

Simulation analysis and physiological and biochemical evaluation of *Sophora flavescens* aboveground against aphids using network pharmacology

Chun-Yan Guo

Qiqihar Medical University

Bao-Chang Zhou

Inner Mongolia Medical University

Yi-Bo Liu

Inner Mongolia Medical University

Hui Niu

Baotou Medical College

Li-Juan Lv

Tianjin Agricultural University

Min-Hui Li

prof_liminhui@yeah.net

Qiqihar Medical University

Research Article

Keywords: Network pharmacology , Molecular dynamics , *Sophora flavescens* Alt. , Aphid , Enzymatic activity

Posted Date: July 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1809565/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Pesticide Biochemistry and Physiology on January 1st, 2023. See the published version at <https://doi.org/10.1016/j.pestbp.2022.105308>.

Abstract

Pests have caused substantial damage to the human environments; therefore, studying insecticidal mechanisms is crucial for improving pest control. However, irreversible secondary damage caused by chemical pesticides must be considered. In this study, we used network pharmacology to determine that *Sophora flavescens* Alt. mainly acts on glucose-6-phosphate 1-dehydrogenase, thymidylate synthase, and translocation protein in aphids, thereby resulting in effective pest control. The stability and reliability of target proteins were analyzed by molecular docking and dynamics simulations. Enzyme activity assays validated the feasibility of network pharmacology to obtain actionable targets. We used interdisciplinary integration to study pest control and used network pharmacology to identify how *Sophora flavescens* Alt. resists aphids. The results show that network pharmacology increases the accuracy and specificity of predicted molecules targeted by insecticides. Our approach will facilitate improved, environmentally friendly pest control.

1. Introduction

In recent years, pests have negatively impacted human life and greatly reduced the overall quality of life (Islam et al. 2020). Owing to parthenogenesis and alternation of generations, aphid elimination is a worldwide problem. Aphids feed on plant sap using their sharp mouthparts, resulting in plaque and wilt, reducing plant growth, and ultimately lead to the death of the plant (Ling et al. 2020). For many years, insect pests were mainly controlled using chemical pesticides. However, the use of highly toxic and persistent chemical pesticides leads to excessive pesticide accumulation in farmland and agricultural products, and can result in poisoning of livestock and consumers (Lambert et al. 2020; Shaalan and Canyon 2018). Thus, it is urgently necessary to replace chemical insecticides with better alternatives. Compared with chemical pesticides, most botanical pesticides are non-destructive and have low green residue and high selectivity (Ling et al. 2020). Thus, botanical pesticides can be considered an environmentally friendly alternative to chemical pesticides in controlling agricultural pests.

Sophora flavescens Alt (*Sophora flavescens*). is a plant that is often used in traditional Chinese medicine to treat a variety of diseases. This species was first described in the Compendium of Shennong Materia Medica and has a long history of use (Chen et al. 2020). Owing to its insecticidal activity and environmental friendliness, *Sophora flavescens* is among the most widely used plant insecticides. For example, Cao et al. (2019) investigated the indoor and field insecticidal activities of *Phyllostachys pubescens* leaves, while Xu et al. (2020) studied the mechanism of matrine-cholesterol derivatives. Additional studies investigated the effects of two types of matrine on pea aphids (Ling et al. 2020). In our previous study (2018-2020), we investigated farms in eastern Inner Mongolia where local vegetation was sprayed with the aboveground part of *Sophora flavescens* in water to control aphids. Most people only pay attention to the value of the *Sophora flavescens* root and seldom use the aboveground part. Although the alkaloids in the aboveground part of *Sophora flavescens* are valuable, people do not attach great importance to them, leading to a serious waste of resources. Therefore, we investigated the aboveground part of *Sophora flavescens* for potential applications in environmental protection and energy recycling.

Currently, most research on the insecticidal mechanism of *Sophora flavescens* focuses on enzymatic activity (Al-Ayedh et al. 2016; Almeida et al. 2021; Clark 2018). Traditional methods cannot keep pace with environmental degradation. Network pharmacology is widely used in humans, mice, rats, and other organisms; however, the lack of pest labeling data and the subjectivity of artificially tagged data have greatly impacted agricultural development. Furthermore, investigating the mechanism of action of harmful insects is challenging. Thus, we investigated the insecticidal mechanism of the aboveground part of *Sophora flavescens* using network pharmacology, molecular docking, and molecular dynamics simulation. We provide the first complete and comprehensive description of how *Sophora flavescens* targets aphids. Subsequently, we tested how glucose-6-phosphate 1-dehydrogenase (G6PD), thymidylate synthase (TS), and translocation protein (TSPO) activity changes in aphids treated with the aboveground part of *Sophora flavescens*.

2. Materials and methods

2.1 Plant material

The aboveground part of *Sophora flavescens* was dried, crushed, filtered through a 0.25-mm (60 mesh) sieve, mixed with 60% ethanol containing 0.02% ammonia (1:6), and stirred overnight at 60 °C. The extract was sonicated using 19-kHz ultrasonic waves, and filtered. The filtrate was collected and the solid portion was re-extracted as described above. Subsequently, the water in the filtrate was removed by vacuum rotary evaporation. The crude extract of the aboveground part of *Sophora flavescens* was obtained by centrifugation at 3,000 rpm for 10 minutes.

2.2 Aphids and reagents

Myzus persicae (Sulzer) was reared at 25 °C and 70% humidity (Akca et al. 2015; Mseddi et al. 2022). The host plants *Brassica campestris* L. ssp. *chinensis* Makino (var. *communis* Tsen et Lee) (A) and *Datura stramonium* Linn. (B) were reared at low density. The photoperiod was set at 12:12 (light:dark) (Colizzi et al. 2021; Nakazato et al. 2021). *M. persicae* grown on pakchoi and mandarin were used as the experimental insects. Uniform *M. persicae* were selected for the experiment. The crude extract of the aboveground part of *Sophora flavescens*, matrine, oxymatrine, sophocarpine, and sophoridine (95% purity) (Chengdu Desite Biotechnology Co., Ltd.) were dissolved in acetone solution containing 20% (Colizzi et al. 2021; Nakazato et al. 2021). The compounds were tested at 100 µM, 1 mM, 10 mM, and 100 mM to determine the activity of G6PD, TS, and TSPO using a kit (Jiangsu enzyme industry Co., Ltd) (Ma et al. 2020). Each experimental compound or blank (5 µL per aphid) was locally applied to early third instar aphids. Sixty aphids were treated per experiment, and each experiment was repeated 3 times. Aphids in the control group were only exposed to the solvent (20% acetone). G6PD activity was examined 12 and 24 h after treatment; TS activity was tested 0.5, 24, and 36 h after treatment, and TSPO activity was tested at 0.5, 12, and 24 h. The collected samples were frozen in liquid nitrogen for 10 minutes and stored in a freezer at -80 °C.

2.3 High-performance liquid chromatography/mass spectrometry

Sophora flavescens powder (100 mg) was added to 1 mL water. The mixture was sonicated and centrifuged at high speed for 10 min. Chromatographic analysis of crude *Sophora flavescens* extracts was performed using SCIEX X500 QTOF system (SCIEX, USA). The high performance liquid chromatography (HPLC) conditions were mobile phase A: 0.05% formic acid in H₂O and B 0.05% formic acid in methanol:acetonitrile (1:1). Gradient elution was set for 30 minutes at a flow rate of 0.3 mL/min and an injection volume of 1 µL. Separation was performed using a T3 column (100 × 2.1 mm, 1.7 µm). Table 1 shows the specific time and flow rate equalization parameters for gradient elution. Subsequently, we set the mass spectrometer (MS) to scan *m/z* 100–1300 (MS1), MS/MS mode to 10 ms/MS, MS2 to scan *m/z* 50–1300, and dynamic background subtraction. The source gas parameters were set to 55 psi for ion source gas 1 and 55 psi for ion source gas 2. Curtain gas was set at 30 psi, and the temperature was set at 550 °C.

Table 1. Flow program of HPLC.

	Time	Flow	A-phase	B-phase
1	0.00	0.3000	100.0	0.0
2	2.00	0.3000	100.0	0.0
3	5.00	0.3000	95.0	5.0
4	15.00	0.3000	80.0	20.0
5	21.00	0.3000	50.0	50.0
6	23.00	0.3000	10.0	90.0
7	25.00	0.3000	10.0	90.0

2.4 Screening common targets of active components in *Sophora flavescens* and protein-protein interaction network construction

Compounds identified by HPLC-MS/MS were searched on the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>). Subsequently, the 2D structures and chemical formulae were imported into SwissADME (<http://www.swissadme.ch/>). If the drug-likeness score was ≥3, the compound was imported into SwissTargetPrediction to predict possible targets (<http://www.swisstargetprediction.ch/>). The predicted target information was compared to aphid target information from the Uniprot database (<https://www.uniprot.org/>) to identify the common target (Szkarczyk et al. 2021). The obtained target protein was input into the string webtool (<https://string-db.org/>) to obtain the protein interaction diagram for each compound in *Sophora flavescens* (Boutet et al. 2016). We set the lowest correlation coefficient of protein interactions to 0.15 to construct an interaction network to identify the pathways targeted by *Sophora flavescens* compounds. The core targets were visualized using Cytoscape software.

2.5 Construction of *Sophora flavescens*-aphid interaction networks

The chemical composition-target interaction network between aphid disease and the aboveground part of *Sophora flavescens* was constructed using Cytoscape 3.7.2 software to explore the potential pharmacological mechanism of the identified compounds. In the network map, a node represents a drug, diseases, active ingredient, or target. Edges represent the relationship between active aphids, the aboveground parts of *Sophora flavescens*, and the molecular targets.

2.6 Molecular docking simulations

The chemical formula of each active compound was downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and used as input for the Swiss model to predict the tertiary structure of large molecules in aphids targeted by the compound. For target sequences with template sequence consistency >30%, PyMOL software was used to remove water and phosphate from the protein (Pinzi and Rastelli 2019). AutoDockTools 1.5.6 software was used to convert the PDB format of drug active ingredients and core protein gene files to PDBQT format. Subsequently, the structures of the binding pockets were retrieved. Finally, a Vina script was used to calculate the molecular binding energy.

2.7 Molecular dynamics simulations

GROMACS v2021.03 (a Theoretical Biophysics, Science for Life Laboratory, KTH Royal Institute of Technology, Solna, Sweden) molecular dynamics simulation software was used to model compound/target binding (<http://creativecommons.org/licenses/by/4.0/>). The predicted protein complex from the docking analysis was used as the initial conformation. All-atom molecular dynamics simulations of action were used to verify the reliability of the binding mode (Van Der Spoel et al. 2005). Amber99SB-ILDN force field parameters were used for the receptor protein. Topology of the gaff-2 type ligand molecules were generated using AnteChamber PYthon Parser interfacE and Antechamber programs. The dodecahedral solvation box was selected. The closest distance between the system boundary and the complex was set to 1.1 nm. The TIP3P water model was selected and 15 Na⁺ were randomly added to the system according to the verlet cut-off method to counteract the protein charge. Subsequently, the system energy was minimized, the canonicalensemble temperature was controlled, and the constant-pressure, constant-temperature was set to maintain the system temperature at 300 K and the pressure at 101.325 kPa. Subsequently, a 0-100 ns free kinetic simulation was performed for this equilibrium system. The binding free energy between protein and ligand was calculated using the Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) method (Kumari et al. 2014).

2.8 Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

We used the clusterProfiler in R (cluster Profiler GO.R package in <https://www.r-project.org/>) to analyze the common targets of active components and aphid proteins. The corresponding KEGG signal pathway map of aphid resistance to compounds in the aboveground part of *Sophora flavescens* was generated using the David 6.8 database (<https://david.ncifcrf.gov/tools.jsp>).

2.9 Complex network topology analysis

Degree value calculation was performed using the cytohubba plug-in. These values were combined using Maximal Clique Centrality (MCC) operation mode in the Local-based method. Closeness, radiality, and betweenness in the global-based method was used to identify the common target genes and verify the KEGG analysis results.

2.10 Enzyme assays and statistical analysis

The samples were prepared as described in Section 2.3. We used the double antibody sandwich method to measure G6PD, TS, and TSPO in *M. persicae*. *Detailed operation procedures are described in the kit instructions.*

Data were analyzed using single factor ANOVA in IBM SPSS (version 20.0) ($p<0.05$). GraphPad Prism 9.0 software was used to analyze the differences between treatment time, drugs, and host plants ($p<0.05$).

3. Results

3.1 Determination of chemical composition

Sixty-four compounds were identified by HPLC-MS/MS analysis. The chemical composition of *Sophora flavescens* was determined by retention time, main fragment ions, and literature analysis. The total ion chromatogram of *Sophora flavescens* in positive ion mode is shown in Fig. 1. The chromatogram suggested the presence of 64 compounds (Table 2). We identified 11 chemical constituents of *Sophora flavescens* alkaloids and used them to find target information in the TCMSP database (Chen L et al. 2020; Ding et al. 2006; Liu et al. 2006; Liu et al. 2010; Liu et al. 2011; Wan et al. 2009; Wang et al. 2012; Weng et al. 2016; Zhao DR et al. 2018). Next, we compared the target information with aphid target information to find common targets for network pharmacological analysis. We also found several alkaloids (matrine, oxymatrine, sophocarpine, and sophoridine) that are commonly used in insecticides and are abundant in *Sophora flavescens*. The fragmentation patterns of these key alkaloids are shown in Fig. 2.

Table 2 The compounds in *Sophora flavescens* were identified by LC-MS/MS POS model

Peak no.	Identification	Adducts	Predicted formula	Retention Time (min)	[M+H] ⁺ (m/z)
1	Betaine	[M+H] ⁺	C ₅ H ₁₁ NO ₂	0.98	118.086
2	Trigonelline	[M+H] ⁺	C ₇ H ₇ NO ₂	1.09	138.055
3	Stachydrine	[M+H] ⁺	C ₇ H ₁₃ NO ₂	1.16	144.102
4	L-valine	[M+H] ⁺	C ₅ H ₁₁ NO ₂	1.44	118.086
5	Pipecolinic acid	[M+H] ⁺	C ₆ H ₁₁ NO ₂	1.61	130.086
6	Nicotinic acid	[M+H] ⁺	C ₆ H ₅ NO ₂	1.89	124.039
7	Nicotinamide	[M+H] ⁺	C ₆ H ₆ N ₂ O	2.5	123.055
8	Isoleucine	[M+H] ⁺	C ₆ H ₁₃ NO ₂	2.7	132.102
9	6-Hydroxypurine	[M+H] ⁺	C ₅ H ₄ N ₄ O	3.02	137.046
10	Oxymatrine	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O ₂	3.25	265.191
11	N-methylcytisine	[M+H] ⁺	C ₁₂ H ₁₆ N ₂ O	3.29	205.134
12	N-oxy Sophocarpine	[M+H] ⁺	C ₁₅ H ₂₂ N ₂ O ₂	3.90	263.175
13	Cytisine	[M+H] ⁺	C ₁₁ H ₁₄ N ₂ O	4.03	191.118
14	Phenprobamate	[M+H] ⁺	C ₉ H ₁₁ NO ₂	7.52	166.086
15	5- α -Hydroxy Sophocarpine	[M+H] ⁺	C ₁₅ H ₂₂ N ₂ O ₂	7.72	263.175
16	Adenosine	[M+H] ⁺	C ₁₀ H ₁₃ N ₅ O ₄	8.65	268.104
17	Sophoramine	[M+H] ⁺	C ₁₅ H ₂₀ N ₂ O	9.39	245.165
18	Cordycepin	[M+H] ⁺	C ₁₀ H ₁₃ N ₅ O ₃	9.51	252.109
19	Sophoranol	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O ₂	9.59	265.191
20	Guanosine	[M+H] ⁺	C ₁₀ H ₁₃ N ₅ O ₅	9.64	284.099
21	(+)-9 α -Hydroxymatrine	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O ₂	10.06	265.291
22	Sophocarpine	[M+H] ⁺	C ₁₅ H ₂₂ N ₂ O	11.83	247.18
23	Thymine	[M+H] ⁺	C ₅ H ₆ N ₂ O ₂	11.85	127.05
24	Sophoridine	[M+H] ⁺		11.94	249.196

C ₁₅ H ₂₄ N ₂ O					
25	(-)-14beta-Hydroxymatrine	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O ₂	13.29	265.191
26	Hymecromone	[M+H] ⁺	C ₁₀ H ₈ O ₃	13.87	177.055
27	Protocatechuic Aldehyde	[M+H] ⁺	C ₇ H ₆ O ₃	14.23	139.039
28	Matrine	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O	14.37	249.196
29	Chlorogenic acid	[M+H] ⁺	C ₁₆ H ₁₈ O ₉	14.91	355.102
30	Scopoletin	[M+H] ⁺	C ₁₀ H ₈ O ₄	15.06	193.05
31	Anisaldehyde	[M+H] ⁺	C ₈ H ₈ O ₂	15.09	137.060
32	Syringin	[M+H] ⁺	C ₁₇ H ₂₄ O ₉	15.16	390.176
33	6-Methylcoumarin1	[M+H] ⁺	C ₁₀ H ₈ O ₂	15.31	161.06
34	Angelicin	[M+H] ⁺	C ₁₁ H ₆ O ₃	15.31	187.039
35	Cinnamaldehyde	[M+H] ⁺	C ₉ H ₈ O	15.31	133.065
36	L(-)-Carvone	[M+H] ⁺	C ₁₀ H ₁₄ O	15.67	151.112
37	Isoferulicacid	[M+H] ⁺	C ₁₀ H ₁₀ O ₄	16.08	195.065
38	Oroxin B	[M+H] ⁺	C ₂₇ H ₃₀ O ₁₅	16.41	595.166
39	Genistin	[M+H] ⁺	C ₂₁ H ₂₀ O ₁₀	16.43	704.312
40	Genipin	[M+H] ⁺	C ₁₁ H ₁₄ O ₅	16.60	227.091
41	Dimethoxycoumarin	[M+H] ⁺	C ₁₂ H ₁₄ O ₂	16.72	191.107
42	I-somatrine	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O	16.81	249.196
43	Vitamin B2	[M+H] ⁺	C ₁₇ H ₂₀ N ₄ O ₆	17.1	377.146
44	Ligustilide	[M+H] ⁺	C ₁₂ H ₁₄ O ₂	17.66	191.107
45	Daidzin	[M+H] ⁺	C ₂₁ H ₂₀ O ₉	17.83	417.118
46	Astragalin	[M+H] ⁺	C ₂₁ H ₂₀ O ₁₁	17.89	449.108
47	Calycosin	[M+H] ⁺	C ₁₆ H ₁₂ O ₅	18.2	285.076
48	Eleutheroside E	[M+H] ⁺	C ₃₄ H ₄₆ O ₁₈	18.31	760.302

49	Coumarin	[M+H] ⁺	C ₉ H ₆ O ₂	18.53	147.044
50	Menthone	[M+H] ⁺	C ₁₀ H ₁₆ O	18.65	153.127
51	Calycosin Oglucoside	[M+H] ⁺	C ₂₂ H ₂₂ O ₁₀	18.69	447.129
52	Isorhamnetin	[M+H] ⁺	C ₁₆ H ₁₂ O ₇	18.79	317.066
53	Vitexin	[M+H] ⁺	C ₂₁ H ₂₀ O ₁₀	18.8	433.113
54	6-hydroxydaidzein	[M+H] ⁺	C ₁₅ H ₁₀ O ₅	19.14	271.06
55	Sophoricoside	[M+H] ⁺	C ₂₁ H ₂₀ O ₁₀	19.14	433.113
56	Kaempferide	[M+H] ⁺	C ₁₆ H ₁₂ O ₆	19.4	301.071
57	Ononin	[M+H] ⁺	C ₂₂ H ₂₂ O ₉	20.73	431.134
58	Artemisinin	[M+H] ⁺	C ₁₅ H ₂₂ O ₅	21.27	283.154
59	Maackiain	[M+H] ⁺	C ₁₆ H ₁₂ O ₅	21.41	285.076
60	6''-O-xylosyl-glycitin	[M+H] ⁺	C ₂₇ H ₃₀ O ₁₄	21.64	579.171
61	(+)-Tetrandrine	[M+H] ⁺	C ₃₈ H ₄₂ N ₂ O ₆	22.16	623.312
62	Pedunculoside	[M+H] ⁺	C ₃₆ H ₅₈ O ₁₀	22.3	668.437
63	Senkyunolide A	[M+H] ⁺	C ₁₂ H ₁₆ O ₂	23.18	193.122
64	Sedanolid	[M+H] ⁺	C ₁₂ H ₁₈ O ₂	23.37	195.138

3.2 Construction of interaction networks and *Sophora flavescens* anti-aphid interaction networks

We obtained 57 aphid proteins targeted by the aerial parts of *Sophora flavescens*. The protein-protein interactions and the data in Fig. 1a were imported into Cytoscape 3.7.2 software. Potential core target genes were obtained according to the magnitude of the degree (Fig. 3a) and a *Sophora* active ingredient-aphid target interaction network was constructed. This network included 70 nodes (57 targets and 11 alkaloids) and 4,830 edges (Fig. 3b).

3.3 Molecular docking simulations

The three-dimensional structure of the core protein targeted by *Sophora flavescens* extract was predicted using the SwissProt database (Fig. 4b). The binding energy of core protein to the active component was lower than -5.0 kcal·mol⁻¹, which indicates a stable binding energy of the core protein. The three-dimensional macroscopic and microscopic molecular docking with protein ligands was determined to be

successful using compounds identified by the Vina script. The four alkaloids with the lowest binding energy and the corresponding protein binding effects are shown in Fig. 4a.

3.4 Molecular dynamics verification

3.4.1 Molecular dynamics simulations

Molecular dynamics simulations were used to verify the influence of root mean square deviation (RMSD), rotation radius (Rg), root mean square fluctuation (RMSF), hydrogen bond change curve, and the superposition of the first and last simulation frames (Fig. 5). The RMSD value (Fig. 5A) and Rg value (Fig. 5B) of the complex fluctuated slightly, which indicates that sophoridine- and matrine-ACPyl001689-PA complexes fluctuated at 25 ns and stabilized at 0.3 nm. Sophorine-ACPyl001689-PA fluctuated at 70 ns and stabilized at 0.4 nm, while oxymatrine-ACPyl006453 fluctuated at 70 ns and stabilized at 0.25 nm. The atomic RMSD measures the stability of the system. Rg indicates the structural tightness. The fluctuation at S corresponds to that of RMSD and indicates that the system is in a transitional state between multiple unstable states. We then converted RMSF to b factor values (Fig. 5C). The root-mean-square values of the four compounds are shown in Fig. 5D, which shows 3–5 large fluctuations. These fluctuations all occurred on the protein surface. The hydrogen bond curves showed that the hydrogen bond contact numbers of the four compounds fluctuated from 0–1 (Fig. 5E). Finally, we superimposed the first and last simulation frames. We observed high overlap, which further indicated that the four-pair complex was stable (Fig. 5F).

3.4.2 Binding free energy calculation

Binding free energy analysis showed that the total binding energies of the four complexes were all negative, which indicates that the proteins and small molecules in the four complexes may interact with each other. For each small molecule, the measured values and electrostatic energy values of the complexes were less than 0, indicating that hydrophobic and electrostatic interactions contribute to bonding. However, the polar solvation energies of the four complexes were all more than 40.596, indicating that polar solvation was not conducive to bonding. We further analyzed the energy of each residue (Fig. 6). Sophoridine-ACPyl001689-PA complex binding involved three steps, of which Leu-912 and Tyr-966 had the lowest energy (approximately -9 kJ/mol). The matrine-ACPyl006829 complex was also divided into three states. Ile-611 and Tyr-625 had the lowest energy (approximately -9 kJ/mol). The binding energy of oxymatrine-ACPyl006453 complexes formed two gradients, of which Asp-34, Asp-204, and Asp-240 had the lowest energy (approximately 6 kJ/mol). Aloperine-ACPyl001689-PA complexes also formed two gradients, in which Phe-943, Phe-1163, Tyr-516, and Phe-562 had the lowest energy (approximately -4 kJ/mol). These results were verified by generating a free energy topographic map. Combined with the results of the lowest energy framework, we determined that the small molecules in the four complexes bound stably in the binding pocket (Fig. 7).

3.5 GO and KEGG enrichment analysis

Next, we analyzed the 57 potential target genes of *Sophora flavescens* for GO and KEGG pathway enrichment. The results showed that 57 potential target genes were mainly enriched in synaptic membranes, striated muscle cell differentiation, response to xenobiotic stimulus, response to vitamin, response to toxic substance, and response to oxygen-containing compounds. GO pathway enrichment analysis showed that the target genes were mainly concentrated in pentose phosphate pathway, glutathione metabolism, metabolic pathway, and carbon metabolism (Fig. 8). KEGG analysis showed that the main targets of *Sophora flavescens* extract in aphids were ACYPI000752 (G6PD), ACYPI006453 (thymidylate synthase-like), and ACYPI004550 (translocator protein-like).

3.6 Analysis of complex network topology

Using the degree value calculation method, we combined local and global methods to more comprehensively analyze common target genes and to better verify the KEGG pathway enrichment using multidimensional analysis (Fig. 9). Complex network topology analysis was used to rank common targets by MCC, betweenness, radiality, and closeness (Fig. 3i). The results showed that ACPYI001689-Pa, ACPYI006453, and ACPYI006829 were very stable in combination with *Sophora flavescens* alkaloids. ACYPI000752 (G6PD) and ACYPI006453 (thymidylate synthase-like) were also among the most reliable binding targets. These proteins play a crucial role in the aphidicidal process of *Radix ginseng*. ACYPI004550 (translocator protein-like) is theoretically less reliable than ACYPI000752 (G6PD) and ACYPI006453 (thymidylate synthase-like).

3.7 Enzyme assays

To support our conclusion that ACYPI000752 (G6PD), ACYPI006453 (thymidylate synthase-like), and ACYPI004550 (translocator protein-like) are the key targets of *Sophora flavescens* extract, we examined the activities of G6PD (at 12 and 24 h), TS (at 0.5, 24, and 36 h), and TSPO (at 0.5, 12, and 24 h) in aphids treated with *Sophora flavescens* extract (Fig. 10). We also examined G6PD, TS, and TSPO activity after treatment with matrine, oxymatrine, sophocarpine, sophorine, and sophoridine in *M. persicae* at 24 h (Fig. 11).

3.7.1 Glucose-6-phosphate 1-dehydrogenase

G6PD (gene: *NagB*) is composed of three sets of dimers linked by disulfide bonds (Altamirano et al. 1993; Horjales et al. 1999; Oliva et al. 1995; Rudiño-Piñera et al. 2002). During n-acetyl-d-glucosamine metabolism, the second cytoplasmic reaction is catalyzed by the deamination of glucose-6-phosphate to ammonia and fructose-6-phosphate, which directly enters glycolysis. The specific reaction mechanism of G6PD in aphids is shown in Fig. 12. When *M. persicae* (A) was treated with crude *Sophora flavescens* extracts for 12 h, there was a significant effect in the treatment and blank groups ($p < 0.001$). G6PD activity in *M. persicae* (A) decreased after treatment for 24 h. When *M. persicae* (B) was treated with crude extract for 12 h, the G6PD activity in the 100 μ M, 1 mM, and 10 mM groups was significantly higher than that in the control group. The 1 mM extract had the most significant effect on *M. persicae*. After 24 h, 100 μ M and 1 mM groups still had a strong effect on aphids.

The effect of oxymatrine, sophoridine, and matrine in aphids (host *Datura*) was induced by inhibiting G6PD, and eventually led to aphid death. When four compounds were used to control *M. persicae* (B), three compounds activated G6PD. In contrast, sophoridine partially inhibited G6PD..

3.7.2 Thymidylate synthase

We measured TS enzyme activity in peach aphids treated with *Sophora flavescens* extract. TS binds to its own mRNA and inhibits translation (Voeller et al. 1995). The specific domain involved in mRNA binding is located at the dimer interface (Voeller et al. 2002). The translation of Thya can also be adjusted through the translation of the upstream LGT ORF (Gan et al. 1995). The specific reaction mechanism of TS in aphid was shown in Fig.13. For *M. persicae* reared on *Datura stramonium* leaves, the responses were the same at 0.5 and 12 h. It was observed that 100 μ M and 1 mM *Sophora flavescens* extract activated TS ($p<0.001$). However, TS activity in the 10 mM group was lower than in the control group ($p<0.001$). Below 10 mM, crude *Sophora flavescens* extract inhibited TS. Subsequently, we treated aphids with crude *Sophora flavescens* extract for 36 h. It was observed that 1 mM, 10 mM, and 100 mM extract significantly inhibited TS activity. The insecticidal effect was 1 mM > 10 mM > 100 mM. For aphids on cabbage leaves, treatment with 100 μ M or 1 mM extract for 0.5 h activated TS. At 24 h, 10 mM extract had the strongest TS inhibition ($p<0.001$). Further, TS was significantly inhibited by 100 μ M, 1 mM, and 10 mM extract after 36 h ($p<0.001$); however, the effect of 100 mM extract was weaker than that of the other groups.

When matrine and sophoridine were used as insecticides against *M. persicae* (A), they showed significant inhibitory activity against aphids (A). The TS levels in the 100 μ M, 1 mM, and 10 mM groups were significantly decreased compared with the CK (blank control) group. Matrine showed a distinct insecticidal effect (inhibition level: 1 mM > 100 μ M). Sophocarpine and oxymatrine conferred an insecticidal effect by similarly inhibiting TS (activation level: 100 mM>100 μ M>10 mM>1 mM).

Treating aphids with sophoridine, sophocarpine, oxymatrine, or matrine inhibited TS; specifically, (1) 24 h sophoridine administration killed aphids. Compared with the blank control group, the TS levels in the 100 μ M and 1 mM groups were significantly decreased. In contrast, TS in the 100 mM group was significantly increased. (2) Compared with the CK group, the effect of 100 μ M sophocarpine was not obvious and the TS levels in the other groups were significantly decreased. (3) Compared with the blank control group, TS levels in each group were significantly decreased by oxymatrine. (4) Compared with the CK group, the TS levels were significantly decreased by 100 μ M oxymatrine, but were significantly increased in the 1 mM, 10 mM, and 100 mM groups (rising level: 100 mM>10 mM>1 mM).

3.7.3. Translocator protein

When treated by *Sophora flavescens* aboveground for 0.5 h, enzyme activity in the blank control was the highest, indicating that TSPO had an inhibitory effect on peach aphids. After 12 h, an insecticidal effect was not observed in the 100 μ M group, but the insecticidal effect in the 100 mM group was significantly higher than that of the control group ($P< 0.05$). Treating aphids with *Sophora flavescens* extract for 24 h

had similar effects to treatment for 12 h. However, treatment with 100 μ M extract for 24 h improved the effects on *M. persicae* (B).

When sophoridine was administered at 100 μ M 24 h after treating *M. persicae* (A) *in vivo*, the effect of sophoridine on *M. persicae* (A). In the 100 mM group, however, TSPO levels were increased, ultimately killing the aphids. TSPO levels in the 100 μ M group were significantly decreased. After 24 h, 1 mM, 10 mM, and 100 mM *Sophora flavescens* extract further increased TSPO levels. TSPO activation in the oxymatrine administration group was 10 mM > 1 mM. In the sophocarpine group, TSPO was activated in all dosage groups, except the 100 mM group, and each concentration killed aphids by inhibiting TSPO.

In the sophoridine group, TSPO levels decreased significantly after 24 h. Indeed, TSPO activity in aphids was inhibited by administration of 100 μ M, 1 mM, and 100 mM. The insecticidal effect of 100 μ M and 1 mM administration was the most significant. When we used oxymatrine to kill *M. persicae* (B), the concentration was set at 100 μ M, 1 mM, or 100 mM. TSPO levels in the aphids were significantly decreased after 24 hours ($P < 0.05$). Finally, we used sophocarpine to treat *M. persicae* (B). The results showed that, compared with the CK group, 100 μ M, 1 mM, 10 mM, and 100 mM sophocarpine activated TSPO, resulting in a sudden increase in TSPO levels in the aphids that was sufficient to kill the aphids.

In summary, we verified that crude *Sophora flavescens* extracts targeted specific enzymes in *M. persicae*. Crude extracts from the aboveground part of *Sophora flavescens* kill aphids by disrupting G6PD, TS, and TSPO function. Therefore, these pathways in aphids are targeted by crude *Sophora flavescens* extract.

3.7.4. Effects of *Sophora flavescens* on G6PD, TS, TSPO in different *M. persicae* strains

We observed a significant difference in the effects of matrine and sophocarpine on G6PD between two *M. persicae* strains, while other compounds had little effect (Fig. 14a-1). Crude extracts from the aboveground part of *Sophora flavescens* and oxymatrine had different effects on TS in *M. persicae*, with the crude *Sophora flavescens* extract having the most significant effect. Further, we observed significant differences in TS after treating *M. persicae* with *Sophora flavescens* extract for 30 min (Fig. 14a-2). This difference was increased after 24 h. We also observed a significant difference in the effect of sophocarpine, oxymatrine, and matrine on TSPO in *M. persicae* (Fig. 14a-3).

3.7.5 Effects of crude *Sophora flavescens* extracts on G6PD, TS, and TSPO in *M. persicae* at different time points

We then compared the effects of crude *Sophora flavescens* extracts on aphids at different time points. The results showed that for TS, there was significant difference between treatment for 0.5 h and 24 h (Fig. 14b-1). For TSPO, there was significant difference between treatment for 0.5, 12, and 24 h (Fig. 14b-2).

3.7.6 Effects of different compound groups on G6PD, TS, and TSPO in *M. persicae*

We analyzed the effects of crude *Sophora flavescens* extracts, matrine, oxymatrine, sophocarpine, and sophoridine. The results showed that there was a significant difference between sophoridine and oxymatrine on TS in *M. persicae* (A) (Fig. 14c-1). Sophoridine exerts its effect by inhibiting TS in peach aphids, while oxymatrine activates TS in *M. persicae* (A). There was a significant difference in insecticidal effects between matrine and oxymatrine (Fig. 14c-2). We also found that oxymatrine had a stronger inhibitory effect on TS than sophocarpine in *M. persicae* (B) (Fig. 14c-3). There was a significant difference between the effects of sophocarpine and oxymatrine on TSPO of *M. persicae* (B). Both oxymatrine and sophoridine achieved insecticidal effects by inhibiting TSPO in *M. persicae* (B), while matrine and sophorine activated TSPO.

4. Discussion

4.1 Mechanism of the same chemical compound in controlling peach aphids reared on different host plants is different

The mechanism of the same chemical compound in controlling *M. persicae* from different host plants would differ (Ge CH 2016). Crude extracts of the aboveground part of *Sophora flavescens* contain various chemical components such as alkaloids, flavonoids, and polysaccharides (Tang et al. 2022; Zhang et al. 2016). The effect of *Sophora flavescens* extract on *M. persicae* from different host plants was quite similar. However, different reaction times, compound concentrations, or active ingredients have different effects on aphids (Li TM et al. 2020; Sun JW and Zhang HY 2020; Yuan EL 2017). We found that pure matrine compounds applied to *M. persicae* from different host plants activated different resistance mechanisms, although insecticidal effects were eventually achieved.

4.2 Increased pesticide concentration is not always associated with increased toxicity

In terms of dose-effect relationships, the highest compound concentration was not the most dangerous. *Sophora flavescens* targets different molecules in aphids. Thus, these molecular targets may have different affinities and downstream effects. In addition, receptor desensitization should also be considered. At high concentrations, many receptors bind to heterophytes, resulting in receptor downregulation (Almasri et al. 2020). Accordingly, we speculate that: (1) the effects of insecticides are produced by acting on multiple target pathways, thereby inducing synergistic or antagonistic effects. We will investigate these effects in future studies. (2) Because the compound interactions may occur with primary or secondary targets, different targets likely have different affinities for the active compounds. The overall end result may be enzyme activation or inhibition of certain components in the pests. (3) The final compound effect may depend on the compound concentration or interactions between pathways *in vivo*, so different mechanisms of action and target pathways may be time- or concentration-dependent. (4) Increasing the compound concentration or treatment time did not always increase the toxicity of the mixture, indicating that toxicity does not arise from a single substance. Rather, cooperating compound effects need to be further explored.

4.3 *Sophora flavescens* aboveground and four alkaloids act on aphids G6PD, TS, and TSPO

We measured how the aboveground parts of *Sophora flavescens* and four alkaloids affect *M. persicae*. We observed significant changes in G6PD, TS, and TSPO activity. For G6PD, (1) the effects of several point mutants affecting n-acetylglucosamine-6-phosphate binding were tested. Glucosamine or n-acetylglucosamine is used as a carbon and energy source *in vivo* (Alvarez-Añorve et al. 2005). This finding is consistent with our research. After treating peach aphids with *Sophora flavescens* extract, we found that *NagB* has a significant effect on peach aphids, which is reflected in G6PD activity. (2) Overexpressing *nagB* inhibits defects in *glmS* mutants, indicating that glucosamine-6-phosphate deaminase plays a role in biosynthesis and provides glucosamine-6-phosphate for uridine diphosphate N-acetylglucosamine synthesis (Vogler and Lengeler 1989). We also show that G6PD is not always activated or continuously inhibited by insecticides. This is consistent with the conclusion that *nagB* mutants cannot grow when using n-acetylglucosamine as the sole carbon and energy source (Rolls and Shuster 1972; White RJ 1968). (3) When *Sophora flavescens* resists aphids, G6PD may increase or decrease, which can disrupt equilibrium. Similar phenomena suggest that this may occur because n-acetyl-d-glucosamine-6-phosphate has variable effects on *NagB* activation (Alvarez-Añorve et al. 2009). Growth is limited by the supply of substrates to *NagB*, not by the lack of N-acetyl-glucosamine-6P (Alvarez-Añorve et al. 2009). Replacing *NagB* with homologous enzymes without allosteric regulation has almost no effect on the growth rate and fitness of *Escherichia coli* (Alvarez-Añorve et al. 2016). Thus, the physiological effect of allosteric regulation of *NagB* activity is not clear and it is necessary to further study the specific mechanism of action on G6PD.

Through molecular simulations and enzyme activity assays, we found that the binding site between *Sophora flavescens* extracts and aphids was very stable. TS also plays a key role in anti-aphid effects. In 1954, Cohen and Barner described "cell death caused by thymine starvation in a thymic deletion mutant (*thyA*)" (Cohen and Barner 1954). In this study, extracts of the aboveground part of *Sophora flavescens* inhibited TS in peach aphids at different times, while other alkaloids from *Sophora flavescens* inhibited TS after 24 h. Although thymine-free death was described in 1954, the exact pathway and mechanism by which it occurs is still under investigation. For example, in this study, four alkaloids were tested at different time points. TS inhibition is only one way for *Sophora flavescens* to kill aphids. There are many related explanations for TS inhibition. For example, thymosin-less death is mediated by the *mazEF* toxin-antitoxin system, which involves the initiation of DNA replication and stalled replication fork repair, resulting in replication starting point destruction and SOS responses. *ThyA* mutants do not grow in thymine-deficient medium and accumulate polyphosphates. Mutations in *deoB* or *deoC* inhibit the high demand for thymine in *thyA* mutants. Previous studies showed that overexpressing *thyA* increases tolerance to furacilin. We found that *Sophora flavescens* sometimes induced a significant increase in peach aphids, which may be due to the corresponding increase of TS caused by the strong resistance of peach aphids. More in-depth studies on TS need to be performed.

We also examined the role of G6PD and TSPO in aphid resistance to *Sophora flavescens*. TSPO is a transmembrane protein which mainly exists in the outer mitochondrial membrane and is involved in various processes, including regulation of cell death, cholesterol transport, steroid biosynthesis, mitochondrial respiration and oxidation, and mitochondrial protein input. This protein may be part of

multiple pathways. Although it usually cannot operate alone, TSPO has auxiliary functions that may explain why TSPO is an important target for killing aphids.

5. Conclusions

Network pharmacology is a multidisciplinary approach that integrates bioinformatics, systems biology, network biology, and computational biology. This approach can generate complex interaction networks using target molecules, biological functions and bioactive compounds. These networks can be introduced into agriculture for screening natural product pesticides. Precision agriculture clearly has potential development space. From a more comprehensive viewpoint, future multi-dimensional research will explain the complex relationships between biological systems and drugs, and will identify synergistic effects between many drugs. In summary, three biological indices (G6PD, TS and TSPO) derived from network pharmacology were shown to be effective ways to resist aphids using the aboveground parts of *Sophora flavescens*. This method can help in the formulation of *Sophora flavescens* insecticides and will inform future studies investigating the mechanism of action of many pesticides.

Declarations

Ethics approval and consent to participate

Ethical approval The animals used in this research are not vertebrates or invertebrates with ethical and care limitations. No human subjects were involved in this study.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and materials The relevant data and materials of this study have been uploaded to the public database and approved, the reader can be access via <https://www.scidb.cn/en/s/rmmUVr>.

Competing interests The authors declare no conflict of interests.

Funding This research was funded by the National Natural Science Foundation of China, grant number M2042003; and the Science and Technology Planning Project of the Inner Mongolia Autonomous Region, China, grant number 2021GG0377.

Authors' contributions All authors contributed to the study conception and design. CYG, BCZ, YBL, LJL, MHL conceived and design the study. CYG performed the experiments. CYG, BCZ, YBL and HN wrote the manuscript with considerable help from LJL and MHL.

Acknowledgements We are grateful for the assistance of all the members in the Inner Mongolia Academy of Chinese and Mongolian Medicine, Hohhot, China.

References

1. Akca I, Ayvaz T, Yazici E, Smith CL (2015) Demography and population projection of *Aphis fabae* (Hemiptera: Aphididae): with additional comments on life table research criteria. J Econ Entomol 108:1466–1478. <https://doi.org/10.1093/jee/tov187>
2. Al-Ayedh H, Rizwan-ul-Haq M, Hussain A, Aljabr AM (2016) Insecticidal potency of RNAi-based *catalase* knockdown in *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae): *Catalase* knockdown in *Rhynchophorus ferrugineus*. Pest Manag Sci 72:2118–2127. <https://doi.org/10.1002/ps.4242>
3. Alikhanian SI, Iljina TS, Kaliaeva ES, Kameneva SV, Sukhodolec VV (1966) A genetical study of thymineless mutants of *E. coli* K12. Genet Res 8:83–100. <https://doi.org/10.1017/s0016672300009939>
4. Almasri H, Tavares DA, Pioz M, Sené D, Tchamitchian S, Cousin M, Brunet J, Belzunces L (2020) Mixtures of an insecticide, a fungicide and a herbicide induce high toxicities and systemic physiological disturbances in winter *Apis mellifera* honey bees. Ecotoxicol Environ Saf 203:111013. <https://doi.org/10.1016/j.ecoenv.2020.111013>
5. Almeida AC, Jesus FG, Heng-Moss T, Lanna A, Barrigossi J (2021) Evidence for rice tolerance to *Tibraca limbativentris* (Hemiptera: Pentatomidae). Pest Manag Sci 77:4181–4191. <https://doi.org/10.1002/ps.6455>
6. Altamirano MM, Plumbridge JA, Barba HA, Calcagno ML (1993) Glucosamine-6-phosphate deaminase from *Escherichia coli* has a trimer of dimers structure with three intersubunit disulphides. Biochem J 295 (Pt 3):645–648. <https://doi.org/10.1042/bj2950645>
7. Alvarez-Añorve LI, Bustos-Jaimes I, Calcagno ML, Plumbridge J (2009) Allosteric regulation of glucosamine-6-phosphate deaminase (NagB) and growth of *Escherichia coli* on glucosamine. J Bacteriol 191:6401–6407. <https://doi.org/10.1128/JB.00633-09>
8. Alvarez-Añorve LI, Calcagno ML, Plumbridge J (2005) Why does *Escherichia coli* grow more slowly on glucosamine than on N-acetylglucosamine? Effects of enzyme levels and allosteric activation of GlcN6P deaminase (NagB) on growth rates. J Bacteriol 187:2974–2982. <https://doi.org/10.1128/JB.187.9.2974-2982.2005>
9. Álvarez-Añorve LI, Gaugué I, Link H, Marcos-Viquez J, Díaz-Jiménez DM, Zonszein S, Bustos-Jaimes I, Schmitz-Afonso I, Calcagno M, Plumbridge J (2016) Allosteric activation of *Escherichia coli* Glucosamine-6-Phosphate Deaminase (NagB) *In Vivo* justified by intracellular amino sugar metabolite concentrations. J Bacteriol 198:1610–1620. <https://doi.org/10.1128/JB.00870-15>
10. Amado L, Kuzminov A (2009) Polyphosphate accumulation in *Escherichia coli* in response to defects in DNA metabolism. J Bacteriol 191:7410–7416. <https://doi.org/10.1128/JB.01138-09>
11. Austin CJD, Kahlert J, Kassiou M, Rendina LM (2013) The translocator protein (TSPO): a novel target for cancer chemotherapy. Int J Biochem Cell Biol 45:1212–1216. <https://doi.org/10.1016/j.biocel.2013.03.004>

12. Belfort M, Pedersen-Lane J (1984) Genetic system for analyzing *Escherichia coli* thymidylate synthase. J Bacteriol 160:371–378. <https://doi.org/10.1128/jb.160.1.371-378.1984>
13. Boutet E, Lieberherr D, Tognolli M, Schneider M, Bansal P, Bridge A, Poux S, Bougueleret L, Xenarios L (2016) UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: how to use the entry view. In: Plant Bioinformatics. Springer, New York, 23–54
14. Chen L, Qu LY, Liu XY, Wang HY, Zhang K, Wang JH (2020) Qualitative analysis of chemical constituents of alkaloids in *Sophora alopecuroides* L. based on UPLC-TQ-MS. J Shihezi Univ (Nat Sci) 38:121–127 <https://doi.org/10.13880/j.cnki.65-1174/n.2019.22.014>
15. Chen M, Ding Y, Tong Z (2020) Efficacy and safety of *Sophora flavescens* (Kushen) based traditional chinese medicine in the treatment of ulcerative colitis: Clinical evidence and potential mechanisms. Front Pharmacol 11:603476. <https://doi.org/10.3389/fphar.2020.603476>
16. Clark RD (2018) Predicting mammalian metabolism and toxicity of pesticides *in silico*: Predicting pesticide metabolites and toxicity. Pest Manag Sci 74:1992–2003. <https://doi.org/10.1002/ps.4935>
17. Clarke R, Kehoe MA, Broughton S, Jones RAC (2020) Host plant affiliations of aphid vector species found in a remote tropical environment. Virus Research 281:197934. <https://doi.org/10.1016/j.virusres.2020.197934>
18. Cohen SS, Barner HD (1954) Studies on unbalanced growth in *Escherichia coli*. Proc Natl Acad Sci U S A 40:885–893. <https://doi.org/10.1073/pnas.40.10.885>
19. Colizzi FS, Beer K, Cuti P, Deppisch P, Martínez TD, Yoshii T, Helfrich-Förster C (2021) Antibodies against the clock proteins period and cryptochrome reveal the neuronal organization of the circadian clock in the pea aphid. Front Physiol 12:705048. <https://doi.org/10.3389/fphys.2021.705048>
20. Ding PL, Liao ZX, Huang H, Zhou P, Chen DF (2006) (+)-12 α -Hydroxysophocarpine, a new quinolizidine alkaloid and related anti-HBV alkaloids from *Sophora flavescens*. Bioorg Med Chem Lett 16:1231–1235. <https://doi.org/10.1016/j.bmcl.2005.11.073>
21. Doucet D, Frisco C, Cusson M, Bauce É, Palli SR, Tomkins B, Arif B, Retnakaran A (2007) Diapause disruption with tebufenozide for early-instar control of the spruce budworm, *Choristoneura fumiferana*. Pest Manag Sci 63:730–736. <https://doi.org/10.1002/ps.1400>
22. Gan K, Sankaran K, Williams MG, Aldea M, Rudd KE, Kushner SR, Wu HC (1995) The *umpA* gene of *Escherichia coli* encodes phosphatidylglycerol: prolipoprotein diacylglycerol transferase (lgt) and regulates thymidylate synthase levels through translational coupling. J Bacteriol 177:1879–1882. <https://doi.org/10.1128/jb.177.7.1879-1882.1995>
23. Gao Q, Shi Y, Liao M, Xiao JJ, Li XX, Zhou LJ, Liu CW, Liu P, Cao HQ (2019) Laboratory and field evaluation of the aphidicidal activity of moso bamboo (*Phyllostachys pubescens*) leaf extract and identification of the active components. Pest Manag Sci 75:3167–3174. <https://doi.org/10.1002/ps.5434>
24. Gatliff J, Campanella M (2012) The 18 kDa translocator protein (TSPO): a new perspective in mitochondrial biology. Curr Mol Med 12:356–368. <https://doi.org/10.2174/1566524011207040356>

25. Ge CH (2016) Comparisons of life histories, feeding behaviors and activities of detoxification enzymes for sitobion avenae on different host plants. Master, Northwest A&F University
26. Glebes TY, Sandoval NR, Reeder PJ, Schilling KD, Zhang M, Gill RT (2014) Genome-wide mapping of furfural tolerance genes in *Escherichia coli*. PLoS One 9:e87540. <https://doi.org/10.1371/journal.pone.0087540>
27. Guzmán EC, Martín CM (2015) Thymineless death, at the origin. Front Microbiol 6:499. <https://doi.org/10.3389/fmicb.2015.00499>
28. Hanawalt PC (2015) A balanced perspective on unbalanced growth and thymineless death. Front Microbiol 6:504. <https://doi.org/10.3389/fmicb.2015.00504>
29. Horjales E, Altamirano MM, Calcagno ML, Garratt RC, Oliva G (1999) The allosteric transition of glucosamine-6-phosphate deaminase: the structure of the T state at 2.3 Å resolution. Structure 7:527–537. [https://doi.org/10.1016/S0969-2126\(99\)80069-0](https://doi.org/10.1016/S0969-2126(99)80069-0)
30. Islam W, Tayyab M, Khalil F, Hua Z, Huang ZQ, Chen HYH (2020) Silicon-mediated plant defense against pathogens and insect pests. Pestic Biochem Physiol 168:104641. <https://doi.org/10.1016/j.pestbp.2020.104641>
31. Joloba ML, Rather PN (2003) Mutations in deoB and deoC alter an extracellular signaling pathway required for activation of the gab operon in *Escherichia coli*. FEMS Microbiol Lett 228:151–157. [https://doi.org/10.1016/S0378-1097\(03\)00754-7](https://doi.org/10.1016/S0378-1097(03)00754-7)
32. Kumari R, Kumar R, Open Source Drug Discovery Consortium, Lynn A (2014) *g_mmpbsa* –A GROMACS tool for High-throughput MM-PBSA calculations. J Chem Inf Model 54:1951–1962. <https://doi.org/10.1021/ci500020m>
33. Kuong KJ, Kuzminov A (2010) Stalled replication fork repair and misrepair during thymineless death in *Escherichia coli*. Genes Cells 15:619–634. <https://doi.org/10.1111/j.1365-2443.2010.01405.x>
34. Kuong KJ, Kuzminov A (2012) Disintegration of nascent replication bubbles during thymine starvation triggers RecA- and RecBCD-dependent replication origin destruction. J Biol Chem 287:23958–23970. <https://doi.org/10.1074/jbc.M112.359687>
35. Lambert WT, Buysse AM, Wessels FJ (2020) Discovery of novel insecticidal 3-aminopyridyl ureas. Pest Manag Sci 76:497–508. <https://doi.org/10.1002/ps.5537>
36. Li S, Zhang B (2013) Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med 11:110–120. [https://doi.org/10.1016/S1875-5364\(13\)60037-0](https://doi.org/10.1016/S1875-5364(13)60037-0)
37. Li TM, Zhao RN, Zhou W, et al (2020) Study on preference, growth and oviposition of *lipaphis erysimi* on different host plants. J Mount Agric Bio 39:88–92
38. Ling SQ, He B, Zeng DQ, Tang WW (2020) Effects of botanical pesticide itol A against the tobacco cutworm, *Spodoptera litura* (Fab.). Environ Sci Pollut Res 27:12181–12191. <https://doi.org/10.1007/s11356-020-07824-2>
39. Liu G, Dong J, Wang H, et al (2011) Characterization of alkaloids in *Sophora flavescens* Ait. by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. J Pharmaceut Biomed 54:1065–1072. <https://doi.org/10.1016/j.jpba.2010.12.024>

40. Liu Q, Zhang H, Liu Y, Zheng Y (2006) Analysis of chemical components in Compound Kushen Injection by HPLC-MSⁿ. *Chin Tradit Pat Med* 1488–1491
41. Liu XJ, Cao MA, Li WH, Shen CS, Yan SQ, Yuan CS (2010) Alkaloids from *Sophora flavescens* Aiton. *Fitoterapia* 81:524–527. <https://doi.org/10.1016/j.fitote.2010.01.008>
42. Lomax MS, Greenberg GR (1968) Characteristics of the deo operon: Role in thymine utilization and sensitivity to deoxyribonucleosides. *J Bacteriol* 96:501–514. <https://doi.org/10.1128/jb.96.2.501-514.1968>
43. Ma T, Shi X, Ma S, Ma ZQ, Zhang X (2020) Evaluation of physiological and biochemical effects of two *Sophora alopecuroides* alkaloids on pea aphids *Acyrtosiphon pisum*. *Pest Manag Sci* 76:4000–4008. <https://doi.org/10.1002/ps.5950>
44. Martín CM, Guzmán EC (2011) DNA replication initiation as a key element in thymineless death. *DNA Repair (Amst)* 10:94–101. <https://doi.org/10.1016/j.dnarep.2010.10.005>
45. Mseddi J, Ben Farhat-Touzri D, Azzouz H (2022) Selection and characterization of thermotolerant *Beauveria bassiana* isolates and with insecticidal activity against the cotton-melon aphid *Aphis gossypii* (Glover) (Homoptera: Aphisidae). *Pest Manag Sci* 78:2183–2195. <https://doi.org/10.1002/ps.6844>
46. Nakazato BM, Macoris M de L da G, Urbinatti PR, Lima-Camara TN (2021) Locomotor activity in *Aedes aegypti* with different insecticide resistance profiles. *Rev saúde pública* 55:18. <https://doi.org/10.11606/s1518-8787.2021055002809>
47. Oliva G, Fontes MR, Garratt RC, Altamirano MM, Calcagno ML, Horjales E (1995) Structure and catalytic mechanism of glucosamine 6-phosphate deaminase from *Escherichia coli* at 2.1 Å resolution. *Structure* 3:1323–1332. [https://doi.org/10.1016/s0969-2126\(01\)00270-2](https://doi.org/10.1016/s0969-2126(01)00270-2)
48. Ostrer L, Hamann BL, Khodursky A (2015) Perturbed states of the bacterial chromosome: a thymineless death case study. *Front Microbiol* 6:363. <https://doi.org/10.3389/fmicb.2015.00363>
49. Pinzi L, Rastelli G (2019) Molecular docking: Shifting paradigms in drug discovery. *IJMS* 20:4331. <https://doi.org/10.3390/ijms20184331>
50. Portilla M (2020) A laboratory diet-overlay bioassay to monitor resistance in *lygus lineolaris* (Hemiptera: Miridae) to insecticides commonly used in the Mississippi delta. *J Insect Sci* 20:4. <https://doi.org/10.1093/jisesa/ieaa067>
51. Rolls JP, Shuster CW (1972) Amino sugar assimilation by *Escherichia coli*. *J Bacteriol* 112:894–902. <https://doi.org/10.1128/jb.112.2.894-902.1972>
52. Rudiño-Piñera E, Morales-Arrieta S, Rojas-Trejo SP, Horjales E (2002) Structural flexibility, an essential component of the allosteric activation in *Escherichia coli* glucosamine-6-phosphate deaminase. *Acta Crystallogr D Biol Crystallogr* 58:10–20. <https://doi.org/10.1107/s0907444901016699>
53. Sangurdekar DP, Hamann BL, Smirnov D, et al (2010) Thymineless death is associated with loss of essential genetic information from the replication origin. *Mol Microbiol* 75:1455–1467. <https://doi.org/10.1111/j.1365-2958.2010.07072.x>

54. Sangurdekar DP, Zhang Z, Khodursky AB (2011) The association of DNA damage response and nucleotide level modulation with the antibacterial mechanism of the anti-folate drug trimethoprim. BMC Genomics 12:583. <https://doi.org/10.1186/1471-2164-12-583>
55. Sat B, Reches M, Engelberg-Kulka H (2003) The *Escherichia coli* mazEF suicide module mediates thymineless death. J Bacteriol 185:1803–1807. <https://doi.org/10.1128/JB.185.6.1803-1807.2003>
56. Shaalan EAS, Canyon DV (2018) Mosquito oviposition deterrents. Environ Sci Pollut Res 25:10207–10217. <https://doi.org/10.1007/s11356-017-0408-1>
57. Sun JW, Zhang HY (2020) Effects of different host plants on the growth and reproduction of peach aphid. Heilongjiang Agric Sci 83–86
58. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva N, Legeay M, Fang T, Bork P, Jensen L, Von MC (2021) The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 49:D605–D612. <https://doi.org/10.1093/nar/gkaa1074>
59. Tang Q, Liu Y, Peng X, Wang BJ, Luan F, Zeng N (2022) Research progress in the pharmacological activities, toxicities, and pharmacokinetics of sophoridine and its derivatives. Drug Des Devel Ther 16:191–212. <https://doi.org/10.2147/DDDT.S339555>
60. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark A, Berendsen HJC(2005) GROMACS: Fast, flexible, and free. J Comput Chem 26:1701–1718. <https://doi.org/10.1002/jcc.20291>
61. Voeller DM, Changchien LM, Maley GF, Maley F, Takechi T, Turner RE, Montfort WR, Allegra CJ, Chu E (1995) Characterization of a specific interaction between *Escherichia coli* thymidylate synthase and *Escherichia coli* thymidylate synthase mRNA. Nucleic Acids Res 23:869–875. <https://doi.org/10.1093/nar/23.5.869>
62. Voeller DM, Zajac-Kaye M, Fisher RJ, Allegra CJ (2002) The identification of thymidylate synthase peptide domains located in the interface region that bind thymidylate synthase mRNA. Biochem Biophys Res Commun 297:24–31. [https://doi.org/10.1016/s0006-291x\(02\)02080-6](https://doi.org/10.1016/s0006-291x(02)02080-6)
63. Vogler AP, Lengeler JW (1989) Analysis of the nag regulon from *Escherichia coli* K12 and *Klebsiella pneumoniae* and of its regulation. Mol Gen Genet 219:97–105. <https://doi.org/10.1007/BF00261163>
64. Wan C, Liu M, Sun H, Liu WJ, Zhang LL (2009) Qualitative and quantitative analyses of 13 constituents of alkaloids from *Sophora alopecuroides* by GC-MS. West China J Pharm Sci 24:587–590
65. Wang H, Guo S, Qian D, Qian YF, Duan JA (2012) Comparative analysis of quinolizidine alkaloids from different parts of *Sophora alopecuroides* seeds by UPLC–MS/MS. J Pharmaceut Biomed 67–68:16–21. <https://doi.org/10.1016/j.jpba.2012.04.024>
66. Weng ZB, Duan JA, Guo S, et al (2016) Evaluation analysis of alkaloids in seed of *Sophora flavescens* from Shanxi province and exploration of its utilization value. China J Chin Mater Medica 41:3265–3271
67. White RJ (1968) Control of amino sugar metabolism in *Escherichia coli* and isolation of mutants unable to degrade amino sugars. Biochem J 106:847-58. <https://doi: 10.1042/bj1060847>.

68. Wu CW, Lu L, Liang SW, Chen C, Wang SM (2016) Application of drug-target prediction technology in network pharmacology of traditional Chinese medicine. *China J Chin Mater Medica* 41:377–382. <https://doi.org/10.4268/cjcmm20160303>
69. Xu HY, Liu ZM, Fu Y, Zhang YQ, Yu JJ, Guo FF, Tang SH, Lv CY, Su J, Cui RY, Yang HJ (2017) Exploiture and application of an internet-based computation platform for integrative pharmacology of traditional Chinese medicine. *China J Chin Mater Medica* 42:3633–3638. <https://doi.org/10.19540/j.cnki.cjcmm.2017.0141>
70. Xu J, Sun Z, Hao M, Lv M, Xu H (2020) Evaluation of biological activities, and exploration on mechanism of action of matrine–cholesterol derivatives. *Bioorg Chem* 94:103439. <https://doi.org/10.1016/j.bioorg.2019.103439>
71. Yuan EL (2017) Host adaptation of pea aphids from different host races. Master, Hebei University
72. Zhang HQ, Zhu ZH, Qian DW, Weng ZB, Guo S, Duan JA, Lei ZH, Li AP (2016) Analysis and evaluation of alkaloids and flavonoids in flower of *Sophora flavescens* from Shanxi province. *China J Chin Mater Medica* 41:4621–4627. <https://doi.org/10.4268/cjcmm20162422>
73. Zhang R, Zhu X, Bai H, Ning K (2019) Network pharmacology databases for traditional Chinese medicine: Review and assessment. *Front Pharmacol* 10:123. <https://doi.org/10.3389/fphar.2019.00123>
74. Zhao DR, Xue Y, Chen XQ, Liu D, Li RT, Li HM (2018) A study on the quinolizidine alkaloids of *Sophora flavescen*. *J Kunming Univ Sci Technol (Nat Sci)* 43:93–97. <https://doi.org/10.16112/j.cnki.53-1223/n.2018.04.013>
75. Zheng H, Wang X, Yomano LP, Shanmugam KT, Ingram LO (2012) Increase in furfural tolerance in ethanologenic *Escherichia coli* LY180 by plasmid-based expression of thyA. *Appl Environ Microbiol* 78:4346–4352. <https://doi.org/10.1128/AEM.00356-12>

Figures

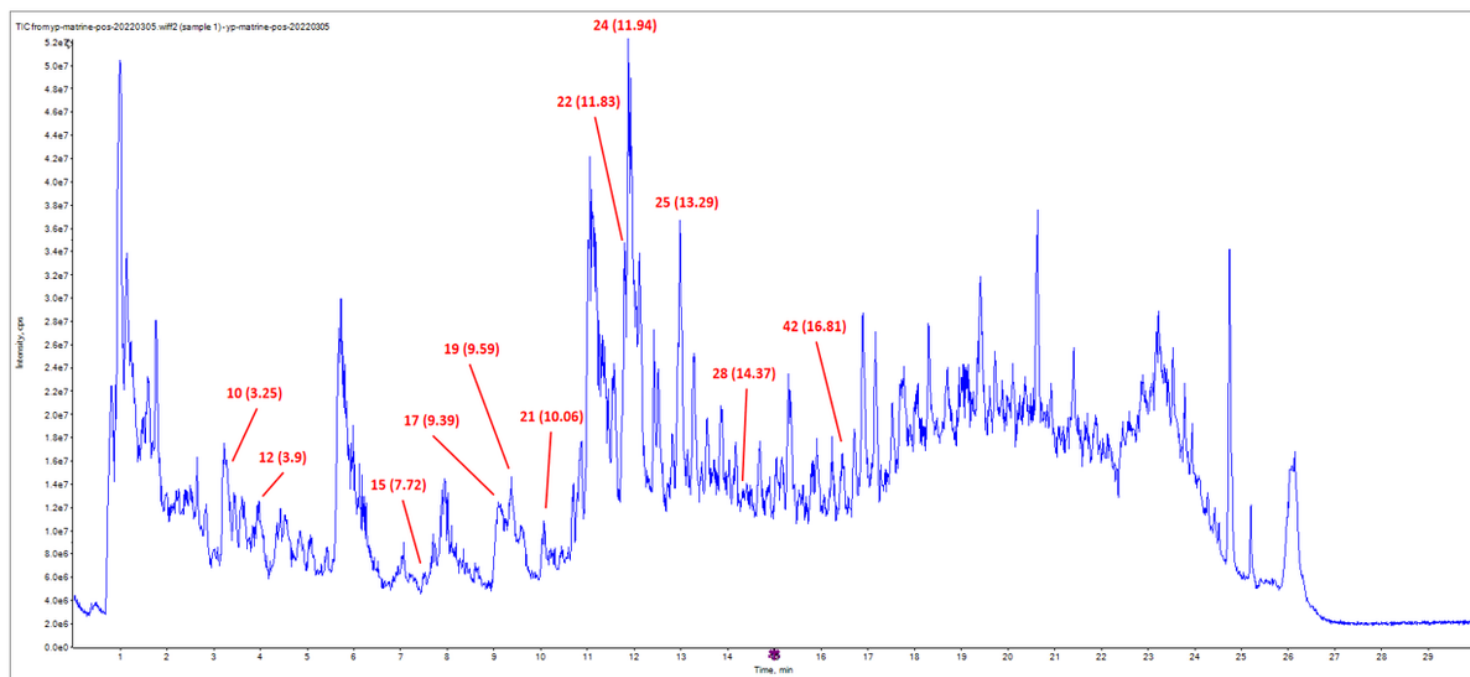


Figure 1

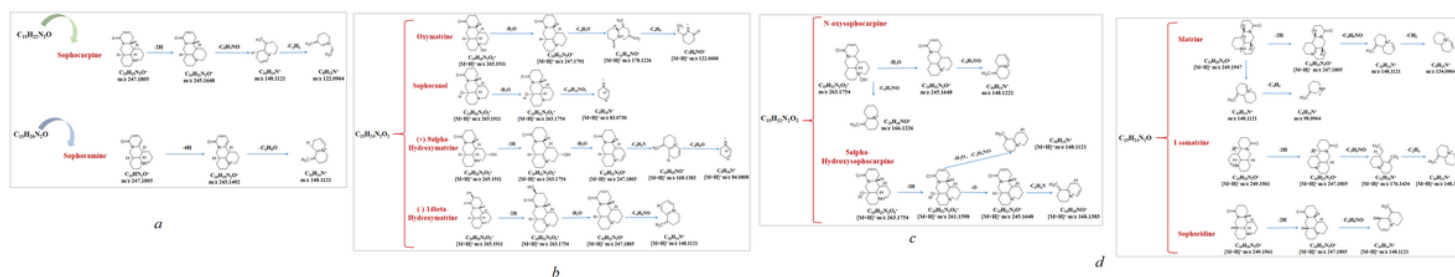
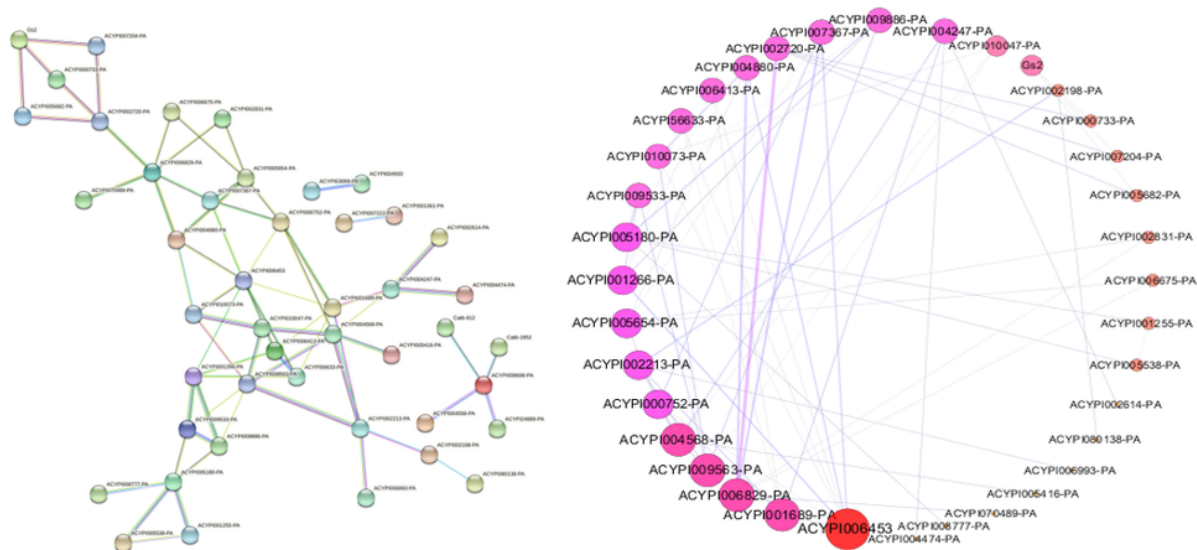
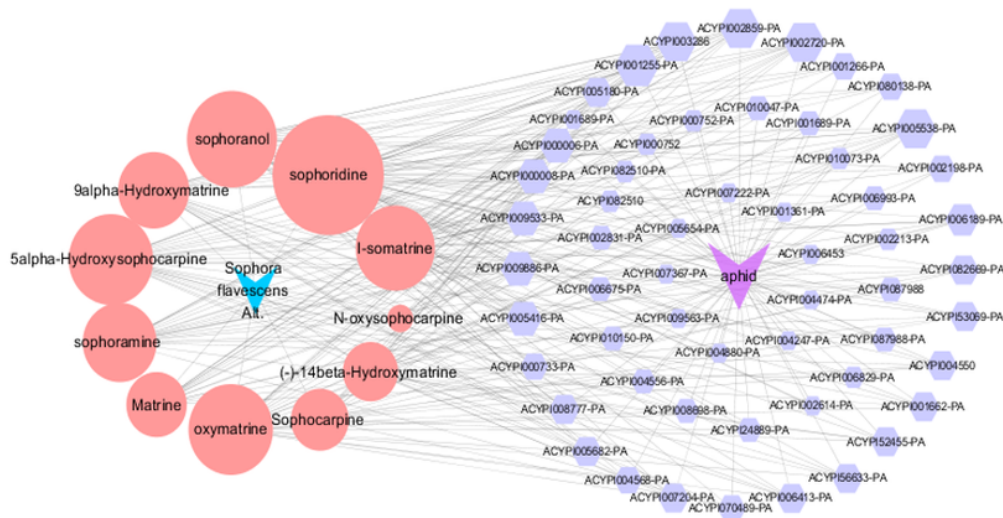


Figure 2



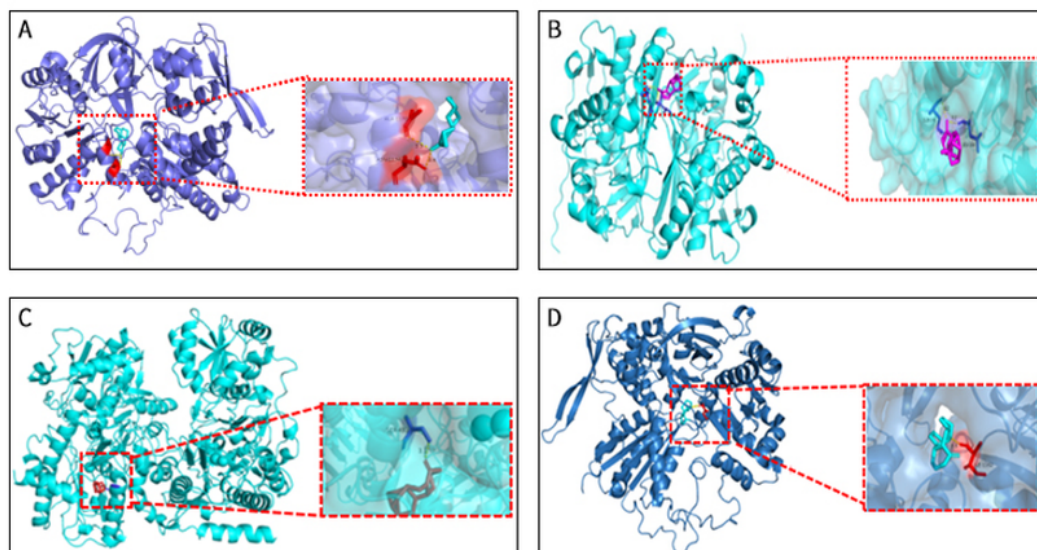
a



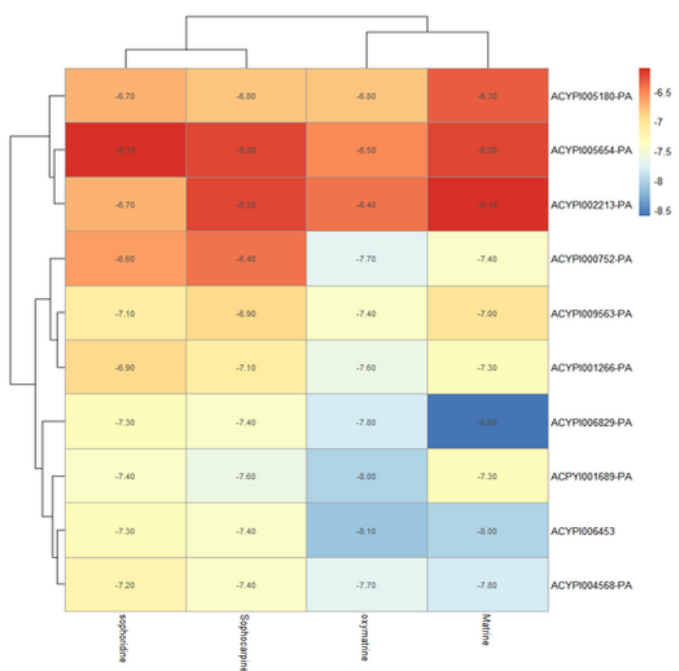
b

Figure 3

a Screening common targets and construction of protein-protein interaction networks; **b** Construction of *Sophora flavescens* anti-aphid-target interaction network



a



b

Figure 4

a Molecular docking and **b** Heat map of *Sophora* alkaloids

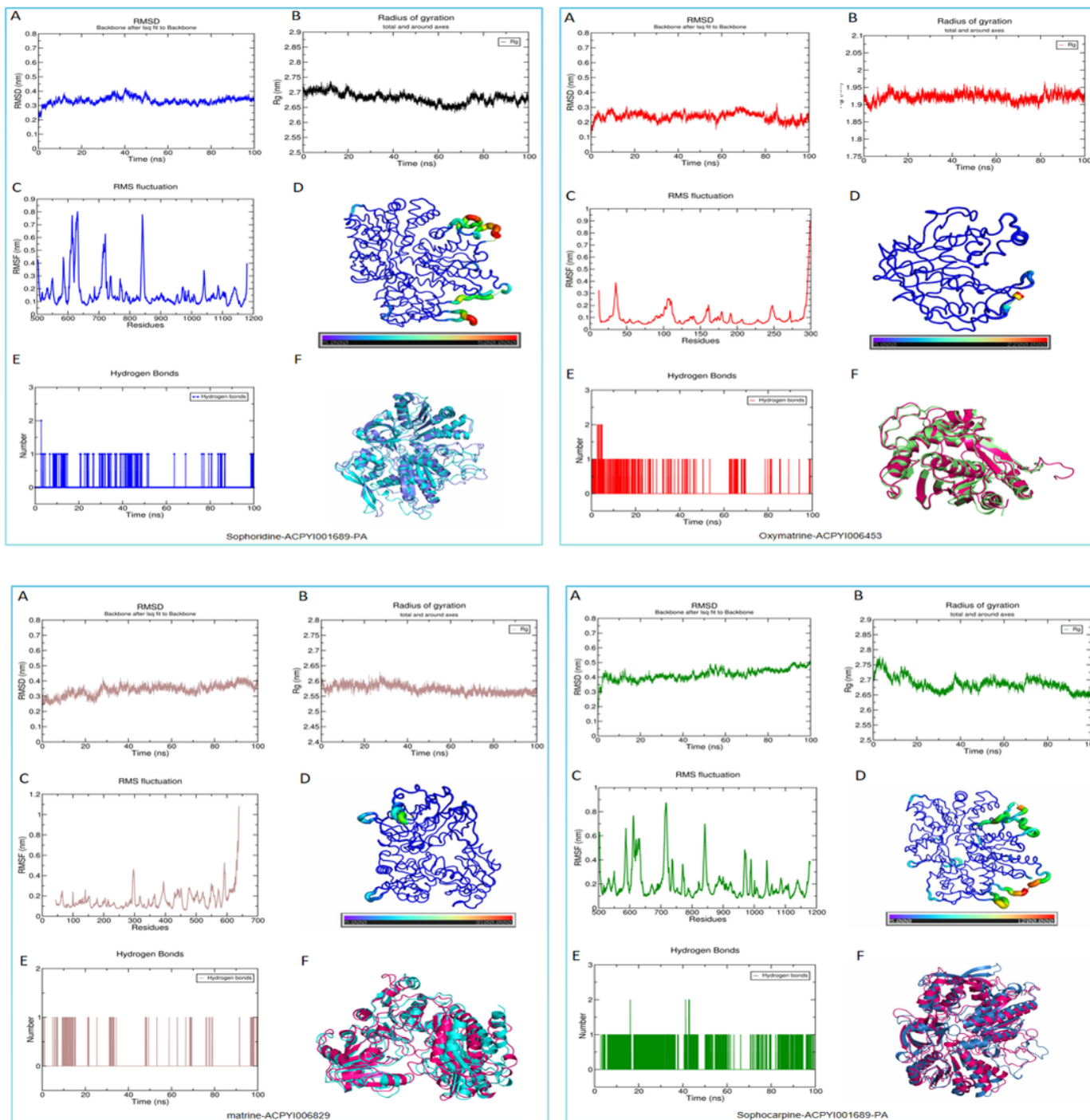


Figure 5

Molecular dynamics simulation of *Sophora* alkaloids

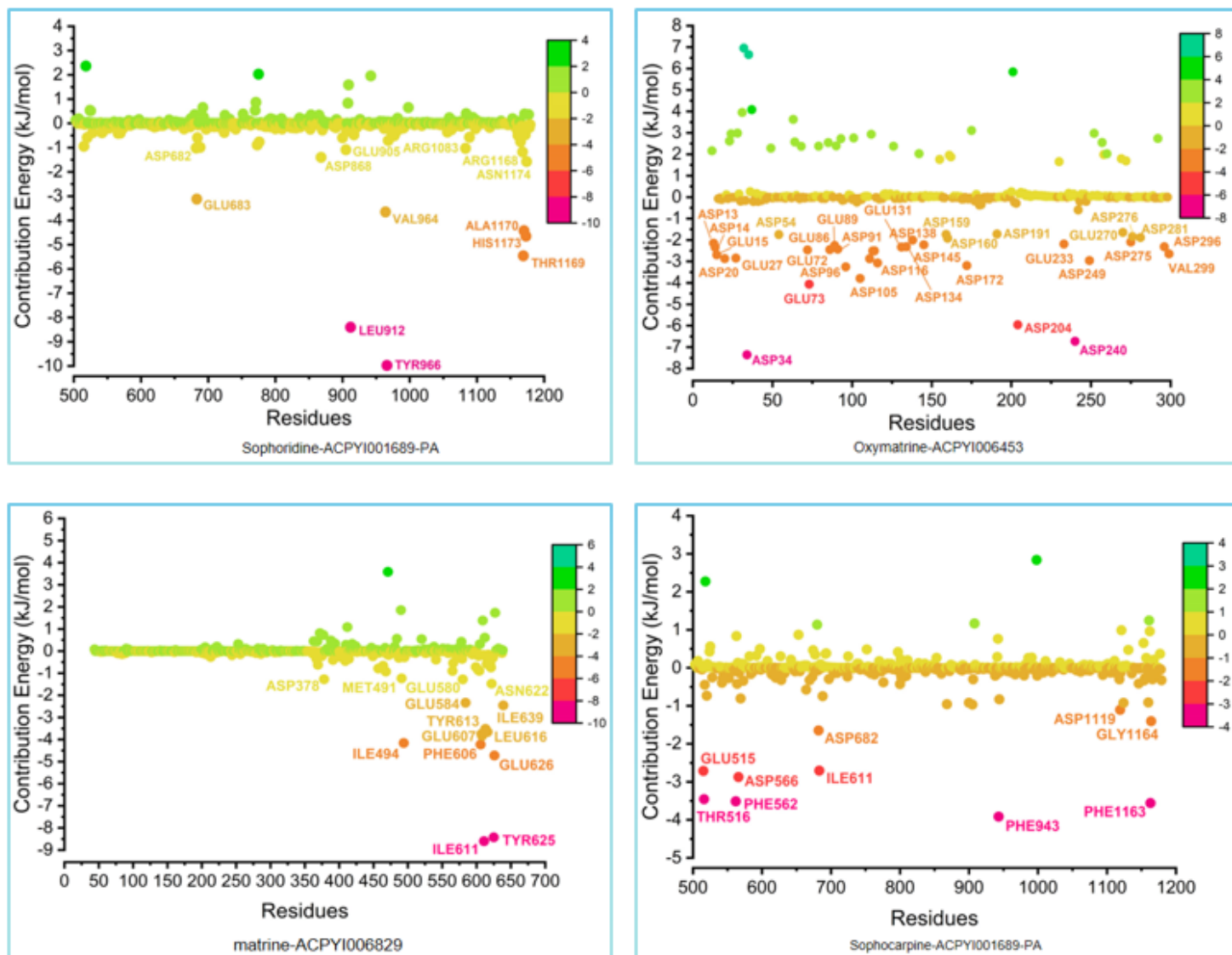


Figure 6

Binding free energy of *Sophora* alkaloids

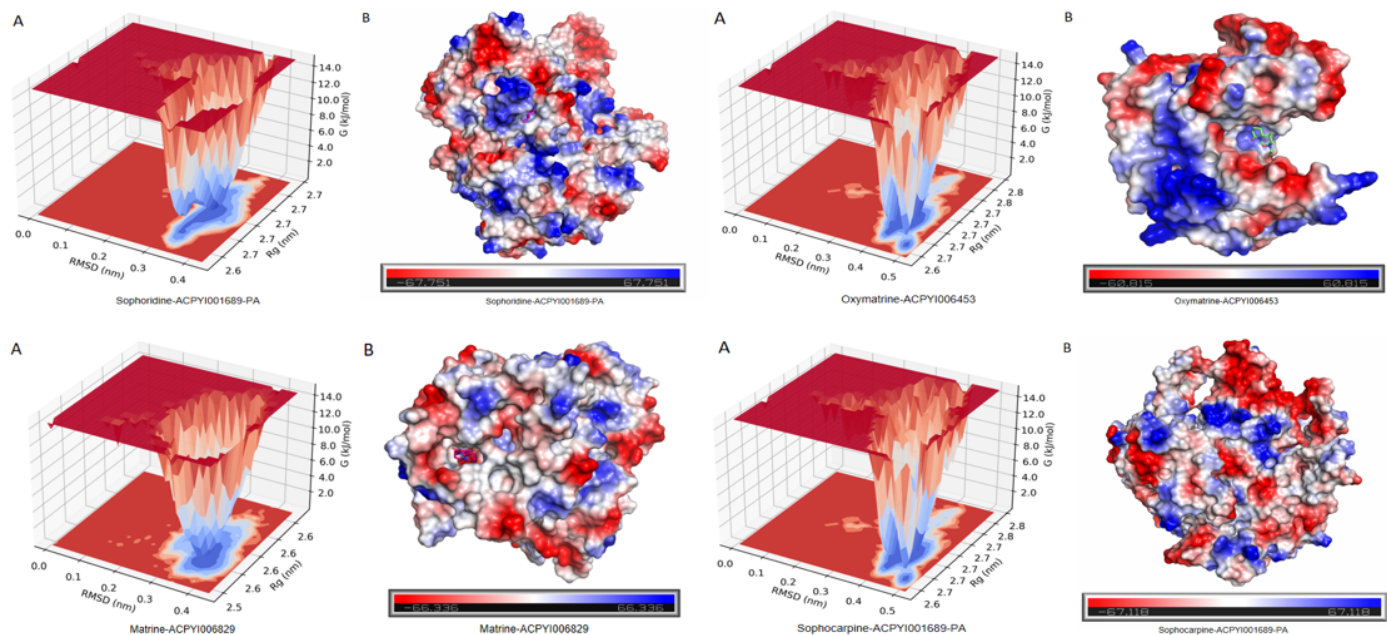


Figure 7

A Gibbs energy landscape and B 3D display of the binding site with the lowest energy for *Sophora* alkaloids

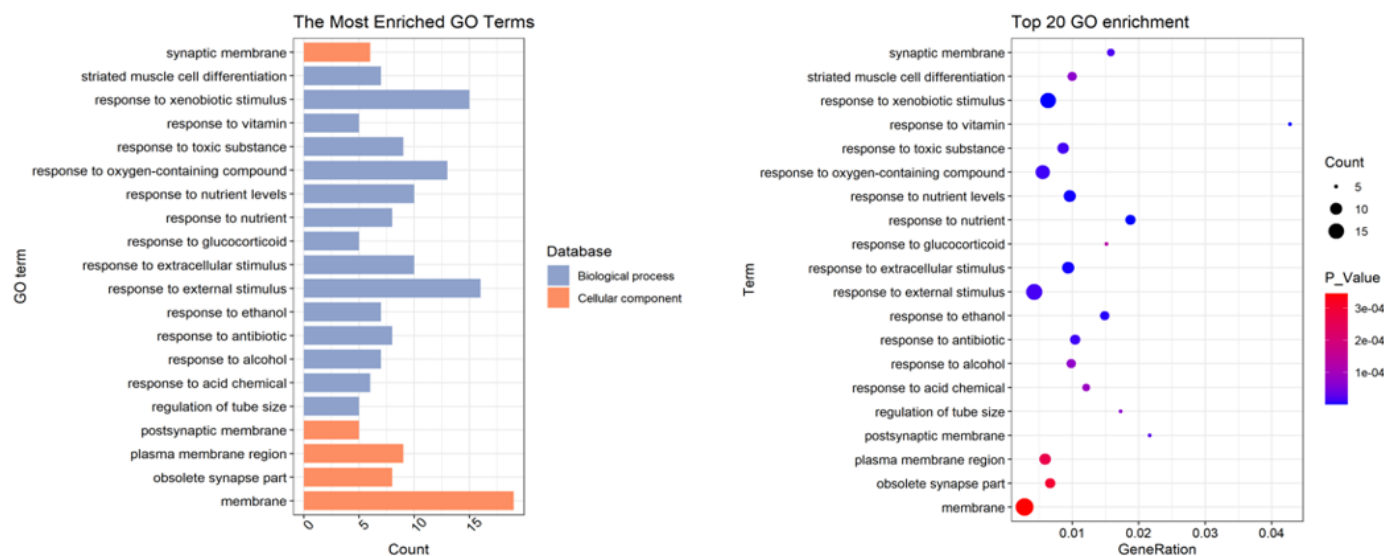


Figure 8

GO function analysis bar chart and bubble charts of genes targeted by *Sophora flavescens* extract

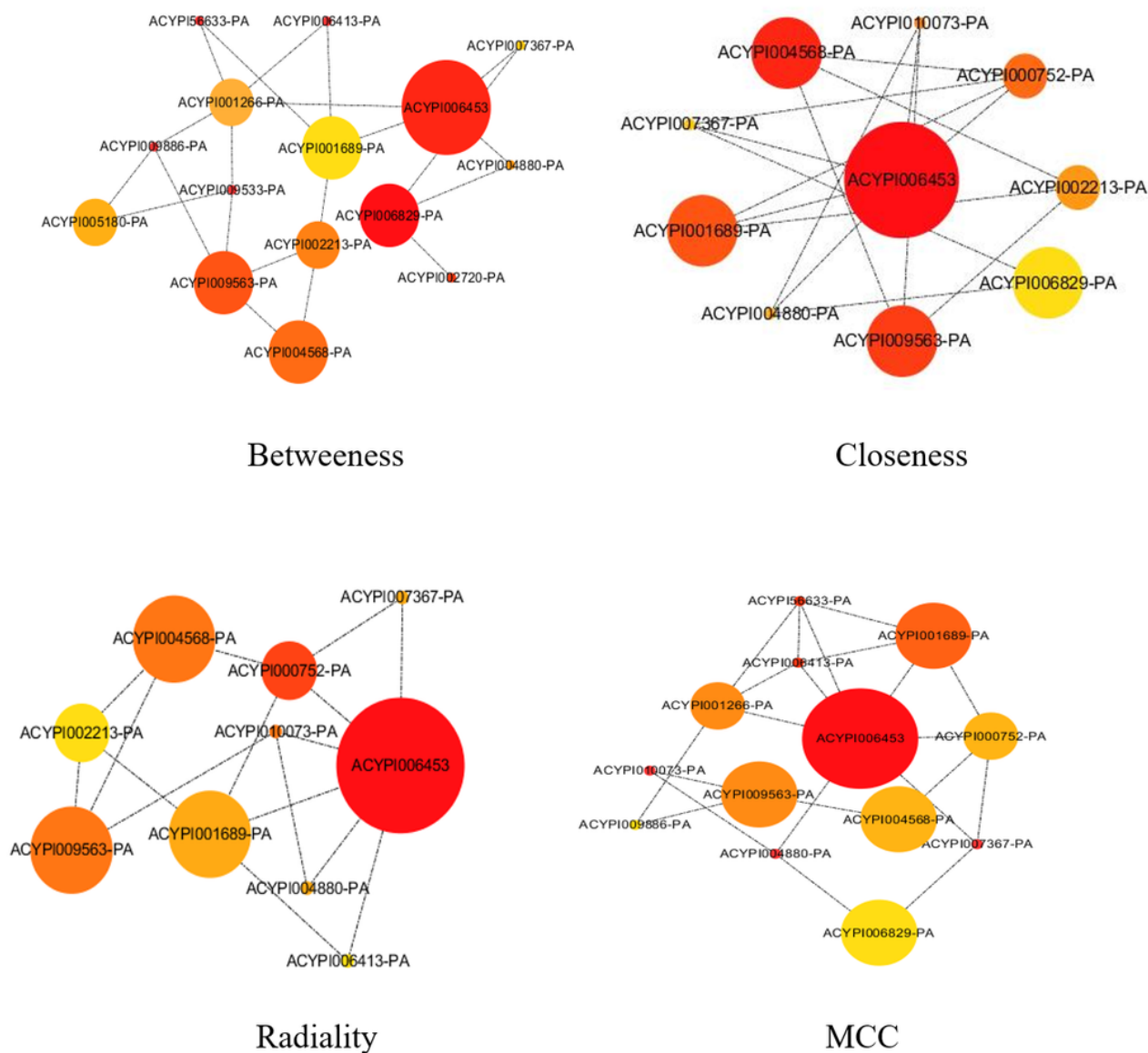


Figure 9

Network topology analysis of joint action targets of *Sophora flavescens* under different screening conditions

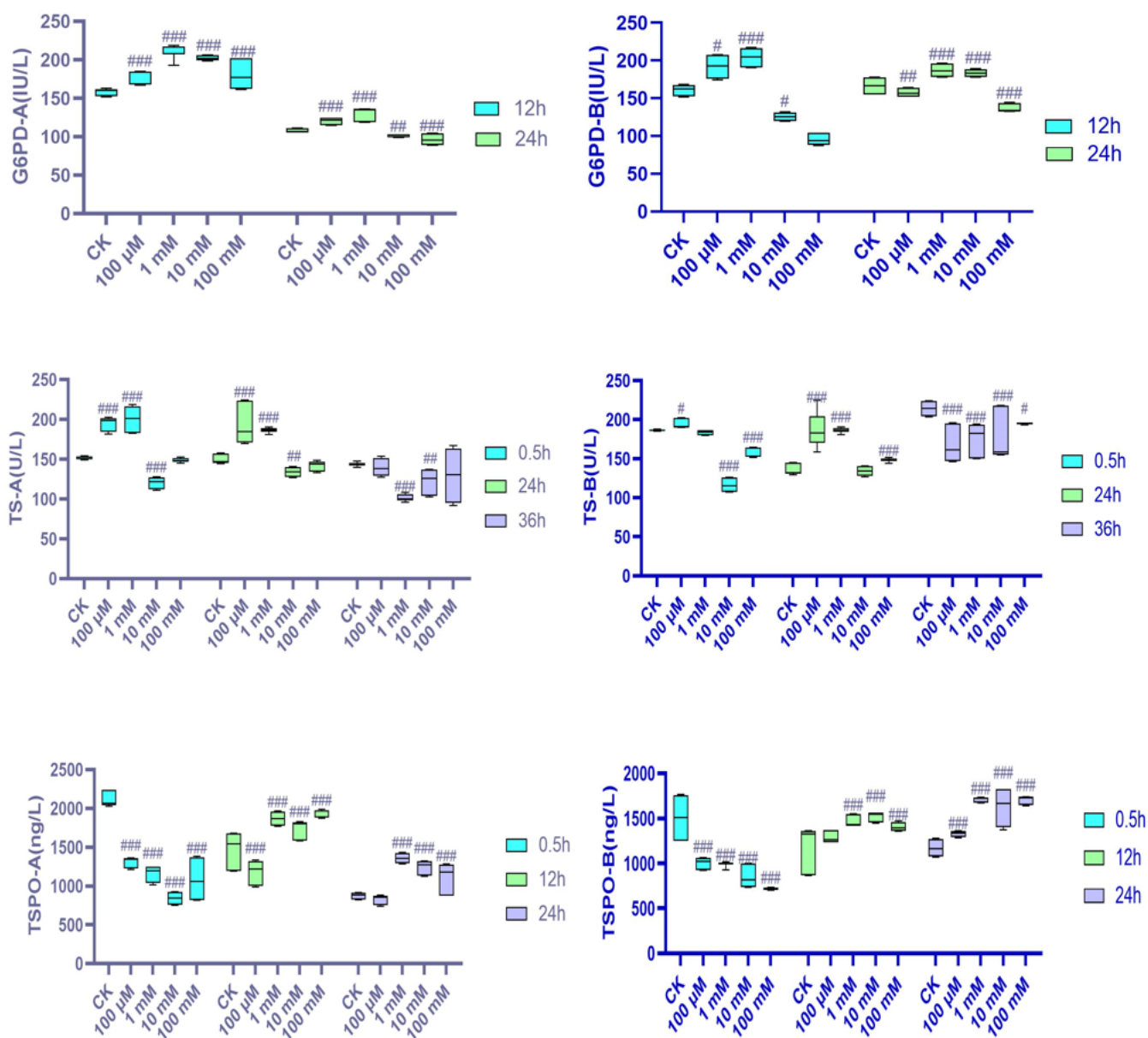


Figure 10

Verification of enzyme activity of crude extract from aboveground part of *Sophora flavescens* against *M. persicae* (host plant A: datura) and *M. persicae* (host plant B: pakchoi) (# $P>0.05$, ## $P>0.01$, ### $P>0.0001$)

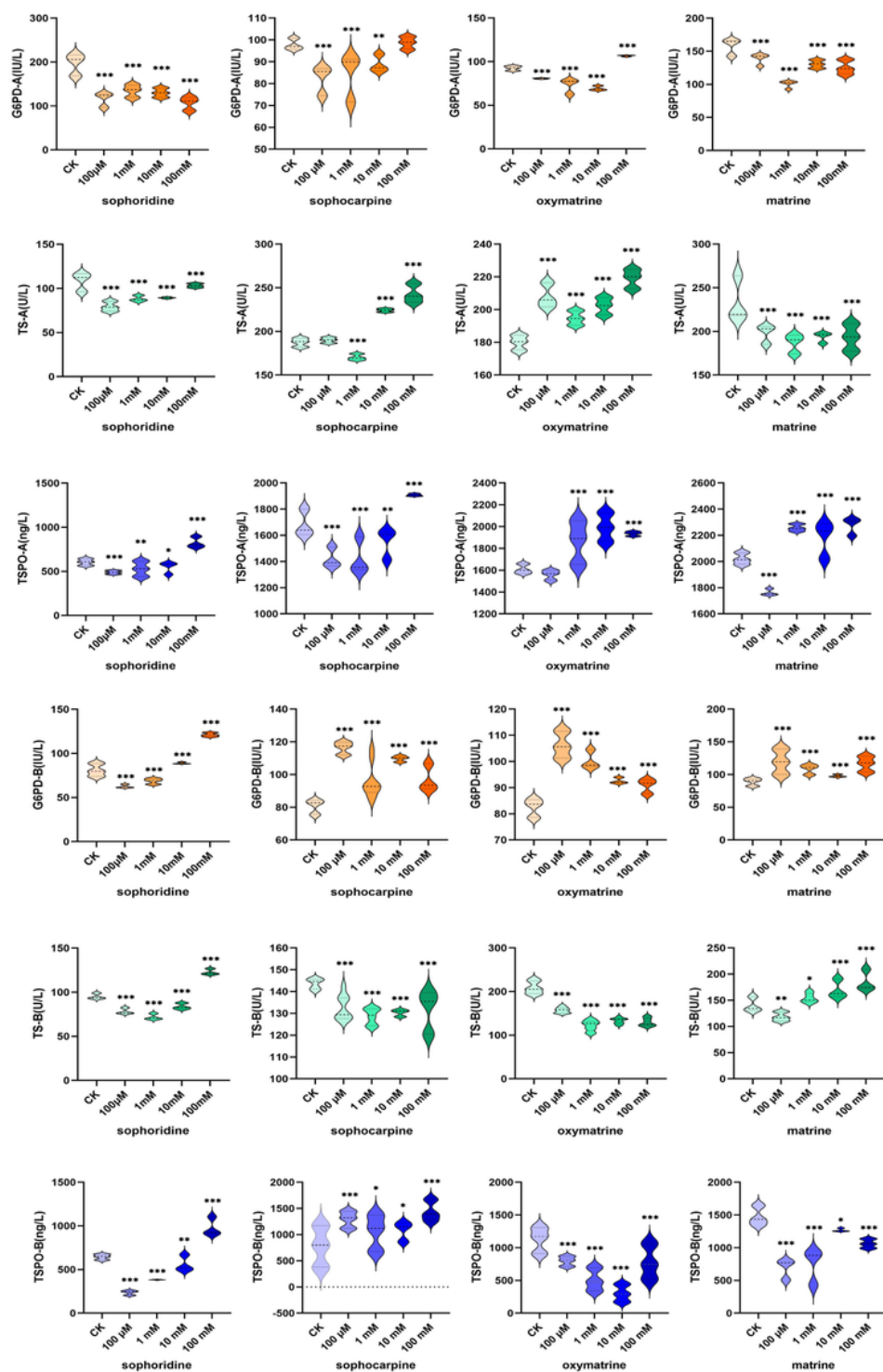


Figure 11

Verification of enzyme activity of matrine, oxymatrine, sophorine, and sophoridine against *M. persicae* (host plant A: datura) and *M. persicae* (host plant B: pakchoi) (* $P > 0.05$, ** $P > 0.01$, *** $P > 0.0001$)

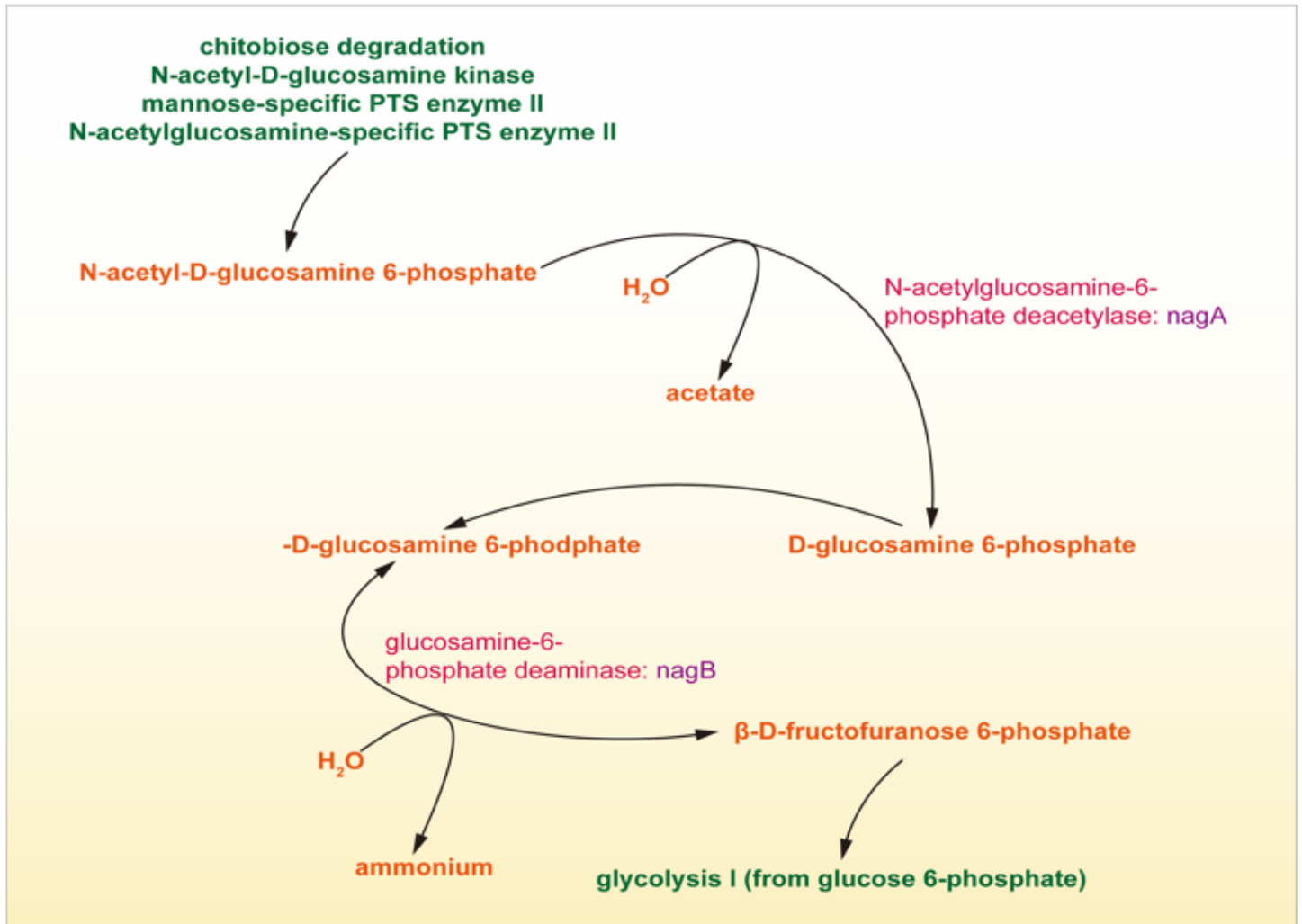
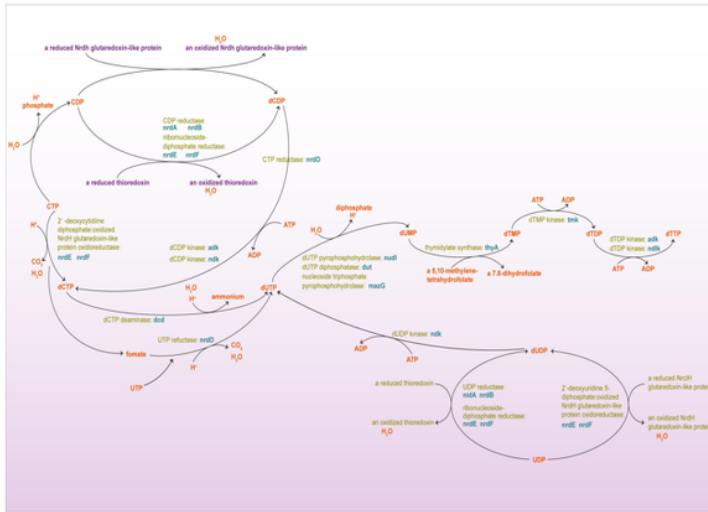
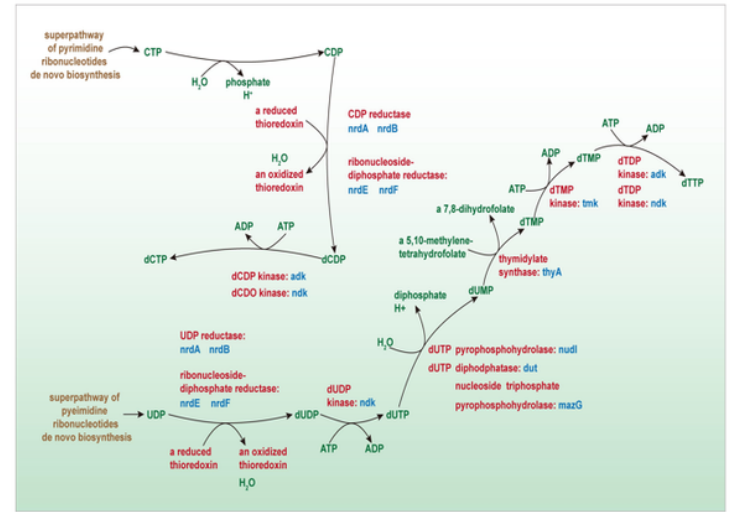


Figure 12

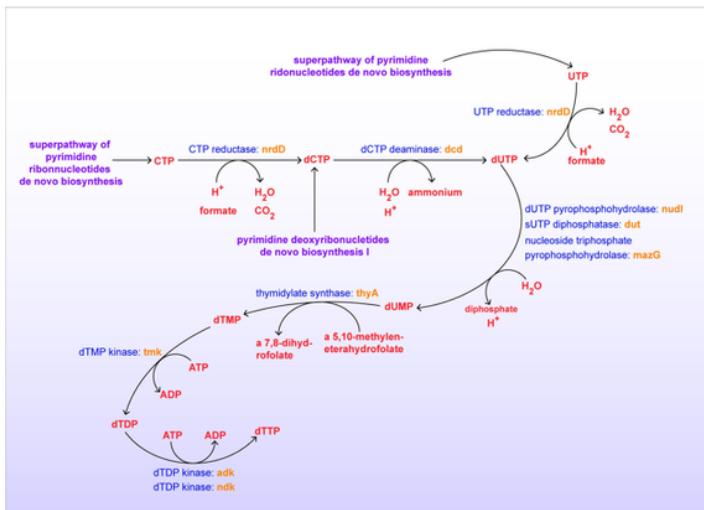
G6PD pathway in aphids (N-acetylglucosamine degradation I).



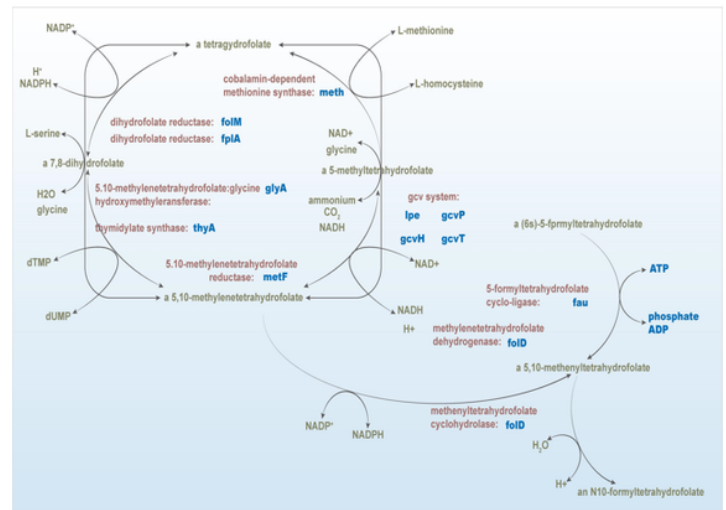
a



b



c



d

Figure 13

TS pathway in aphids. (**a** Superpathway of *de novo* pyrimidine deoxyribonucleotide biosynthesis; **b** *De novo* pyrimidine deoxyribonucleotide biosynthesis I; **c** *De novo* pyrimidine deoxyribonucleotide biosynthesis II; **d** Folate transformation III)

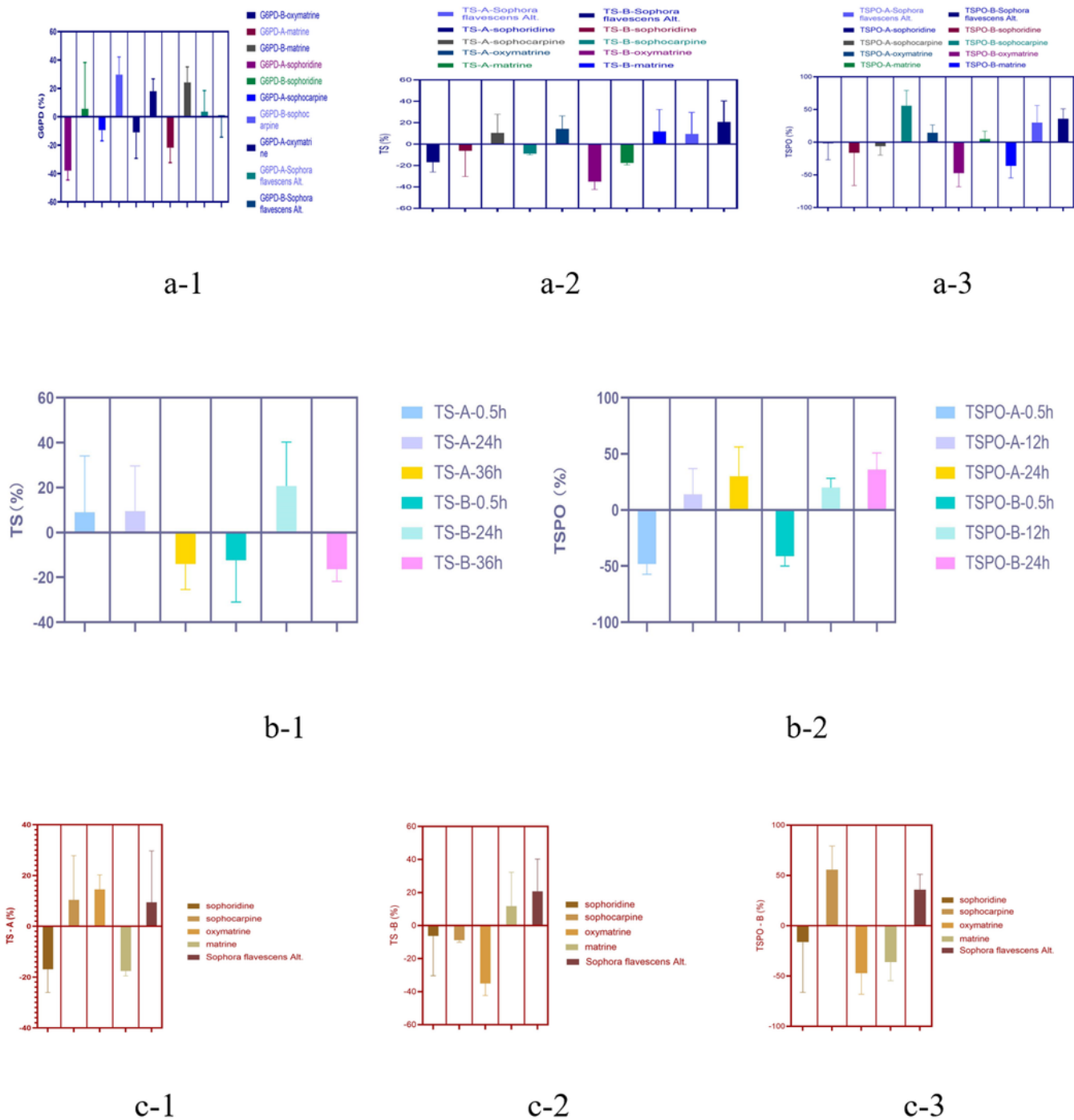


Figure 14

Other factors influencing G6PD, TS, and TSPO in *M. persicae* (host plant A: datura) and *M. persicae* (host plant B: pakchoi)