a total

mean basis the highest parasitoid population/ 10 plants were recorded in mustard + wheat (13.47) and barley (13.14) which are at par with fennel (12.99) and coriander (12.83) and showed significant difference with other intercrop combinations along with untreated control (6.17).

During *Rabi* 2020-21 (table 4.36), at 35 and 50 DAS mustard intercropped with coriander and fennel have supported the higher abundance of parasitoids *i.e.*, 4.21 to 8.71 and 4.16 to 8.62/ 10 plants respectively. Remarkably at 65, 80 and 95 DAS maximum population of parasitoids were recorded in mustard + wheat (19.15, 25.41, 10.79) followed by mustard + barley (17.68, 23.88, 9.87). Similarly, on total mean basis maximum parasitoids/ 10 plants were recorded in mustard + wheat (13.02) followed by mustard + fennel (12.18), mustard + coriander (11.80) and mustard + barley (11.66) which are statistically at par and showed significant difference with other intercrop combinations along with mustard sole crop (5.47).

Based on the pooled mean data of *Rabi* 2019-20 and 2020-21, presented in Table 4.38 and depicted in Figure 4.22, it is understand that on cumulative mean basis mustard intercropped with wheat supported a greater number of parasitoids (14.01/ 10 plants) followed by mustard + barley (13.34/ 10 plants), mustard + coriander (13.11/ 10 plants) and mustard + fennel (12.88/ 10 plants) which are statistically at par and showed significant difference with other intercrop combinations along with untreated control (6.12/ 10 plants).

Spiders

It is obvious from Table 4.37 that during *Rabi* 2020, at 35, 50, 65, 80 and 95 DAS mustard + wheat (2.01, 3.01, 4.87, 8.41, 5.57/ 10 plants) have accommodated a higher number of spider populations followed by mustard + barley (1.95, 2.76, 4.37, 8.08, 5.15/ 10 plants) and mustard + pea (1.89, 2.15, 3.56, 7.04, 4.54/ 10 plants) respectively. Similarly, on total mean basis spider population/ 10 plants was observed maximum in mustard + wheat (4.77) followed by mustard + barley (4.50), mustard + pea (3.84) which are at par and showed a significant difference with other intercrop combinations and mustard sole crop (1.73).

Correspondingly, during *Rabi*, 2020-21 (table 4.37) the maximum mean number of spiders per 10 plants at 35, 50, 65, 80, 95 DAS was also recorded in mustard + wheat (1.68, 2.51, 3.98, 7.33, 4.54/ 10 plants) followed by mustard + barley (1.55, 2.38, 3.87, 7.04, 4.75/ 10 plants) and mustard + pea (1.49, 2.33, 3.54, 6.56, 4.12/ 10 plants) respectively. Similarly, on total mean basis spider population/ 10

plants was highest in mustard + wheat (4.01) followed by mustard + barley (3.92) and mustard + pea (3.53) which are at par and showed a significant difference with other intercrop combinations and untreated control (1.48).

Based on the pooled mean data of *Rabi*, 2019-20 and 2020-21 presented in Table 4.38 and depicted in Figure 4.23, it is obvious that different intercrop combinations with mustard had a significant effect on spider population. Based on cumulative mean, among all the intercrop combinations, mustard + wheat supported a highest number of spiders (4.39/ 10 plants) followed by barley (4.21/ 10 plants) which are at par and showed a significant difference to other intercrop combinations and mustard sole crop (1.61/10 plants).

4.4.3 Effect of different intercrops on the mustard seed yield (kg/ha) during *Rabi*, 2019-2020 & 2020-2021

From table 4.39, it is revealed that during *Rabi*, 2019-20 mustard intercropped with different crops have influenced the crop yield. Results illustrated that the highest seed yield (1178 kg/ha) was recorded in mustard + wheat followed by mustard + barley (1166 kg/ha) over mustard sole crop (871 kg/ha). Similar trend was observed with comparatively greater yield during *Rabi*, 2020-21 where mustard intercropped with wheat has shown the highest yield (1249 kg/ha) over mustard sole crop (885 kg/ha). Based on the pooled mean data of two years, it is comprehend that intercrops had a significant effect on mustard seed yield. Among all the intercrop combinations, mustard intercropped with wheat had showed highest yield (1213 kg/ha) followed by mustard + barley (1201 kg/ha) over mustard sole crop (878 kg/ha).

The current study clearly demonstrated that, introducing intercrops in mustard resulted in less insect pest infestation and an abundance of natural enemies, resulting in a positive influence. Among different intercrops, mustard intercropped with wheat, barley, fennel and coriander found to be superior in the minimization of aphid infestation with increased activity of natural enemies than intercropping with other crops and sole mustard in both the years. The strong pungency of spice crops like fennel and coriander in the intercropped plots may have repelled aphids and thus protected the mustard crop from pest attack. However, mustard intercropped with wheat and barley had a higher diversity and abundance of natural enemies, such as coccinellids, syrphids, parasitoids, and spiders. This could be due to the high incidence of aphids on the intercrops, which release semiochemicals and attract natural enemies. The combined operation of natural enemies and some physical

barrier effects can be linked to the increased effectiveness of mustard intercropped with wheat, barley, and fennel system, which recorded lower levels of aphid population.

Previous researchers have demonstrated that intercropping of mustard with other crops descends aphid infestation when compared to sole mustard (Sarker *et al.* 2007). Lasker *et al.* (2004) also found that intercropping mustard with wheat and barley reduced aphid incidence. Hoverfly population density was increased and aphid numbers were reduced when buckwheat was intercropped between rows of peach trees (Mizutani *et al.* 2010). Whereas, in a wheat-oilseed rape intercropping field, Wang *et al.* (2008) observed an elevated level of aphid parasitoids. Intercropping cotton with wheat reduced aphid abundance, with a significant difference in mummified cotton aphids among the treatments (Ma *et al.*, 2006). According to Men *et al.* (2004); Ma *et al.*, (2006), wheat can serve as a reservoir for predators such as ladybird beetles, spiders, lacewings, and spiders and relay their predators to nearby fields. Wheat intercropped with cotton significantly increased the population of natural enemies while minimising cotton aphid populations, according to Parajulee *et al.* (1997); Velders *et al.* (2002). Similarly, Ali (2002); Bedoussac *et al.* (2010); Lopes *et al.* (2015) have observed that intercropping of pea with wheat reduced the aphid population. The rapid growth habits of wheat and sorghum may have served as insect pest barrier crops (Hagargi and Patil, 2018).

Halepyatic *et al.* (1987) believed that intercropping of spice crops reduces the population of various target pests. The ability of coriander to repel aphids, spider mites, and potato beetles in potato crops has long been known (Anonymous, 2004). Allelochemicals released by the fennel may have discouraged aphid colonisation, according to Singh and Kothari (1997), and phenotypic characteristics of the fennel inflorescence resulted in natural cover across the mustard canopy's inflorescence. This arrangement may have slowed aphid population growth by forming an allelochemical barrier film that served as an alarm pheromone, similar to what has been observed in wild potato aphids (Gibson and Pickett, 1983) and *Plutella xylostella* in cauliflower (Ali *et al.*, 2020). Monika *et al.* (2005) also found that mustard grown with coriander had the lowest aphid population. Bowie *et al.* (1995) reported that cabbage intercropped with coriander attracts adult hoverflies and reduces the number of aphids. According to Vaidya and Banskota (2005), aphid populations are lowest in cauliflower interplanted with coriander, followed by fenugreek.

Intercropping of mustard with other crops has also shown an advantage on yield over mustard sole crop as aphid densities above a certain threshold can have a negative influence on yield and result in economic losses. Rosenheim *et al.* (1995) and Sarkar (2018) have observed the highest mustard seed yield on intercrop combination with coriander. The literature available on competitive function and monetary advantage of intercrops with respect to mustard is scanty, thus the present finding gives very conspicuous facts for the future research.

At the field level, mustard plants aged 60-85 DAS were found to be vulnerable to aphid infestation, according to the findings. Furthermore, crop habitat manipulation may be able to promote or enhance natural control efficacy. According to Baliddawa (1985), the natural enemy effect accounts for up to 40% of pest reduction in intercropping systems.

Table 4.31: Effect of different intercrops on the aphid population in mustard during *Rabi*, 2019-20 and 20-21

Treatments	2019-20						2020-21					
	Mean number/top 10 cm inflorescence of 10 plants						Mean number/top 10 cm inflorescence of 10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean
Mustard + Wheat (3:1)	32.88 ^b	50.41 ^b	71.94 ^a	44.54 ^a	27.85 ^a	45.52 ^a	24.11 ^{bc}	41.37 ^b	62.24 ^a	36.02 ^a	23.26 ^a	37.40 ^a
Mustard + Barley (3:1)	34.03 ^{bcd}	52.18 ^b	73.84 ^a	46.35 ^a	28.09 ^a	46.89 ^a	23.17 ^{bc}	43.01 ^b	64.72 ^a	37.52 ^a	25.09 ^a	38.70 ^a
Mustard + Chickpea (3:1)	36.96 ^{bcd}	75.42 ^d	109.45 ^d	80.08 ^b	66.55 ^c	73.69 ^c	22.72 ^{bc}	61.98 ^{cd}	99.21 ^{bc}	74.53 ^b	59.82 ^c	63.65 ^c
Mustard + Lentil (3:1)	38.66 ^{cd}	72.83 ^d	116.22 ^d	93.47 ^c	74.31 ^d	79.10 ^{cd}	25.68 ^c	65.86 ^d	101.83 ^c	82.35 ^c	68.11 ^d	68.77 ^{cd}
Mustard + Peas (3:1)	34.13 ^{bc}	60.41 ^c	98.64 ^b	75.34 ^b	51.78 ^b	64.06 ^b	22.04 ^b	50.65 ^{Bc}	88.41 ^b	68.15 ^b	50.64 ^b	55.98 ^b
Mustard + Linseed (3:1)	39.59 ^d	80.54 ^e	122.05 ^d	99.64 ^c	79.74 ^d	84.31 ^d	26.14 ^c	68.68 ^d	108.47 ^c	89.81 ^c	73.21 ^{de}	73.26 ^e
Mustard + Coriander (3:1)	22.62 ^a	38.48 ^a	83.54 ^a	54.95 ^a	37.11 ^a	47.34 ^a	16.75 ^a	30.32 ^a	73.56 ^a	45.04 ^a	28.32 ^a	38.79 ^a
Mustard + Fennel (3:1)	24.04 ^a	42.77 ^a	87.68 ^a	55.43 ^a	35.88 ^a	49.02 ^a	18.15 ^a	34.87 ^a	74.11 ^a	42.21 ^a	26.54 ^a	39.18 ^a
Mustard sole (Untreated control)	45.37 ^e	95.84 ^e	160.12 ^e	121.24 ^d	88.64 ^e	102.24 ^e	30.67 ^d	80.54 ^e	145.36 ^d	105.64 ^d	65.64 ^e	85.57 ^f
C.D.	4.556	8.020	12.709	8.844	6.411	8.085	3.180	6.670	11.221	7.683	5.515	6.788
SE(m)	1.507	2.652	4.203	2.938	2.120	2.674	1.052	2.206	3.711	2.541	1.824	2.245
C.V.	7.599	7.276	7.098	6.875	6.825	7.062	7.801	7.203	7.081	6.815	6.764	6.983

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.32: Effect of different intercrops on the aphid population in mustard (data given are the pooled value of two years *i.e.*, Rabi, 2019-20 and 2020-21)

Treatments	Mean number/top 10 cm inflorescence of 10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Cumulative mean
Mustard + Wheat (3:1)	28.50 ^b	45.89 ^b	67.09 ^a	40.28 ^a	25.56 ^a	41.46 ^a
Mustard + Barley (3:1)	29.55 ^b	47.80 ^b	70.68 ^a	42.64 ^a	27.19 ^a	42.79 ^a
Mustard + Chickpea (3:1)	29.84 ^{bc}	68.70 ^d	104.33 ^{bc}	77.31 ^b	63.19 ^c	68.67 ^c
Mustard + Lentil (3:1)	32.17 ^{bc}	69.35 ^d	109.03 ^c	87.91 ^c	71.21 ^d	73.93 ^{cd}
Mustard + Peas (3:1)	28.09 ^{bc}	55.53 ^c	93.53 ^b	71.75 ^b	51.21 ^b	60.02 ^b
Mustard + Linseed (3:1)	32.87 ^c	74.61 ^d	115.26 ^c	94.73 ^c	76.48 ^d	78.79 ^d
Mustard + Coriander (3:1)	19.69 ^a	34.40 ^a	78.55 ^a	48.80 ^a	30.97 ^a	43.06 ^a
Mustard + Fennel (3:1)	21.35 ^a	38.83 ^a	79.79 ^a	47.97 ^a	29.91 ^a	44.10 ^a
Mustard sole (Untreated control)	38.02 ^d	88.19 ^e	152.74 ^d	113.44 ^d	77.14 ^d	93.91 ^e
C.D.	3.890	7.327	11.965	8.300	5.960	7.449
SE(m)	1.286	2.423	3.957	2.745	1.971	2.463
C.V.	7.729	7.225	7.090	6.860	6.793	7.038

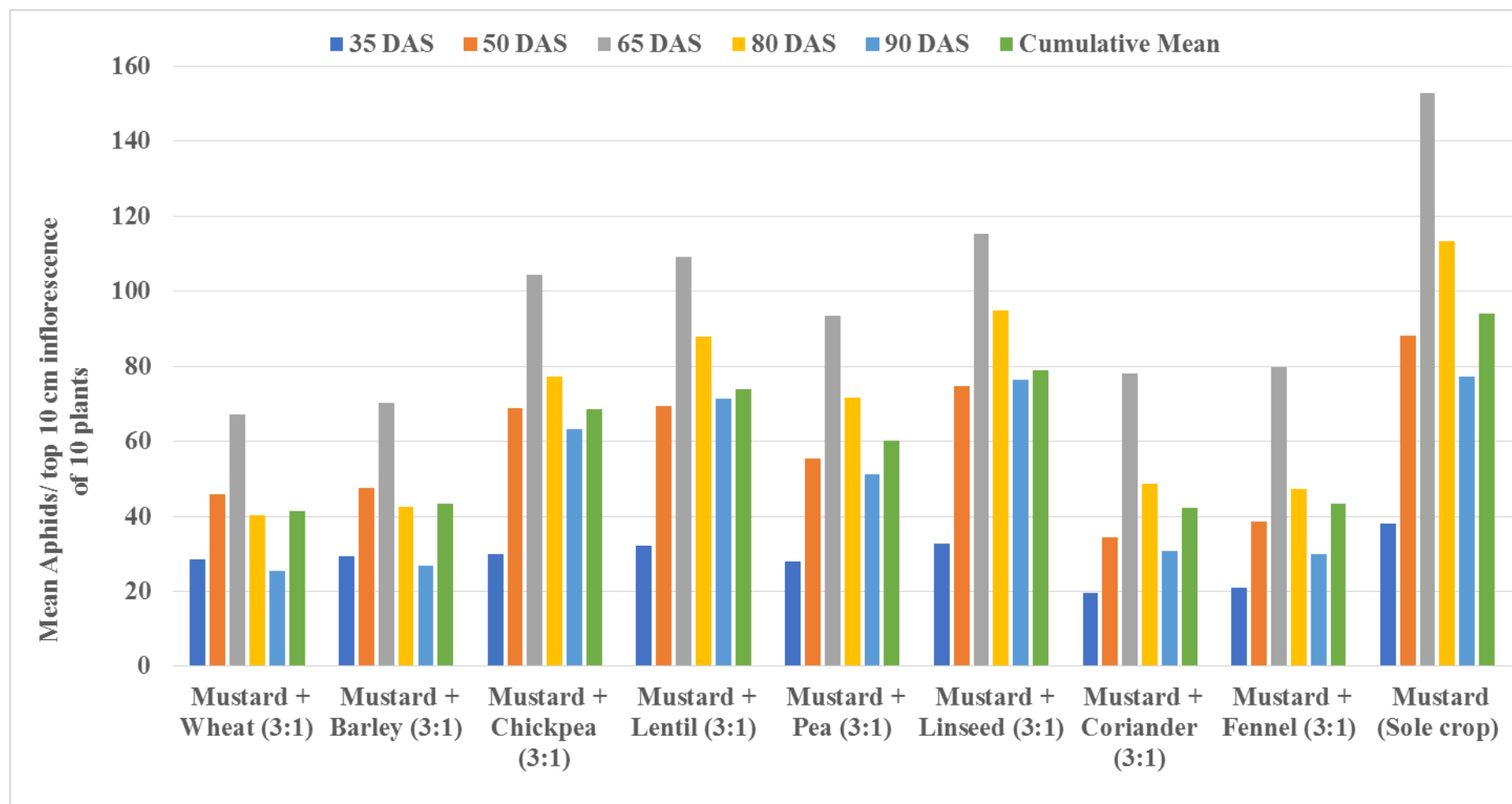


Figure 4.19: Effect of different intercrops on the mustard aphid population in mustard (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)

Table 4.33: Effect of different intercrops on the Coccinellids population in mustard during Rabi, 2019-20 and 20-21

Treatments	2019-20						2020-21					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean
Mustard + Wheat (3:1)	2.31 ^b	5.66 ^b	9.88 ^a	16.14 ^a	8.72 ^a	8.54 ^a	1.88 ^b	4.76 ^b	9.87 ^a	14.88 ^a	8.84 ^a	8.04 ^a
Mustard + Barley (3:1)	2.25 ^b	5.09 ^b	9.59 ^a	14.97 ^a	8.39 ^a	8.06 ^a	1.85 ^b	4.59 ^b	8.94 ^a	13.96 ^a	7.98 ^a	7.46 ^a
Mustard + Chickpea (3:1)	2.12 ^b	4.35 ^{cd}	6.01 ^c	9.06 ^b	4.91 ^b	5.29 ^b	1.69 ^b	3.71 ^{cd}	5.96 ^b	8.98 ^b	4.58 ^b	4.98 ^b
Mustard + Lentil (3:1)	2.09 ^b	4.22 ^{cd}	5.98 ^c	8.79 ^b	4.83 ^b	5.18 ^b	1.63 ^b	3.68 ^{cd}	5.67 ^b	8.42 ^b	4.41 ^b	4.76 ^b
Mustard + Peas (3:1)	2.28 ^b	4.91 ^{bc}	7.38 ^b	9.88 ^b	5.21 ^b	5.93 ^b	1.84 ^b	4.31 ^{bc}	6.43 ^b	9.56 ^b	4.96 ^b	5.42 ^b
Mustard + Linseed (3:1)	2.01 ^b	3.99 ^d	5.85 ^c	8.58 ^b	4.62 ^b	5.01 ^b	1.59 ^b	3.63 ^d	5.55 ^b	8.29 ^b	4.27 ^b	4.67 ^b
Mustard + Coriander (3:1)	3.42 ^a	6.12 ^a	8.68 ^a	13.84 ^a	8.02 ^a	7.92 ^a	3.11 ^a	5.65 ^a	7.96 ^a	12.68 ^a	6.85 ^a	7.25 ^a
Mustard + Fennel (3:1)	3.21 ^a	6.01 ^a	8.89 ^a	13.55 ^a	7.86 ^a	7.88 ^a	2.86 ^a	5.49 ^a	7.81 ^a	12.56 ^a	6.69 ^a	7.08 ^a
Mustard sole (Control)	1.56 ^c	2.85 ^e	3.91 ^d	5.21 ^c	3.22 ^c	3.35 ^c	1.21 ^c	2.35 ^e	3.56 ^e	4.96 ^d	2.98 ^d	3.01 ^c
C.D.	0.338	0.694	1.148 ^e	1.793	1.001	0.994	0.288	0.623	1.055	1.647	0.881	0.893
SE(m)	0.112	0.230	0.380	0.593	0.331	0.329	0.095	0.206	0.349	0.545	0.291	0.295
C.V.	8.195	8.357	9.009	9.310	9.304	9.046	8.408	8.501	9.044	9.143	9.174	8.928

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.34: Effect of different intercrops on the Syrphids population in mustard during *Rabi*, 2019-20 and 20-21

Treatments	2019-20						2020-21					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean
Mustard + Wheat (3:1)	1.41 ^b	3.48 ^b	8.92 ^a	13.25 ^a	7.98 ^a	7.01 ^a	1.21 ^b	3.34 ^b	7.69 ^a	12.56 ^a	5.81 ^a	6.12 ^a
Mustard + Barley (3:1)	1.39 ^b	3.39 ^b	8.76 ^{ab}	12.89 ^a	7.66 ^a	6.82 ^a	1.15 ^{bc}	3.21 ^b	7.52 ^{ab}	11.98 ^a	5.58 ^a	5.89 ^a
Mustard + Chickpea (3:1)	1.34 ^b	2.81 ^c	5.17 ^{de}	6.89 ^{bc}	3.99 ^c	4.05 ^{bc}	1.09 ^{bc}	2.26 ^c	4.56 ^{cd}	5.91 ^{cd}	2.98 ^c	3.36 ^{bc}
Mustard + Lentil (3:1)	1.26 ^b	2.64 ^{cd}	5.02 ^{de}	6.42 ^{bc}	3.91 ^c	3.85 ^{bc}	1.03 ^{bc}	2.13 ^c	4.22 ^d	5.49 ^d	2.61 ^c	3.10 ^c
Mustard + Peas (3:1)	1.38 ^b	3.46 ^b	5.95 ^d	7.98 ^b	4.23 ^c	4.60 ^b	1.25 ^b	2.86 ^b	5.12 ^c	7.21 ^c	3.87 ^b	4.06 ^b
Mustard + Linseed (3:1)	1.22 ^b	2.38 ^{de}	4.85 ^e	6.12 ^c	3.76 ^c	3.67 ^c	1.01 ^{bc}	2.01 ^c	3.95 ^d	5.25 ^d	2.78 ^c	3.00 ^c
Mustard + Coriander (3:1)	2.51 ^a	4.96 ^a	7.84 ^{bc}	12.39 ^a	5.87 ^b	6.71 ^a	2.24 ^a	4.15 ^a	6.98 ^{ab}	9.81 ^b	5.17 ^a	5.67 ^a
Mustard + Fennel (3:1)	2.38 ^a	4.41 ^a	7.28 ^c	12.68 ^a	6.06 ^b	6.56 ^a	2.02 ^a	3.85 ^a	6.63 ^b	10.01 ^b	5.41 ^a	5.58 ^a
Mustard sole (Control)	0.99 ^c	1.96 ^e	3.01 ^f	3.98 ^d	1.91 ^d	2.37 ^d	0.89 ^c	1.75 ^d	2.45 ^e	3.52 ^e	1.78 ^d	2.08 ^d
C.D.	0.197	0.447	0.998	1.522	0.862	0.816	0.194	0.457	0.876	1.379	0.676	0.715
SE(m)	0.065	0.148	0.330	0.503	0.285	0.270	0.064	0.151	0.289	0.456	0.224	0.237
C.V.	7.332	7.744	9.055	9.501	9.792	9.235	8.392	9.202	9.192	9.907	9.703	9.460

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.35: Effect of different intercrops on the Coccinellids and Syrphids population (data given are the pooled value of two years i.e., Rabi, 2019-20 and 2020-21)

Treatments	Coccinellids						Syrphids					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Cumulative mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Cumulative mean
Mustard + Wheat (3:1)	2.10 ^b	4.92 ^b	9.32 ^a	14.71 ^a	8.08 ^a	8.29 ^a	1.31 ^b	3.41 ^b	8.31 ^a	12.91 ^a	6.90 ^a	6.57 ^a
Mustard + Barley (3:1)	2.05 ^b	4.79 ^b	9.17 ^a	14.47 ^a	7.84 ^a	7.76 ^a	1.27 ^b	3.30 ^b	8.14 ^a	12.44 ^{ab}	6.62 ^a	6.35 ^a
Mustard + Chickpea (3:1)	1.91 ^b	4.03 ^{cd}	5.99 ^{bc}	9.02 ^b	4.75 ^b	5.14 ^b	1.22 ^b	2.54 ^c	4.87 ^{cd}	6.40 ^{cd}	3.49 ^c	3.70 ^{bc}
Mustard + Lentil (3:1)	1.86 ^b	3.95 ^d	5.83 ^{bc}	8.61 ^b	4.62 ^b	4.97 ^b	1.15 ^{bc}	2.39 ^c	4.62 ^{cd}	5.96 ^d	3.26 ^c	3.47 ^c
Mustard + Peas (3:1)	2.06 ^b	4.61 ^{bc}	6.91 ^b	9.72 ^b	5.09 ^b	5.68 ^b	1.32 ^b	3.16 ^b	5.54 ^c	7.60 ^c	4.05 ^c	4.33 ^b
Mustard + Linseed (3:1)	1.80 ^b	3.81 ^d	5.70 ^c	8.44 ^b	4.45 ^b	4.84 ^b	1.12 ^{bc}	2.20 ^{cd}	4.40 ^d	5.69 ^d	3.27 ^c	3.33 ^c
Mustard + Coriander (3:1)	3.22 ^a	5.84 ^a	8.63 ^a	12.96 ^a	7.37 ^a	7.59 ^a	2.38 ^a	4.56 ^a	7.41 ^{ab}	11.10 ^b	5.52 ^b	6.19 ^a
Mustard + Fennel (3:1)	3.04 ^a	5.75 ^a	8.25 ^a	13.31 ^a	7.50 ^a	7.45 ^a	2.15 ^a	4.13 ^a	6.87 ^b	11.21 ^b	5.61 ^b	5.98 ^a
Mustard sole (Control)	1.39 ^c	2.60 ^e	3.74 ^d	5.09 ^c	3.10 ^c	3.18 ^c	0.94 ^c	1.86 ^d	2.73 ^e	3.75 ^e	1.85 ^d	2.22 ^d
C.D.	0.320	0.655	1.113	1.709	0.934	0.943	0.219	0.457	0.957	1.444	0.739	0.752
SE(m)	0.106	0.217	0.368	0.565	0.309	0.312	0.072	0.151	0.316	0.478	0.244	0.249
C.V.	8.500	8.385	9.029	9.154	9.120	8.936	8.808	8.562	9.320	9.658	9.395	9.194

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

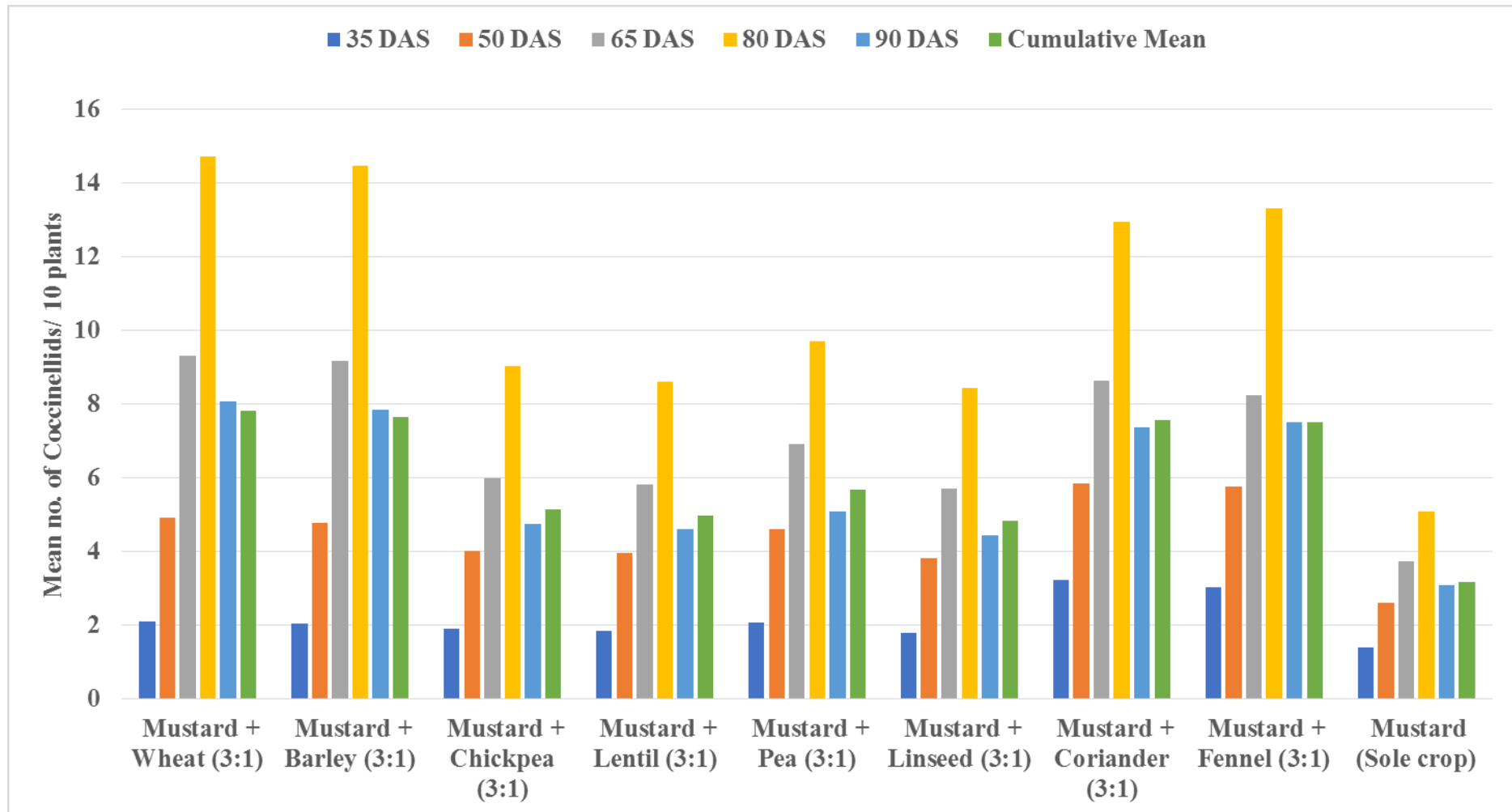


Figure 4.20: Effect of different intercrops on population of Coccinellids (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)

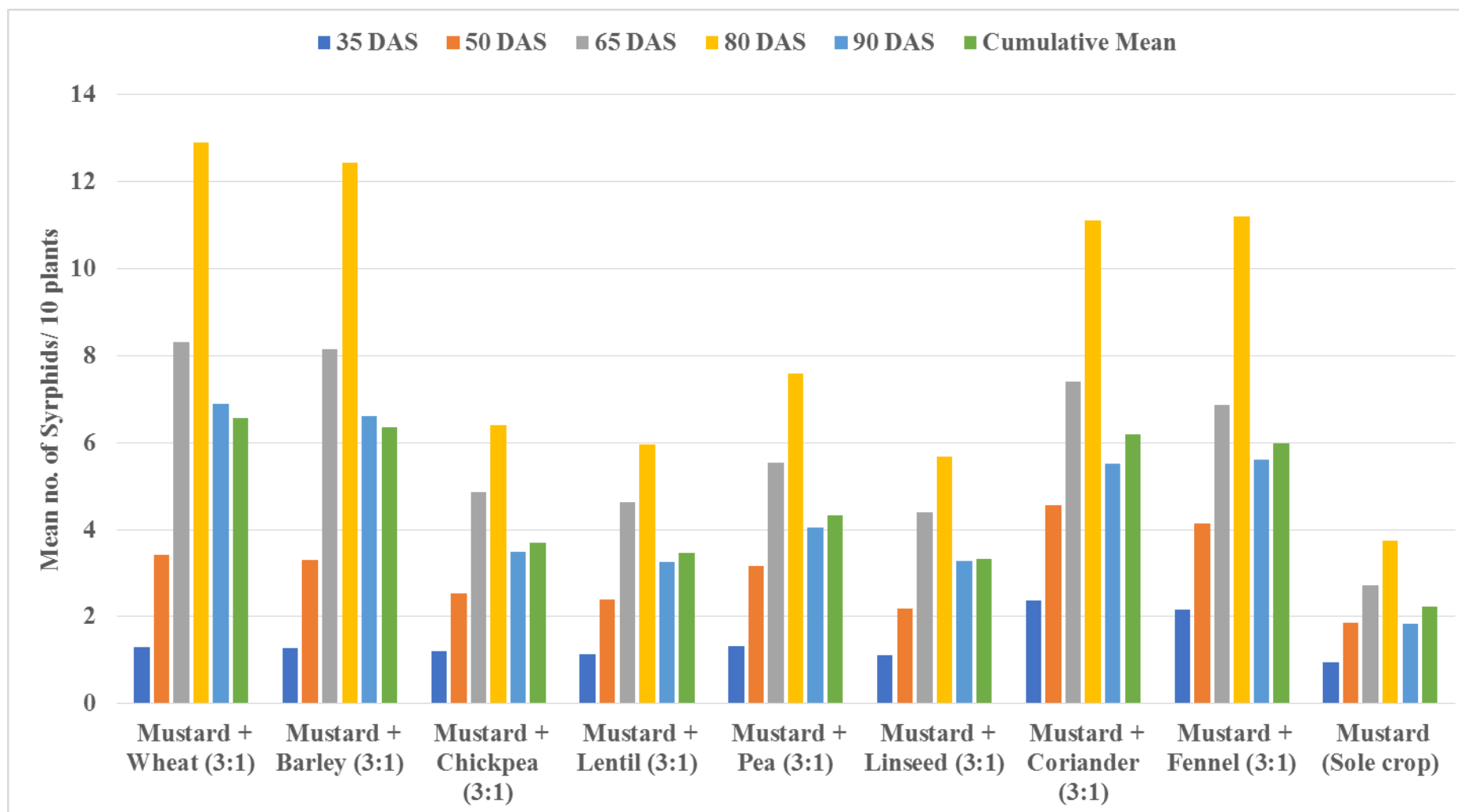


Figure 4.21: Effect of different intercrops on the Syrphids population (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)

Table 4.36: Effect of different intercrops on the Parasitoids population in mustard during *Rabi*, 2019-20 and 20-21

Treatments	2019-20						2020-21					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean
Mustard + Wheat (3:1)	2.95 ^b	8.21 ^b	19.41 ^a	25.66 ^a	13.82 ^a	14.01 ^a	2.62 ^b	7.15 ^b	19.15 ^a	25.41 ^a	10.79 ^a	13.02 ^a
Mustard + Barley (3:1)	2.89 ^b	8.06 ^b	18.58 ^{ab}	23.98 ^a	13.18 ^a	13.34 ^a	2.59 ^b	6.86 ^{bc}	17.68 ^{ab}	23.88 ^{ab}	9.87 ^{ab}	12.18 ^a
Mustard + Chickpea (3:1)	2.78 ^b	6.36 ^c	12.06 ^{cd}	15.55 ^b	7.54 ^{cd}	8.86 ^b	2.63 ^b	5.92 ^{cd}	10.96 ^d	14.28 ^d	6.31 ^{cd}	8.02 ^b
Mustard + Lentil (3:1)	2.69 ^b	5.98 ^c	11.73 ^{cd}	14.98 ^b	7.34 ^{cd}	8.54 ^b	2.57 ^b	5.88 ^{cd}	10.81 ^d	14.05 ^d	6.18 ^d	7.90 ^b
Mustard + Peas (3:1)	2.81 ^b	6.87 ^c	13.95 ^c	16.22 ^b	8.65 ^c	9.70 ^b	2.65 ^b	6.21 ^{cd}	12.69 ^d	15.32 ^d	7.49 ^c	8.87 ^b
Mustard + Linseed (3:1)	2.57 ^b	5.79 ^c	10.55 ^{de}	13.89 ^b	6.94 ^{de}	7.95 ^b	2.53 ^b	5.65 ^d	10.45 ^d	13.96 ^d	6.02 ^d	7.72 ^b
Mustard + Coriander (3:1)	4.66 ^a	9.43 ^a	17.87 ^{ab}	22.78 ^a	10.82 ^b	13.11 ^a	4.21 ^a	8.71 ^a	15.97 ^{bc}	20.85 ^c	9.25 ^b	11.80 ^a
Mustard + Fennel (3:1)	4.41 ^a	9.02 ^{ab}	16.69 ^b	23.12 ^a	11.15 ^b	12.88 ^a	3.88 ^a	8.15 ^a	15.08 ^c	21.65 ^{bc}	9.54 ^{ab}	11.66 ^a
Mustard sole (Control)	1.98 ^c	4.01 ^d	8.45 ^e	10.76 ^c	5.42 ^e	6.12 ^c	1.46 ^c	3.05 ^e	7.11 ^e	8.98 ^e	4.18 ^e	4.96 ^c
C.D.	0.467	1.059	2.283	2.965	1.554	1.662	0.397	0.925	2.163	2.856	1.230	1.527
SE(m)	0.154	0.350	0.755	0.981	0.514	0.550	0.131	0.306	0.715	0.945	0.407	0.505
C.V.	8.689	8.561	9.101	9.160	9.438	9.068	8.156	8.291	9.304	9.295	9.102	9.146

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.37 Effect of different intercrops on Spiders population in mustard during *Rabi*, 2019-20 and 20-21

Treatments	2019-20						2020-21					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Total mean
Mustard + Wheat (3:1)	2.01 ^a	3.01 ^a	4.87 ^a	8.41 ^a	5.57 ^a	4.77 ^a	1.68 ^a	2.51 ^a	3.98 ^a	7.33 ^a	4.54 ^a	4.01 ^a
Mustard + Barley (3:1)	1.95 ^a	2.76 ^a	4.55 ^a	8.08 ^a	5.15 ^a	4.50 ^a	1.55 ^{ab}	2.38 ^{ab}	3.87 ^a	7.04 ^a	4.75 ^{ab}	3.92 ^a
Mustard + Chickpea (3:1)	1.31 ^c	1.89 ^{bcd}	2.98 ^{cd}	4.43 ^c	3.01 ^c	2.72 ^c	1.04 ^c	1.75 ^{de}	2.74 ^d	3.91 ^c	2.98 ^d	2.48 ^c
Mustard + Lentil (3:1)	1.23 ^c	1.75 ^{cd}	2.84 ^{cd}	4.18 ^c	2.98 ^c	2.60 ^c	1.03 ^c	1.69 ^{de}	2.62 ^d	3.49 ^c	2.81 ^d	2.33 ^c
Mustard + Peas (3:1)	1.89 ^{ab}	2.15 ^b	3.56 ^b	7.04 ^b	4.54 ^b	3.84 ^b	1.49 ^b	2.33 ^{ab}	3.54 ^{ab}	6.56 ^{ab}	4.12 ^{bc}	3.53 ^{ab}
Mustard + Linseed (3:1)	1.13 ^c	1.65 ^d	2.75 ^d	4.11 ^c	2.88 ^c	2.50 ^c	0.93 ^c	1.58 ^e	2.58 ^d	3.35 ^{cd}	2.78 ^d	2.24 ^c
Mustard + Coriander (3:1)	1.65 ^b	2.05 ^{bc}	3.28 ^{bcd}	6.47 ^b	4.08 ^b	3.51 ^b	1.09 ^c	2.01 ^{cd}	3.05 ^{cd}	6.12 ^b	3.87 ^c	3.23 ^b
Mustard + Fennel (3:1)	1.73 ^b	2.11 ^b	3.43 ^{bc}	6.88 ^b	4.29 ^b	3.69 ^b	1.01 ^c	2.18 ^{bc}	3.23 ^{bc}	6.44 ^{ab}	4.01 ^{bc}	3.37 ^b
Mustard sole (Control)	0.75 ^d	1.16 ^e	1.98 ^e	2.81 ^d	1.95 ^d	1.73 ^d	0.56 ^d	0.95 ^f	1.78 ^e	2.56 ^d	1.58 ^e	1.48 ^d
C.D.	0.234	0.329	0.532	0.958	0.630	0.536	0.185	0.312	0.462	0.837	0.545	0.482
SE(m)	^{0.077}	0.109	0.176	0.317	0.208	0.177	0.061	0.103	0.153	0.277	0.180	0.159
C.V.	8.847	9.129	9.086	9.433	9.440	9.240	9.241	9.240	8.696	9.232	8.914	9.319

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.38: Effect of different intercrops on the Parasitoids and Spiders population (data given are the pooled value of two years i.e., Rabi, 2019-20 and 2020-21)

Treatments	Parasitoids						Spiders					
	Mean number/10 plants						Mean number/10 plants					
	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Cumulative mean	35 DAS	50 DAS	65 DAS	80 DAS	95 DAS	Cumulative mean
Mustard + Wheat (3:1)	2.79 ^b	7.68 ^{bc}	19.28 ^a	25.54 ^a	12.31 ^a	14.01 ^a	1.85 ^a	2.76 ^a	4.43 ^a	7.87 ^a	5.06 ^a	4.39 ^a
Mustard + Barley (3:1)	2.74 ^b	7.46 ^{cd}	18.13 ^{ab}	23.93 ^{ab}	11.53 ^{ab}	13.34 ^a	1.75 ^a	2.57 ^{ab}	4.21 ^a	7.56 ^{ab}	4.95 ^a	4.21 ^a
Mustard + Chickpea (3:1)	2.71 ^b	6.14 ^e	11.51 ^{cd}	14.92 ^c	6.93 ^{de}	8.86 ^b	1.17 ^{cd}	1.82 ^{de}	2.86 ^{cd}	4.17 ^d	3.00 ^c	2.60 ^c
Mustard + Lentil (3:1)	2.63 ^b	5.93 ^e	11.27 ^{cd}	14.52 ^c	6.76 ^{de}	8.54 ^b	1.13 ^{cd}	1.72 ^{de}	2.73 ^d	3.84 ^d	2.90 ^c	2.46 ^c
Mustard + Peas (3:1)	2.73 ^b	6.54 ^{de}	13.32 ^c	15.77 ^c	8.07 ^d	9.70 ^b	1.69 ^a	2.24 ^{bc}	3.55 ^b	6.80 ^{bc}	4.33 ^b	3.72 ^b
Mustard + Linseed (3:1)	2.55 ^b	5.72 ^e	10.50 ^d	13.93	6.48 ^e	7.95 ^b	1.03 ^d	1.62 ^e	2.67 ^d	3.73 ^d	2.83 ^c	2.37 ^c
Mustard + Coriander (3:1)	4.44 ^a	9.07 ^a	16.92 ^b	21.82 ^b	10.04 ^c	13.11 ^a	1.37 ^{bc}	2.03 ^{cd}	3.17 ^{bcd}	6.30 ^c	3.98 ^b	3.37 ^b
Mustard + Fennel (3:1)	4.15 ^a	8.59 ^{ab}	15.89 ^b	22.39 ^b	10.35 ^{bc}	12.88 ^a	1.41 ^b	2.15 ^c	3.33 ^{bc}	6.66 ^{bc}	4.15 ^b	3.53 ^b
Mustard sole (Control)	1.72 ^c	3.53 ^f	7.78 ^e	9.87 ^d	4.80 ^f	6.12 ^c	0.66 ^e	1.06 ^f	1.88 ^e	2.69 ^e	1.77 ^d	1.61 ^d
C.D.	0.415	1.011	2.210	2.944	1.380	1.662	0.210	0.332	0.508	0.886	0.563	0.489
SE(m)	0.137	0.334	0.731	0.973	0.456	0.550	0.070	0.110	0.168	0.293	0.186	0.162
C.V.	8.050	8.600	9.147	9.332	9.206	9.059	9.015	9.542	9.089	9.199	8.807	8.909

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.39: Effect of different intercrops on the mustard seed yield (kg/ha) during Rabi, 2019-20 and 2020-21

Treatments	2019-20	2020-21	Pooled mean
Mustard + Wheat (3:1)	1178 ^a	1249 ^a	1213 ^a
Mustard + Barley (3:1)	1166 ^a	1235 ^a	1201 ^a
Mustard + Chickpea (3:1)	935 ^{bcd}	951 ^{cd}	943 ^{bc}
Mustard + Lentil (3:1)	914 ^{cd}	928 ^d	921 ^c
Mustard + Peas (3:1)	965 ^{abcd}	981 ^{bcd}	973 ^{abc}
Mustard + Linseed (3:1)	908 ^{cd}	921 ^d	915 ^c
Mustard + Coriander (3:1)	1073 ^{ab}	1116 ^{ab}	1095 ^{ab}
Mustard + Fennel (3:1)	1059 ^{abc}	1095 ^{abc}	1077 ^{ab}
Mustard sole (Control)	871 ^d	885 ^d	878 ^c
CD (P=0.05)	141.321	146.354	143.822
SEm(±)	46.736	48.401	47.563
CV (%)	8.182	8.244	8.213

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

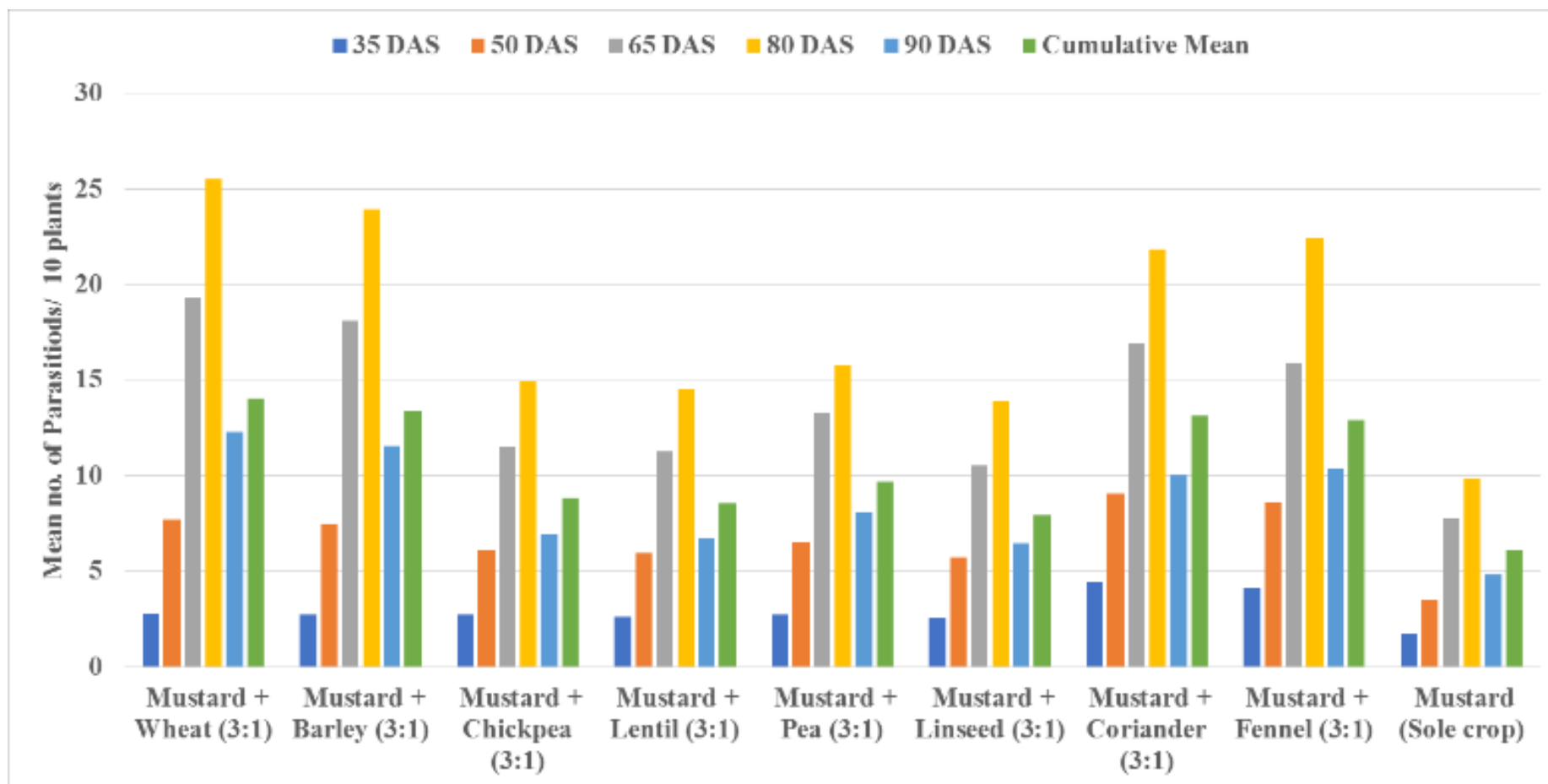


Figure: 4.22: Effect of different intercrops on the Parasitoids population (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)

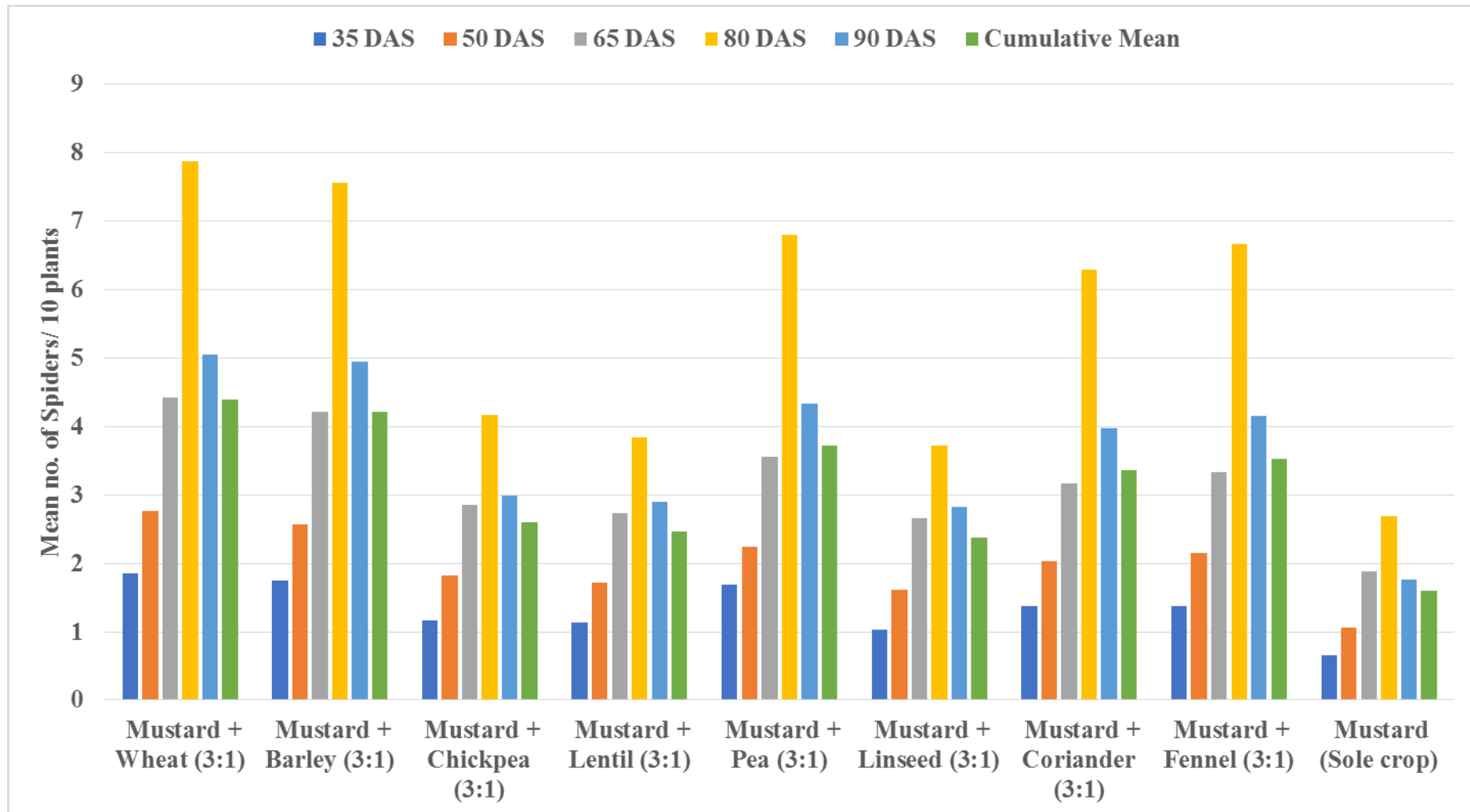


Figure 4.23: Effect of different intercrops on the Spiders population (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)

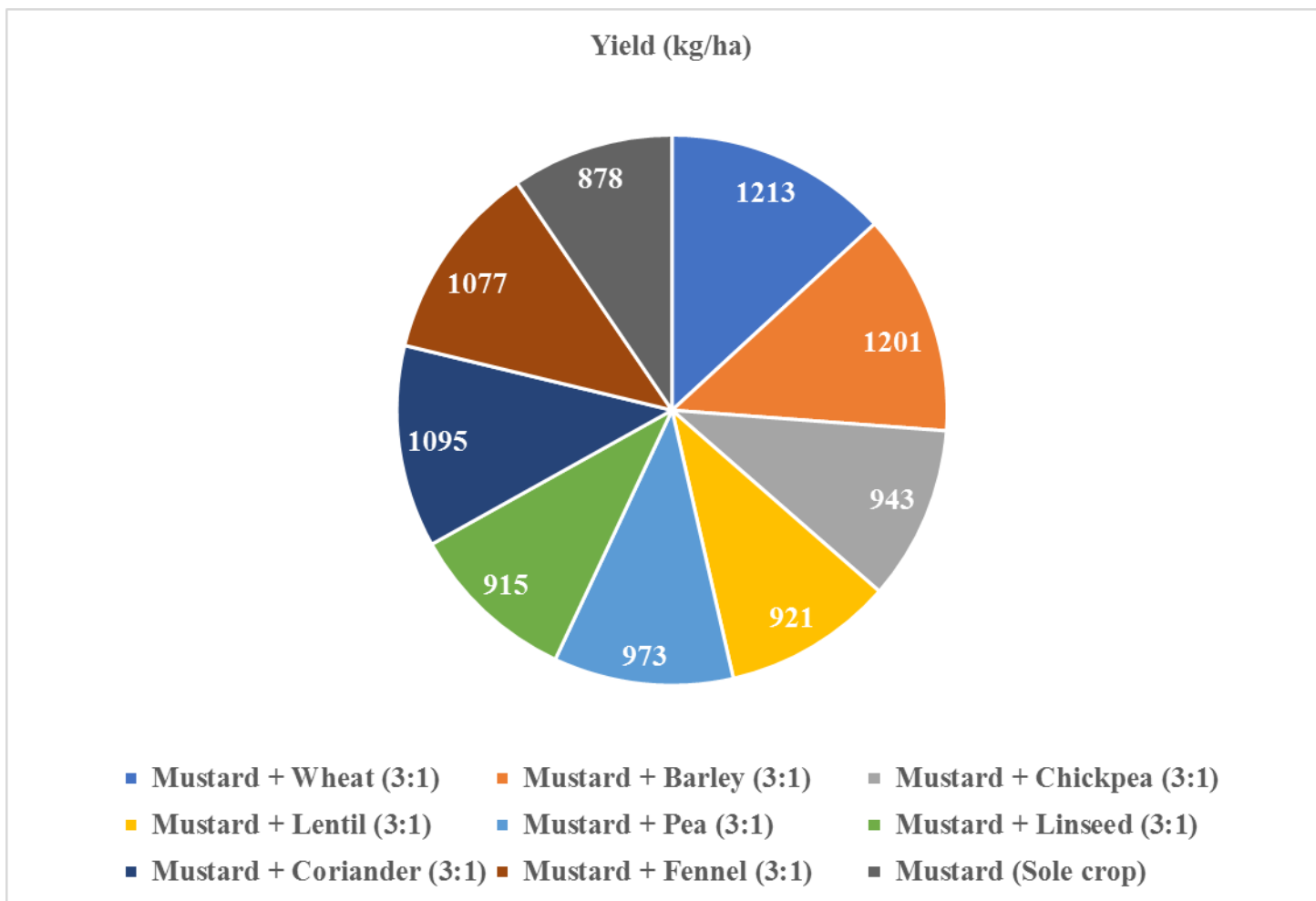


Figure 4.24: Effect of different intercrops on mustard seed yield (kg/ha) (based on the pooled mean of *Rabi*, 2019-20 and 2020-21)



Coccinella septempunctata



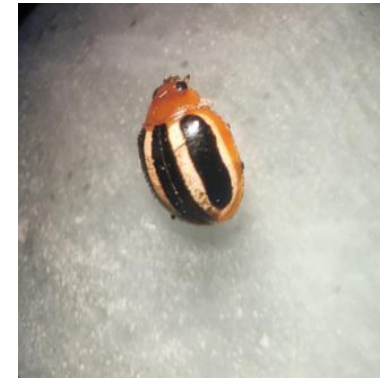
Cheilomenes sexmaculata



Coccinella transversalis



Propylea sp



Brumoides suturalis



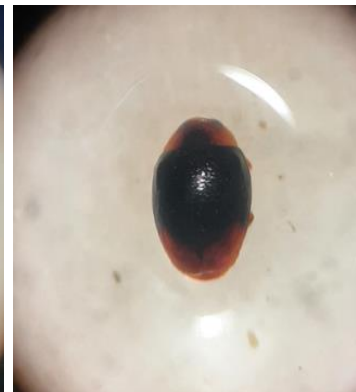
Cheilomenes sexmaculata



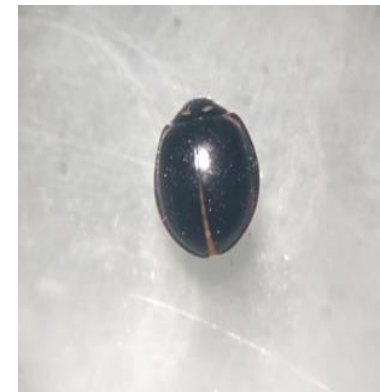
Propylea dissecta



Propylea dissecta



Cryptolaemus montrouzieri



Chilocoris nigrinus

Plate 4.7: Diversity of Coccinellids in mustard intercropped with *Rabi* crops



Episyrphus sp



Sphaerophoria sp



Ishciodon sp



Syrphid larvae



Parasitized aphids



Diaeretiella rapae



Chalcididae



Eulophidae

Plate 4.8: Diversity of Syrphids and Parasitoids in mustard intercropped with *Rabi* crops



Heteropoda sp



Telamonia dimidiata



Oxyopes sp



Thomisus sp



Leucauge decorata



Araneidae



Oxyopidae



Neoscona sp

Plate 4.9: Diversity of Spiders in mustard intercropped with *Rabi* crops

4.4. Effect of botanicals on mustard aphid and its natural enemies under field conditions

To ensure eco-friendly management of mustard aphid an experiment was conducted to assess the efficacy of various indigenously available plant parts along with a chemical Dimethoate 30 EC @ 0.003 % (check) and an untreated control. The first spraying was done at 50 % flowering stage, while the second spraying was done at fortnight interval from the first. The foliar application of treatments showed differential effect on the prevalence of mustard aphid and its natural enemies during two subsequent crop seasons *i.e.*, 2019-20 & 2020-21 and the results are summarized and elaborated below.

4.4.1 Effect of botanicals on mustard aphid during *Rabi*, 2019-20 & 2020-21

It can be deduced from Table 4.40 that during *Rabi*, 2019-2020, mean aphid population on one day before first spray was not significantly varied in all the treatment plots. After first and second spray application, the minimum aphid population (14.09, 5.05/ top 10 cm inflorescence of 10 plants) was observed with Dimethoate 30 EC @ 0.003 % respectively, followed by Neem Seed Kernel Extract (NSKE) @ 5.0% (27.53, 19.33), Yam Bean Seed Extract (YBSE) @ 5% (32.60, 22.60), and Bhang Leaf Extract (BLE) @ 5% (34.37, 25.42) treated plots. Whereas, the maximum aphid population was observed in Field Bindweed Leaf Extract (FBLE) @ 5% (54.95, 77.01) followed by Lamb's Quarter Leaf Extract (LQLE) @ 5% (51.75, 69.97) in comparison to (92.38, 153.76) in untreated plots, which showed statistically significant difference with other botanical and chemical treatments. Congruently, on cumulative mean basis of two sprays, the minimum aphid population (14.09) was recorded in Dimethoate 30 EC @ 0.003 % treatment followed by NSKE @ 5% (27.53), YBSE @ 5% (32.60) and BLE @ 5% (34.37). Whereas, the maximum aphid population was observed in FBLE @ 5% (54.95). The % reduction in aphid population over control after first and second spray was also found maximum (84.74, 96.70 % respectively) with Dimethoate 30 EC @ 0.003 % followed by NSKE @ 5% (70.19, 87.37 %), YBSE @ 5% (64.71, 85.23%) and BLE (62.51, 83.39 %) respectively. Conversely, minimum reduction in aphid population over untreated control was recorded with FBLE @ 5% (40.51, 50.01 %) followed by LQLE @ 5% (43.98, 54.29 %).

It is evident from Table 4.41 that during *Rabi*, 2020-2021, mean aphid population on one day before first spray was not varied significantly in all the treatment plots. After first and second spray application, the minimum aphid population (11.15 & 3.35/ top 10 cm inflorescence of 10 plants) was observed with Dimethoate 30 EC @ 0.003 % respectively, followed by NSKE @ 5.0% (23.08, 16.11), YBSE @ 5% (25.23, 17.05), and BLE @ 5% (27.35, 18.57) treated plots. Whereas, the maximum aphid population was observed in FBLE @ 5% (45.19, 64.10) followed by LQLE @ 5% (41.77, 58.82) in comparison to (80.28, 140.92) in untreated plots, which showed statistically significant difference with other chemical and botanical treatments. Congruently, on cumulative mean basis of two sprays, the minimum aphid population (3.57) was recorded in Dimethoate 30 EC @ 0.003 % treatment followed by NSKE @ 5% (15.36), YBSE @ 5% (17.87) and BLE @ 5% (19.32). Whereas, the maximum aphid population was observed in FBLE @ 5% (64.82). The % reduction in aphid population over control after first and second spray was also found to be maximum (86.11 & 96.85 %) with Dimethoate 30 EC @ 0.003 % followed by NSKE @ 5% (71.25, 88.29 %), YBSE @ 5% (68.57, 86.32 %) and BLE (65.93, 84.78 %) respectively. Conversely, minimum reduction in aphid population over untreated control was recorded with FBLE @ 5% (43.71, 42.41 %) followed by LQLE @ 5% (47.96, 47.86 %).

Comparative perusal of the pooled data *i.e.*, 2019-20 & 2020-21 (Table 4.42) after first and second spray application revealed that the minimum aphid population (12.62, 4.42/ top 10 cm inflorescence of 10 plants) was recorded with Dimethoate 30 EC @ 0.003 % respectively followed by NSKE @ 5% (25.30, 17.72), YBSE @ 5% (28.92, 20.65) and BLE @ 5% (30.86, 22.75). Whereas, the maximum aphid population (50.07, 71.27) was observed in FBLE @ 5%. Similarly, on cumulative mean basis the minimum aphid population (6.57) was recorded with Dimethoate 30 EC @ 0.003 % followed by NSKE @ 5% (19.39), YBSE @ 5% (22.74) and BLE @ 5% (24.61). Whereas, the maximum aphid population (65.40) was observed in FBLE @ 5%. All the treatments proved their superiority in reducing aphid population over untreated control. The % reduction in aphid population over control after first and second spray was also found to be maximum (85.38, 97.02 %) with Dimethoate 30 EC @ 0.003 % followed by NSKE @ 5% (70.69, 87.97 %), YBSE @ 5% (66.58, 86.06 %) and BLE (64.25, 84.56 %) respectively. Conversely, minimum reduction in

aphid population over untreated control was recorded with FBLE @ 5% (42.06, 51.62 %) followed by LQLE @ 5% (45.83, 55.77 %).

4.4.2 Effect of botanicals on natural enemies during *Rabi*, 2019-20 & 2020-21

From the data presented in Table 4.43 it is quite obvious that the natural enemies are remarkably greater in all the botanical treatments and control except in chemical treatment. During both the years *viz.*, *Rabi*, 2020 & 2021 the coccinellids were found maximum (2.81 to 2.55/10 plants) in untreated control respectively and are statistically at par with all the botanical treatments and minimum (0.41 to 0.34/10 plants) in the Dimethoate 30 EC @ 0.003 % treatment respectively. Similarly, the population of syrphids, parasitoids and spiders were found maximum (2.21 to 2.01, 5.21 to 4.74 and 1.85 to 1.69/10 plants) in untreated control respectively, and are statistically at par with all the botanical treatments. Whereas minimum population (0.32 to 0.22, 0.29 to 0.24 and 0.67 to 0.52/10 plants) respectively was recorded in the Dimethoate 30 EC @ 0.003 % treatment and shown a significant difference with all the treatments. Based on the pooled mean of *Rabi* 2019-20 & 2020-21 (table 4.43) it is apparent that the population of coccinellids, syrphids, parasitoids and spiders were found maximum (2.68, 2.11, 4.98, 1.77/ 10 plants respectively) in untreated control and are statistically at par with all the botanical treatments and minimum (0.38, 0.27, 0.25, 0.60/ 10 plants respectively) with Dimethoate 30 EC @ 0.003 % treatment and shown a significant difference with all the treatments. It was clear from the above results that botanicals have no negative effects on the abundance of natural enemies unlike chemical treatment.

4.4.3 Effect of botanicals on mustard seed yield and Economics

Besides suppressing the population of mustard aphid, the insecticide/ botanicals also influenced the yield of crop. During 2019-20 and 2020-21, with regard to yield (Table 4.44) all the treatments enumerated higher yield *i.e.*, 1585 to 1129 and 1630 to 1198 kg/ha respectively than the untreated control (988 to 1056 kg/ha). During both the years, the maximum seed yield was recorded with Dimethoate 30 EC @ 0.003 % treated plot *i.e.*, 1585 to 1630 kg/ha respectively, followed by NSKE @ 5% (1485 to 1517 kg/ha), YBSE @ 5% (1478, 1498 kg/ha), BLE @ 5% (1463, 1487 kg/ha) and minimum seed yield was recorded in FBLE @ 5% (1129, 1198 kg/ha) in comparison to untreated plots (988 to 1056 kg/ha) respectively. Similarly, on pooled mean of two

years the maximum seed yield was recorded with Dimethoate 30 EC @ 0.003 % treated plot (1608 kg/ha) followed by NSKE @ 5% (1501 kg/ha), YBSE @ 5% (1488 kg/ha), BLE @ 5% (1475 kg/ha) and minimum seed yield was recorded in FBLE @ 5% (1164 kg/ha) in comparison to untreated plots (1022 kg/ha).

The overall performance of different treatments was finally assessed on the basis of benefit realised in monetary terms during both years under investigation, and relevant data related to economic parameters are presented in Table 4.44. The net profit derived from various treatments was influenced by the costs associated with these treatments ranged from a minimum (Rs. 4610.00/ ha) with BLE @ 5% and LQLE @ 5% to the maximum of Rs 8640.00/ ha with YBSE @ 5%. The net profit obtained from the various treatments varied greatly, and it was the highest (Rs. 42100.00/ ha) in case of Dimethoate 30 EC @ 0.003 % followed by BLE @ 5% (Rs. 31630.00/ ha), NSKE @ 5% (Rs. 30020.00/ha), YBSE @ 5% (Rs. 28640.00/ha) and the lowest (Rs. 6120.00/ ha) in FBLE @ 5%. The benefit-cost ratio of different treatments differed remarkably with highest (8.80:1) Dimethoate 30 EC @ 0.003 % followed by Bhang leaf extract (6.86:1), NSKE @ 5% (3.61:1), YBSE @ 5% (3.31:1), LQLE @ 5% (2.14:1) and FBLE @ 5% (1.16:1).

According to the findings of the current study, spraying insecticides/botanicals resulted in the reduction of population of mustard aphid to an extent during both the years of experimentation (2019-20 and 2020-21). The current results are in accordance with Srivastava and Guleria (2003), Singh (2007), Singh *et al.* (2011), and Nagar *et al.* (2012) who found that NSKE @ 5% was the most efficient in reducing mustard aphid population. Similarly, Chandel *et al.* (2012) reported highest nymphal and adult mortality of mustard aphid with NSKE @ 5% which possesses a wide range of insect-related activities, including repellent, antifeedant, anti-ovipositional, fecundity, insect growth regulator (IGR) and fitness-reducing properties (Schmutterer, 1990).

The literature on the efficacy of yam bean seed extract and bhang leaf extract on mustard aphid and its natural enemies under field conditions is scanty. However, here we have discussed the possible ways of their effect under laboratory conditions and with closely related literature available. Yam bean seeds have been found to be effective botanical insecticides against a variety of insect pests in toxicity studies (Tangchitphinitkan *et al.*, 2007; Yongkhamcha and Indrapichate, 2012). Yam bean

seed extract @ 5% had proved most efficacious in minimizing aphid population on mustard and were found on par with chemical dimethoate after first and second spraying (Singh *et al.*, 2019). Walker and Anderson (1943), Adjahossou and Sogbenon (1994) observed the control of aphids and diamond-back moth larvae on collards and kale with yam bean seed extract. According to Yang and Tang (1988), rice leafhoppers, were effectively controlled by the insecticides derived from yam bean seed. Yam bean seed extract @ 5% resulted in high mortality (over 95 %) at 5 DAT of field pests such as *Aphis craccivora*, *Spodoptera litura* and *Pericallia ricini* (Naskar, 2009). Low volatility yam bean seed extract outperformed volatile compounds as an oviposition deterrent against insect pests, though this could be offset by controlled release formulation (Culter, 1988). Another common generic feature is the presence of an insecticidal compound known as rotenone (C₂₃H₂₂O₆) which can be found in hazardous concentrations in the ripe seeds, but not in the tuber or any other portion of the plant. Scramin (1994); Villar and Valio (1994) put forward that the presence of rotenoid compounds are likely to have a protective effect against insect pests.

Bedini *et al.* (2016) and Benelli *et al.* (2018) found that the cannabis leaf extract is effective against aphids, moths, flies. Srivastava and Guleria (2003) tested 34 leaf extracts, including bhang, against mustard aphid and observed that all the treatments exhibited insecticidal activity against aphid. *Cannabis* leaf extracts were toxic to all stages of the spider mite, according to Fenili and Pegazzano (1974). To reduce oviposition of the spotted stalk borer Bajpai and Sharma (1992) sprayed a 20 % leaf extract of bhang. The various plant parts of *Azadirachta indica* and *Cannabis sativa* are commonly used in field and stored crop protections (Tavares *et al.*, 2021). According to Dhakal *et al.* (2019), neem products and cannabis leaf extract have a substantial effect in reducing cow pea aphid population when compared to other plant-based extracts and control. Ahmed *et al.* (2020) also demonstrated the aphicidal activity of *Chenopodium ficifolium* extracts. Furthermore, the plant-based chemical compounds in extracts are target specific which ensures safety on non-target organisms especially the beneficial organisms including natural enemies and pollinators (Biswas, 2013; Dougoud *et al.*, 2019; Lengai *et al.*, 2020). Adults and eggs of numerous natural enemies, such as coccinellids, syrphids, parasitoids and others are unaffected by neem seed extracts (Schmutterer, 1997; Satti *et al.*, 2003; Nboyine *et*

al., 2013). Bedini *et al.* (2016) sprayed bhang leaf extracts on cabbage leaves to test against non-target species (ladybird beetle), at various EO concentrations. They have observed that adults showed no mortality, and 3rd instar larvae showed only 3.3 % mortality at the highest concentration. The present results regarding yield with insecticide/ botanical treatments find ample support from the findings of Mandal *et al.* (2012), Pal *et al.* (2018), Kumar *et al.* (2020). The present findings on B:C ratio of NSKE @ 5% and Dimethoate 30 EC @ 0.003 % are in partial conformity with the finding of Agarwal *et al.* (2008) and Ahlawat *et al.* (2018) respectively. The literature on B:C ratio of other botanicals used in the current study are very scanty.

Table 4.40: Efficacy of botanicals against mustard aphid and their effect on mustard seed yield (kg/ha) during Rabi, 2019-20

Treatments	First Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Second Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Cumulative Mean	Yield (kg/ha)
	1DBS	3DAS	5DAS	7DAS	10DAS	Total Mean	% reduction over control	1DBS	3DAS	5DAS	7DAS	10DAS	Total Mean	% reduction over control		
T1-Neem Seed Kernel Extract @ 5%	56.54 ^a	34.67 ^b	25.05 ^b	20.16 ^b	30.24 ^b	27.53 ^b	70.19	57.14 ^b	30.82 ^b	17.05 ^b	12.56 ^{ab}	16.89 ^b	19.33 ^b	87.37	23.43 ^b	1485
T2- Lambs Quarter Leaf Extract @ 5%	54.45 ^a	48.68 ^c	43.22 ^c	47.64 ^c	67.47 ^c	51.75 ^c	43.98	86.62 ^c	75.14 ^c	67.33 ^c	65.15 ^c	72.31 ^c	69.97 ^c	54.29	60.86 ^c	1177
T3- Field Bindweed Leaf Extract @ 5%	57.05 ^a	52.04 ^c	47.01 ^c	50.27 ^c	70.48 ^c	54.95 ^c	40.51	95.12 ^c	82.42 ^c	75.36 ^c	71.57 ^c	78.67 ^c	77.01 ^c	50.01	66.01 ^c	1129
T4- Bhang Leaf Extract @ 5%	56.84 ^a	40.87 ^b	31.03 ^b	27.04 ^b	38.52 ^b	34.37 ^b	62.51	65.25 ^b	35.51 ^b	23.64 ^b	19.41 ^b	23.13 ^b	25.42 ^b	83.39	29.89 ^b	1463
T5-Yam Bean Seed Extract @ 5%	60.08 ^a	39.52 ^b	29.45 ^b	25.21 ^b	36.22 ^b	32.60 ^b	64.71	60.61 ^b	32.09 ^b	20.63 ^b	16.11 ^b	21.56 ^b	22.60 ^b	85.23	27.60 ^b	1478
T6-Dimethoate 30 EC (check)	58.58 ^a	21.37 ^a	13.46 ^a	8.38 ^a	13.14 ^a	14.09 ^a	84.74	29.03 ^a	6.78 ^a	5.02 ^a	3.15 ^a	5.25 ^a	5.05 ^a	96.70	9.57 ^a	1585
T7-Untreated control	55.64 ^a	69.31 ^d	81.35 ^d	97.55 ^d	121.31 ^d	92.38 ^d	-	160.85 ^d	170.45	165.45 ^d	151.12 ^d	128.02 ^d	153.08 ^d	-	123.07 ^d	988
CD (P=0.05)	N/A	6.530	6.293	7.035	9.274	7.248		12.746	11.890	11.209	10.383	9.616	10.722		8.897	201.797
SEm(±)	2.621	2.096	2.020	2.258	2.977	2.326		4.091	3.817	3.598	3.333	3.086	3.442		2.856	64.773
CV (%)	7.962	8.293	9.051	9.908	9.562	9.167		8.944	10.683	11.650	11.918	10.822	11.183		10.170	8.088

DBS – Day Before Spray, DAS – Days After Spray

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.41: Efficacy of botanicals against mustard aphid and their effect on mustard seed yield (kg/ha) during Rabi, 2020-21

Treatments	First Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Second Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Cumulative Mean	Yield (kg/ha)
	1DBS	3DAS	5DAS	7DAS	10DAS	Total Mean	% reduction over control	1DBS	3DAS	5DAS	7DAS	10DAS	Total Mean	% reduction over control		
T1-Neem Seed Kernel Extract @ 5%	51.14 ^a	28.34 ^b	21.08 ^b	18.84 ^b	24.04 ^b	23.08 ^b	71.25	45.14 ^b	22.15 ^b	15.41 ^b	11.12 ^b	15.77 ^b	16.11 ^b	88.29	15.36 ^b	1517
T2- Lambs Quarter Leaf Extract @ 5%	48.74 ^a	40.21 ^c	35.64 ^c	35.56 ^c	55.67 ^c	41.77 ^c	47.96	78.68 ^c	66.51 ^c	56.79 ^c	53.05 ^c	65.08 ^c	60.36 ^c	47.86	59.59 ^c	1228
T3- Field Bindweed Leaf Extract @ 5%	50.78 ^a	43.87 ^c	38.15 ^c	38.61 ^c	60.14 ^c	45.19 ^c	43.71	84.21 ^c	71.25 ^c	62.25 ^c	58.28 ^c	70.34 ^c	65.53 ^c	42.41	64.82 ^c	1198
T4- Bhang Leaf Extract @ 5%	49.85 ^a	32.67 ^b	25.14 ^b	22.12 ^b	29.47 ^b	27.35 ^b	65.93	52.25 ^b	26.08 ^b	20.04 ^b	16.04 ^b	18.13 ^b	20.07 ^b	84.78	19.32 ^b	1487
T5-Yam Bean Seed Extract @ 5%	50.02 ^a	30.45 ^b	23.06 ^b	20.03 ^b	27.38 ^b	25.23 ^b	68.57	50.56 ^b	25.26 ^b	18.05 ^b	14.52 ^b	16.94 ^b	18.69 ^b	86.32	17.87 ^b	1498
T6-Dimethoate 30 EC (check)	52.08 ^a	17.68 ^a	10.76 ^a	6.12 ^a	10.02 ^a	11.15 ^a	86.11	21.07 ^a	5.59 ^a	4.01 ^a	2.15 ^a	3.44 ^a	3.80 ^a	96.85	3.57 ^a	1630
T7-Untreated control	48.66 ^a	58.78 ^d	72.33 ^d	83.45 ^d	106.54 ^d	80.28 ^d	-	138.45 ^d	151.12 ^d	158.85 ^d	143.67	110.05 ^d	140.43 ^d	-	139.65 ^d	1056
CD (P=0.05)	N/A	5.419	5.417	5.826	7.934	6.120		11.005	10.461	10.442	9.529	8.443	9.688		9.554	206.111
SEm(±)	2.621	1.739	1.739	1.870	2.547	1.965		3.532	3.358	3.352	3.059	2.710	3.110		3.067	66.158
CV (%)	7.962	8.370	9.323	10.093	9.857	9.377		9.105	11.063	12.118	12.408	10.960	11.582		11.613	8.099

DBS – Day Before Spray, DAS – Days After Spray

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.42: Efficacy of botanicals on mustard aphid and their effect on mustard seed yield (kg/ha) (data given are the pooled mean of Rabi, 2019-20 & 2020-21)

Treatments	First Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Second Spray (Mean no. of aphids/ 10 cm inflorescence of 10 plants)							Cumulative mean	Yield (kg/ha)
	1DBS	3DAS	5DAS	7DAS	10DAS	Total mean	% reduction over control	1DBS	3DAS	5DAS	7DAS	10DAS	Total mean	% reduction over control		
T1-Neem Seed Kernel Extract @ 5%	53.84 ^a	31.51 ^b	23.07 ^b	19.50 ^b	27.14 ^b	25.30 ^b	70.69	51.14 ^b	26.49 ^b	16.23 ^b	11.84 ^b	16.33 ^b	17.72 ^b	87.97	19.39 ^b	1501
T2- Lambs Quarter Leaf Extract @ 5%	51.60 ^a	44.45 ^c	39.43 ^c	41.60 ^c	61.57 ^c	46.76 ^c	45.83	82.65 ^c	70.83 ^c	62.06 ^c	59.08 ^c	68.70 ^c	65.16 ^c	55.77	60.22 ^c	1203
T3- Field Bindweed Leaf Extract @ 5%	53.92 ^a	47.96 ^c	42.58 ^c	44.44 ^c	65.31 ^c	50.07 ^c	42.06	89.67 ^c	76.84 ^c	68.81 ^c	64.93 ^c	74.51 ^c	71.27 ^c	51.62	65.40 ^c	1164
T4- Bhang Leaf Extract @ 5%	53.35 ^a	36.77 ^b	28.09 ^b	24.58 ^b	34.00 ^b	30.86 ^b	64.25	58.75 ^b	30.80 ^b	21.84 ^b	17.73 ^b	20.63 ^b	22.75 ^b	84.56	24.61 ^b	1475
T5-Yam Bean Seed Extract @ 5%	55.05 ^a	34.99 ^b	26.26 ^b	22.62 ^b	31.80 ^b	28.92 ^b	66.58	55.59 ^b	28.68 ^b	19.34 ^b	15.32 ^b	19.25 ^b	20.65 ^b	86.06	22.74 ^b	1488
T6-Dimethoate 30 EC (check)	55.33 ^a	19.53 ^a	12.11 ^a	7.25 ^a	11.58 ^a	12.62 ^a	85.38	25.05 ^a	6.19 ^a	4.52 ^a	2.65 ^a	4.35 ^a	4.42 ^a	97.02	6.57 ^a	1608
T7-Untreated control	52.15 ^a	64.05 ^d	76.84 ^d	90.50 ^d	113.93 ^d	86.33 ^d	-	149.65	160.79	162.15	147.40	119.04	147.34		131.36 ^d	1022
CD (P=0.05)	N/A	5.975	5.855	6.453	8.611	6.651		11.857	11.165	10.829	9.911	9.014	10.221		9.221	203.965
SEm(±)	2.484	1.918	1.879	2.072	2.764	2.135		3.806	3.584	3.476	3.181	2.893	3.281		2.960	65.469
CV (%)	8.026	8.331	9.172	10.024	9.707	9.217		9.004	10.848	11.874	12.091	10.870	11.387		10.866	8.094

DBS – Day Before Spray, DAS – Days After Spray

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.43: Efficacy of botanicals on natural enemies of mustard aphid during *Rabi*, 2019-20 and 2020-21

Treatments	2019-20				2020-21				Pooled Mean			
	Mean number/10 plants				Mean number/10 plants				Mean number/10 plants			
	Coccinellids	Syrphids	Parasitoids	Spiders	Coccinellids	Syrphids	Parasitoids	Spiders	Coccinellids	Syrphids	Parasitoids	Spiders
T1-Neem Seed Kernel Extract @ 5%	2.61 ^a	1.96 ^a	4.78 ^a	1.65 ^a	2.25 ^a	1.74 ^a	4.45 ^a	1.49 ^a	2.43 ^a	1.85 ^a	4.62 ^a	1.57 ^a
T2- Lambs Quarter Leaf Extract @ 5%	2.65 ^a	2.01 ^a	4.84 ^a	1.68 ^a	2.39 ^a	1.77 ^a	4.49 ^a	1.51 ^a	2.52 ^a	1.89 ^a	4.67 ^a	1.60 ^a
T3- Field Bindweed Leaf Extract @ 5%	2.75 ^a	2.12 ^a	5.01 ^a	1.79 ^a	2.41 ^a	1.87 ^a	4.65 ^a	1.62 ^a	2.58 ^a	2.00 ^a	4.83 ^a	1.71 ^a
T4- Bhang Leaf Extract @ 5%	2.72 ^a	2.08 ^a	4.96 ^a	1.75 ^a	2.35 ^a	1.82 ^a	4.58 ^a	1.59 ^a	2.54 ^a	1.95 ^a	4.77 ^a	1.67 ^a
T5-Yam Bean Seed Extract @ 5%	2.68 ^a	1.99 ^a	4.89 ^a	1.69 ^a	2.29 ^a	1.79 ^a	4.49 ^a	1.53 ^a	2.49 ^a	1.89 ^a	4.69 ^a	1.61 ^a
T6-Dimethoate 30 EC (check)	0.41 ^b	0.32 ^b	0.29 ^b	0.67 ^b	0.34 ^b	0.22 ^b	0.20 ^b	0.52 ^b	0.38 ^b	0.27 ^b	0.25 ^b	0.60 ^b
T7-Untreated control	2.81 ^a	2.21 ^a	5.21 ^a	1.85 ^a	2.55 ^a	2.01 ^a	4.74 ^a	1.69 ^a	2.68 ^a	2.11 ^a	4.98 ^a	1.77 ^a
CD (P=0.05)	0.362	0.265	0.625	0.228	0.312	0.228	0.622	0.220	0.306	0.246	0.627	0.247
SEm(±)	0.116	0.085	0.201	0.073	0.100	0.073	0.200	0.071	0.098	0.079	0.201	0.079
CV (%)	8.475	8.156	8.101	8.053	8.304	7.933	8.734	8.573	7.595	8.006	8.467	9.134

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test

Table 4.44: Economics of botanicals and synthetic insecticide used as foliar spray against mustard aphid (data given are the pooled mean of Rabi, 2019-20 & 2020-21)

Treatments	Dose/concentration per ha	Yield (kg/ha)	Additional yield over control (kg/ha)	Price of additional yield (Rs./ha)	Cost of treatment (Rs./ha)	Net profit over control (Rs./ha)	B:C Ratio
T1-Neem Seed Kernel Extract	5%	1501	479	38320	8300	30020	3.61:1
T2- Lambs Quarter Leaf Extract	5%	1203	181	14480	4610	9870	2.14:1
T3- Field Bindweed Leaf Extract	5%	1164	142	11360	5240	6120	1.16:1
T4- Bhang Leaf Extract	5%	1475	453	36240	4610	31630	6.86:1
T5-Yam Bean Seed Extract	5%	1488	466	37280	8640	28640	3.31:1
T6-Dimethoate 30 EC (check)	500 ml	1608	586	46880	4780	42100	8.80:1
T7-Untreated control	-	1022	-	-	-	-	-
CD (P=0.05)	-	198.365	-	-	-	-	-
SEm(±)	-	63.672	-	-	-	-	-
CV (%)	-	8.143	-	-	-	-	-

Price of mustard- Rs 80/kg, Labour charge- Rs 330/ per day per person, Number of labours required- 3 persons/ha/spray

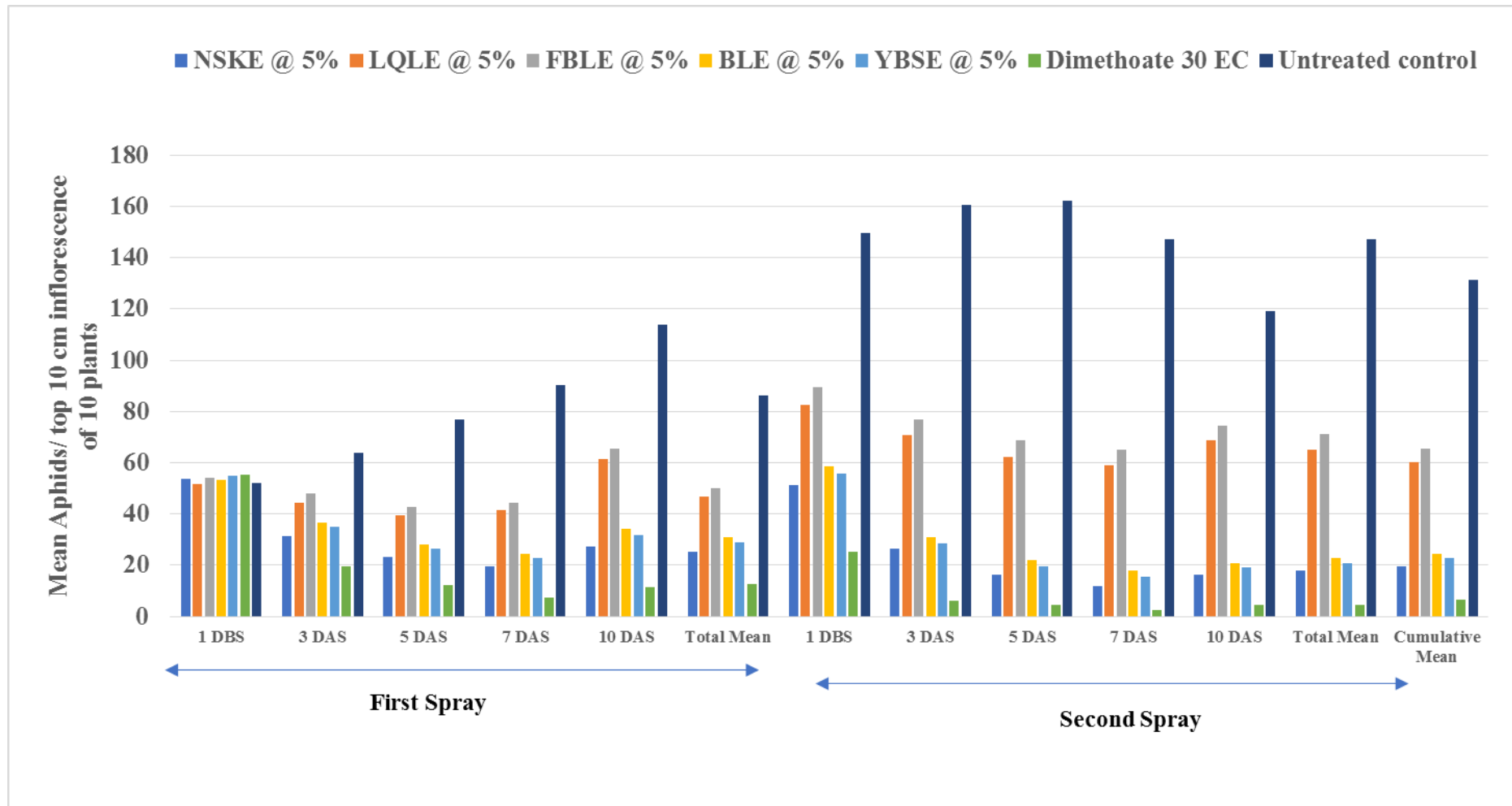


Figure 4.25: Efficacy of botanicals on mustard aphid and their effect on mustard seed yield (kg/ha) (data given are the pooled mean of Rabi, 2019-20 & 2020-21)

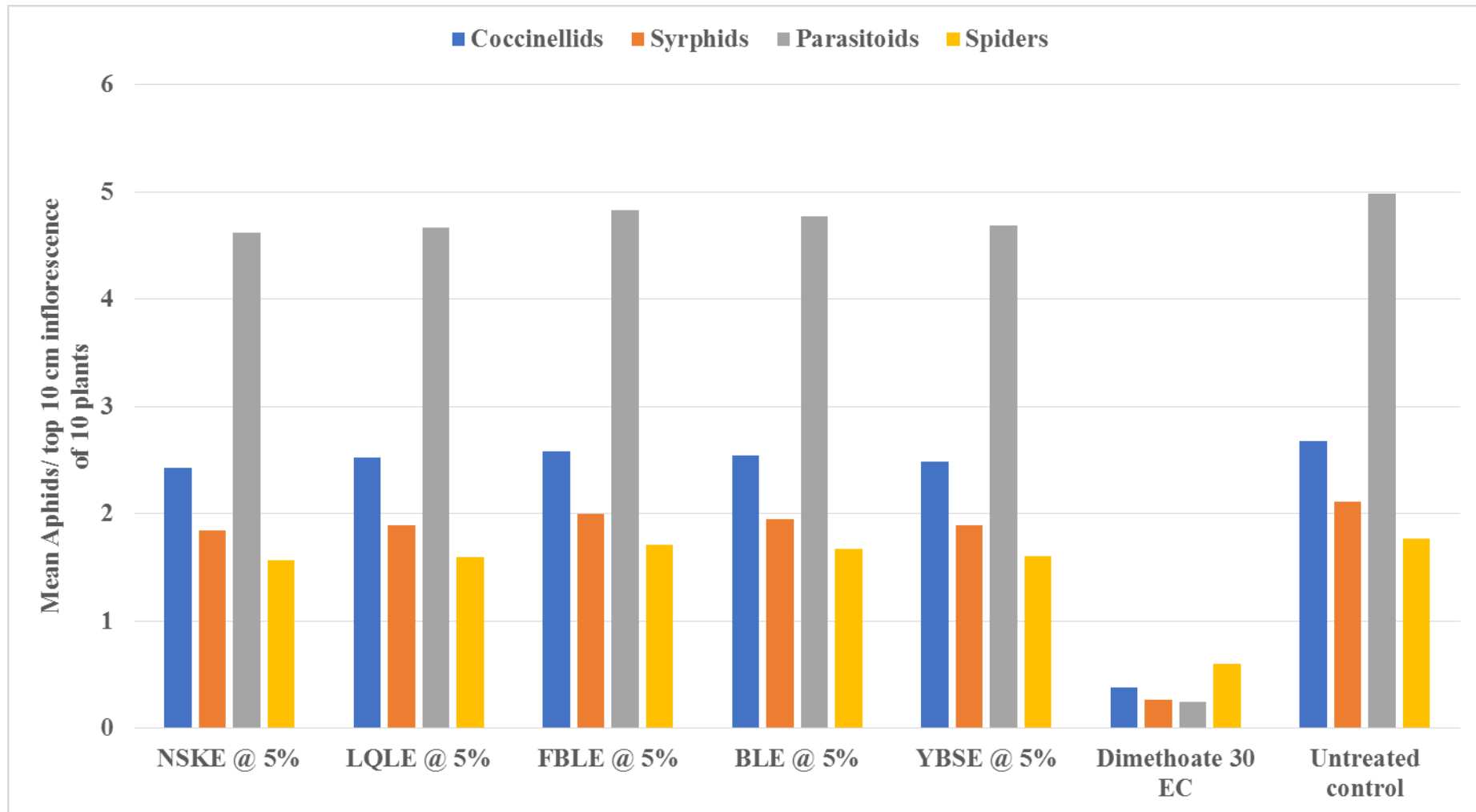


Figure 4.26: Efficacy of botanicals on natural enemies of mustard aphid during *Rabi*, 2019-20 and 2020-21

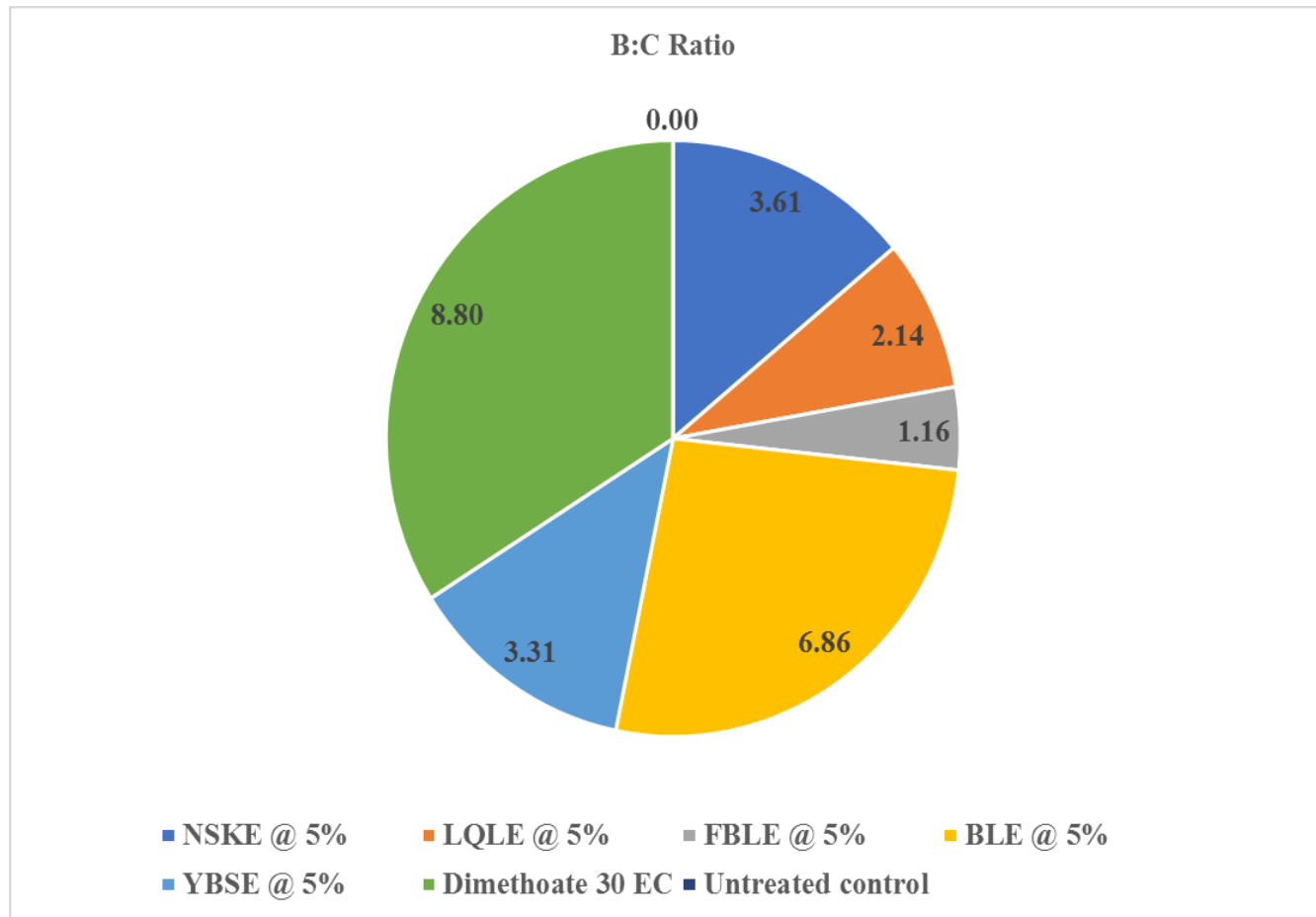


Figure 4.27: Economics of botanicals and synthetic insecticide used as foliar spray against mustard aphid (data given are the pooled mean of *Rabi*, 2019-20 & 2020-21)

4.4.4 Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis of methanol extracts of Yam bean seed and Bhang leaf

From the current study, three botanicals (NSKE, YBSE and BLE @ 5% respectively) were found to be promising against mustard aphid. Since, there are lot of studies and literature on insecticidal properties of NSKE; we selected YBSE and BLE further for GC-MS analysis to determine the presence of biologically active components. The results of the extracts' GC-MS analysis led to the identification of several biological compounds. The main active chemicals, their retention time, peak area (%), molecular formula and molecular weight (g mol^{-1}) of methanolic extracts of yam bean seed and bhang leaf were given in the Tables 4.45 & 4.46.

The chemical profiling of YBSE obtained through GC-MS analysis revealed the presence of thirty-five compounds, accounting for 99.94% of the total extract. Among which 9,12-Octadecadienoic acid (Z,Z)- (40.50%), n-Hexadecanoic acid (23.37%), Mome inositol (7.40%) and Coumarine, 6-(7-hydroxycoumarin-8-yl)-7-methoxy- (5.71%) were the major compounds identified, and rest of the other minor compounds with lower relative peak areas ranging from 0.07– 4.11% were identified. It was believed that the insecticidal properties of YBSE were due to presence of compound rotenone. In the present study we have identified the rotenone compounds viz., Coumarine, 6-(7-hydroxycoumarin-8-yl)-7-methoxy-, 2H-1-benzopyran-2-one, 7-(diethylamino)-3-[4-(dimethylamino)benzoyl]- etc. which have insecticidal properties against herbivores. Rotenone deterred the oviposition of *C. maculatus* (Necha *et al.*, 2004). However, Basukriadi and Wilkins (2014) observed that pure rotenone had no effect on the oviposition of diamondback moth adults, implying that the deterrent effect of yam bean seed extract is likely due to factors other than the seeds' rotenone content (Sørensen, 1996) there could be compounds other than rotenone which have insecticidal properties.

The compounds like 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-octadecatrienoic acid methyl ester, n-Hexadecanoic acid, 3-arylcoumarin or phenylfuranocoumarin, pachyrrhizid etc. were also responsible for the activity against insects (Phrutivorapongkul *et al.*, 2002; Jiang *et al.*, 2018). Lepage *et al.* (1946) evaluated the toxic effect of ethanolic seed extract of yam bean on aphids (*Brevicoryne brassicae*). Sørensen (1990) studied the insecticidal compounds and their properties of yam bean seed against various insect pests. Further, Lackhan

(1994), Scramin (1994) exploited the insecticidal properties of yam bean seed commercially against insect pests.

The chemical profiling of crude bhang leaf extract obtained through GC-MS analysis revealed the presence of thirty one compounds, accounting for 99.94% of the total extract; Among which (E)- β -caryophyllene (26.94%), Limonene (11.54%), β -Sesquiphellandrene (8.43%), α -Pinene (6.17%), Myrcene (5.06%), β -Selinene (4.80%), α -Terpinolene (4.78) were the major terpenoid compounds identified, and rest of the minor compounds with lower relative peak areas ranging from 0.10– 2.87% were identified.

The chemical profiling through GC-MS was dominated by terpenoids *i.e.*, (E)-caryophyllene (45.4%), myrcene (25.0%) and α -pinene (17.9%). The insecticidal activities of extracts from cannabis were highly toxic to *M. persicae* aphids and non-toxic to the beneficial organisms (Benelli *et al.*, 2018). This supported our findings, indicating the effectiveness of the cannabis crude extract against mustard aphid. Jin *et al.* (2020) performed GC-MS to identify the chemical components in a crude cannabis extract and found the abundant compounds *i.e.*, β -myrcene (16.78% to 23.57%), α -Pinene (4.26% to 36.07%), β -Pinene (3.04% to 7.12%), Limonene (3.79% to 16.42%), Linalool (2.10% to 2.99%), β -Caryophyllene (6.71% to 45.25%). Terpenoids, which are well-known for their insecticidal and repellent properties are the principal chemical compound group of bhang, target insect octopamine or GABA receptors and are responsible for deterrence (Benelli *et al.*, 2018). Three terpenoids from bhang *i.e.*, limonene, linalool, and pinene are marketed as insecticides (McPartland and Sheikh, 2018). Kashyap *et al.* (1991), Ross and ElSohly (1996) reported that volatile compounds present in the bhang leaves can act as a powerful insect repellent. Turner *et al.* (1980) reported that bhang leaves are reservoir of hundreds of volatile compounds, such as terpenes, esters and ketones and have high insecticidal properties. However, very few studies on the efficacy of bhang crude extract against crop pests have been conducted to date, and the literature on mustard aphid is exceptionally limited.

Table 4.45: GC-MS analysis of Yam bean seed extract

Peak#	R.Time	Area	Area%	Name	Chemical Formula	Mol. Wt
1	4.460	18297956	1.16	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144.12
2	5.655	4810497	0.31	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11
3	12.020	1372432	0.09	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37
4	13.206	116678079	7.40	Mome inositol	C ₇ H ₁₄ O ₆	194.18
5	13.615	7186326	0.46	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
6	14.347	368468876	23.37	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42
7	15.268	10674359	0.68	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
8	15.325	5591025	0.35	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.5
9	15.558	1042306	0.07	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
10	16.144	638698865	40.50	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.4
11	16.267	16915189	1.07	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5
12	17.579	2064639	0.13	9-Eicosenoic acid, (Z)-	C ₂₀ H ₃₈ O ₂	310.5
13	17.764	1929764	0.12	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312.5
14	18.420	1682908	0.11	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
15	18.545	3055191	0.19	(Z)6,(Z)9-Pentadecadien-1-ol	C ₁₅ H ₂₈ O	224.38
16	20.112	4231099	0.27	Oleoyle chloride	C ₁₈ H ₃₃ ClO	300.9
17	20.253	2839644	0.18	Eicosanoic acid, tetradecyl ester	C ₃₄ H ₆₈ O ₂	508.9
18	21.027	767311	0.05	Nicotinic acid, 5-[5-(3,4-dimethoxyphenyl)-[1,3,4]oxadiazol-2-yl]-2,6-dimethyl-, hydrazide	C ₁₈ H ₁₉ N ₅ O ₄	369.5
19	21.697	2386211	0.15	3-Hydroxy-4-methoxycinnamic acid	C ₁₀ H ₁₀ O ₄	194.18
20	21.819	1541512	0.10	Acetamide, N-(3,4,5-trimethoxyphenethyl)-	C ₁₃ H ₁₉ NO ₄	253.29
21	22.083	35241155	2.23	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-	C ₁₂ H ₂₅ B ₂ NO ₂	237.5
22	22.522	18660424	1.18	1,2,3,4-Tetrahydroisoquinolin-6-ol, 1-benzyl-7-methoxy-	C ₁₇ H ₁₉ NO ₂	269.34
23	22.859	91471277	3.00	Neocuproine	C ₁₄ H ₁₂ N ₂	208.26
24	23.619	4954614	0.31	1,6-dimethylamino-2-(3,4-dimethoxyphenyl)-7-methoxynaphthalene	C ₂₁ H ₂₄ N ₂ O ₃	352.4
25	23.975	64752319	4.11	5H-Dibenzof[a,d]cycloheptene	C ₁₅ H ₁₂	192.25
26	24.235	41866660	5.71	Coumarine, 6-(7-hydroxycoumarin-8-yl)-7-methoxy-	C ₁₉ H ₁₂ O ₆	336.3
27	24.790	63608646	4.03	5-Methyl-3-phenyl-1H-indazole	C ₁₄ H ₁₂ N ₂	208.5
28	24.957	2827743	0.18	(3-Methoxy-phenyl)-(6-methyl-4-phenyl-quinazolin-2-yl)-amine	C ₂₂ H ₁₉ N ₃ O	341.2
29	25.184	3974852	0.25	Strychnidin-10-one, 2,3-dimethoxy-	C ₂₃ H ₂₆ N ₂ O ₄	394.5
30	25.531	1179587	0.07	γ-Sitosterol	C ₂₉ H ₅₂ O ₂	432.7
31	26.369	7343245	0.47	2,4-dimethyl-9-mesitylindano[3,2-a]indene	C ₂₇ H ₂₆	351.2
32	27.194	3812378	0.24	Stigmast-4-en-3-one	C ₂₉ H ₄₈ O	412.7
33	29.180	1490010	0.95	2H-1-benzopyran-2-one, 7-(diethylamino)-3-[4-(dimethylamino)benzoyl]-	C ₂₂ H ₂₄ N ₂ O ₃	364.2
34	30.337	2407126	0.15	Neronine, 4.beta.,5-dihydro-	C ₁₈ H ₂₁ NO ₆	347.7
35	31.223	5480785	0.35	1H-Pyrrolo[3,2-g]quinoline, 9-methoxy-1,2,3-trimethyl-5,7-diphenyl-	C ₂₇ H ₂₄ N ₂ O	392.6
		1576942222	100.00			

Table 4.46: GCMS analysis of Bhang leaf extract

Peak#	R.Time	Area	Area%	Name	Chemical formulae	Mol. wt
1	9.868	456382	1.69	3',5'-Dimethoxyacetophenone	C ₁₀ H ₁₂ O ₃	180.2
2	11.494	270405	0.50	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂	242.4
3	13.613	1425183	2.70	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.5
4	14.079	3394243	5.06	Myrcene	C ₁₀ H ₁₆	136.23
5	15.247	3218218	4.80	β-Selinene	C ₁₅ H ₂₄	204.35
6	15.307	3206641	4.78	α-Terpinolene	C ₁₀ H ₁₆	136.23
7	15.449	1063515	1.59	Phytol	C ₂₀ H ₄₀ O	296.5
8	15.542	508240	0.76	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5
9	15.801	18077422	26.94	(E)-β-caryophyllene	C ₁₅ H ₂₄	204.35
10	15.982	580494	0.87	Undec-10-ynoic acid, undecyl ester	C ₂₂ H ₄₀ O ₂	336.6
11	16.606	419019	0.62	1,E-8,Z-10-Tetradecatriene	C ₁₄ H ₂₄	192.34
12	16.949	417307	0.92	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
13	17.070	1596397	2.72	α-Pinene	C ₁₀ H ₁₆	136.35
14	17.236	180626	0.27	2-Dodecylcyclobutanone	C ₁₆ H ₃₀ O	238.41
15	17.303	240608	0.36	Tetradecanedioic acid, monomethyl ester	C ₁₅ H ₂₈ O ₄	272.38
16	18.366	1852050	2.76	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	213.32
17	18.403	1789131	2.67	Fumaric acid, 2-dimethylaminoethyl nonyl ester	C ₁₇ H ₃₁ NO ₄	313.4
18	18.515	5657332	8.43	β-Sesquiphellandrene	C ₁₅ H ₂₄	204.35
19	18.552	7741358	11.54	Limonene	C ₁₀ H ₁₆	136.23
20	18.750	745128	1.11	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
21	19.905	1922510	2.87	Linolool oxide	C ₁₃ H ₂₆ O ₂ Si	242.43
22	20.115	1387019	2.07	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	266.5
23	20.829	330948	0.82	Myristin, 1,3-diaceto-2-	C ₂₁ H ₃₈ O ₆	386.5
24	21.746	64282	0.10	Glycidyl palmitate	C ₁₉ H ₃₆ O ₃	312.5
25	22.037	124633	0.19	Phytyl decanoate	C ₃₀ H ₅₈ O ₂	450.8
26	23.444	210684	0.31	α-Tocopherol	C ₂₉ H ₅₀ O ₂	430.7
27	25.522	826601	1.73	γ -Sitosterol	C ₂₉ H ₅₀ O	414.7
28	27.083	511298	0.76	3-Cyclohexylnonadecane	C ₂₅ H ₅₀	350.7
29	27.667	4142829	6.17	β-Bisabolene	C ₁₅ H ₂₄	204.23
30	30.266	1857140	2.77	Viridiflorol	C ₁₅ H ₂₆ O	222.37
31	35.849	820312	1.22	Hexadecanoic acid, 1,3-propanediyl ester	C ₃₅ H ₆₈ O ₄	552.9
		67097750	100.00			

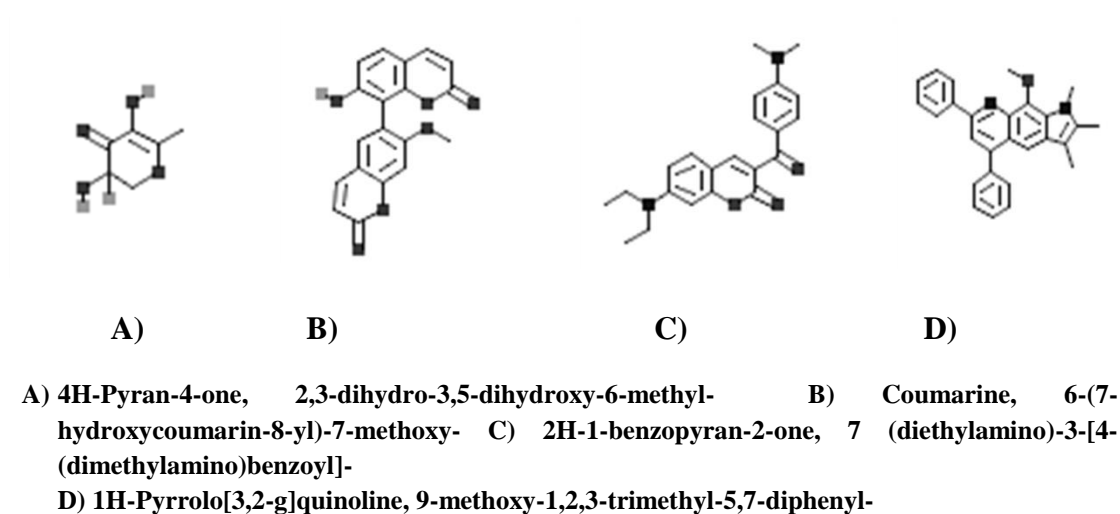


Figure 4.28: Chemical structures of specific compounds identified in Yam bean seed extract through GC-MS analysis

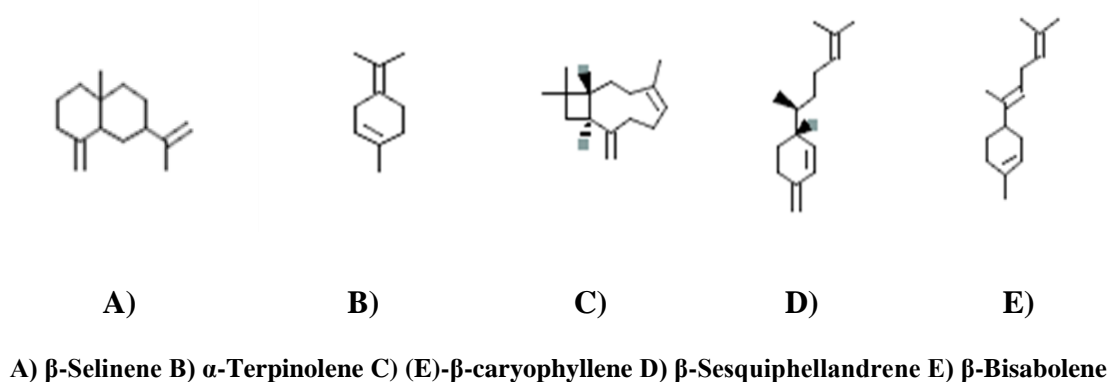


Figure 4.29: Chemical structures of specific compounds identified in Bhang leaf extract through GC-MS analysis

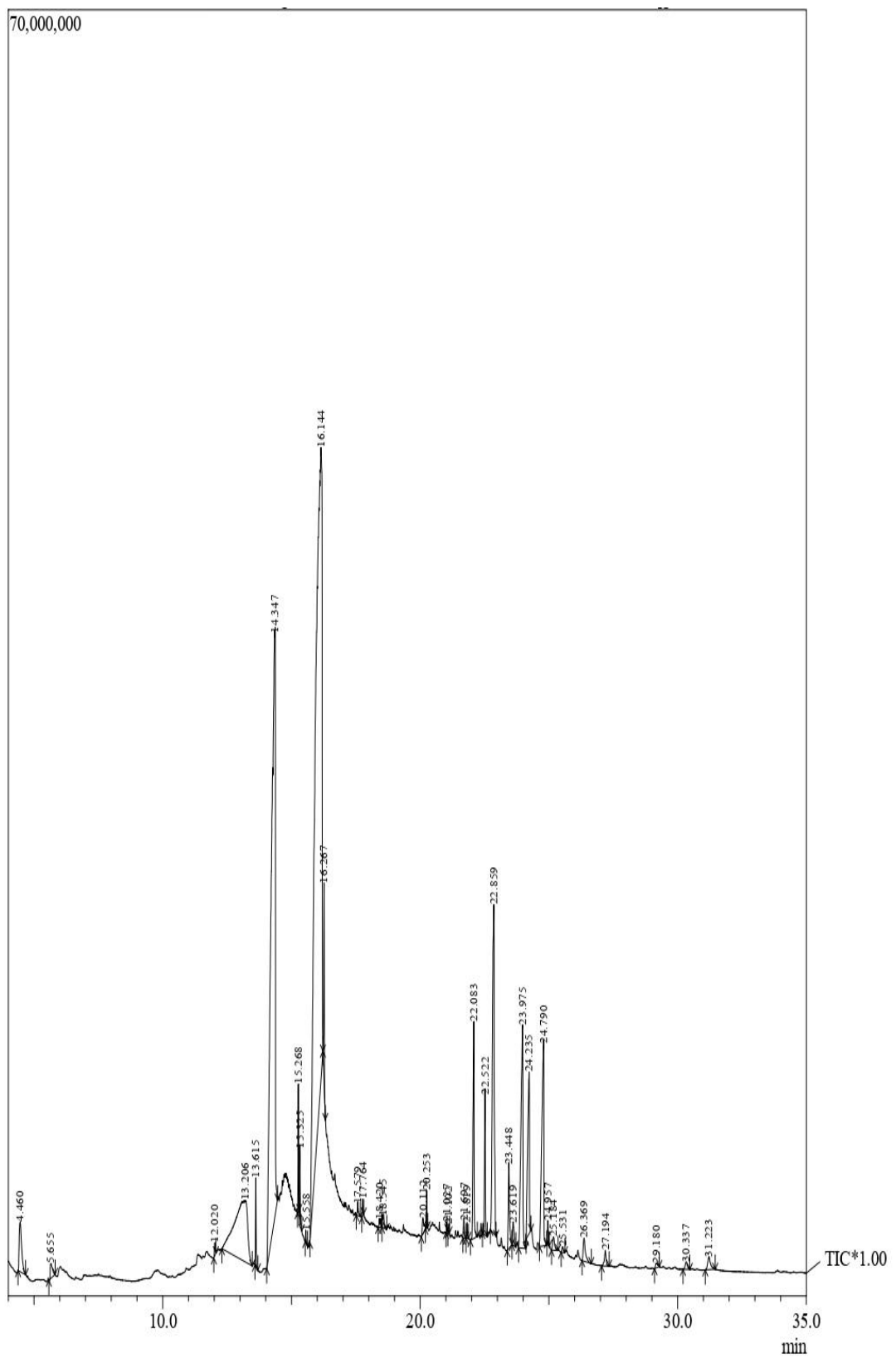


Figure 4.30: GC-MS chromatogram of Yam bean seed extract

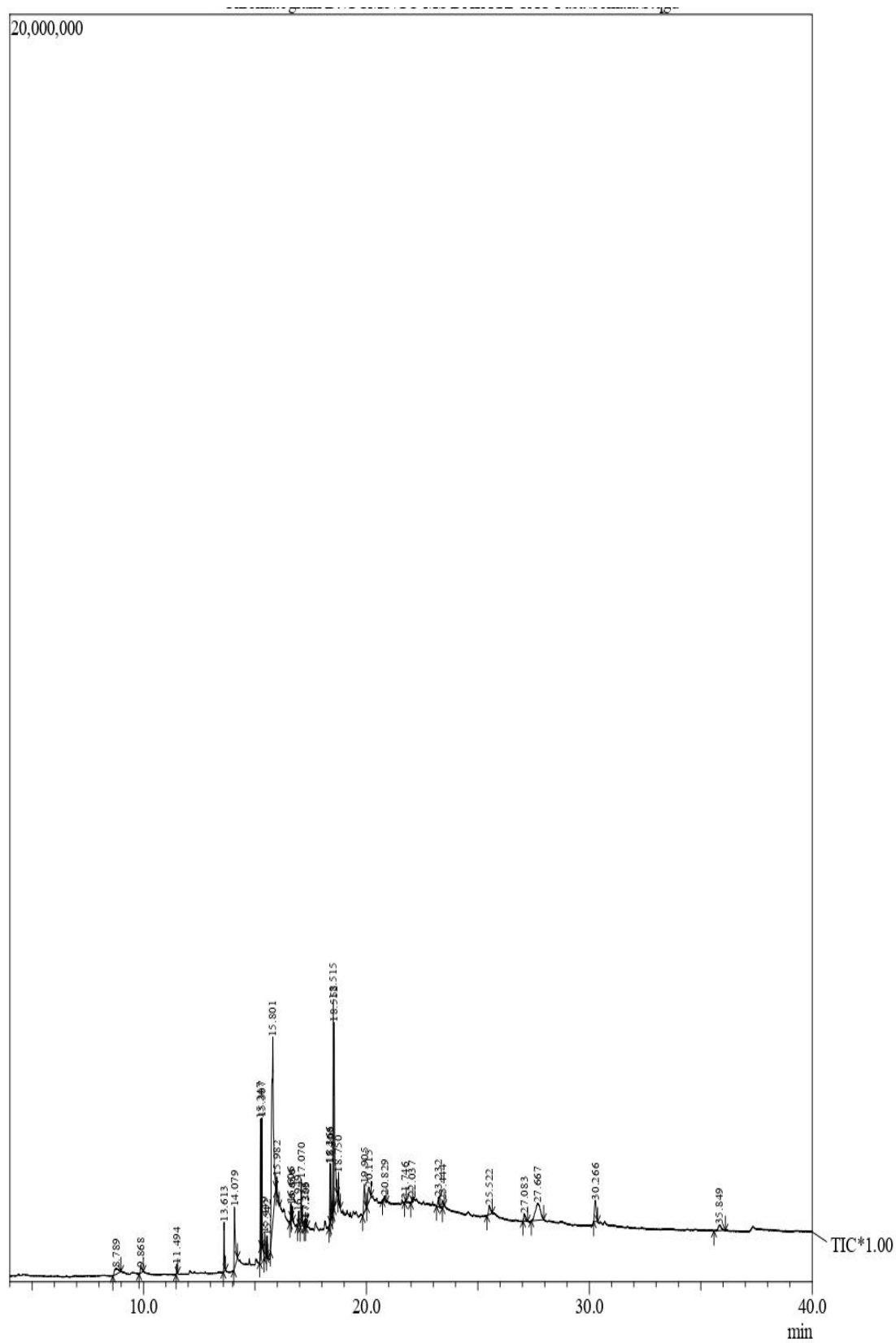
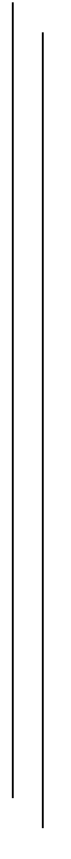


Figure 4.31: GC-MS chromatogram of Bhang leaf extract





CHAPTER - V



SUMMARY AND CONCLUSION



The present study entitled “Screening of Rapeseed-mustard Genotypes and Eco-friendly Management against Mustard Aphid, *Lipaphis erysimi* (Kalt.)” was carried out at Research farm, TCA, Dholi, Department of Entomology, Department of Genetics and Plant Breeding, and Department of Biochemistry, PG College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar and Advance Research Instrumental Facilities (AIRF), Jawaharlal Nehru University (JNU), New Delhi. The investigation had been carried out on molecular characterization and genetic diversity of mustard specialist aphid and its endosymbionts in the Indian subcontinent, monitoring of mustard alate aphids and its natural enemies in relation to abiotic factors, to find out the interaction of different Rapeseed-mustard genotypes and their resistance mechanism with mustard aphid and to develop eco-friendly tactics for management of mustard aphid by using different modules such as intercropping and botanicals. The investigation's key findings are summarised below:

Surveys were conducted to collect the mustard aphid from different parts of rapeseed-mustard growing regions in India during 2019-2020. A total of 16 locations viz., Pantnagar, Ludhiana, Jodhpur, Jhansi, Ranchi, Mohanpur, Raipur, Dharwad, Chintapalli, Barapani, Imphal, Dholi, Dariapur, Alipur Bihta, Delhi and Jorhat belonging to 13 states and one Union Territory of India were surveyed. The collected mustard aphids were morphologically confirmed for *Lipaphys erysimi* species. It was found that *Lipaphys erysimi* species was distributed throughout the 16 locations ranging from 40.42 m (Dariapur) to 980.80 m (Barapani) altitude.

Further, the genetic diversity studies were carried out with 10 RAPD and 09 SSR primers. Among all the primers, RAPD primers (OPA-02, OPA-04, F2) and SSR primers (ApH04M, Ap-01 and ApH08M) with polymorphic band percentage (>70), higher PIC, EMR, MI and RP were considered as greater informativeness for genetic diversity studies.

Barcoding of all the 16 locations with universal primer (LCO1490- Forward and RHCO2- Reverse) at 675 bp of *mtCOI* gene has confirmed the presence of *Lipaphis erysimi pseudobrassicae*. The Multiple sequence alignment of nucleotides of

16 locations revealed 438 fully conserved residues and 141 Single Nucleotide Polymorphisms (SNPs). The Tamura 3-parameter with Gamma distribution (T92+G) was found to be the finest DNA model for current nucleotide substitution studies based on low Bayesian Information Criterion (BIC). The phylogenetic tree shows the clustering of all 16 locations with *L. e. pseudobrassicae* (reference sequence). Among all the locations, Jorhat of North-Eastern India was found to be highly diverged with higher branch distance (0.13) in the phylogenetic tree and similar results were observed in RAPD and SSR dendrograms with 30.0 and 31.0 % dissimilarity respectively. Tajima's neutrality test of 16 locations resulted in a negative *D* value with excess of low-frequency polymorphisms, relative to expectation and representing population size expansion.

The association of bacterial endosymbionts with *L. e. pseudobrassicae* from all the 16 locations were identified with specific primers. The results show that *Buchnera* sp, a primary endosymbiont was found in all the locations. Whereas in secondary endosymbionts, *Wolbachia* sp, *Rickettsia* sp, and *Hamiltonella* sp dominated the most with 81.25 % and *Cardinium* sp least (31.25 %). Among all the locations, Jodhpur and Ranchi had a 100 % incidence of endosymbionts and lower incidence was found in Ludhiana, Raipur, Dharwad, Alipur Bihta, Delhi, Jorhat (42.85 %).

The effect of prevailing weather parameters on population fluctuation of alate mustard aphid and its natural enemies were assessed during two successive *Rabi* seasons (2019-20 and 2020-21). The population of alate mustard aphids commenced first on the 52nd SW (8.32/4 traps) during first crop season and 51st SW (3.98/4 traps) during second crop season and reached maximum on 8th SW in both the years with population of 168.66 and 145.62/4 traps respectively. The correlation analysis revealed that during first crop season, the population of alate mustard aphids showed positive correlation with maximum temperature ($r=0.508$), minimum temperature ($r=0.183$), morning relative humidity ($r=0.084$), rainfall ($r=0.063$), sunshine hrs ($r=0.637^*$) and negative correlation with evening relative humidity ($r=-0.354$). However, during second crop season, the population of alate mustard aphids showed positive correlation with maximum temperature ($r=0.491$), minimum temperature ($r=0.295$), significant positive correlation with sunshine hrs ($r=0.540$) and negative correlation with morning and evening relative humidity ($r=-0.497$, $r=-0.427$).

respectively). During both the seasons, all the prevailing weather parameters viz., maximum and minimum temperature, morning and evening relative humidity, rainfall and sunshine hrs collectively governed $R^2 = 84.10$ and 64.00 % contribution respectively towards alate mustard aphid population.

The population of natural enemies i.e., adult coccinellids, syrphids and parasitoids were commenced first on the 1st SW (0.42, 0.31, 0.68/ 4 traps respectively) and reached their peak on 8th SW (6.22, 5.63, 15.44/ 4 traps respectively). However, during second crop season they commenced first on 52nd SW (0.45, 0.32, 1.29/ 4 traps respectively) and reached their peak on 8th SW (6.22, 5.63, 15.44/ 4 traps respectively). The correlation analysis revealed that during first crop season, the population of adult coccinellids, syrphids and parasitoids showed significant positive correlation with maximum temperature ($r=0.634^*$, $r=0.620^*$, $r=0.625^*$ respectively) and sunshine hrs ($r= 0.646^*$, 0.638^* , 0.630^*) and positive correlation with minimum temperature ($r=0.416$, $r=0.401$, $r=0.412$), morning relative humidity ($r=0.226$, $r=0.199$, $r=0.198$) and rainfall ($r=0.209$, $r=0.187$, $r=0.218$). Whereas, negative correlation with evening relative humidity ($r=-0.347$, $r=-0.335$, $r=-0.335$). All the prevailing weather parameters collectively governed $R^2 = 83.85$, 84.00 , 80.00 % contribution towards adult coccinellids, syrphids and parasitoids respectively. Similarly, during second crop season also the population of adult coccinellids, syrphids and parasitoids showed significant positive correlation with maximum temperature ($r=0.560^*$, $r=0.552^*$, $r=0.596^*$) and sunshine hrs ($r= 0.579^*$, 0.575^* , 0.619^* respectively) and positive correlation with minimum temperature ($r=0.395$, $r=0.390$, $r=0.460$ respectively). Contrarily it showed negative correlation with morning relative humidity ($r=-0.546$, $r=-0.536$, $r=-0.541$) and evening relative humidity ($r=-0.434$, $r=-0.431$, $r=-0.453$ respectively). All the prevailing weather parameters collectively governed $R^2 = 66.89$, 63.80 , 61.28 % contribution towards adult coccinellids, syrphids and parasitoids respectively

In regarding to studies of resistance mechanism of rapeseed-mustard genotypes against mustard aphids field trials were conducted. Thirty genotypes were preliminary screened against mustard aphid with three checks, Yellow sarson 66-197-3 (Susceptible check), Kranti (National resistant check) and BSH-1 (National susceptible check). Based on Aphid Resistance Index (ARI) of both the years, Six genotypes viz., RTM 314 (0.90), RTM 1624 (1.51), RH 7846 (1.71), AKGS 19-8

(1.76), GSH 1699 (1.78) and GSH 2180 (1.81) were found promising against mustard aphid. These six genotypes were further evaluated for the biochemical and GC-MS analysis.

The biochemical tests revealed the glucosinolates and phenols are present highest in RTM 314 (109.87 $\mu\text{mole/g}$ and 6.51 mg/g) respectively, followed by RTM 1624 (95.76 $\mu\text{mole/g}$ and 5.42 mg/g), RH 7846 (91.21 $\mu\text{mole/g}$ and 5.01 mg/g), AKGS 19-8 (89.68 $\mu\text{mole/g}$ and 4.52 mg/g), GSH 1699 (86.34 $\mu\text{mole/g}$ and 4.39 mg/g) and GSH 2180 (83.66 $\mu\text{mole/g}$ and 4.18 mg/g) respectively. However, total soluble sugars has shown a reverse trend with lowest in RTM 314 (6.42 mg/g) followed by RTM 1624 (7.08 mg/g), RH 7846 (8.15 mg/g), AKGS 19-8 (9.12 mg/g), GSH 1699 (9.56 mg/g) and GSH 2180 (10.04 mg/g).

Whereas, the GCMS analysis revealed that all the promising genotypes has shown a common phytochemical constituents viz., Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; (E)-Phytol which have insecticidal properties. However, 2-(hydroxymethyl)-2-nitro-1,3-Propanediol; 5-(Methylsulfinyl) pentanenitrile and 1-Isothiocyano-4-(methylsulfinyl) butane are present only in the genotype RTM 314 which could be the reason for resistance against mustard aphid and can be used for future in IPM programme against mustard aphid in farmer's field.

The effect of different intercrops (wheat, barley, chickpea, lentil, pea, linseed, coriander and fennel) combinations in mustard was evaluated to study the incidence of mustard aphid and its natural enemies. Based on the two years study, among different intercrop combinations, lowest cumulative mean aphid population was recorded in mustard + wheat (41.46/ top 10 cm inflorescence of 10 plants) followed by mustard + barley (42.79/ top 10 cm inflorescence of 10 plants), mustard + coriander (43.06/ top 10 cm inflorescence of 10 plants) and mustard + fennel (44.10/ top 10 cm inflorescence of 10 plants) which are statistically at par and have shown a significant difference with other intercrop combinations along with mustard sole crop (93.91/ top 10 cm inflorescence of 10 plants). Similarly, mustard + wheat intercrop combination also served as a reservoir of number of natural enemies *i.e.*, coccinellids, syrphids and parasitoids (8.29, 6.57, 14.01/10 plants respectively) followed by mustard + barley (7.26, 6.35, 13.34/ 10 plants), mustard + coriander (7.59, 6.19, 13.11/ 10 plants) and mustard + fennel (7.45, 5.98, 12.88/ 10 plants) respectively

which are at par and shown a significant difference with other intercrop combinations and mustard sole crop (3.18, 2.22, 6.12/ 10 plants). Whereas, spider population was abundant in mustard + wheat (4.39/ 10 plant) followed by mustard + barley (4.21/ 10 plant) in comparison to the other intercrop combinations and mustard sole crop (1.61/10 plants). It is also comprehend that intercrops had a significant effect on mustard seed yield. Among all the intercrop combinations, mustard intercropped with wheat had showed highest yield (1213 kg/ha) followed by mustard + barley (1201 kg/ha) over mustard sole crop (878 kg/ha).

The field efficacy of botanicals against mustard aphid was evaluated in comparison with standard chemical check (Dimethoate 30 EC @ 0.003 %). The observations made on overall mean aphid population after both first and second spray applications clearly indicated a sharp reduction in aphid population over untreated control. Based on cumulative mean of two years, the aphid population after both sprays applications was recorded minimum (6.57/top 10 cm inflorescence of 10 plants) in Dimethoate 30 EC @ 0.003 % treatment followed by NSKE @ 5% (19.39), YBSE @ 5% (22.74), BLE @ 5% (24.61), LQLE @ 5% (60.22) and FBLE @ 5% (65.40) in comparison to untreated control (131.36). Similarly, the % reduction in aphid population over control after first and second spray was also found to be maximum (85.38, 97.02 %) with Dimethoate 30 EC @ 0.003 % followed by NSKE @ 5% (70.69, 87.97 %), YBSE @ 5% (66.58, 86.06 %), BLE (64.25, 84.56 %), LQLE @ 5% (45.83, 55.77 %) and FBLE @ 5% (42.06, 51.62 %) respectively.

The effect of botanicals/chemical treatment against natural enemies *i.e.*, coccinellids, syrphids, parasitoids and spiders were also evaluated. It was clear that maximum population of coccinellids, syrphids, parasitoids and spiders (2.68, 2.11, 4.98, 1.77/ 10 plants respectively in untreated control and are statistically at par with all the botanical treatments. Whereas, minimum population (0.38, 0.27, 0.25, 0.60/ 10 plants respectively) was observed with Dimethoate 30 EC @ 0.003 % treatment which showed a significant difference with all the treatments.

The maximum seed yield was recorded with Dimethoate 30 EC @ 0.003 % treated plot *i.e.*, 1608 kg/ha followed by NSKE @ 5% (1501 kg/ha), YBSE @ 5% (1488 kg/ha), BLE @ 5% (1475 kg/ha) and minimum seed yield was recorded in FBLE @ 5% (1164 kg/ha) in comparison to untreated plots (1022 kg/ha). The benefit-cost ratio of different treatments differed remarkably with highest (8.80:1) in case of

Dimethoate 30 EC @ 0.003 % followed by Bhang leaf extract (6.86:1), NSKE @ 5% (3.61:1), YBSE @ 5% (3.31:1), LQLE @ 5% (2.14:1) and FBLE @ 5% (1.16:1).

GC-MS analysis of methanolic extract of yam bean seed revealed the presence of thirty five compounds corresponding to 99.94% of the total extract; Among which 9,12-Octadecadienoic acid (Z,Z)- (40.50%), n-Hexadecanoic acid (23.37%), Mome inositol (7.40%) and Coumarine, 6-(7-hydroxycoumarin-8-yl)-7-methoxy- (5.71%) were the major compounds identified, and rest of the other minor compounds with lower relative peak areas ranging from 0.07– 4.11% were identified. It was believed that the insecticidal properties of YBSE were due to presence of compound rotenone. In the present study we have identified the rotenone compounds viz., Coumarine,6-(7-hydroxycoumarin-8-yl)-7-methoxy-; 2H-1-benzopyran-2-one,7-(diethylamino)-3-[4-(dimethylamino)benzoyl]- etc. which have insecticidal properties against herbivores.

Similarly, GC-MS analysis of methanolic extract of bhang leaf revealed the presence of thirty one compounds corresponding to 99.94% of the total extract; Among which (E)- β -caryophyllene (26.94%), Limonene (11.54%), β -Sesquiphellandrene (8.43%), α -Pinene (6.17%), Myrcene (5.06%), β -Selinene (4.80%), α -Terpinolene (4.78) were the major terpenoid compounds identified which have insecticidal properties against herbivores and other minor compounds with lower relative peak areas ranged from 0.10– 2.87%.

Conclusions:

- Surveys conducted in 16 locations viz., Pantnagar, Ludhiana, Jodhpur, Jhansi, Ranchi, Mohanpur, Raipur, Dharwad, Chintapalli, Barapani, Imphal, Dholi, Dariapur, Alipur Bihta, Delhi and Jorhat ranging from 40.42 m (Dariapur) to 980.80 m (Barapani) altitude, confirmed the presence of *L. e. pseudobrassicae*
- The RAPD primers OPA-02, OPA-04, F2 and SSR primers ApH04M, Ap-01, ApH08M were considered as higher informativeness in genetic diversity studies.
- Among all the locations, Jorhat of North-Eastern India was found to be highly diverged with higher branch distance (0.13) in the phylogenetic tree and similar results were observed in RAPD and SSR dendrograms with 30.0 and 31.0 % dissimilarity respectively.

- Genetic variations between the populations could be due to latitudinal, altitudinal range of species, insecticide usage, and adaptations to local abiotic conditions.
- The *Buchnera* sp, a primary endosymbiont with 100.0 percent incidence was found in all the locations followed by secondary endosymbionts viz., *Wolbachia* sp, *Rickettsia* sp, and *Hamiltonella* sp with 81.25 % incidence. Astonishingly, among all the locations, Jodhpur and Ranchi had the greatest diversity (100.00 %) of associated bacterial endosymbionts.
- Accurate identification of aphids is critical in agriculture due to differences in insecticide susceptibility and virus transmission between species, biotypes and strains. This provides quick insights into the most effective control approach as well as a deeper grasp of the evolutionary history.
- Thoughtfulness of the role and diversity of *L. e. pseudobrassicae* associated endosymbionts also aids in the development of effective pest management strategies.
- During both the years the population of alate mustard aphids and natural enemies i.e., adult coccinellids, syrphids and parasitoids reached their peak on 8th SMW i.e., 19th to 25th February.
- Both alate mustard aphids and natural enemies i.e., adult coccinellids, syrphids and parasitoids showed strong positive correlation with maximum temperature and sunshine hrs in comparison to other weather parameters.
- Among all the genotypes, RTM 314 had shown low AII, ADI and ARI and also accumulated high amount of glucosinolates and phenols in response to aphid infestation.
- GC-MS analysis revealed that RTM 314 consisting of three toxic compounds viz., 2-(hydroxymethyl)-2-nitro-1,3-Propanediol; 5-(Methylsulfinyl)pentanenitrile and 1-Isothiocyanto-4-(methylsulfinyl)butane have been identified unlike in other genotypes which could be the reason for resistance against mustard aphid
- The incidence of mustard aphid was lower and the activity of natural enemies like coccinellids, syrphids, parasitoids and spiders was found to be greater in mustard + wheat (3:1) followed by mustard + barley (3:1) with greater yield. Provisioning

of natural enemies with these intercrops will help in maintaining their population in the agricultural habitats and enhance biological control of insect pests.

- Our results on the efficacy of botanicals against mustard aphid and its natural enemies shed light on the possible utilization of the crude extracts of locally easily available plants.
- Neem seed kernel extract @ 5%, bhang leaf extract @ 5% and yam bean seed extract @ 5% can be used effectively against mustard aphid, which are also safer and maintain biological diversity of beneficial organisms, reduce environmental contamination and human health hazards. Although the application of Dimethoate 30 EC has reduced the aphid population significantly in comparison to crude extracts of yam bean seed and bhang leaf, GC-MS analysis revealed that both yam bean seed and bhang leaf crude extracts had significant insecticidal compounds and could be used as botanical insecticides with further research on the purification and identification of active components responsible for mustard aphid mortality, as well as comparisons to other insect pests.





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