Wild *Solanum* species exhibit feeding antixenosis against ash weevil, *Myllocerus subfasciatus* Guerin-Meneville (Coleoptera: Curculionidae)

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This study was aimed at identifying host plant resistance sources of *Solanum* species against the dreaded brinjal pest, ash weevil. *Myllocerus subfasciatus* Guerin-Meneville. A total of 84 brinjal genotypes (both cultivated and wild) were screened for feeding preference/non-preference against the ash weevil under field as well as *in vitro* conditions. All the cultivated and five wild genotypes (bitter brinjal, *Solanum gilo* Raddi; black nightshade, *Solanum indicum* L.; African eggplant, *Solanum macrocarpon* L.; Ethiopian eggplant, *Solanum aethiopicum* L. and Dutch eggplant, *Solanum acculeatissimum* Jacq.) were found highly susceptible to the ash weevil. The other wild species, namely tropical soda apple, *Solanum viarum* Dunal; nipple fruit (= cow's udder) *Solanum mammosum* L.; European nightshade, *Solanum nigrum* L.; cockroach berry, *Solanum capsicoides* Allioni; Brazilian nightshade, *Solanum seaforthianum* Andrews; Turkey berry, *Solanum torvum* Sw. and sticky nightshade, *Solanum sisymbriifolium* Lam exhibited complete resistance to the ash weevil with leaf feeding damage ranging from zero to <1.00 (when scored on 0.00–10.00 scale). This study helped identify feeding antixenosis (feeding non-preference) as the major component of resistance in these wild genotypes against *M. subfasciatus*. Response of the ash weevil to these wild/cultivated genotypes and their volatiles has also been discussed in detail.

Keywords: Host plant resistance, *Myllocerus subfasciatus*, olfactometer assays, plant volatiles, *Solanum* species.

BRINJAL or aubergine (*Solanum melongena* L.) is the most widely used vegetable in several countries like Central, South and southeast Asia, and parts of Africa and Central America[1]; it is cultivated throughout the year. Brinjal is prone to several insect pests, of which ash weevils and *Myllocerus* spp. (Coleoptera: Curculionidae) are the most widespread and notorious ones. Several species of the genus *Myllocerus* occur worldwide; more than >90 species occur in India[2]. Of these, *Myllocerus subfasciatus* Guerin-Meneville is becoming an emerging threat to brinjal.

Adult weevils feed on the foliage of brinjal by making characteristic notches (like half-moon-shaped markings) along the leaf margins. Under favourable conditions they have the potential to cause 100% yield loss[3]. The adult weevils lay eggs in the soil close to the plant and the grubs exclusively feed on the roots causing stunting/wilting of the plant. This subterranean nature of the grubs makes the management of ash weevils difficult. Farmers often depend on the soil as well as foliar insecticide applications to manage grubs and adult weevils.

Exploring the host plant resistance (HPR) to prevent insect pest losses in crops has been revamped in recent decades as insecticidal resistance has become a major drawback in the fight against phytophagous insect pests[4]. HPR refers to the ability of a plant to withstand or compensate for insect pest attacks. Management of insect pests through integrated pest management (IPM) strategies mainly advocates HPR coupled with other methods, as the former is an effective, economic and environment-friendly component in IPM[4–6].

Crop cultivars with resistance against insect pests can form sustainable IPM components in the long run. However, limited work has been done on economically important crops to explore HPR, particularly against coleopteran insects. To cite a few examples, different *Musa* spp. against banana rhizome weevil, *Cosmopolites sordidus* (Germar) were screened and it was found that the wild diploid banana (Calcutta-4), diploid banana hybrids (TMB2 × 6142-1, TMB2 × 8075-7 and TMB2 × 7197-2) as well as cultivars (Yangambi-Km5 and Cavendish) showed very high levels of resistance. It has been suggested that they may be exploited as genetic sources for locating resistance genes against this economically important pest of banana[7]. In the case of date palm, laboratory studies revealed that the Canary Island date

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palm and Washington palm were the most suitable host plants against red palm weevil, *Rhychophorus ferrugineus* (Olivier) and silver date palm was the least suitable plant[8]. Wild *Solanum* species screening against Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomeliadae) revealed that the traits like lower levels of foliar glycoalkaloids and absence of glandular trichomes in wild potato species, namely *Solanum trifidum* (Correll), *Solanum raphanifolium* Cárdenas and Hawkes, and *Solanum circaeifolium*. Bitter contributed to substantial resistance against this economically important pest compared to cultivated potato, *Solanum tuberosum* L. (Potato)[9]. In the present study, detailed experiments have been conducted to identify resistance sources to ash weevil *M. subfasciatus* in cultivated as well as wild relatives of brinjal.

Materials and methods

Field screening

Field evaluation of selected *Solanum* genotypes was conducted consecutively for two years (2019 and 2020, *rabi* season) at the vegetable experimental block of ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, Karnataka (77°28′40″E and 13°8′18″N). A total of 84 *Solanum* genotypes comprising 12 wild species (tropical soda apple, *Solanum viarum* Dunal; nipple fruit (=cow's udder), *Solanum mammosum* L.; European nightshade, *Solanum nigrum* L.; cockroach berry, *Solanum capsicoides* Allioni; Brazilian nightshade, *Solanum seaforthianum* Andrews; Turkey berry, *Solanum torvum* Sw.; sticky nightshade, *Solanum sisymbriifolium* Lam; bitter brinjal, *Solanum gilo* Raddi; black nightshade, *Solanum indicum* L..; African eggplant, *Solanum macrocarpon* L.; Ethiopian eggplant, *Solanum aethiopicum* L. and Dutch eggplant, *Solanum acculeatissimum* Jacq.) and 72 cultivated types were screened for ash weevil damage under field conditions (Supplementary Table 1). Feeding damage was assessed by visually scoring leaf damage on the 0.00–10.00 scale (where 0.00 indicates no damage; 1.00, 10%; 2.00, 20% 3.00, 30%; 4.00, 40%; 5.00, 50%; 6.00, 60%; 7.00, 70%; 8, 80%; 9.00, 90% and 10.00 indicates 100% leaf damage). The observations were recorded at different crop phenological stages, namely the vegetative (35 days after transplanting (DAT)) and reproductive (70 DAT) stages on leaf damage and the number of adult weevils present per plant (during the observation period). Totally ten plants per genotype were observed and in each plant, ten leaves were scored randomly (n = 10) for leaf damage by ash weevil. The mean leaf damage data of different *Solanum* genotypes were further grouped into different host plant resistance categories based on the feeding preference of ash weevil, namely 'resistant' (genotypes that scored '0.00' on the visual damage scale, with zero leaf damage); 'tolerant' (genotypes that scored between 0.10 and 3.00 on the visual damage scale, with less leaf damage); 'susceptible' (genotypes that scored between 3.10 and 8.00 on the visual damage scale) and 'highly susceptible' (genotypes that scored between 8.10 and 10.00 on the visual damage scale, with high leaf damage). The data were subjected to the pooled ANOVA across the years and results were interpreted.

Insects

Adult ash weevils were collected directly from the infested brinjal plants in the experimental fields of ICAR-IIHR. The collected weevils were brought to the laboratory and maintained on brinjal leaves under ambient conditions (24–27°C and 60–70% RH) in plastic cage dishes (10 cm width × 4 cm height) having a small mesh opening (4 cm dia.) on the top for aeration. Two layers of filter paper were placed inside these dishes to keep the insects away from the moisture produced by the frass. In each cage, 10 pairs of ash weevils were released and provided with fresh brinjal leaves *ad libitum* for feeding on a daily basis. These ash weevils were used for all the *in vitro* studies.

Host plants

All 12 wild *Solanum* species, along with the highly susceptible check *Solanum melongena* (cv. Harshitha) were chosen for detailed *in vitro* studies. The seeds of *S. melongena* (cv. Harshitha) and wild *Solanum* spp., viz. *S. gilo*, *S. indicum*, *S. macrocarpon*, *S. aethiopicum*, *S. aculeatissimum*, *S. viarum*, *S. mammosum*, *S. nigrum*, *S. capsicoides*, *S. seaforthianum*, *S. torvum* and *S. sisymbriifolium* were sown in portrays. After 45 days, the seedlings were transplanted to plastic pots (2′ height) containing a potting mixture of well-drained, fertile silt loam soil (pH 5.5–6.0) and well-decomposed farmyard manure (1 : 1 ratio). The plants were grown inside the shade net and 75-day-old plants with good vegetative foliage were used for all the *in vitro* studies. The pots were drenched with copper oxychloride (@ 2 g/l) as a prophylactic measure to prevent fungal diseases and no insecticidal sprays were administered.

In vitro screening

The 12 wild *Solanum* species, along with the cultivated *S. melongena* (cv. Harshitha), were further subjected to choice and no-choice feeding assays using a detached leaf as well as whole plant methods to confirm the weevil feeding preference.

Detached leaf method

Fresh leaves along with petioles without any external damage symptoms were carefully detached from the plant. The leaf petiole was covered with a wet cotton at the base to keep

the leaf fresh for longer periods. Such prepared detached leaves were used for both no-choice and choice assays. The experiment was replicated 10 times (n = 10).

No-choice assays: The detached leaves of selected 12 wild *Solanum* species were placed individually in a mesh cage (45 cm width × 45 cm height) and adult weevils starved for 2–3 h (20♂ : 20♀) were released. After 24 h, observations were recorded on leaf feeding damage by weevils (as mentioned earlier). The experiment was replicated 10 times (n = 10). The data were subjected to one-way ANOVA using GraphPad Prism (v 9.3.1).

Choice assays: For conducting choice assays, the detached leaves of each selected wild *Solanum* species were placed individually along with the cultivated genotype *S. melongena* (cv. Harshitha, as control) next to each other in a nylon mesh cage (45 cm height × 45 cm width) at a distance of 5 cm from one another. Twenty pairs of pre-starved (for 2–3 h) ash weevil adults were released into the cage. Observations were recorded after 24 h on the extent of leaf feeding (as explained earlier). The experiment was replicated 10 times (n = 10) and data were analysed using a paired-t test and GraphPad prism (v 9.3.1).

Whole-plant method

In whole-plant method, potted plants of selected genotypes were exposed to adult weevils in both no-choice and choice assays as mentioned below.

No-choice assays: For conducting no-choice assays, 25 pairs of ash weevils were released into cages (5′ × 5′) containing the potted plants (n = 5) of individual genotypes. After 24 h of exposure, observations were recorded on the extent of leaf feeding by weevils (as explained earlier). In each plant, 10 randomly selected leaves were observed and the experiment was replicated 10 times (n = 10). The data were subjected to ANOVA using GraphPad prism (v 9.3.1).

Choice assays: For conducting choice assays, a similar experimental set-up was used as that of no-choice assays with minor modifications. Here, with each selected wild *Solanum* spp. (n = 12), the cultivated *S. melongena* plant (as control) was exposed to weevils together. After 24 h in each plant, five randomly selected leaves were observed for damage by weevils (as mentioned above). Data were analysed using paired t-test and GraphPad prism software (v 9.3.1).

Repeat choice assays with selected wild Solanum spp.

Based on the data generated in the above experiments, to confirm ash weevil preference for the selected wild-resistant *Solanum* species (*S. viarum*, *S. mammosum*, *S. seaforthianum*, *S. capsicoides*, *S. torvum*, *S. nigrum* and *S. sisymbriifolium*), detailed choice assays were performed with and without a susceptible check (*S. melongena* cv. Arka Harshitha) using detached leaf method. Two series of assays were carried out. In the first series, only the selected wild *Solanum* species were exposed to ash weevil in the no-choice assay. Secondly, all the selected wild *Solanum* species were exposed to ash weevil along with susceptible check in the multiple choice-assay.

Briefly, the detached leaves of the above-selected genotypes were placed inside individual nylon mesh cages (45 cm height × 45 cm width) and a total of 20 pairs of pre-starved adult ash weevils were released into the cages. Each assay was replicated ten times. Observations were recorded after 24 h on the feeding damage caused by weevils, as mentioned earlier. The data were subjected to ANOVA using GraphPad prism (v 9.3.1).

Host plant volatile collection

Headspace volatiles from 12 different wild *Solanum* species, along with *S. melongena* (cv. Arka Harshitha), were collected using a customized air entrainment system during their vegetative stage (35 DAT). The plants were draped inside an autoclaved polythene bag (41 × 32.5 cm) with both outlet and inlet ports inside the cover. The polythene cover was then tightly closed at the base using rubber bungs to avoid air movement. Volatiles were collected on Porapak Q columns (50 mg, 60/80 mesh; Supel co, Sigma Aldrich, Bengaluru, India) for 6 h and the columns were eluted with 750 µl of redistilled diethyl ether (DEE, Merck 99.7%). The collected volatile samples were stored in a freezer (–20°C) until further use[10].

Y-tube olfactometer assays

Y-tube olfactometer assays were carried out to determine the response of ash weevil adults to 12 wild species of *Solanum* along with *S. melongena* (cv. Arka Harshitha). Before performing the assays, both sexes of ash weevils were separated and starved for 3 h. A total of 30 insects from each sex were subjected to the bioassays. The Y-tube olfactometer consists of a Y-shaped glass tube with stem length of 15 cm and two arms at the top (9 cm each, with an internal diameter of 1 cm). The bottom end of the Y-tube was used to release the adult weevils.

The test samples of different plant volatiles were pipetted out (10 µl) onto a filter paper strip (Whatman No. 1). The filter paper was then placed at the end of the treated arm. Filter paper strips with solvent (10 µl of diethyl ether) served as control in the other arm in case of no-choice assays. Whereas, in the case of dual-choice assays, one arm of this Y-tube contained the selected wild *Solanum* plant volatiles and the other arm contained the volatiles of *S. melongena* as control. The entire Y-tube olfactometer was

Table 1. Grouping of wild and cultivated *Solanum* species based on leaf damage by *Myllocerus subfasciatus* under field conditions

Visual feeding damage score* (on 0.00–10.00 scale)			
0.00 (resistant)	0.10–3.00 (tolerant)	3.10–8.00 (susceptible)	8.10–10.00 (highly susceptible)
Solanum viarum	*Solanum capsicoides*	–	*Solanum gilo*
Solanum mammosum	*Solanum torvum*		*Solanum aethiopicum*
Solanum nigrum			*Solanum acculeatissimum*
Solanum seaforthianum			*Solanum macrocarpon*
Solanum sisymbriifolium			*Solanum indicum*
			Solanum melongena (72 genotypes)

*Leaf damage scored on 0–10 scale, where 0.00 indicates no damage, 1.00, 10%; 2.00, 20%; 3.00, 30%; 4.00, 40%; 5.00, 50%; 6.00, 60%; 7.00, 70%; 8, 80%; 9.00, 90% and 10.00 indicates 100% leaf damage.

connected to the air entrainment system and purified air was drawn through activated charcoal into the glass chambers and Y-tube arms at a rate of 600 ml min^{-1}. A total of thirty (n = 30) replicates were done for each assay. The response of both male and female ash weevil adults to the odour sources was recorded separately based on their movement. The movement of each insect was observed for 5 min. The weevils which did not respond for 2 min were discarded. The observation was terminated when the insect reached the far end (finish line) of one of the arms. A new filter paper strip with test volatiles was used for each bioassay. The Y-tube glass apparatus was replaced for every five bioassays and a sterilized glass tube was used. The experiments were conducted at ambient room temperature (27° ± 1°C; 60–70% RH). Before each experiment, all glassware were washed with liquid detergent, rinsed with acetone and distilled water, and baked in an oven overnight at 180°C. The chi-square test was used to determine the significant differences in the attraction of male and female ash weevils to the odour sources of different *Solanum* species using GraphPad prism v 9.3.1.

Gas chromatography coupled with mass spectrometry

The volatiles eluted from Porapak Q columns were analysed using gas chromatography coupled with mass spectrometry (GC-MS), and the Agilent 7890B GC system equipped with a mass spectrometer (Agilent 5977 MSD). A capillary column (Agilent J&W, HP-5 MS UI) of 30 m length, 0.25 mm diameter and 0.25 µm film thickness was used to examine the samples. The thermal programme was initially set at 60°C for 1 min and later ramped at 7°C/min up to 240°C and held for 2 min. Helium was used as the carrier gas at a flow rate of 1 ml/min. MS was in full-scan mode (70 eV) and amu ranged from 40 to 450 m/z. One microlitre of the sample was injected in split-less mode (40 ml min^{-1}) with an injection temperature of 270°C. Total volatile emissions were estimated by the sum of all GC-FID peak areas in the chromatogram and individual compounds were quantified as relative per cent area. The compounds were identified by GC retention time, mass spectrum and KOVATS index (C_7 to C_{30} homologous series of *n*-alkenes as standard, Sigma-Aldrich, USA; Kovats, 1965) using NIST 14 software. The identified compounds were authenticated by co-injecting standard synthetic compounds along with the samples. Volatile organic compounds for which standards are unavailable were identified tentatively based on NIST-14 spectral library[10].

Results and discussion

HPR herbivores mostly comprise antixenosis, antibiosis and tolerance as components[11–14]. Among these, antixenosis often referred as non-preference by the insects, a plant characteristic that renders it unattractive for oviposition, feeding or shelter[15]. Thus, plants with antixenosis traits may have an effect on insect pests upon their arrival/first attack on the plant due to morphological traits such as colour, hairiness, trichome diversity/density and waxy surface. Several wild crop relatives exhibit varied types of resistance mechanisms to insect pests and identifying such resistance sources help us to exploit them for designing HPR-dependent pest management strategies.

In the present study, significant differences were observed among the genotypes screened against *M. subfasciatus* with respect to feeding damage ($F_{83,738}$ = 705.54; P < 0.0001). All the 72 cultivated *S. melongena* genotypes were highly susceptible to ash weevil and exhibited zero feeding non-preference (feeding antixenosis). The leaf feeding damage in all the *S. melongena* genotypes was maximum and ranged from 9.53 to 9.95 (mean ± SE; 9.78 ± 0.00). The mean number of ash weevil adults per plant in the cultivated *S. melongena* genotypes ranged from 1.00 to 21.00 (mean ± SE; 3.90 ± 0.30; Table 1 and Supplementary Table 1).

Grouping of genotypes into three major categories (resistant, tolerant and susceptible) based on visual feeding damage data revealed that among the 12 wild *Solanum* species, *S. viarum*, *S. mammosum*, *S. nigrum*, *S. seaforthianum* and *S. sisymbriifolium* recorded zero feeding damage and exhibited complete feeding non-preference (feeding antixenosis) for ash weevil. Further, no adult weevil presence was recorded on these wild species. Two other wild *Solanum* species, namely *S. capsicoides* and *S. torvum* were found to be tolerant to ash weevil and recorded <1.00 feeding damage (0.78 and 0.36 respectively). Though no ash weevil

presence was recorded on *S. torvum*, a mean of 1.00 weevil per plant was noted in case of *S. capsicoides.* The wild *Solanum* species, namely *S. indicum*, *S. macrocarpon*, *S. gilo*, *S. aethiopicum* and *S. acculeatissimum* were highly susceptible to ash weevils with high leaf feeding damage ranging from 9.43 to 9.88 under field conditions, similar to the cultivated genotypes.

Significant positive relationship was observed ($r = 0.48$; $P < 0.0001$; d$f = 83$) between the number of adult *M. subfasciatus* weevils and leaf feeding damage. The variability in leaf damage due to the adult weevil numbers was explained to the tune of 69% through a simple linear regression model ($y = 0.391x + 0.44$, $R^2 = 0.69$). This indicates that leaf damage is directly related to the number of adult ash weevils on the plant.

In no-choice assays (detached leaf method), the ash weevils did not prefer to feed on *S. viarum*, *S. mammosum*, *S. seaforthianum*, *S. nigrum* and *S. sisymbriifolium*, and exhibited strong feeding antixenosis towards these wild *Solanum* species. No visual feeding damage was observed in these species (with zero score), indicating the existence of a complete feeding antixenosis against ash weevil. In the case of *S. torvum* and *S. capsicoides*, feeding damage was minimal, i.e. <1.00 (mean feeding damage ± SE, 0.36 ± 0.18 (*S. torvum*); 0.78 ± 0.10 (*S. capsicoides*)). No ash weevil adults were noticed on *S. torvum* during the observation period. However, in the case of *S. capsicoides*, a mean number of 1.00 (± 0.16 SE) weevil per plant was recorded. However, the remaining wild *Solanum* species, namely *S. gilo* (9.58 ± 0.01), *S. aethiopicum* (9.94 ± 0.1), *S. acculeatissimum* (9.43 ± 0.00), *S. indicum* (9.80 ± 0.00) and *S. macrocarpon* (9.00 ± 0.04) recorded the highest leaf feeding damage and higher number of ash. They were statistically on par with the cultivated species *S. melongena* cv. Arka Harshitha (9.88 ± 0.00; $P = 0.45$; Supplementary Figure 1).

The data on individual choice assays using the detached leaf method between each wild *Solanum* species and cultivated *S. melongena*, revealed significant differences with respect to leaf feeding damage, particularly in the case of *S. nigrum* vs *S. melongena* ($P < 0.0001$, $t = 63.50$, d$f = 9$); *S. sisymbriifolium* vs *S. melongena* ($P < 0.0001$, $t = 58.79$, d$f = 9$); *S. viarum* vs *S. melongena* ($P < 0.0001$, $t = 58.70$, d$f = 9$); *S. mammosum* vs *S. melongena* ($P < 0.0001$, $t = 63.50$, d$f = 9$); *S. capsicoides* vs *S. melongena* ($P < 0.0001$, $t = 96.93$, d$f = 9$); *S. seaforthianum* vs *S. melongena* ($P < 0.0001$, $t = 96.93$, d$f = 9$) and *S. torvum* vs *S. melongena* ($P < 0.0001$, $t = 36.88$, d$f = 9$). However, the data on individual choice assays using the detached leaf method between each of the remaining wild *Solanum* species and cultivated *S. melongena*, revealed no significant difference with respect to leaf feeding damage, indicating that the wild species *S. indicum* ($P = 0.43$, $t = 1.60$, d$f = 9$), *S. macrocarpon* ($P = 0.45$, $t = 1.172$, d$f = 9$), *S. gilo* ($P = 0.39$, $t = 1.71$, d$f = 9$), *S. acculeatissimum* ($P = 0.45$, $t = 1.44$, d$f = 9$) and *S. aethiopicum* ($P = 0.28$, $t = 2.36$, d$f = 9$) were equally susceptible to ash weevils as cultivated *S. melongena* (Figure 1 *a* and *b*).

The individual choice assays using the whole-plant method between each selected wild species and cultivated *S. melongena* showed significant differences in leaf feeding damage by ash weevil and the data exhibited a similar trend as observed in the detached leaf method. The wild *Solanum* species, namely *S. sisymbriifolium*, S. *seaforthianum*, *S. torvum*, *S. nigrum*, *S. capsicoides*, *S. mammosum* and *S. viarum* exhibited strong feeding antixenosis against ash weevil (*S. sisymbriifolium*, $P < 0.0001$, $t = 264.9$, d$f = 4$; *S. seaforthianum*, $P < 0.0001$, $t = 509.2$, d$f = 4$; *S. torvum*, $P < 0.0001$, $t = 322.7$, d$f = 4$; *S. nigrum*, $P < 0.0001$, $t = 462.10$, d$f = 4$; *S. capsicoides*, $P < 0.0001$, $t = 425.5$, d$f = 4$; *S. mammosum*, $P < 0.0001$, $t = 426.5$, d$f = 4$ and *S. viarum*, $P < 0.0001$, $t = 421.5$, d$f = 4$; Figure 2 *a*).

The other wild *Solanum* species, namely *S. macrocarpon*, *S. indicum*, *S. acculeatissimum*, *S. aethiopicum* and *S. gilo* were highly susceptible to ash weevil and no significant difference was found when compared to *S. melongena* (*S. macrocarpon*, $P = 0.43$, $t = 18.75$, df = 4; *S. indicum*, $P = 0.16$, $t = 1.12$, d$f = 4$; *S. acculeatissimum*, $P = 0.15$, $t = 3.26$, d$f = 4$; *S. aethiopicum*, $P = 0.17$, $t = 1.04$, d$f = 4$ and *S. gilo*, $P = 0.12$, $t = 3.93$, d$f = 4$; Figure 2 *a*).

No-choice assays using the whole-plant method also showed a similar trend as observed earlier and significant differences were noticed in leaf feeding damage by ash weevil ($F_{12,130} = 254.80$; $P < 0.0001$; Figure 1 *b*). The wild species *S. viarum*, *S. mammosum*, *S. nigrum*, *S. seaforthianum* and *S. sisymbriifolium* were not preferred by ash weevils for feeding. The mean leaf feeding damage scores were zero and these species were found resistant to ash weevil as they exhibited a strong feeding antixenosis. The wild species, namely *S. torvum* (0.34 ± 0.02) and *S. capsicoides* (0.76 ± 0.03) showed <1.00 feeding damage score and were found to be tolerant. The mean leaf-feeding damage in other wild species, namely *S. gilo*, *S. aethiopicum*, *S. acculeatissimum*, *S. indicum* and *S. macrocarpon* was statistically on par with the cultivated species *S. melongena* ($F_{5,80} = 0.00015$; $P > 0.59$) and they were found to be highly susceptible to ash weevil (Figure 2 *b*).

Repeat choice assays using the detached leaf method with only resistant/tolerant wild *Solanum* species (*S. viarum*, *S. mammosum*, *S. nigrum*, *S. seaforthianum*, *S. sisymbriifolium*, *S. torvum* and *S. capsicoides*) revealed no significant differences in their leaf feeding damage, indicating that all these species were equally resistant to ash weevil ($F_{6,95} = 0.00019$; $P > 0.99$). The mean leaf feeding damage scores in these species supported the earlier results that indicated the presence of strong feeding antixenosis in them against ash weevil. The repeat choice assays of these selected wild *Solanum* species with the cultivated species *S. melongena* revealed that the wild species were significantly less preferred over the cultivated species ($F_{7,110} = 865.9$; $P < 0.0001$; Supplementary Figure 2 *a* and *b*).

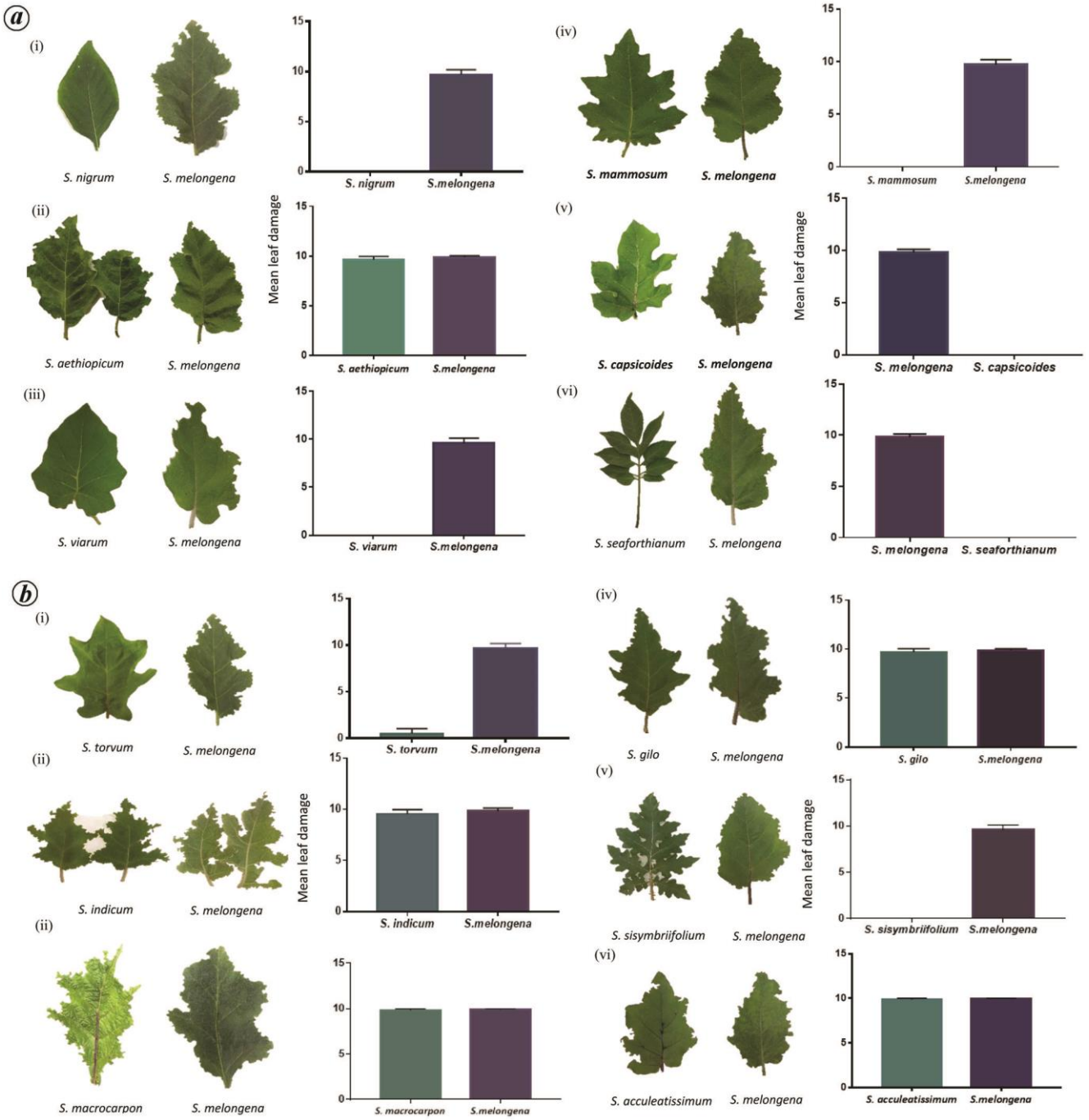

Figure 1. ***a***, Leaf feeding damage by ash weevil, *Myllocerus subfasciatus* in different *Solanum* species in choice-assay (detached leaf method). (i) *S. nigrum* vs *S. melongena*, (ii) *S. aethiopicum* vs *S. melongena*, (iii) *S. viarum* vs *S. melongena*, (iv) *S. mammosum* vs *S. melongena*, (v) *S. capsicoides* vs *S. melongena* and (vi) *S. seaforthianum* vs *S. melongena.* ***b***, Leaf feeding damage by ash weevil. *M. subfasciatus* in different *Solanum* species in choice-assay (detached leaf method). (i) *S. torvum* vs *S. melongena*, (ii) *S. indicum* vs *S. melongena*, (iii) *S. macrocarpon* vs *S. melongena*, (iv) *S. gilo* vs *S. melongena*, (v) *S. sisymbriifolium* and (vi) *S. acculeatissimum* vs *S. melongena.*

Y-tube olfactometer assays with male and female ash weevils to the headspace volatiles of different *Solanum* species showed significant variations in their responses. In no-choice assays the female and male ash weevils preferred the *S. melongena* over the wild species (*S. mammosum*, *S. sisymbriifolium*, *S. nigrum*, *S. seaforthianum*, *S. viarum*, *S. torvum* and *S. capsicoides*; Figure 3 *a* and *b*). Similarly, in dual-choice bioassay, the females and males of *M. subfasciatus* preferred host plant volatiles of *S. melongena* over wild species *S. mammosum*, *S. sisymbriifolium*, *S. nigrum*, *S. seaforthianum*, *S. viarum*, *S. torvum* and *S. capsicoides* (Figure 3 *c* and *d*). However, both sexes of *M. subfasciatus* did not reveal any significant preference when a choice was given between *S. macrocarpon* and *S. melongena*; *S. aethiopicum*

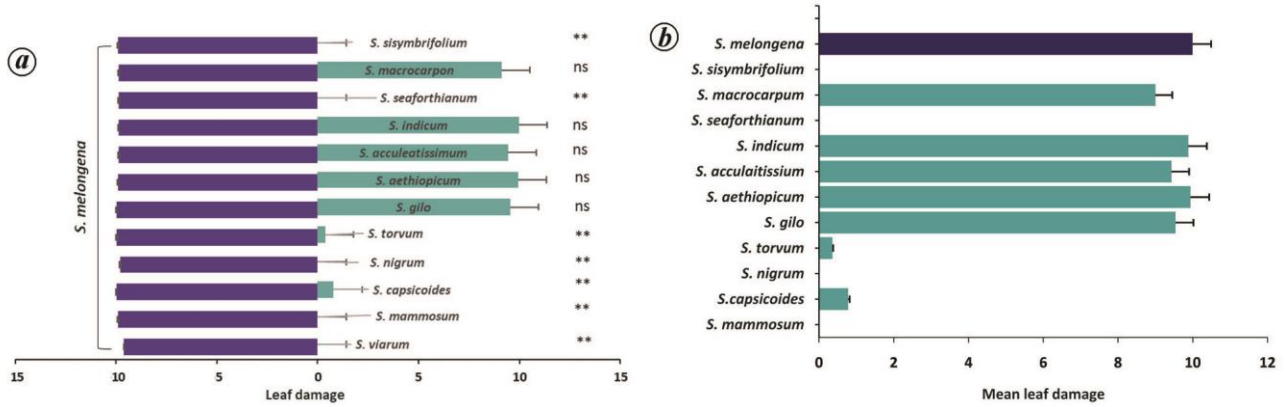

Figure 2. Comparison of leaf feeding damage by adult weevils, *M. subfasciatus* in different wild *Solanum* species vs cultivated species, *S. melongena*. (***a***) Choice assay and (***b***) no-choice assay. Ns, Non-significant; **Significant.

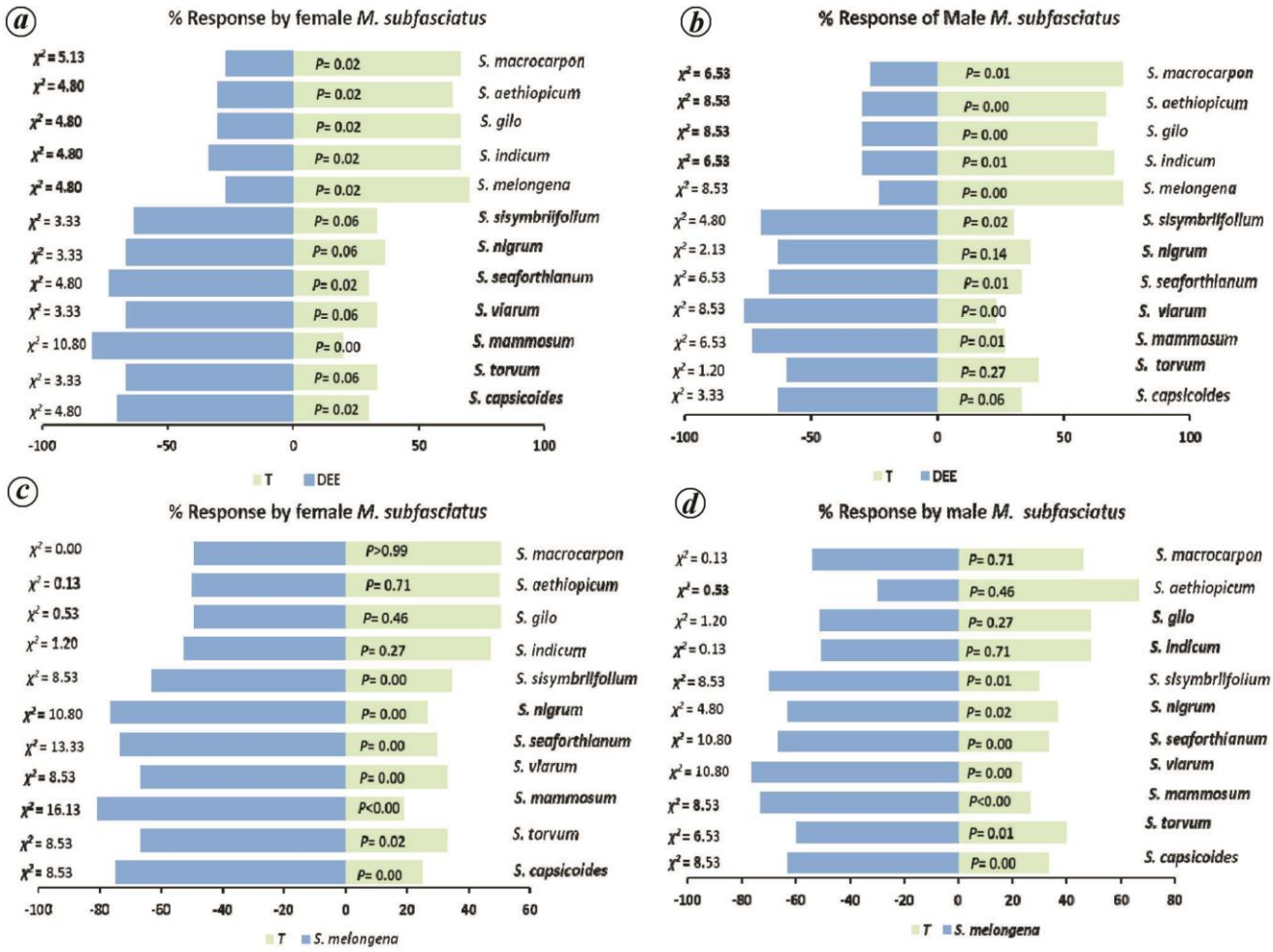

Figure 3. Response of ash weevil to various headspace volatiles of *Solanum* species in Y-tube olfactometer assays. ***a***, ***b***, No-choice assays with (***a***) female and (***b***) male weevils, where *T* is the respective *Solanum* species and DEE (diethyl ether solvent is the control. ***c***, ***d***, Dual-choice assays with (***c***) female and (***d***) male weevils where, T is the respective *Solanum* species *and S. melongena* is the control.

and *S. melongena* and *S. gilo* and *S. melongena*; *S. indicum* and *S. melongena* (Figure 3 *c* and *d*). Thus, the Y-tube olfactometer assays with host plant volatiles of different *Solanum* species conclusively established the presence of non-preference in wild *Solanum* genotypes (*S. mammosum*, *S. sisymbriifolium*, *S. nigrum*, *S. seaforthianum*, *S. viarum*, *S. torvum* and *S. capsicoides*) against *M. subfasciatus*.

Plants produce a number of secondary metabolites, including volatile organic compounds (VOCs), that help directly in herbivore defence by acting as repellents or toxins, as

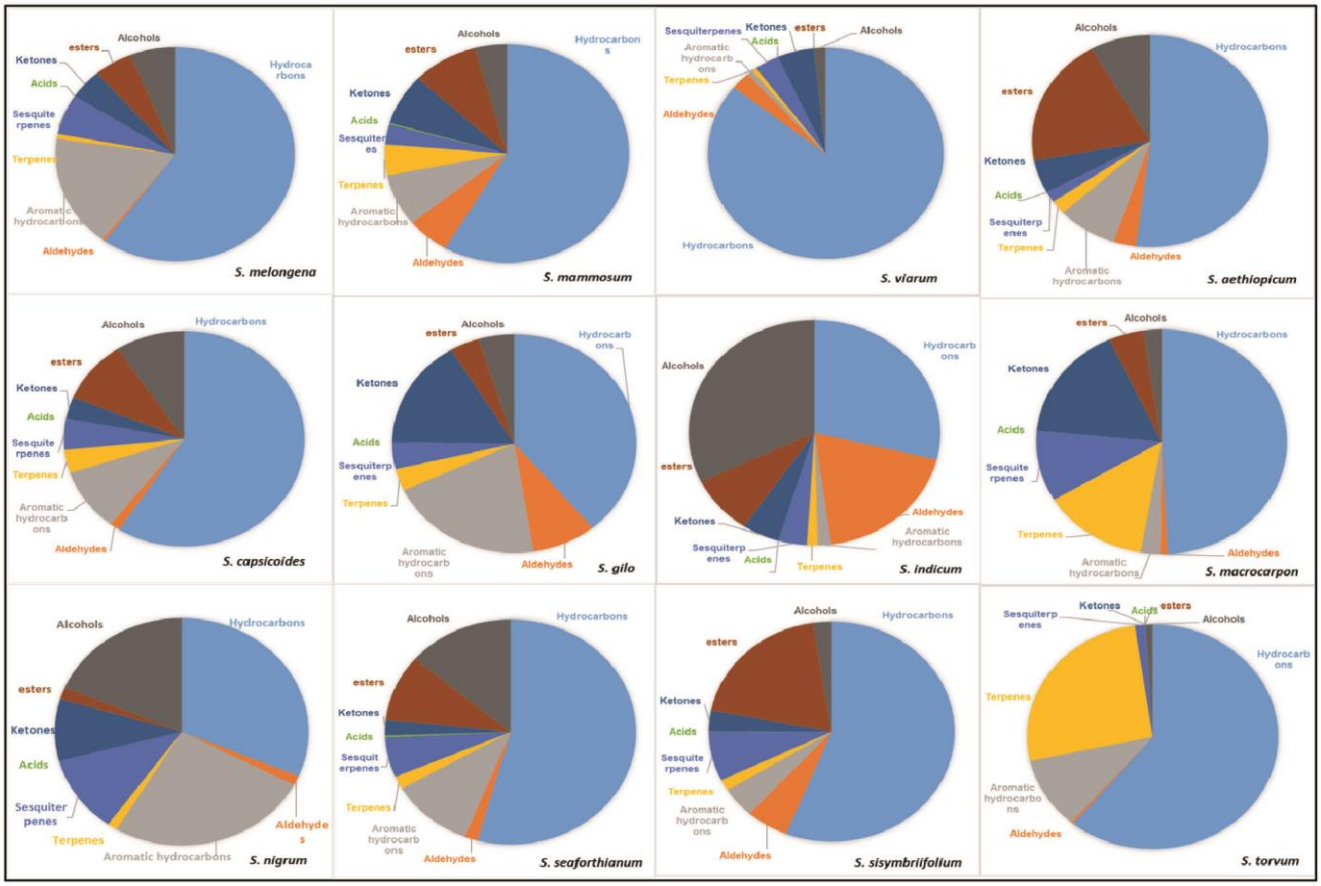

Figure 4. Quantitative proportion of major functional groups of volatile organic compounds (VOCs) in the headspace collections of *Solanum* species.

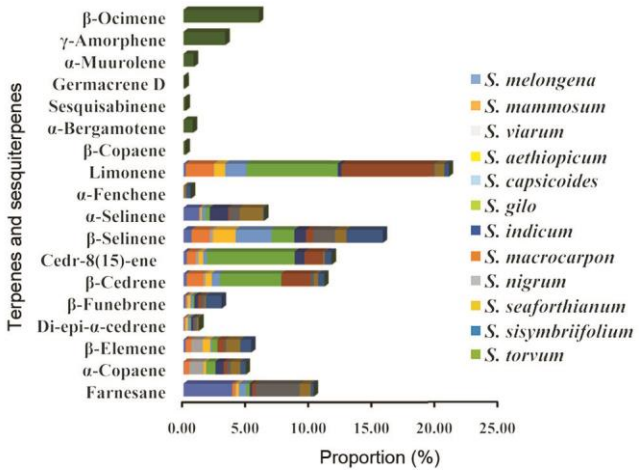

Figure 5. Relative proportion of various terpenes and sesquiterpenes in headspace collections of different *Solanum* species.

well as indirectly by attracting natural enemies[16,17]. In the present study, a total of 75 VOCs were identified their relative abundance varied among the *Solanum* species. The functional groups of VOCs, namely hydrocarbons, aromatic hydrocarbons, terpenes, sesquiterpenes, alcohols, esters, ketones, acids and aldehydes differed significantly among the 12 different *Solanum* spp. ($F = 44.53$; d$f = 8$; $P = 0.009$; Figure 4).

Relative proportions of terpene and sesquiterpene groups were higher in resistant genotypes (*S. mammosum* (7.17%), *S. torvum* (23.70%), *S. seaforthianum*, *S. capsicoides* (7.85%), *S. nigrum* (7.66%)) compared to the susceptible genotypes (*S. melongena* (6.70%), *S. indicum* (4.59%), *S. aethiopicum* (3.44%), *S. gilo* (6.76%)), except for *S. macrocarpon* (Figure 3). Earlier studies revealed that one of the major families of plant-derived VOCs are monoterpenes and sesquiterpenes[16]. Among the various sesquiterpenes, the proportion of *β*-selinene was relatively higher in the resistant genotypes (*S. sisymbriifolium* (2.90%), *S. nigrum* (1.70%), *S. capsicoides* (2.85%), *S. mammosum* (1.45%), *S. torvum* (1.7%)) compared to the susceptible genotypes (*S. melongena* (0.63%), *S. indicum* (0.93%), *S. macrocarpon* (0.29%), *S. indicum* (0.93%), *S. aethiopicum* (0.09%). However, the sesquiterpenes, namely *γ*-amorphene (3.32%), *β*-ocimene (5.93%) and traces of *β*-copaene, *α*-bergamotene, sesquisabinene, germacrene D, *α*-muurolene were found only in the resistant genotype, *S. torvum*; they were absent in other *Solanum* species. In spite of the higher proportions of terpenes/sesquiterpenes, *S. gilo* (limonene (7.20%), cedre-8

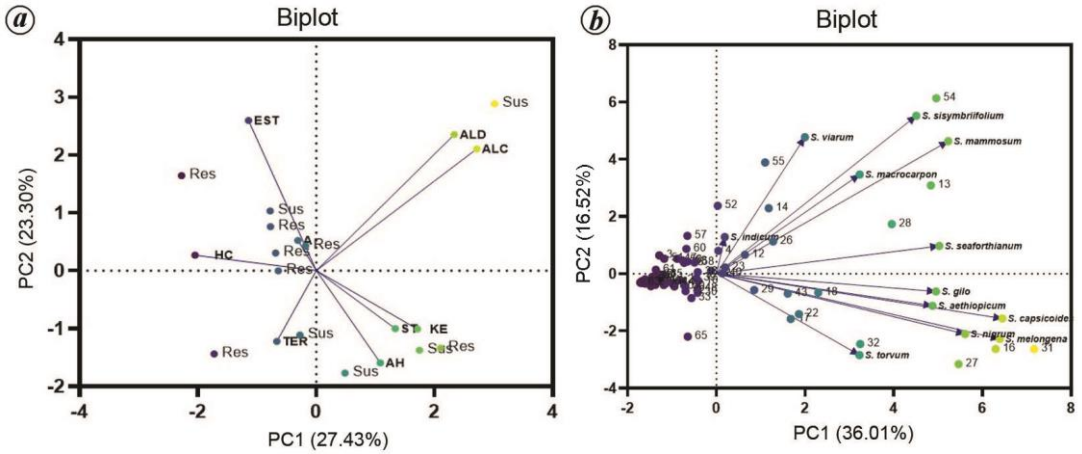

Figure 6. ***a***, Principal component analysis (PCA) showing the association of different functional groups of VOCs with susceptible and resistant *Solanum* species. Res, Resistant; Sus, Susceptible. Functional groups are given in abbreviated forms: TER, Terpenes; AH, Aromatic hydrocarbons; ST, Sesquiterpenes; KE, Ketones; ALD, Aldehydes; ALC, Alcohols; EST, Esters and A, Acids. ***b***, PCA showing the association of different GCMS compounds in susceptible and resistant *Solanum* species. The numerical values in the score plot indicate different chemical compounds as mentioned here – 54: 7,7-Diethylheptadecane, 55: n-eicosane, 13: 4,5-dimethylnonane, 14: trans-1,4-dimethylcyclooctane, 26: p-isopropyl benzaldehyde; 12, limonene, 29: 2-methyl-1-dodecanol; 43: 2-hexyl-1-decanol, 18: 2,4,6-trimethyldecane, 22: ethyl benzoate, 17: 2,6-dimethyldecane, 32: 4,6-dimethyldodecane, 16: n-undecane and 31: 2,3,5,8-tetramethyldecane.

(15) ene (6.94%), *β*-cedrene (4.86%)) and *S. macrocarpon* (limonene (7.26%)), were found to be susceptible to ash weevil (Figure 5 and Supplementary Tables 2 and 3).

Interestingly, principal component analysis (PCA) segregated the overall composition of functional groups of VOCs of different *Solanum* species in relation to their feeding antixenosis with respect to ash weevil damage. The total variance in VOC abundance to the tune of 50.73% was explained by two principal components (PC1, 27.43% and PC2, 23.30%, with significant eigenvalues of 2.4 and 2.09 respectively). The scores on PC1 susceptible genotypes were close to the origin and were positive (mean score = 1.74), while the resistant genotypes were strongly negative (mean score = –2.07). PC1 contributed to *M. subfasciatus* resistance mainly through terpenes, sesquiterpenes and ketones. On the other hand, PC2 mainly contributed to the susceptibility of *M. subfasciatus* through the presence of aldehydes and alcohols. Neither the hydrocarbons, acids or esters established an obvious relationship with either susceptible or resistant genotypes (Figure 6 *a*).

Bi-plot analysis of VOCs related to resistance and susceptibility of various *Solanum* species explained the overall variation to the tune of 52.53% (PC1, 36.01% and PC2, 16.52%). In *S. viarum*, resistance was found to be principally mediated by *n*-eicosane and trans-1,4-dimethyl cyclooctane. Similarly, 7,7-diethyl heptadecane contributed to ash weevil resistance in *S. sisymbriifolium*. In the case of *S. mammosum*, limonene and *p*-isopropyl benzaldehyde, in case of *S. nigrum*, 2-hexyl-1-decanol and 2,4,6-trimethyldecane, in case of *S. torvum*, 4,6-dimethyldodecane, 2,6-dimethyldecane, ethyl benzoate, 2-methyl-1-decanol, and in case of *S. seaforthianum* 4-ethyl acetophenone were the key contributors to resistance. In contrast, several compounds, viz. limonene and 4,5-dimethylnonae in the case of *S. macrocarpon*, 4-methyloctane in the case of *S. indicum*, *n*-undecane, 2,3,5,8-tetramethyldecane in *S. melongena* were found to mainly contribute towards ash weevil susceptibility (Figure 6 *b*).

The present study reveals a considerable variation in the relative susceptibility of different wild and cultivated *Solanum* genotypes to ash weevil *M. subfasciatus*. Since measuring the extent of leaf damage is the most common parameter used to determine genotypic resistance or susceptibility to ash weevil, we found all the 72 cultivated genotypes of *S. melongena* that were evaluated in the field screening suffered heavy feeding damage, indicating their susceptibility (Table 1). The highest levels of feeding non-preference (feeding antixenosis) were observed in the wild *Solanum* species, namely *S. viarum*, *S. mammosum*, *S. nigrum*, *S. sisymbriifolium* and *S. seaforthianum*, as zero feeding damage was noticed. Follow-up experiments involving choice and no-choice assays further established the presence of feeding antixenosis in these wild *Solanum* species against ash weevil. *S. capsicoides* and *S. torvum* were tolerant to ash weevil and recorded <1.00 feeding damage scores. However, *S. acculeatissimum*, *S. aethiopicum*, *S. indicum*, *S. macrocarpon* and *S. gilo* were found to be highly susceptible, similar to the cultivated genotype *S. melongena*. The ash weevil exhibited consistent feeding response towards *Solanum* genotypes both in *in vitro* and *in vivo* studies, confirming the existence of feeding antixenosis in the wild species, namely *S. viarum*, *S. mammosum*, *S. nigrum* and *S. seaforthianum*.

Antixenosis or non-preference is one of the mechanisms of HPR in which the insect is either repelled by or not attracted to the plant. The present study has conclusively

identified the sources of resistance against ash weevil *M. subfasciatus*, as well as phytochemicals associated with it. The genotypes *S. viarum*, *S. mammosum*, *S. nigrum*, *S. sisymbriifolium* and *S. seaforthianum* exhibited complete feeding antixenosis. The observed antixenosis might be associated with physical characteristics such as dense trichomes, trichome diversity, waxy surface[18], or varied VOCs composition as seen in the present study, which would have deterred the ash weevil from feeding. The genotypes composed of VOCs with higher terpenes, sesquiterpenes, esters, aromatic hydrocarbons and acids showed strong feeding antixenosis to ash weevil. We identified that the specific sesquiterpene β-selinene is abundant in resistant genotypes (*S. mammosum*, *S. sisymbriifolium*, *S. nigrum* and *S. capsicoides*). The other sesquiterpenes, namely, γ-amorphene, β-ocimene, β-copaene, α-bergamotene, sesquisabinene, germacrene D and α-muurolene were abundantly found in the resistant wild *Solanum* species. In addition, *n*-eicosane; trans-1,4-dimethyl cyclooctane; 7,7-diethyl heptadecane; p-isopropyl benzaldehyde; 2-hexyl-1-decanol; 2,4,6-trimethyldecane; diemthyl dodecane; 2,6-dimethyl decane; ethyl benzoate; 2-methyl-1-decanol and 4-ethyl acetophenone were found to be the key contributing compounds for resistance against ash weevil. However, detailed confirmation studies need to be carried out to ascertain the role of these identified compounds. Further, for targeted breeding or metabolic engineering approach, the biosynthetic genes which upregulate the identified compounds that might impart feeding antixenosis against *M. subfasciatus* could be identified from these *Solanum* species.

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