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Insect resistance responses of ten *Aster* varieties to damage by *Tephritis angustipennis* in the three rivers source region of China

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Aster **varieties are widely used for medicinal purposes, landscaping, and ecological restoration, but their growth and reproduction are significantly threatened by the seed predator *Tephritis angustipennis* (Diptera: Tephritidae). The cultivation of pest-resistant varieties offers an effective, economical, and eco-friendly approach to managing *T. angustipennis* infestations. This study evaluates the impact of *T. angustipennis* on ten *Aster* varieties in the Three Rivers Source Region (TRSR), with a focus on population density, plant damage rate, and the activity of resistance enzymes and insect-resistant metabolites. The results classified the ten varieties into four resistance groups: one highly resistant variety [HR: *Aster altaicus* (MQAA)], four moderately resistant varieties [MR: *Aster asteroides* (DRAA), *Aster flaccidus* (QLAF), *Aster tongolensis* (BMAT), *Aster poliothamnus* (MQAP)], two moderately susceptible varieties [MS: *Aster diplostephioides* (QLAD), *Aster souliei* (DRAS)], and three highly susceptible varieties [HS: *A. diplostephioides* (MQAD), *Aster yunnanensis var. labrangensis* (MQAY), *Aster farreri* (MQAF)]. Notably, HR and MR varieties exhibited significantly higher activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), trypsin inhibitor (TI), and chymotrypsin inhibitor (CI), as well as higher contents of tannins (TN) and flavonoids (FN), compared to MS and HS varieties. Specifically, the HR variety (MQAA) showed the highest levels of CAT, POD, SOD, and TN, significantly enhancing its resistance to *T. angustipennis*. Statistical analyses further revealed that MDA, TN, FN, and antioxidant enzyme activities were found to be key factors influencing insect resistance across the different varieties and resistance levels. These findings enhance our understanding of the physiological and biochemical mechanisms underlying resistance in *Aster* spp. and offer valuable insights for developing integrated pest management strategies. By identifying and promoting resistant varieties, this study lays the groundwork for effective, sustainable control measures that protect *Aster* crops from *T. angustipennis* damage.**

Keywords *Aster* spp., *Tephritis angustipennis*, Enzymatic activities, Secondary metabolites, Insect resistance

The genus *Aster* belongs to the Asteraceae family[1], comprising rhizomatous plants with a wide distribution[2]. It can be used for medicinal, landscaping, and ecological purposes[3]. Over 1000 Aster species are reported globally[4], with 123 species in China, second only to Poaceae, Cyperaceae, and Fabaceae. This genus of plants plays a significant role in medicine, chemical, and ecological development due to their unique flower and rich chemical substances[5]. Studies on *Aster* spp. include cultivation, secondary metabolites, medicinal uses, drought and salinity tolerance, and ecological functions[6–9]. These studies have provided many suggestions and references for the development, medicinal evaluation, and ecological functions of *Aster* species. Known for vibrant colors and adaptability to harsh conditions, they are considered important grassroots species for ecological restoration, it's also a very important Chinese herbal medicine in China[10]. However, pests like *Tephritis angustipennis*, *Campiglossa loewiana*, Noctuidae sp., and moths have hindered the collection of genetic resources and the breeding of resistant *Aster* varieties, causing significant economic losses, posing major challenges to the

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domestication and large-scale cultivation of *Aster* species in the Three-River Source Region (TRSR) and even in the whole country of China.

T. angustipennis (Diptera, Tephritidae), also known as *Trypeta angustipennis* (synonym)[11], is a widespread phytophagous pest in China, with *Aster* spp. as its main hosts[12]. These holometabolic insects are oviparous with a wide host range, and the larvae are endophagous, feeding internally on plant tissues[13]. *T. angustipennis* parasitizes *Aster* fruit, with 40% of larvae developing in flowers. Eggs are laid in the capitulum, and larvae feed on seeds[14]. They are key seed predators in Asteraceae plants[15]. *T. angustipennis* has been found in many regions, such as Kyrgyzstan[16], Ukraine[17], Iran[18], and China. Our team found *T. angustipennis* causes severe damage to *Aster* inflorescences in the TRSR[19]. This prevents seed formation and maturation, leading to poor yield, ecological losses, and obstacles to Aster genetic resource collection in the TRSR. Therefore, finding effective strategies to control *T. angustipennis* and contribute to agricultural, economic, and social benefits is crucial.

Plants have developed various defense strategies, including morphological, biochemical, and molecular mechanisms, to combat herbivore attack[20]. Host plant defenses are commonly classified into constitutive and induced defenses[21]. Moreover, biochemical-based defense (induced defenses) in plants may directly affect insect growth, development and survival[22], through the production and accumulation of plant defensive substances, including plant secondary metabolites, oxidative enzymes, protease inhibitors (PIs) and other defensive proteins[23]. The release of secondary metabolites by plants affects insect oviposition, larval growth, and pupal mass[24]. Total phenolics (TP), flavones (FN), malondialdehyde (MDA), and tannins (TN) are key secondary metabolites in plants[25]. TP are widespread defensive compounds in plants, important roles in defense against insect herbivores, micro-organisms, and competing plants[26]. Although TP are constitutively produced but increase in response to herbivore attack, as seen in plants like *Arachis hypogaea* (Rosales: Papilionoideae)[27], *Manihot esculenta* Crantz (Euphorbiaceae: Manihot)[28], and *Gossypium hirsutum* (Malvales: Malvaceae)[29]. TP have direct toxic or act as antifeedants as antifeedants[23]. Polyphenols and proteins affect herbivore growth through antisymbiosis, antibiosis, or antixenosis. Polyphenols cause oxidative damage in the insect midgut and thus act as toxic compounds for the pest. TN, as one group of the most abundant plant secondary metabolites, play an important role in plant defense against phytophagous insects. TN can interact with insect midgut proteins and digestive enzymes to limit their activities in insect pests and ultimately reduce insect growth and development[30]. FN inhibit gene expression via the ecdysone receptor in insects[31]. MDA is widely used as a biomarker of cell membrane damage; the content of insect-resistant secondary metabolites (MDA, FN, TN, TP) is positively correlated with plant tolerance to pests and fungi[32].

Another important defense mechanisms are the antioxidant defense system. Insect infestation induces antioxidant enzymes as part of the plant's early defense response. This includes antioxidant enzymes and PIs, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), chymotrypsin inhibitor (CI), and trypsin inhibitor (TI)[33]. SOD converts superoxide radicals (O^{-2}) into H_2O_2[34]; while POD and CAT scavenge H_2O_2 to water using electron donors. Rapid detoxification of both O^{-2} and H_2O_2 are essential to prevent excessive oxidative damage[35]. PPO and PAL synthesis insect-resistant metabolites (phenols and lignin) in response to insect attacks[36]. Several studies have demonstrated that the activity of antioxidant enzymes was positively correlated with plant tolerance to biotic stresses, including pests and fungi[37]. PIs are generated through stable defensive metabolism upon signal perception by the plant, synthesized as needed; they are a part of the plant's proactive defense system[38]. Proteinase inhibitors have been widely employed in pest resistance research in plants[39]. The coordination and interaction of various protective enzymes can effectively inhibit the damage caused by biotic stresses and improve the resistance of plants[40].

Previous studies on *Aster* have mainly focused on species classification[41,42], phylogenetic analysis[43], medicinal components[44], and abiotic stress[45,46]. However, no research has focused on the resistance evaluation and induced defense mechanisms of different *Aster* varieties against *T. angustipennis*, hindering the domestication and cultivation of this medicinal species. Furthermore, this notorious pest's rapid development and high fecundity enable it to adapt quickly and reach high population densities[47]. Therefore, identifying resistant *Aster* spp. varieties to *T. angustipennis* is crucial. In this study, we selected ten *Aster* varieties to evaluate their susceptibility and resistance to *T. angustipennis*. To understand how resistant varieties inhibit *T. angustipennis*, we measured secondary metabolite contents and antioxidant enzyme activities in ten varieties of *Aster* from the TRSR. This study helps clarify the resistant of *Aster* spp. to *T. angustipennis*, highlighting the relationship between antioxidant enzyme activity, secondary metabolite content, and insect resistance. It is of great significance for promoting *Aster* species with high insect resistance and developing pest control strategies for cultivating local grass species.

Materials and methods

Survey of pest occurrence and resistance grading of each variety of *Aster* in TRSR

This study, conducted from July to September 2023 during the growth period of *Aster*, explored the main distribution areas of *Aster* and the occurrence of pests on the species in the TRSR. Through systematic field surveys and detailed site investigations, along with methods such as net trapping, and branch shaking, the research investigated pest outbreaks on asters. During the systematic surveys, plant species in the sampling areas were first identified based on the "*Main Grassland Types and Common Plant Atlas of Qinghai*". Specimens of asters were then collected based on external morphological features and brought back to the laboratory for identification, seeking expert advice from plant taxonomists, and using the *rbcL* barcode technology for accurate species identification. Field records were also taken and photographs were taken. At the end, the study found significant distributions of various asters in Maqin, Dari, and Banma counties of Golog Tibetan Autonomous Prefecture, and Qilian County of Haibei Tibetan Autonomous Prefecture. Based on prior investigations by the research group and the results of this study, a total of 9 species (10 varieties) of *Aster* were identified within the surveyed areas. The specific information on the sampling sites is detailed in the attached Fig. 1 and Table S1.

Further investigation assessed the growth conditions and pest occurrence. The survey used a 5-point sampling method, randomly selecting five points along the diagonal of concentrated asters growth zones. At each point, 45 plants with consistent growth were chosen, with 5 plants collected as specimens for further species identification in the laboratory to minimize sampling errors. Detailed records were made of the growth conditions, habitat vegetation, and areas of pest damage on the plants. Insect species and developmental stages (life stages) were identified by examining morphological features under a dissecting microscope; and pest species, population density, number of pest holes, and the proportion of flower area affected were statistically recorded.

Additionally, in each sampling site, three 1 m×1 m quadrats were randomly set in densely populated areas, and both net trapping and branch shaking methods were used to survey the insect species and quantities on all plants of asters within the quadrats. Particular attention was paid to recording "the number of asters per square meter and the frequency of affected plants" and "the pest population density per square meter at different growth stages." Furthermore, pest samples were collected based on external morphological features and stored in centrifuge tubes as specimens. Live insects were brought back to the laboratory for breeding and *COI* barcode-assisted identification. The final data from both methods were used to calculate the pest species and occurrence on different species of *Aster* and their habitats. Based on field observations, different species were classified according to the period of damage and resistance levels. Field surveys were conducted every 15 days, in clear weather, with delays in case of rainy days. The survey revealed that the primary pest causing significant damage to all asters was the *Tephritis angustipennis*, which caused harm at the flower receptacle[19]. Based on the major pest outbreaks on most plants in the survey area, the damage period was divided as follows: early July was the non-damage period, mid to late July was the initial damage period, early August was the moderate damage period, mid to late August was the severe damage period, and late August to early September marked the end of the damage period. Plant and insect specimens collected in the study are preserved in the laboratory of the Grassland Institute, College of Animal Science and Veterinary Medicine, Qinghai University.

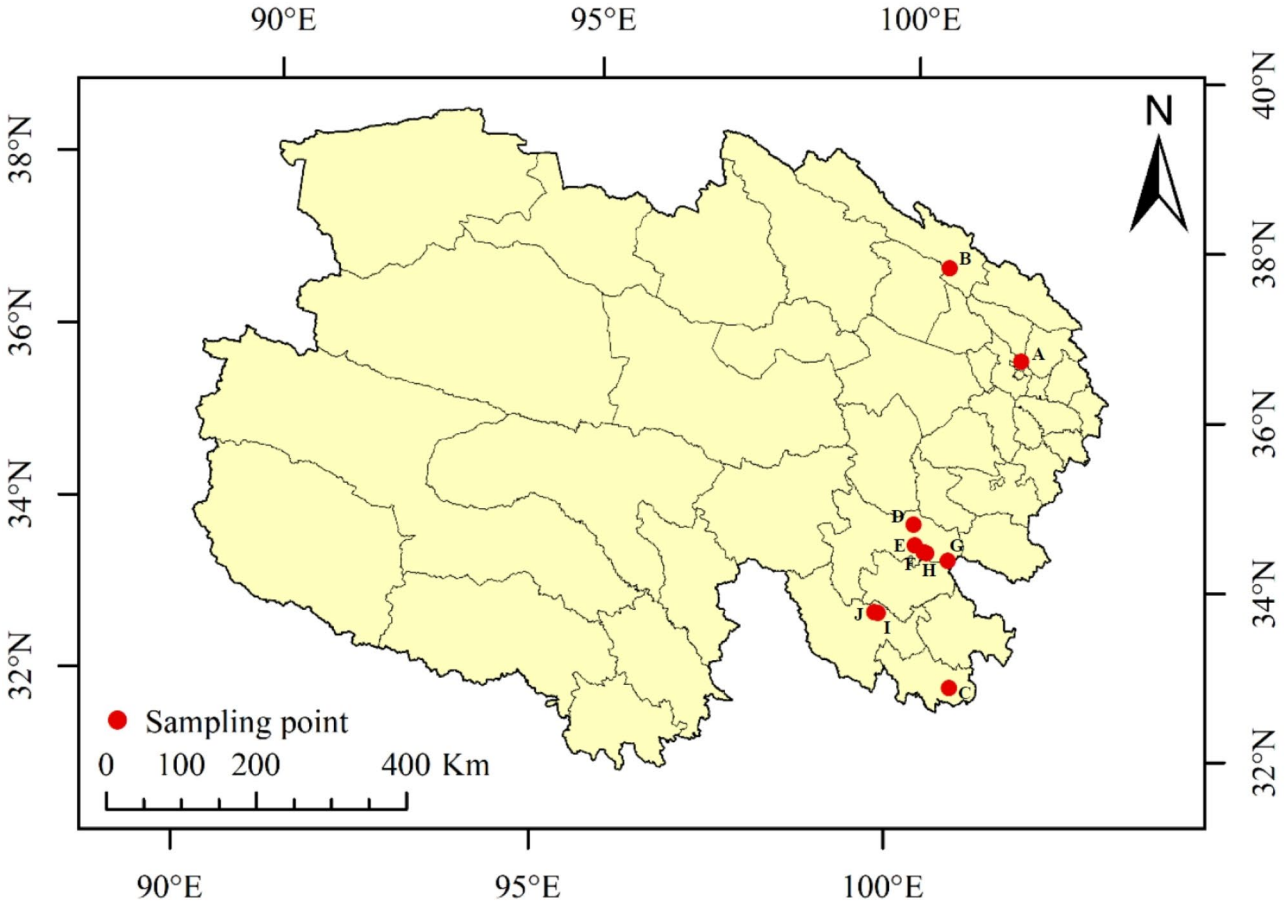

Fig. 1. Sample collection sites different varieties of *Aster* spp. in the Three-River Source Region (TRSR), China. Note: A-J on the map represent the collection sites of *Aster flaccidus* (QLAF), *Aster diplostephioides* (QLAD), *Aster tongolensis* (BMAT), *Aster poliothamnus* (MQAP), *Aster yunnanensis var. labrangensis* (MQAY), *A. diplostephioides* (MQAD), *Aster farreri* (MQAF), *Aster altaicus* (MQAA), Aster souliei (DRAS), *A. asteroides* (DRAA), respectively. See Additional information (Exhibit 1) for specific sampling point information.

Classification of different damage levels (hazards) and sampling of each plant

Based on a detailed analysis of the varieties, pest density, number of pest holes, and the proportion of flower damage on individual asters during the field surveys, the level of pest damage was classified into five damage levels: excellent quality (non-harm period, CK), slight hazard (primary harm period, HP), moderately hazard (mid-term harm period, HM), serious hazard (high harm period, HH), and more serious hazard (end-stage harm period, HE), as defined by specific criteria in Table 1.

It is important to note that due to the varying flowering periods of different asters, sampling was conducted during their peak flowering period (mid to late August), when varying degrees of damage were observed. For each variety, plants with similar growth and damage levels were selected. Each damage level group consisted of 20 plants as one replicate. During sampling, stems, leaves, flowers, and bracts were collected from plants representing each damage level. These samples were mixed, numbered, and wrapped in aluminum foil before being immediately placed in liquid nitrogen for transport. Upon return to the laboratory, the samples were stored at -80 °C for subsequent analyses of enzyme activity and anti-insect compounds. All flower samples for the same varieties with different damage levels (CK, HP, HM, HH, HE) were collected on the same day within the same time frame. The total sampling duration was three days, during which the weather was clear, and plants were healthy, free of dead branches or other diseases. Only plants with damage from *T. angustipennis* were selected for experimental use.

Determination of protective, defense, and inhibitory enzyme activities in *Aster* spp. Flowers

Prior to formal experiments, pre-tests were conducted using samples with expected significant differences to calibrate experimental conditions. The pre-tests were performed following the instructions provided in the reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). These pre-tests determined whether sample dilution was necessary, the appropriate dilution factor, and ensured familiarity with the experimental procedures. Formal experiments were carried out only after completing these calibrations. The following methods describe the measurement of specific enzyme activities:

Determination of CAT, POD, SOD, PPO, and PAL activities: flowers of each *Aster* spp. at different hazard levels were weighed (0.1 g) and placed in a pre-cooled mortar. An appropriate volume of extraction buffer was added, and the samples were homogenized. The homogenates were then centrifuged under specific conditions: CAT (EC number: 1.11.1.6), SOD (EC number: 1.15.1.1), PPO (EC number: 1.10.3.1) were centrifugated at 8000 × g for 10 min at 4℃, POD (EC number: 1.11.1.7) were centrifugated at 3500 r/min for 10 min at 4℃, PAL (EC number: 4.3.1.5) were centrifuged at 10,000 r/min for 10 min at 4℃. The resulting supernatants were collected and used for enzymatic activity assays. Enzymatic activities were measured spectrophotometrically following the methodologies provided in the reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activities of SOD, CAT, POD, PPO, and PAL were determined from the measurement of absorbance at 560, 240, 420, 420, and 290 nm, respectively. These specific wavelengths correspond to the characteristic absorption peaks of the respective enzyme reactions, enabling the calculation of enzyme activity based on the rate of change in absorbance. The results of the aforementioned enzymes were expressed as U g^{-1} FW; Three biological replicates were utilized to make each of the measurements.

Determination of TI, CI activities: flowers from each variety at different hazard levels were weighed (0.1 g) and ground in a pre-cooled mortar with Tris-HCl buffer and quartz sand. The homogenates were placed in an ice bath, thoroughly mixed, and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatants were collected and assayed for TI and CI activities based on modified methods from Wang Qi[48]. The activities of TI (EC number: 3.4.21.4), and CI (EC number: 3.4.21.1) were determined from the absorbance measurement at 256, and 253 nm, respectively. The characteristic absorption peaks of these enzymes at the specified wavelengths were used to calculate their activity. Results were expressed as U g^{-1} FW; and all measurements were performed in triplicate with three biological replicates.

Hazardous period	Classes	Degree of damage	Classification standard of pest survey	Classification	Classification abbreviation
Non-harm period	0	No pest damaged	Plants are free from any pests, plants and flowers are in good condition	Excellent quality	CK
Primary harm period	1	Less than 1/4 of the flowers area are damaged	Occasional, almost do not cause harm, almost no pests; the number of harmful insect holes ≤ 1, the population density ≤ 1 head / plant	Slight hazard	HP
Mid-term harm period	2	1/4 – 1/2 of the flowers area are damaged	The degree of damage is heavier; single plant flower insect holes 2–4, 1 < population density < 5 head / plant, resulting in a large area of the receptacle lack of holes	Moderately hazard	HM
High harm period	3	1/2–3/4 of the flowers area are damaged	Widespread occurrence, the degree of damage is extremely heavy; single flower insect holes greater than 4, The population density of ≥ 5 head / plant, some almost the receptacle to feed overall	Serious hazard	HH
End-stage harm period	4	More than 3/4 of the flowers area are damaged or almost all of it is damaged	1 < population density < 2 head / plant, the late stage of growth is basically in the withering and yellowing stage of plant growth, and most of them are old larvae, very few are adults, larvae feeding on the plant receptacle caused by mutilation, high-aged pests feeding on the plant stems of many plants hollow or even rot	More serious hazard	HE

Table 1. Investigation And classification of damage to *Aster* spp. And evaluation standard of resistant to *Tephritis angustipennis*. Note: The insect population density in the table is determined based on the initial survey results at the time of initial pest infestation, and the results of subsequent surveys are consistent with it.

Determination of biochemical constituents in *Aster* spp. Flowers

The contents of malondialdehyde (MDA), tannins (TN), total phenols (TP), and flavonoids (FN) determined using standard spectrophotometric methods, as detailed below: thiobarbituric acid (TBA)-based spectrophotometric method, activated charcoal adsorption-spectrophotometric method, tungsten molybdate reduction-spectrophotometric method, and nitrite reaction-spectrophotometric method, respectively. Measurement kits were procured from Shanghai Sangon Biotech Co., Ltd. The contents of MDA, tannin, total phenols, and flavonoids were determined based on absorbance readings at 532, 275, 760, and 502 nm, respectively. These wavelengths correspond to the characteristic absorption peaks of the respective metabolites. The content of each compound was reported as micrograms per gram of fresh weight (mg g^{-1} FW or nmol g^{-1} FW). All measurements were conducted in triplicate.

Statistical analysis

Following the software user manual, ArcGIS (V10.8) was used to label 10 varieties' sampling plots, assigning attributes to facilitate subsequent analysis. Prior to analysis, the data distribution was assessed via Q-Q plots and the interquartile range (IQR) method, identifying potential outliers. These outliers, given their biological significance, were retained but addressed through logarithmic transformation and robust regression to minimised their impact. Data were also standardized in redundancy analysis (RDA) to stabilize ordination axes. Sensitivity analysis confirmed that retaining these values did not compromise the overall conclusions' robustness.

To compare antioxidant enzyme activity and toxic secondary metabolite contents at different pest damage stages within a single variety, and to evaluate pest resistance across varieties and damage levels, normality and homogeneity of variance tests were performed. Due to a lack of normality but satisfaction of variance homogeneity, non-parametric tests were used for significance analysis. Kruskal-Wallis tests were employed for comparisons, followed by Dunn's test ($P<0.05$) for multiple comparisons. Multivariate general linear models were applied for between-subject effects, analyzing the influence of pest damage levels and variety. Differences between two treatments were examined using the bootstrap method. Results are presented as means ± standard errors (SE). Spearman correlation analysis and stepwise regression were performed to investigate relationships between variables, while redundancy analysis (RDA) was used to explore key factors influencing pest resistance. All statistical analyses were conducted in SPSS v26.0 (SPSS Inc., Chicago, IL, USA), with tables prepared in Microsoft Excel 2016 and figures created in Origin 2021 and Canoco 5.

Results

Differential analysis of *T. angustipennis* resistance in ten *Aster* spp. and classification of pest damage levels.

The investigation revealed significant differences in population densities of *T. angustipennis* eggs (TaEs) and larvae (TaLs) among ten *Aster* varieties ($P<0.01$) (Table 2). Highly susceptible plants experienced up to 98.5% infestation, while highly resistant varieties were nearly pest-free (Table S2). At the beginning of the infestation, *Aster farreri* (MQAF) had the highest egg and larval densities (37 and 68 heads/m^2, respectively), followed by *Aster yunnanensis var. labrangensis* (MQAY) and *Aster diplostephioides* (MQAD). MQAF maintained the highest density throughout the reproductive level of *T. angustipennis*, peaking at 213 heads/m^2 during the moderately larval stage (Table 2, Figure S1A).

Based on average *T. angustipennis* population densities across various development periods and plant damage incidence from July to September, the ten *Aster* varieties were classified into four groups: highly resistant (HR, represented by *Aster altaicus*, MQAA)); moderately resistant (MR, including *Aster tongolensis* (BMAT), *Aster*

Species (abbreviated)	Population density of *T. angustipennis* (head/m^2)				The type of resistant
	TaE (early July)	TaL1 (early August)	TaL2 (mid-August)	TaL3 (late August)	
Aster altaicus (MQAA)	0	1 ± 0.38 d	2 ± 0.37 e	1 ± 0.48 e	Highly resistant (HR)
Aster tongolensis (BMAT)	0	1 ± 0.21 d	4 ± 1.29 e	8 ± 2.09 de	Moderately resistant (MR)
Aster flaccidus (QLAF)	0	3 ± 0.33 d	8 ± 1.47 e	13 ± 1.54 de	Moderately resistant (MR)
A. asteroides (DRAA)	0	5 ± 0.52 d	15 ± 1.33 e	12 ± 1.40 de	Moderately resistant (MR)
Aster poliothamnus (MQAP)	6 ± 1.11 de	7 ± 0.60 d	17 ± 1.26 e	6 ± 0.67 de	Moderately resistant (MR)
Aster souliei (DRAS)	10 ± 0.60 cd	10 ± 1.05 cd	56 ± 2.41 d	18 ± 2.51 d	Moderately susceptible (MS)
Aster diplostephioides (QLAD)	12 ± 0.42 c	11 ± 0.37 cd	76 ± 5.43 c	31 ± 2.35 c	Moderately susceptible (MS)
A. diplostephioides (MQAD)	23 ± 4.02 b	19 ± 3.79 bc	178 ± 5.88 b	46 ± 8.96 b	Highly susceptible (HS)
Aster yunnanensis var. labrangensis (MQAY)	26 ± 3.17 b	22 ± 1.58 b	205 ± 3.90 a	57 ± 8.79 b	Highly susceptible (HS)
Aster farreri (MQAF)	37 ± 2.66 a	68 ± 10.66 a	213 ± 14.28 a	73 ± 4.62 a	Highly susceptible (HS)

Table 2. Average population density of *Tephritis angustipennis* on different aster species. Note: The data in the table is the average ± standard error; Different letters in the same column of data indicated significant differences between different species ($P<0.05$). TaE, TaL1, TaL2, and TaL3 represent the growth stage of pests at the time of collection, namely egg stage, younger larvae stage, moderately larvae stage, and advanced larvae stag, respectively.

Classes	Classification standards for pest survey and plant damage rate	The type of resistant
0	Few infestations on any part of the plant, plant damage rate < 10%, insect population density of egg and advanced larvae stag ≤ 1 head/m^2	Highly resistant (HR)
1	Plant damage rate of 10-55%, 1 head/m^2 < population density of egg < 10 head/m^2, and 5 head/m^2 < insect population density of advanced larvae stag < 15 head/m^2	Moderately resistant (MR)
2	55%< plant damage rate < 60%; 10 head/m^2 ≤ population density < 15 head/m^2, and 15 head/m^2 ≤ insect population density of advanced larvae stag < 40 head/m^2	Moderately susceptible (MS)
3	Plant damage rate 60-100%; population density ≥ 15 head/m^2, even up to 37 head/m^2; and insect population density of advanced larvae stag ≥ 40 head/m^2, even up to 73 head/m^2	Highly susceptible (HS)

Table 3. Investigation on the damage and resistance classification of *Aster* spp. To different plant species. Note: The plant damage rate is equal to the total number of plants with pest damage per 200 *Aster* plants. The plant damage rate in the table is the average of three survey results for each *Aster* spp. The insect population density is determined based on the initial survey results of the initial pest damage period.

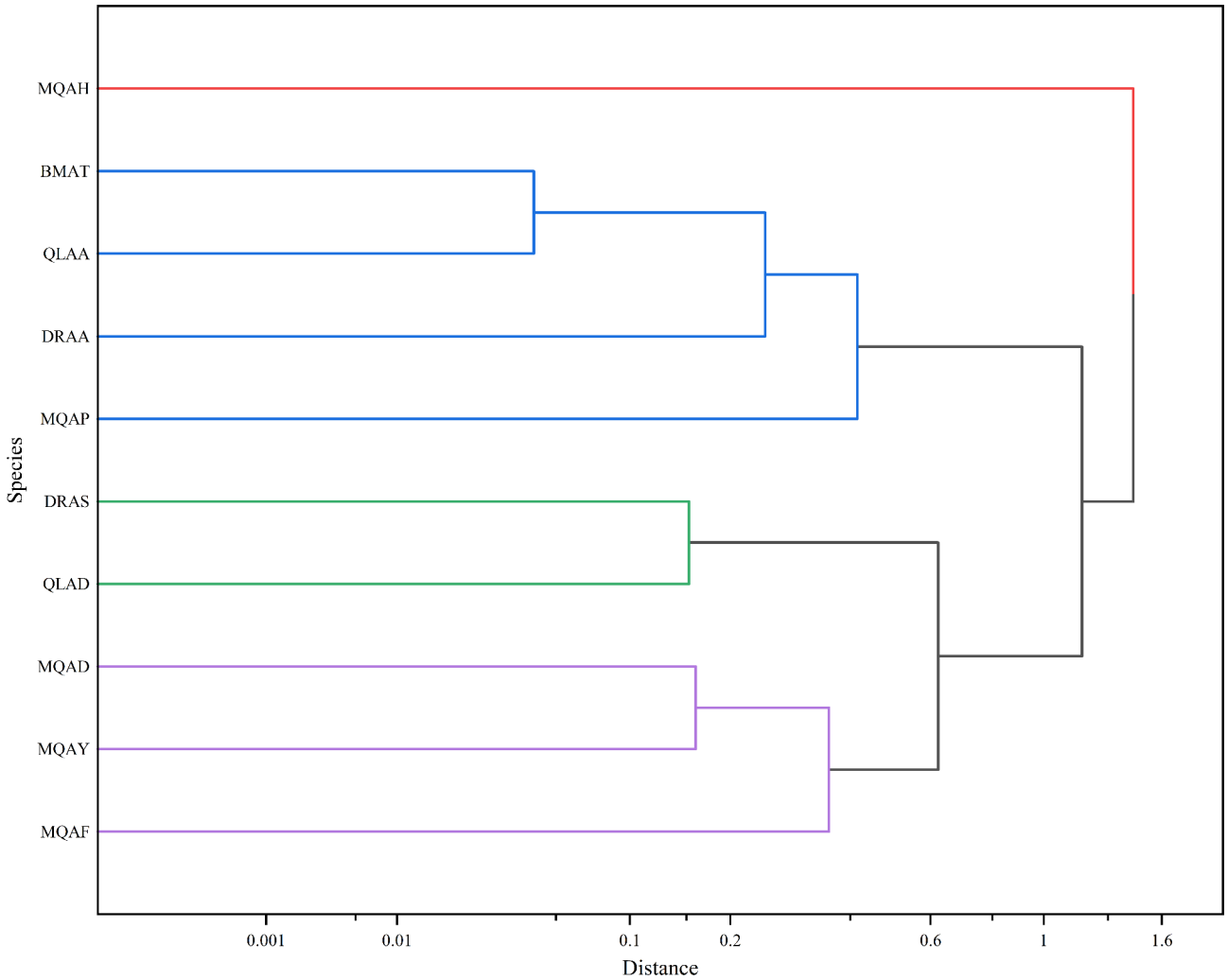

Fig. 2. Results of cluster analysis based on the population density of *Tephritis angustipennis* on different varieties of *Aster* plants.

poliothamnus (MQAP), *Aster asteroides* (DRAA), and *Aster flaccidus* (QLAF)), moderately susceptible (MS, including *Aster souliei* (DRAS) and *Aster diplostephioides* (QLAD)), and highly susceptible (HS, including *A. diplostephioides* (MQAD), *Aster yunnanensis var. labrangensis* (MQAY), and *Aster farreri* (MQAF)) (Tables 2 and 3; Fig. 2).

Changes and differences in SOD, CAT, and POD activities in protective enzyme systems of various resistant varieties

Different varieties of *Aster* spp. and different damaging levels of *T. angustipennis* are significant impact on the activities of CAT, POD, and SOD ($P<0.01$) (Table S3). Overall, HR and MR varieties consistently displayed higher activities than MS and HS varieties, both under non-feeding conditions and during pest infestation (Table S4). Significant differences in CAT, POD, and SOD activities are observed among different *Aster* varieties during different damaging periods and within the same period ($P<0.05$). In non-feeding conditions, CAT and SOD activities in HR and MR varieties are significantly higher than in MS and HS varieties ($P<0.05$), while POD activity shows slight differences or equality with MS and HS varieties, highlighting resistant-related distinctions. However, as pest infestation occurs and feeding time increases, significant fluctuations occur in CAT, POD, and SOD activities across resistant *Aster* varieties, particularly during intense feeding levels, where pest activity notably alters these enzyme activities (Table S5, Figure S1B–D).

Post-damaged by *T. angustipennis*, during most damaging levels, MS and HS varieties exhibit significantly lower CAT, POD, and SOD activities compared to HR and MR types (Table S5). Compared to CK, activities of CAT, POD, and SOD generally increase in various resistant varieties (Figure S1B–D). Specifically, CAT and SOD activities in HR significantly increase ($P<0.05$) during HP, peaking at HH and HP, respectively; SOD shows a higher increase (141.54%) compared to CAT (17.52%), while POD activity significantly decreases during HP ($P<0.05$), but all three enzymes were significantly reduced in the HE (Fig. 3A,C). In MR, CAT, POD, SOD activities significantly increase during HP ($P<0.05$), generally rise during HM with a significant lower or equal to the previous level during HH to HE ($P<0.05$) (Fig. 4A,C). MS and HS varieties showed significant enzyme activity reductions after initial increases during HP ($P<0.05$), and all of them were significantly reduced afterwards; with MS types seeing a significant decrease in POD and SOD activities from HH to HE (Fig. 5A,C), POD and SOD activities of HS type were significantly reduced from HM to HE or did not differ much from one period to another (Fig. 6A,C) These patterns demonstrate that HR and MR varieties generally maintain higher defensive enzyme activities throughout the feeding cycle, contributing to their greater resistance.

Changes and differences in PPO, PAL activities in defense enzyme systems of various resistant varieties

PPO and PAL activities varied significantly across *Aster* varieties and damage levels ($P<0.01$) (Table S3). Generally, HR and MR varieties exhibited higher PPO and PAL activities overall, especially before feeding and during early damage levels (Table S4). As feeding continued, notable fluctuations in PPO and PAL activities occurred, strongly influenced by pest activity. HR varieties generally maintained higher PPO and PAL levels than MS and HS varieties, particularly during intermediate and later damage levels ($P<0.05$) (Table S5, Figure S2A–B).

Specifically, PPO and PAL activity in HR varieties decrease significantly during HP ($P<0.05$), followed by significant increases during HM and HH, respectively; decreased significantly at HE ($P<0.05$) (Fig. 3D,E). PAL activity in MR varieties sharply increases during HP and HE, while PPO shows a significant increase during HM and HE with a delayed response (Fig. 4D,E). In MS varieties, PPO activity decreases significantly during HP and HH, slightly rises during HM, and peaks significantly at HE ($P<0.05$) (Fig. 5D); PAL activity increases significantly from HP to HH ($P<0.05$) and decreases towards HE ($P<0.05$) (Fig. 5E). Similarly, PPO activity in HS varieties significantly increases during HP, peaks during HM or HH, and decreases markedly at HE ($P<0.05$) (Fig. 6D); PAL activity remains stable during HP and HM but significantly increases during HH ($P<0.05$) (Fig. 6E). In summary, HR and MR varieties displayed consistent increases in PPO and PAL activities after pest infestation, with significant differences between varieties at various damage levels. These trends highlight the role of PPO and PAL in resistance, as resistant varieties showed greater and more sustained enzymatic responses to pest pressure.

Changes and differences in TI and CI activities across different resistant varieties' inhibitory enzyme systems

TI and CI activities were significantly affected by Aster variety and infestation level ($P<0.01$) (Table S3). During the control check (CK), significant differences in enzyme activity were observed among different resistant *Aster* spp. at different levels ($P<0.05$), with TI activities higher in HR and MR types than in MS and HS types, while CI activities showed the opposite trend (Figure S2C–D).

After *T. angustipennis* damage, TI and CI activities increased significantly in HR and MR varieties, peaking during intermediate and high damage levels. MS and HS varieties, on the other hand, showed stable or declining TI and CI activities as damage progressed (Table S5). Generally, HR and MR varieties exhibited higher TI and CI activities compared to MS and HS types (Figure S2C–D). Post *T. angustipennis* attack, TI and CI activities of different resistant varieties generally increased compared to CK. Specifically, TI and CI activities in HR varieties (MQAA) increased notably after *T. angustipennis* infestation ($P<0.05$), peaking during HH (Fig. 3F,G). MR varieties showed a rapid response during HP, with significant increases in TI and CI activities in all varieties except BMAT ($P<0.05$), peaking during HM and HH (Fig. 4F,G). TI activities in MS varieties remained stable during HP, slightly increasing in HM, while CI activities decreased significantly ($P<0.05$); trends in TI and CI activities during HH were opposite to those in HM, generally decreasing towards HE (Fig. 5F and G). HS varieties exhibited significant decreases in both TI and CI activities during HP ($P<0.05$), with TI activities stable or slightly increasing in HM, and CI activities stable or slightly increasing during HH, with slight increases or stability towards HE (Fig. 6F,G). In summary, these findings demonstrate that TI and CI activities in resistant varieties play a crucial role in pest resistance, with HR and MR types showing consistently higher levels of these inhibitory enzymes throughout the infestation cycle.

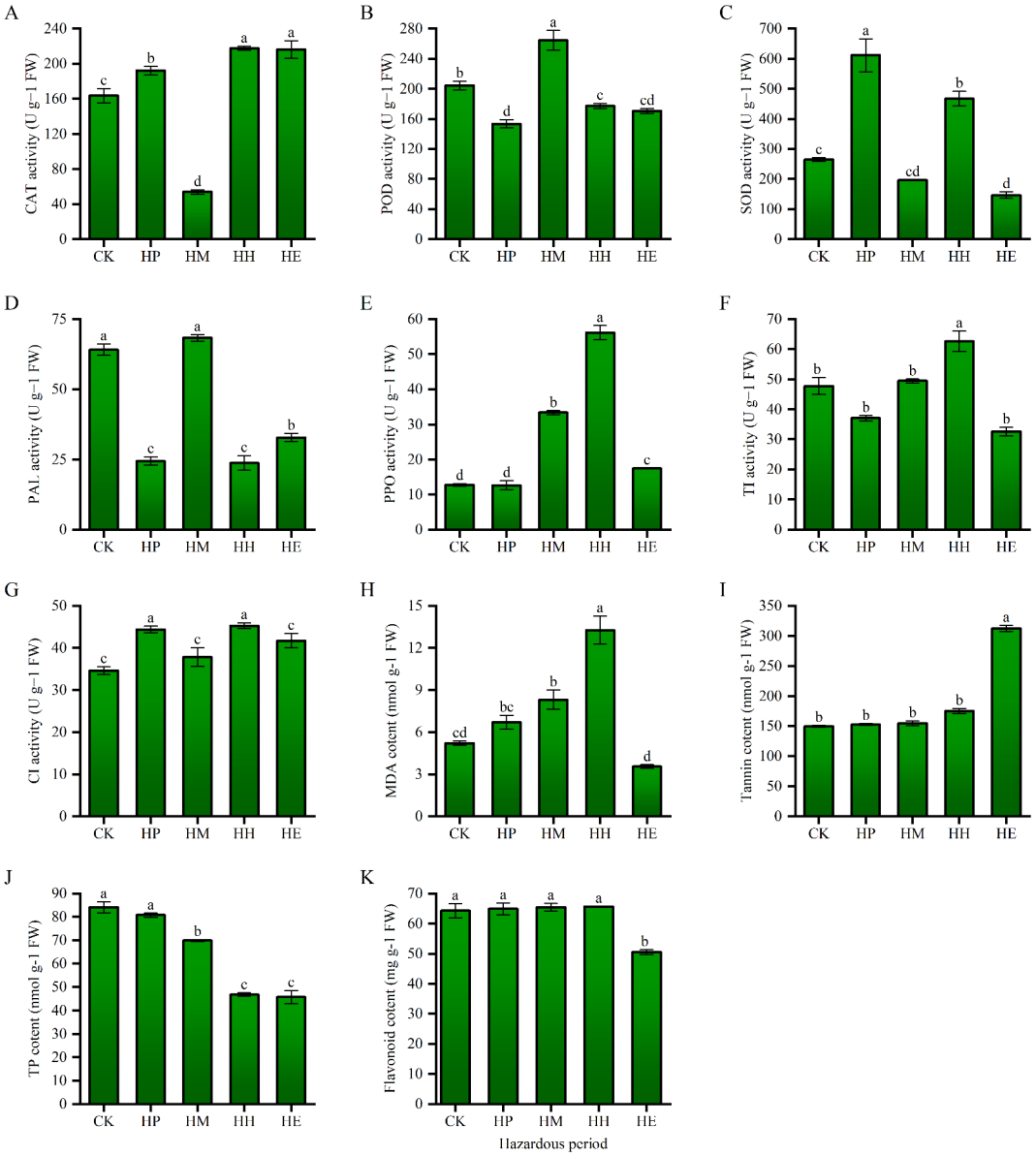

Fig. 3. Effects of feeding by *Tephritis angustipennis* on antioxidant enzyme activities and toxic secondary metabolites contents in flowers of highly resistant (HR) varieties of *Aster* spp. A-K stands for (**A**) catalase (CAT), (**B**) peroxidase (POD), (**C**) superoxide dismutase (SOD), (**D**) polyphenol oxidase (PPO), and (**E**) phenylalanine ammonia lyase (PAL), (**F**) Trypsin inhibitor (TI), (**G**) Chymotrypsin (CI), (**H**) Malondialdehyde (MDA), (**I**) tannin (TN), (**J**) total phenols (TP), and (**K**) flavonoids (FN), respectively. Data represent means ± standard error of three replicates. Different lower-case letters indicate significant differences among the same species at different hazard periods by one-way ANOVA ($P < 0.05$, Duncan's t-test). CK, HP, HM, HH and HE representing different hazard levels (Hazardous period), namely non-harm (excellent quality), slight hazard, moderately hazard, serious hazard and more serious hazard, respectively. The same as below.

Changes and differences in the activity of insect-resistant compounds across different resistant varieties

MDA, TN, TP, and FN contents were significantly influenced by variety and damage level ($P < 0.01$) (Table S3). HR and MR varieties maintained higher TN, TP, and FN levels, while the MDA was lower compared to MS and HS types (Table S6). Before pest feeding, significant differences in MDA, TN, TP, and FN contents are observed among different *Aster* spp. varieties ($P < 0.05$) (Table S5). Specifically, TN, TP, and FN contents in HR and MR varieties are significantly higher, whereas MDA contents are notably lower in MS and HS varieties, indicating resistant-related differences (Figure S3A–D).

After *T. angustipennis* damage, enzyme activities increased significantly in HR and MR varieties, peaking during intermediate and high damage levels. MS and HS varieties, on the other hand, showed stable or declining TI and CI activities as damage progressed (Table S5, Figure S3A–D). Compared to CK, the contents of MDA,

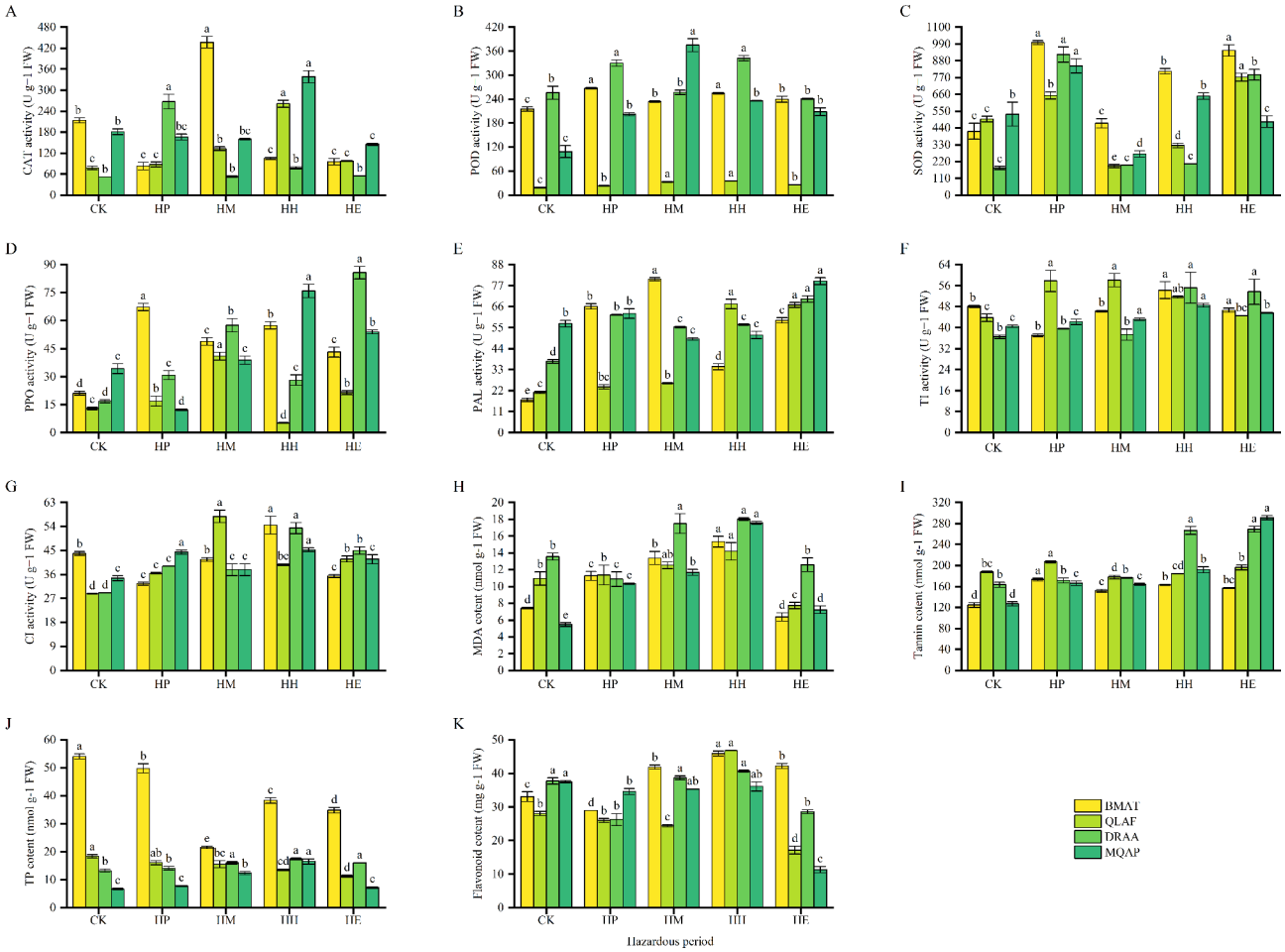

Fig. 4. Effects of feeding by *Tephritis angustipennis* on antioxidant enzyme activities and toxic secondary metabolites contents in flowers of moderately resistant (MR) varieties of *Aster* spp.

TN, TP, and FN in HR and MR varieties significantly increase, though with slightly different extents and trends. Specifically, MDA, TN contents in HR (Fig. 3H–J) and MR (Fig. 4H–J) varieties significantly increase in the HP ($P < 0.05$), peaking in the HH or HE, with rapid changes in TP content in the HP. FN content in HR varieties significantly increase (Fig. 4K), while the trend is small for MR varieties (Fig. 3K); both increase significantly at HH, and significantly decreased towards HE ($P < 0.05$).

In MS (Fig. 5H–K) and HS varieties (Fig. 6H–K), MDA, TN, TP, and FN contents significantly increase in the HP ($P < 0.05$), with TN content showing notably larger increases, peaking during this period for most varieties. TP content in MS and HS varieties either increases slightly in the HP or remains at CK levels (Figs. 5J and 6J). During HM, MDA, TN, and FN contents in MS varieties significantly increase, with FN content peaking, while TP content generally decreases. In the HH, TN, TP, and FN contents in MS varieties significantly decrease ($P < 0.05$) (Fig. 5I–K), while MDA content significantly elevates (Fig. 5H). MDA content in HS varieties is significantly higher from HM to HH and lower as HE (Fig. 6H). TN content in HS varieties consistently decreases from HM to HE (Fig. 6I), while TP content in other HS varieties maintain the trend of first increasing and then decreasing, except for MQAD, which shows significantly higher levels in HM and HE (Fig. 6J). FN content in HS varieties increases significantly only in HM, but decreases significantly in HH and HE, showing a relatively lagging response (Fig. 6K). These patterns indicate that resistant varieties rely on elevated secondary metabolite levels and reduced oxidative damage (as indicated by MDA content) to maintain resilience. In contrast, MS and HS varieties showed lower metabolite levels and higher MDA content, underscoring their greater susceptibility.

Relationships between resistance indicators and population density of *Aster* spp. across different levels of damage

Redundancy analysis between insect density and resistant indicators

Redundancy analysis showed that MDA, TN, TP, FN, and various enzyme activities strongly influenced insect population density at different damage levels. For HR varieties, MDA content explained 23% of the variation, while TP content was most influential in MR types, and PPO activity was key for HS varieties. These results highlight the importance of multiple biochemical markers in determining pest population density and plant resistance (Fig. 7).

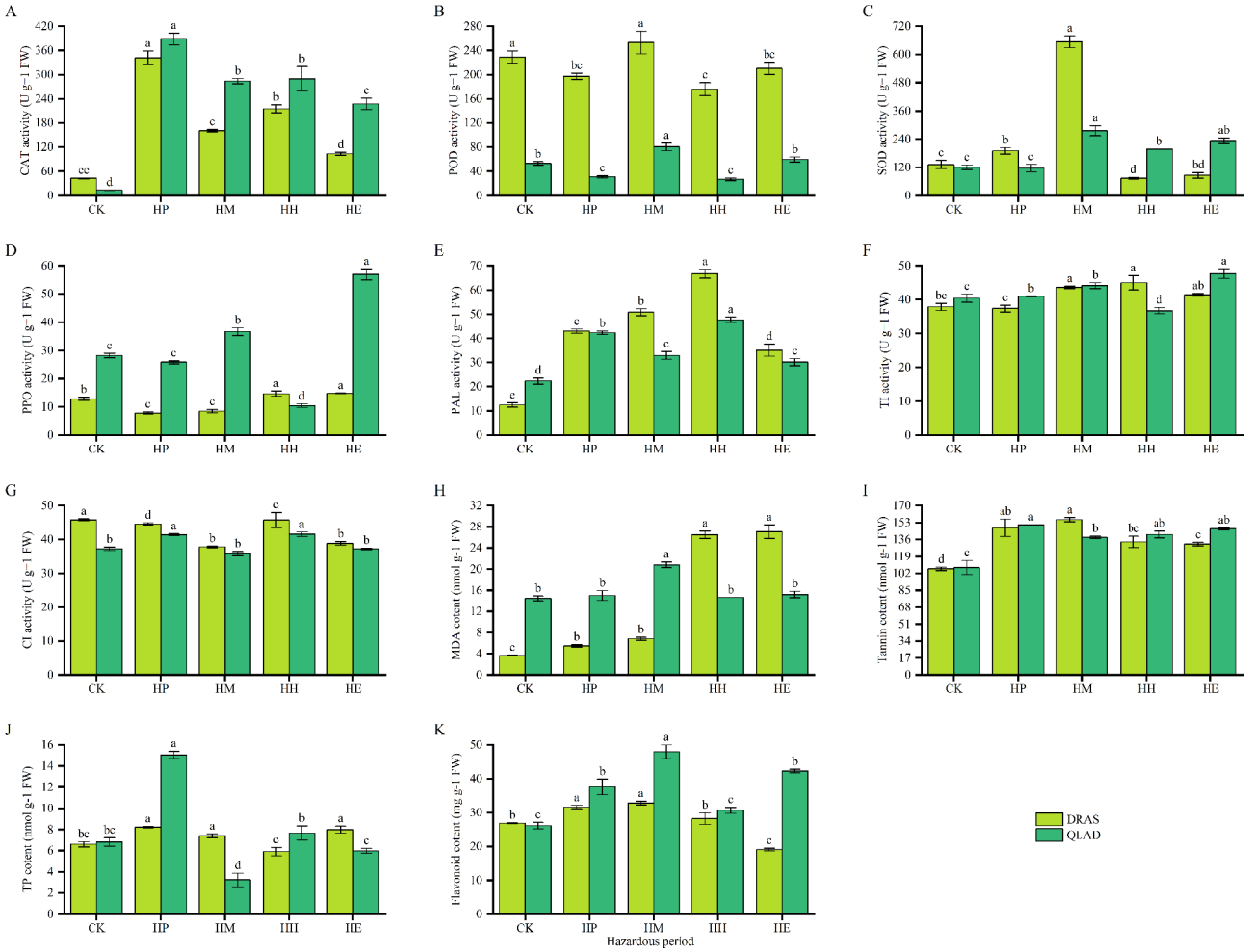

Fig. 5. Effects of feeding by *Tephritis angustipennis* on antioxidant enzyme activities and toxic secondary metabolites contents in flowers of moderately susceptible (MS) varieties of *Aster* spp.

Correlation analysis

T. angustipennis population density was significantly negatively correlated with defensive enzymes and secondary metabolites ($P < 0.05$), but positively correlated with MDA levels at all damage levels ($P < 0.001$) (Fig. 8A). The PD of *T. angustipennis* at CK is significantly negatively correlated with SOD, CAT, PPO, PAL activities and TN, TP, FN contents (Fig. 8B). At HP, PD is significantly negatively correlated with SOD activity and TP content ($P < 0.001$) (Fig. 8C). At HM, PD is significantly negatively correlated with PAL, TI activities and TN, TP, FN contents ($P < 0.01$)(Fig. 8D). At HH, PD is significantly negatively correlated with SOD, TI activities and TN, TP, FN contents ($P < 0.05$) (Fig. 8E). At HE, PD is significantly negatively correlated with POD activity and TN, TP, FN contents ($P < 0.05$) (Fig. 8F). These correlations confirm the importance of maintaining high enzyme activity and low oxidative damage to achieve resistance.

Stepwise regression analysis

In the stepwise regression analysis, each influencing factor as a whole was analyzed against the environmental factor (PD) (Table 4). The results showed that PD on ten varieties was closely related to MDA, FN, and TN contents, with the strongest association observed between PD and MDA content and FN content (Fig. 9A–B). Further regression analyses indicated different main influencing factors at distinct hazard levels, such as FN and MDA contents at CK and HP, FN and TN contents at HH and HE, and TN content at the HM (Fig. 9C–G). These results showed that MDA, FN, and TN contents as the key factors influencing pest density across damage levels. FN and MDA contents were particularly significant in the early levels, while TN played a larger role in later levels. This further emphasizes the combined effects of oxidative stress mitigation and secondary metabolite production in determining resistance.

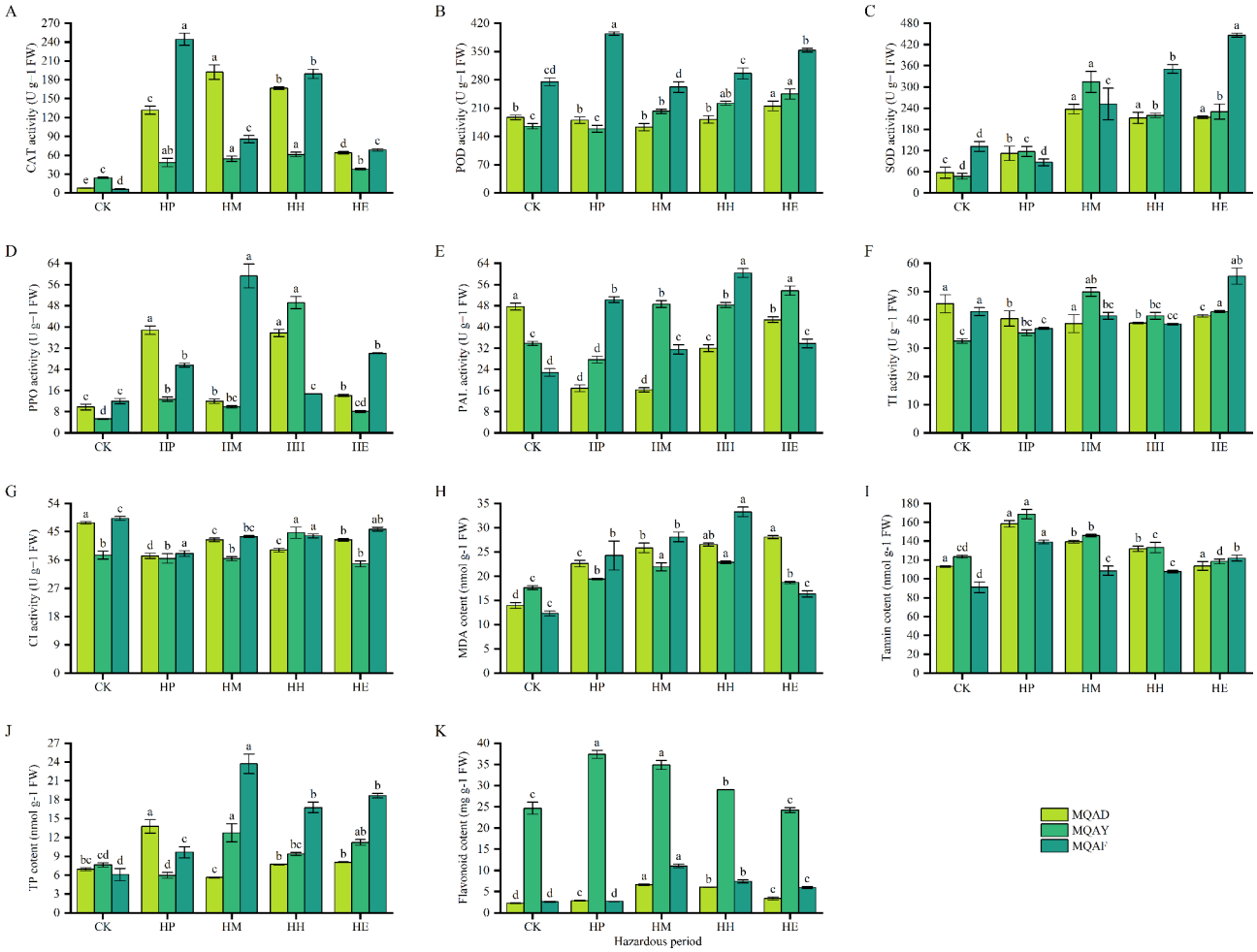

Fig. 6. Effects of feeding by *Tephritis angustipennis* on antioxidant enzyme activities and toxic secondary metabolites contents in flowers of highly susceptible (HS) varieties of *Aster* spp.

Discussion

Relationship between variety, population density and damage level of ten varieties and its resistance to insects

Development and reproduction are crucial factors in how pests adapt to host plants[49]. Previous studies show that herbivorous insects respond not only to variations between different plant species but also to differences within varieties of the same species[50,51]. For instance, significant variations in growth, development, reproduction, and population density of *Paracoccus marginatus* have been observed among different cassava varieties[28]. Our study revealed that population densities of TAEs and TaLs varied across *Aster* varieties, with significantly higher densities on susceptible varieties than on resistant ones. Notably, none of the non-susceptible varieties (MQAA) showed susceptibility, leading us to classify MQAA as highly resistant (HR), while BMAT, MQAP, QLAF, and MQAA were categorized as moderately resistant (MR). Conversely, QLAD from Qilian and DRAS from Dari moderately susceptible (MS), and MQAD, MQAY, and MQAF from Maqin County (Guoluo Tibetan Autonomous Prefecture) were highly susceptible (HS). These classifications highlight a negative correlation between plant resistance and pest population density, underscoring the use of insect population density at different developmental stages as a critical indicator for evaluating *Aster* species' susceptibility and resistance to *T. angustipennis*.

Protective enzymes and their role in insect resistance

Insect infestations trigger the production of reactive oxygen species (ROS) in plants, causing potential cellular damage[52]. In response, plants activate antioxidative enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) to mitigate ROS damage, maintain oxidative balance, and initiate defense signaling cascades[53]. These enzymes are critical in protecting plant cells and enhancing overall resistance to biotic stress[54]. Our study revealed that resistant *Aster* varieties consistently exhibited higher levels of CAT, POD, and SOD activities throughout various pest feeding levels, particularly in the early levels of infestation. This early and sustained enzyme activity appears to be a key factor in their stronger resistance to pests compared to susceptible varieties.

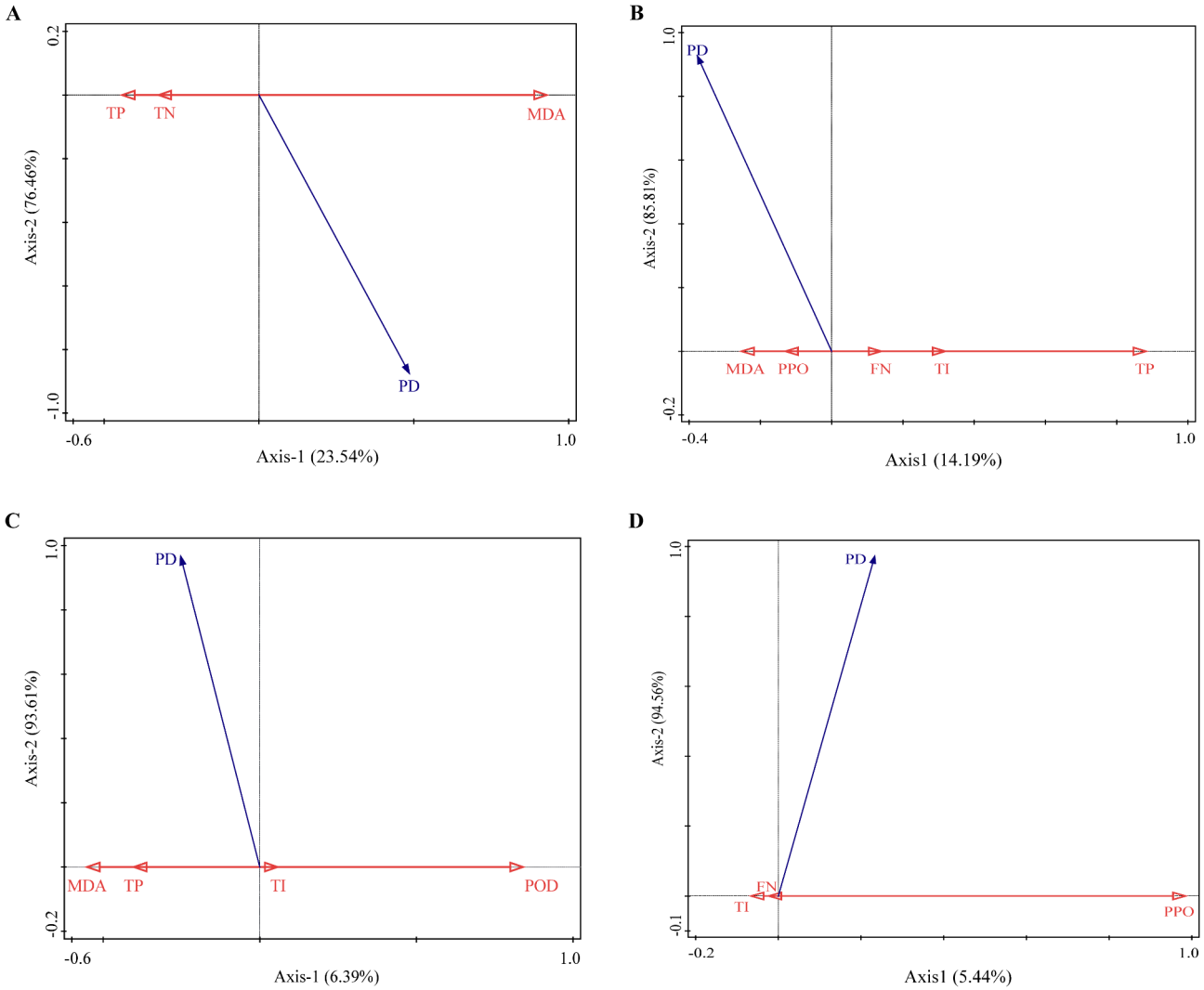

Fig. 7. Redundancy analysis (RDA) of different resistant *Aster* samples used 11 resistant indicators, and one environmental parameter. In the end, indicators with low contribution rates were deleted and only those with high contribution rates were retained for the final analysis. The red vectors in the graph indicate resistant indicators: peroxidase (POD), Polyphenol oxidase (PPO), Trypsin inhibitor (TI), Malondialdehyde (MDA), tannin (TN), total phenols (TP), and flavonoids (FN). The blue vectors indicate the environmental variables: insect population density (PD). (**A**–**D**) are the results of RDA analyses of highly resistant, moderately resistant, moderately susceptible, and highly susceptible types, respectively.

Our research shows that these enzymes show distinct temporal patterns. During control conditions (CK), CAT and SOD activities were generally higher in SR and MR varieties compared to MS and HS varieties, whereas POD activity was slightly lower or comparable to susceptible varieties This suggests that initial CAT, POD, and SOD levels correlate with *Aster* spp. resistant. With pest occurrence and prolonged feeding, CAT, POD, and SOD activities varied significantly in different resistant *Aster* spp. HR and MR varieties consistently exhibited higher CAT, POD, and SOD activities during most stages of infestation and development, indicating their resistance is linked to induced expression strength during pest infestation levels, the enzyme systems that play a major role at different hazard levels are different. CAT activity often peaks during initial infestation (HP or HM), like studies on protective enzymes in small onion (*Allium cepa*) during different tobacco thrips damage levels[55] and after *Tetranychus truncatus* damage on *Solanum tuberosum*[56]; reflecting its role in rapidly detoxifying H_2O_2. SOD, being a first-line defense, quickly scavenges superoxide radicals, while POD, which adapts to metabolic and environmental shifts, maintains longer-term oxidative stability. This sequence suggests a well-coordinated, time-dependent antioxidant response that aligns with pest feeding pressures. This finding aligns with studies on bean leaves and cotton leaves, suggesting *T. angustipennis* feeding induces oxidative stress and secondary metabolite pathways in *Aster* spp. development[57], but the exact mechanism needs to be further validation.

Furthermore, changes in CAT, POD, and SOD activities indicate a stress response, closely tied to pest damage; this stress induces plant responses, with *T. angustipennis* feeding effectively stimulating protective enzymes, particularly the antioxidant system, to mitigate ROS and enhance physiological defense against insect stress in

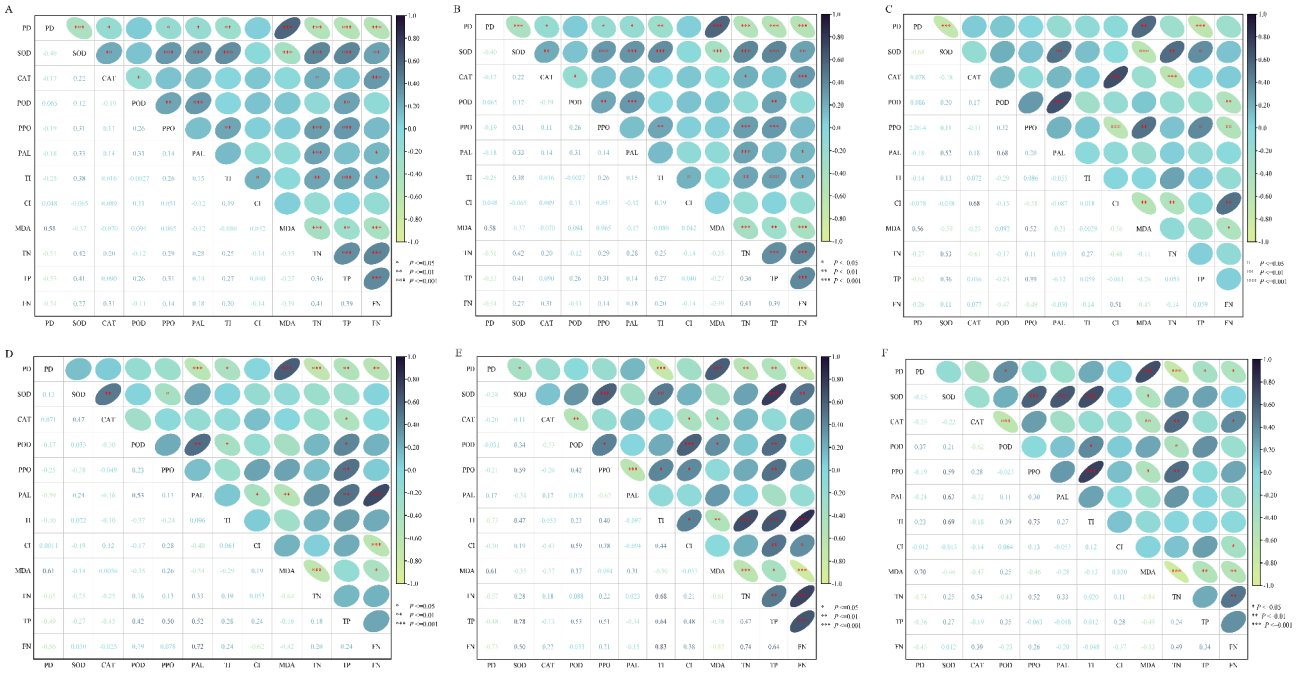

Fig. 8. Correlation analysis of enzyme activities and secondary metabolite contents of different damage levels of *Aster* spp. with insect population density. (**A**) Exemplify the comprehensive data analysis for individual indicators, (**B**–**F**) represents the results of correlation analysis of five different levels, namely CK, HP, HM, HH and HE, respectively.

Category	Dependent variables	Model	Predictors	*R*	R^2	F	*P*
CK	PD	1	FN	0.512	0.262	9.936	0.004
HP	PD	1	MDA	0.683	0.466	24.472	0.000
HM	PD	1	TN	0.596	0.355	15.435	0.001
		2	TN, FN	0.665	0.443	10.724	0.000
HH	PD	1	FN	0.621	0.385	17.536	0.000
HE	PD	1	TN	0.489	0.239	8.806	0.006
ALL	PD	1	MDA	0.491	0.241	47.326	0.000
		2	MDA, FN	0.556	0.309	33.141	0.000
		3	MDA, FN, TN	0.578	0.334	24.610	0.000

Table 4. Model summary and ANOVA of the Stepwise regression analysis in the 11 resistance indicators and environmental factors at different hazard levels. Note: CK, HP, HM, HH and HE representing different hazard levels (Hazardous period), namely non-harm (excellent quality), slight hazard, moderately hazard, serious hazard and more serious hazard, respectively. ALL exemplify the comprehensive data analysis for individual indicators. PD, MDA, TN, FN stand for insect population density, Malondialdehyde, tannin, and flavonoids content, respectively.

asters. The results of the correlation analysis confirm this. Distinct temporal patterns in enzyme activity suggest a coordinated response: CAT activity peaks early to detoxify H_2O_2, SOD quickly neutralizes superoxide radicals, and POD sustains long-term oxidative stability. Together, these enzymes form a multi-layered defense system that reduces ROS-induced stress and helps suppress pest growth. Such findings suggest that breeding programs could prioritize Aster varieties with robust and persistent antioxidant enzyme activities, leading to crops with greater resilience against pest infestations.

Defensive enzymes and structural defenses

Defensive enzymes such as polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) are central to secondary metabolite production and structural reinforcements against insect feeding[58][98–100]. These enzymes contribute to structural defenses by driving the production of lignin, flavonoids, and phenolic compounds. These metabolites reinforce plant cell walls, form physical barriers, and deter further pest feeding[58]. During control conditions (CK), PPO activity was lowest in HS varieties of *Aster* spp. During *T. angustipennis* infestation's high feeding (HP) level, activities of PPO and PAL in resistant varieties increased more rapidly than in susceptible

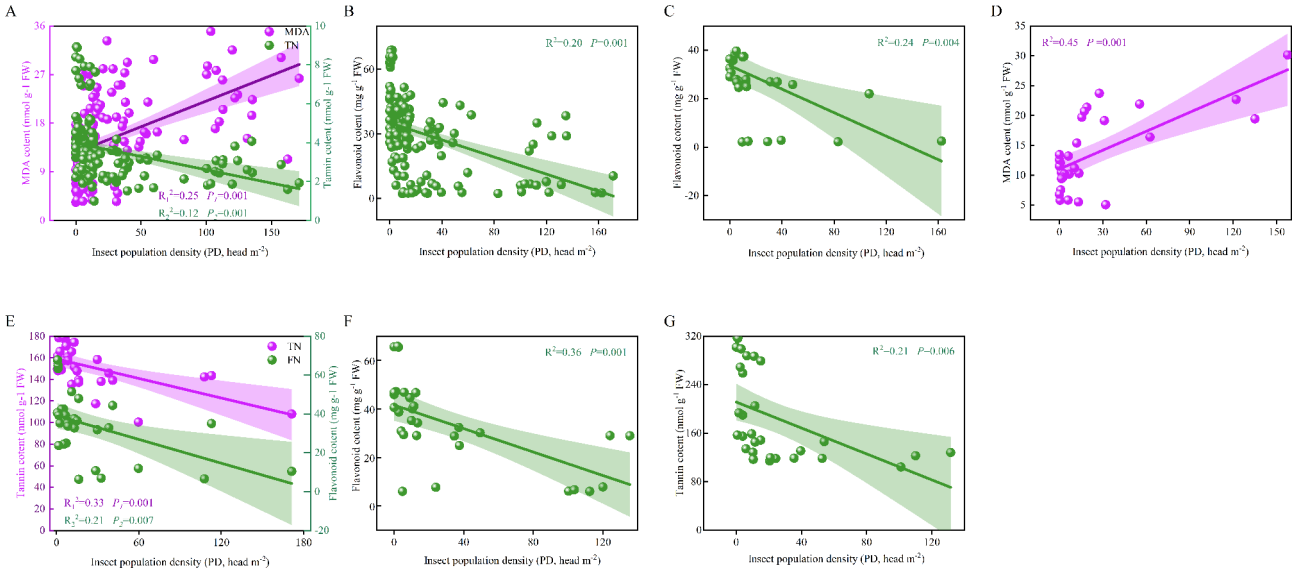

Fig. 9. Optimized fitted curves from stepwise regression analysis were employed to correlate 11 resistant indicators with environmental factors such as insect population density (PD) across various hazard levels. (**A**–**B**) Exemplifies the comprehensive data analysis for individual indicators, whereas (**C**–**G**) signifies the stepwise regression analysis outcomes for five distinct levels, denoted as CK, HP, HM, HH, and HE.

ones, with MR varieties showing notably higher enzyme activities than others. Notably, our experiment showed that HR and MR varieties exhibited significantly higher PPO and PAL activities compared to susceptible ones, particularly during high pest feeding stages (such as HM, HH, HE). The induction of these enzymes was more rapid and sustained in resistant varieties (such as MQAA, QLAF, BMAT, DRAA, and MQAP), emphasizing their pivotal role in early and long-term defense responses. This indicates that a prompt and sustained induction of these enzymes helps establish effective structural defenses. Consequently, this results consistent with reports of significantly elevated PPO and PAL activities due to thrips feeding on resistant alfalfa varieties[59].

PPO, known for its role in polymerizing phenolic compounds into defensive barriers. Compared to PAL, in this study, the feeding-induced PPO response to *T. angustipennis* were displayed a lagged but sustained response in resistant varieties. PAL activity in HR varieties significantly increased ($P < 0.05$) during the HM, whereas PPO activity peaked only during the HH. In MR varieties, PAL activity rose significantly during the HP, and peaked during HM and HE, whereas PPO activity significantly increased during HM to HE. This pattern parallels findings by Tang et al. on apple fruit's phenolic metabolism under varying gray mold disease resistant[60]. This delayed peak may be tied to the time required for cross-linking phenolic compounds into a physical barrier.

PAL, on the other hand, rose quickly in response to initial feeding, underscoring its function in producing precursor compounds for downstream defenses. In our study, TP content significantly decreased in both HR and MR varieties during HP caused by pests, minimum during HM or HH, while PAL activity for TP synthesis in resistant species notably decreased during HH. This underscores TP as the primary insect-resistant substances in *Aster* spp., with PAL and TP crucial in the plant's anti-corrosive response. However, PAL may preferentially synthesize phenolic acid defense substances like TP in HR and MR, potentially leading to less synthesis than consumption, thereby showing a decreasing trend in PAL activity during HH. In both MS and HS types, PAL contents increased significantly during the HH period, but TP content decreased significantly, indicating that depletion was markedly greater than synthesis, so TP contents play a crucial role in the insect resistant of *Aster* plants, especially in the insect-sensitive *Aster* spp., as evidenced by related analyses. These results indicate that PPO's role in polymerizing phenolic compounds into protective barriers occurs after an initial rise in PAL activity, which provides precursor compounds. This temporal sequence ensures that early chemical defenses quickly counter pest intrusion, while longer-lasting structural defenses maintain protection over time. By focusing on *Aster* varieties that exhibit both rapid PAL responses and durable PPO activities, breeding efforts can enhance resistance mechanisms and reduce reliance on chemical pest control methods.

Inhibitory enzymes and their direct impact on pests

Protease inhibitors such as chymotrypsin inhibitor (CI), and trypsin inhibitor (TI) disrupt insect digestion, slowing growth and reproduction[61]. It is apparent that high CI and TI activity during non-damaged levels correspond to highly resistant in this study, which may be related to the nature of the plants themselves. Following damage by *T. angustipennis*, concurrent with pest infestation and increased feeding time, significant changes in TI and CI activities were observed across various resistant *Aster* spp., with enzyme activities notably increasing overall and showing significant variations over time with pest damage ($P < 0.05$). Specifically, HR and MR varieties consistently maintained higher levels of CI and TI activities compared to susceptible varieties (MS and HS), with activity peaks aligning closely with intense pest feeding periods. HR varieties exhibited a pattern of declining, then increasing inhibitor activity, while MR varieties showed sustained rises during heavy feeding

levels. This suggests that the timing and magnitude of protease inhibitor activity are critical for maintaining pest resistance.

In MS and HS varieties, TI and CI activities showed slight increases during the HM and HH, but not throughout the damage period, suggesting that resistant types are more capable of responding rapidly than susceptible types of *Aster* and eliciting an anorexic response in insects by regulating the accumulation of PIs. These findings suggest that *Aster* spp. may adjust resource allocation to activate more effective defense mechanisms during levels of heightened insect density. Furthermore, TI showed a greater increase than CI in HR and MR varieties during the HM and HH, suggesting a potentially greater role of TI in *Aster* resistant. In a word, the dynamic of CI and TI activity changes reflects a strategic defense mechanism. Initial reductions in activity might allow for rapid resource allocation toward immediate defenses, while subsequent increases strengthen the plant's longer-term resistance. This adaptive response not only hinders pest digestion but also delays reproduction cycles, effectively reducing pest populations over time. Breeding efforts can target *Aster* varieties with strong and well-timed TI and CI responses, improving pest management strategies and minimizing the need for external chemical treatments.

Secondary metabolites as integral components of resistance

Secondary metabolites such as tannins (TN), phenols (TP), and flavonoids (FN) play crucial roles in deterring pests and safeguarding plant tissues[62–64]. Additionally, malondialdehyde (MDA), a key indicator of oxidative stress and membrane damage, provides valuable insights into the intensity of pest-induced stress[65]. Our results demonstrated that HR and MR varieties consistently maintained higher levels of TN, TP, and FN compared to susceptible varieties, while showing lower MDA accumulation. This suggests that the ability to limit membrane damage under pest pressure is closely associated with increased pest resistance. The relationship between various metabolites and plant insect resistance is discussed separately below:

Relationship between plant phenols (TP) and insect resistance of Aster spp

In our study, we observed significant variations in total phenol (TP) content among different *Aster* varieties, in the pre-infestation period, resistant varieties exhibit significantly higher TP content compared to susceptible varieties, especially evident in MR varieties, where TP content decreases significantly post *T. angustipennis* infestation with a strong negative correlation to PD; conversely, susceptible varieties show a notable increase in TP content. The decrease in TP content correlated with reduced insect growth, development, and fecundity, whereas the elevated TP content in susceptible plants indicates a defensive response to insect damage. with resistant types showing a pronounced increase in TP following *T. angustipennis* infestation compared to susceptible ones. This increase in TP content correlated with reduced insect growth, development, and fecundity. Despite high TP content in HS varieties, TP levels decreased significantly post-pest damage, whereas PAL activity, crucial for TP synthesis, increased during this period, indicating PAL's role in HS variety resistance. PAL likely synthesizes defense compounds like phenolic acids, potentially exceeding their utilization, thus showing increased activity during peak damage levels.

Changes in phenols and oxidative enzyme activities are common defenses against herbivorous insects[66]. Our study revealed significant increases in antioxidant enzyme activities (CAT, POD, SOD, PPO, and PAL) and TP content in most resistant *Aster* spp. following *T. angustipennis* infestation, highlighting their role in cyclic reduction of ROS and subsequent activation of defense mechanisms[67]. Antioxidant enzyme activity analysis provided insights into plant-pest interactions, showing *Aster* spp.'s ability to enhance TP content and antioxidant enzyme activities in response to *T. angustipennis*, with HS and MS varieties exhibiting substantial increases during the HP, significantly higher than resistant types. In contrast, MR and HR varieties showed marginal decreases during HP, but TP content in all types of periwinkles significantly increases during the HH, while PD on plants at various stages of pest infestation shows a significant negative correlation with TP content, indicating stronger responsiveness to pests, and it also suggests that resistant to *T. angustipennis* in resistant *Aster* spp. is strongly correlated with the plant itself.

Relationship between tannins (TN) and insect resistance of Aster spp

Our study demonstrated significant effects of species and infestation levels on TN content ($P < 0.01$), with resistant *Aster* varieties exhibiting higher TN contents post-pest infestation compared to susceptible ones, suggesting TN's role in conferring resistant. In addition to TN, reductions in digestive enzyme activities (amylase, trypsin) in *Frankliniella occidentalis* when transferred to less-preferred hosts underscored the role of secondary metabolites in influencing insect fitness[68].

Relationship between malondialdehyde (MDA) and insect resistance of Aster spp

MDA content serves as a key indicator of oxidative damage in plant cell membranes under biotic and abiotic stress[69]. Our study revealed significant effects of variety and damage period on MDA content ($P < 0.01$), analyzing the relationship between *Aster* resistant and PD of *T. angustipennis* on *Aster* varieties revealed that population density on *Aster* species was closely related to MDA. With susceptible *Aster* types exhibiting higher MDA content than resistant types post-pest infestation, indicating greater membrane damage in susceptible plants. Similar trends have been observed in other studies on alfalfa and groundnut, where susceptible varieties showed higher MDA contents under insect infestation[65]. MDA was a major influence on population density during the HR classes of *Aster* and the HP of pest damage. It is also worth noting that despite significant increases in MDA content across all *Aster* types during HH, MS and HS types exhibited lower increases compared to SR and HR types (Figure S3A), indicating less severe cell membrane damage in resistant types during heavy *T. angustipennis* attack, and this result also showed the damage to the cell membrane system of susceptible plants was less than

that of resistant plants, which may be related to the rapid response of resistant types of *Aster* varieties in the early stage of damage to avoid the damage of pests, consistent with previous research findings.

Relationship between flavonoids (FN) and insect resistance of Aster spp

Flavonoids (FN), essential products of plant secondary metabolism, are widely distributed and play critical roles in plant responses to stressors[70]. Our research observed significant variations in FN content among *Aster* spp., with HR and MR types displaying an increase in FN content post-infestation, notably during the HH compared to MS and HS varieties. Apart from the HP, PD of *T. angustipennis* showed a significant negative correlation with FN content at all other infestation stages. Particularly in the CK and HH, FN content emerged as the primary influencing factor on PD, highlighting the significant association between insect resistant in plants and initial FN content. Furthermore, during high infestation periods (under severe infestation levels), a higher insect density corresponded to lower FN content, indicating a more severe level of damage, underscoring the critical role of FN in insect resistant among *Aster* varieties. What is noteworthy is that this slower but sustained increase in FN content in resistant types contrasts with the rapid but short-lived response in susceptible types, highlighting the adaptive advantage of resistant *Aster* spp. against *T. angustipennis* infestations.

Overall, the multiple analyses of this study revealed that TN levels peak at later stages of infestation, providing long-term toxic deterrence. TP acts earlier, reducing pest growth and reproduction through metabolic disruption, while FN shows a more sustained increase, contributing to long-term oxidative stress mitigation. Meanwhile, resistant varieties displayed lower MDA levels across all infestation stages, indicating less oxidative damage and stronger cellular integrity. In contrast, susceptible varieties exhibited higher MDA levels, reflecting more severe cellular damage and reduced resilience. By integrating these findings, it becomes clear that the coordinated actions of TN, TP, FN, and the suppression of MDA contribute to a multi-faceted resistance strategy in *Aster* plants. For pest management, understanding these biochemical responses provides new avenues for monitoring pest pressures and identifying the most resilient varieties of *Aster*. In breeding applications, selecting varieties that combine elevated secondary metabolite levels with reduced MDA accumulation can yield crops with more robust and sustained pest resistance. This, in turn, supports more sustainable agricultural practices by reducing reliance on chemical controls and promoting the long-term health and productivity of *Aster* varieties. Future research could focus on the molecular pathways regulating resistance enzyme activity and secondary metabolite levels to further elucidate their roles in pest resistance and improve the selection criteria for developing more resilient varieties of *Aster*.

Conclusion

This study examined the physiological and biochemical responses of ten asters to *T. angustipennis* by evaluating insect population densities, plant damage rate, enzymatic activities, and insect-resistant metabolites. Significant differences were found in the population densities of *T. angustipennis* eggs and larvae, with highly resistant varieties exhibiting minimal pest infestation. enzyme activities of CAT, POD, SOD, PPO, PAL, TI, and CI were notably higher in HR and MR varieties, indicating stronger resistance, while MDA levels were higher in susceptible types.

The analysis revealed that pest feeding significantly influenced the activity of these enzymes and the content of insect-resistant metabolites across different varieties of *Aster*. Specifically, HR and MR varieties showed enhanced activities of defensive enzymes and higher contents of insect-resistant compounds like TN, TP, and FN, indicating stronger pest resistance. In contrast, MDA content was elevated in MS and HS varieties, suggesting greater susceptibility. Furthermore, the relationship between enzyme activities and *T. angustipennis* population density was analyzed, revealing negative correlations with several enzyme activities and metabolite contents, and a strong positive correlation with MDA levels. Stepwise regression analysis identified MDA, FN, and TN contents as key factors influencing pest density across different infestation levels. In conclusion, the study highlights the biochemical markers, such as enzyme activities and metabolite levels, that are essential for assessing the resistance of *Aster* spp. to *T. angustipennis*. These findings contribute to understanding plant resistance mechanisms and offer valuable insights for identifying and breeding more resistant asters varieties for pest management and ecological conservation.

Data availability

The data that support the findings of this study are openly available in Mendeley Data at https://data.mendeley.com/datasets/ty5cy4y5hn/1. And the datasets generated and analyzed during the current study are also available from the corresponding author on reasonable request.

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Author contributions

L.-J.Z. (first author) collected the materials, conception and designed the experiment. Y.-L.W., Y.M., L.-L.X., and X.-Y.W. participated in the collection of experimental materials and the whole experimental process. And L.-J.Z. analyzed, interpretation of the data, and drafting of the manuscript. After that, Y.-S.M. and Y.L. revised it critically for intellectual content of this article and agreed to the final approval to be published. All authors reviewed the manuscript and agreed to be accountable for all aspects of the work.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This research does not contain any experiments using any animal species that require ethical approval, and research on plant protection enzymes and secondary metabolites does not require ethical approval.

Consent to participate

Consent was given by all participants included in the study.

Consent for publication

All authors consent to the publication of this manuscript in Scientific Reports.

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