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RESEARCH

A novel monocarbonyl curcumin analog synergizes pesticide efficiency via cytochrome P450 inhibition in the whitefly *Bemisia tabaci*

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Abstract

Synthetic insecticides remain the cornerstone of pest management in both agriculture and public health. However, their extensive application has led to the emergence of insecticide resistance, while regulatory standards are shifting toward less toxic insecticidal compounds, of natural origin (green chemicals). This study investigates the inhibitory effects of various natural products, polyphenols and synthetic curcuminoids, on cytochrome P450 CYP6CM1 from the whitefly *Bemisia tabaci* (*Bt*CYP6CM1). This major agricultural pest exhibits significant insecticide resistance, which is largely mediated by the overexpression of *Bt*CYP6CM1. Among the tested natural products, curcumin emerged as the strongest inhibitor of *Bt*CYP6CM1 (IC_{50}: 6.82 ± 0.54 μM). Additionally, a synthetic library of thirteen monocarbonyl curcumin analogs was screened in search of compounds with improved inhibitory potency toward *Bt*CYP6CM1. The monocarbonyl curcumin derivative DM96 exhibited significantly enhanced inhibitory potency against *Bt*CYP6CM1, with an IC_{50} value of 2.41 ± 0.24 μM. Furthermore, DM96 was evaluated as a potential synergist with imidacloprid in bioassays with adult whiteflies. The results demonstrated a substantial synergistic effect, as the combination of the curcumin derivative and imidacloprid increased *B. tabaci* mortality rates by up to fourfold. These findings suggest that DM96 could be a promising candidate for the development of more effective pesticide formulations, with lower toxicity and resistance breaking potential. Overall, the work contributes to the ongoing search for eco-friendly bio-pesticides and synergists that can mitigate resistance and reduce the reliance on chemical insecticides, thus supporting more sustainable agricultural practices.

Keywords Cytochrome P450 · Inhibition potency · Curcumin · Curcumin analogs · Synergism insecticide resistance · *Bemisia tabaci*

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Introduction

Synthetic insecticides remain the primary strategy for insect pest management in both crop protection and public health. However, the emergence of insecticide resistance because of widespread utilization, along with adverse effects on non-target species, has highlighted the need for alternative control strategies and improved pesticide formulations (Schneider et al. 2023; Sureshbabu et al. 2023; Ye et al. 2022; Sparks et al. 2020; Maronne 2019). While alternative approaches, such as genetically modified (GM) crops and augmentative biological control, offer potential solutions, they encounter significant challenges related to regulation, scalability, efficacy and prolonged timelines for implementation (Van Lenteren 2012). Consequently, for the near future, the protection of food sources and human health from insects may remain reliant on chemical insecticides. Therefore, there is a pressing need to develop improved insecticide formulations that are highly efficacious, are not compromised by pre-existing resistance, and exhibit greater selectivity and safety for mammals, pollinators and other non-target species.

Cytochrome P450 monooxygenases (CYPs) are a superfamily of enzymes that catalyze the oxidative transformation of a wide range of exogenous and endogenous substrates in most organisms (Nauen et al. 2022). P450-based insecticide resistance can develop through elevated P450 expression and/or qualitative changes, such as amino acid alterations, that enhance the metabolic breakdown of insecticides (Li et al. 2024; Qin et al. 2023; Nauen et al. 2022; Ye et al. 2022; Liu et al. 2015). The molecular mechanisms regulating P450 expression include the roles of *cis/trans*- regulatory factors, gene amplification or duplication, thus leading to increased availability of P450 enzymes for insecticide metabolism (Zeng et al. 2023; Nauen et al. 2022; Zimmer et al. 2018). Less commonly, resistance can also arise from downregulation of P450s that are responsible for activating pro-pesticides (Nauen et al. 2022; Vlogiannitis et al. 2021). Insecticide selectivity in pollinators has also been linked to differences in metabolic ability of P450s against certain neonicotinoids (Bass et al. 2024; Reid et al. 2020; Manjon et al. 2018). Additionally, insect P450 genes can be induced by exposure to xenobiotics, particularly plant chemicals, resulting in temporary tolerance due to the non-heritable nature of this response (Cui et al. 2016; Liu et al. 2015). This is less likely with pesticides, since high dosages do not allow sufficient time for enzyme upregulation before causing irreversible toxicity (Nauen et al. 2022). However, previous work on pests, such as *Bemisia tabaci,* has shown that different host plants may influence P450's expression and thus insecticide sensitivity (Pym et al. 2023; Dermauw et al. 2013). Non-insecticidal chemicals, such as herbicides and pollutants, can also induce P450s, resulting in moderate enhancement of resistance to subsequent insecticide exposure (Poupardin et al. 2008).

The cotton or sweet potato whitefly, *Bemisia tabaci*, is a persistent and destructive pest with substantial agricultural significance due to the major losses it causes across a wide range of crops (Abubakar et al. 2022; Barman et al. 2022; Perring et al. 2018). *B. tabaci* has developed resistance to most classes of chemical insecticides typically employed for its control, primarily as a result of adaptive evolutionary mechanisms that increase detoxifying enzyme activity before the compound reaches its target site (Lira et al. 2023; Du et al. 2023; Xie et al. 2018; Basit 2019; Ghanim and Kliot 2013; Pietri and Liang 2018; Bass et al. 2015). In the last decades, neonicotinoid insecticides, have largely replaced organophosphates, organochlorine insecticides and pyrethroids in the management of *B. tabaci* worldwide; however, resistance and tolerance to neonicotinoids has been reported in several countries (Naveen et al. 2017; Jeschke and Nauen 2008). Among the several CYPs associated with neonicotinoid resistance, *Bt*CYP6CM1 is the most frequently acknowledged (Pym et al. 2023; Yang et al. 2020; Hamada et al. 2019; Nauen et al. 2015; Karunker et al. 2008). *Bt*CYP6CM1 was initially characterized by its ability to metabolize the neonicotinoid imidacloprid into less toxic compounds, thus reducing the pest's efficacy (Karunker et al. 2008, 2009). Subsequently, *Bt*CYP6CM1 has been linked to resistance against thiamethoxam, acetamiprid, clothianidin and thiacloprid (Barman et al. 2022; Rao et al. 2012; Roditakis et al. 2011). Over-expression of *Bt*CYP6CM1 has also been associated to cross-resistance between neonicotinoids and pymetrozine in *B. tabaci* (Nauen et al. 2013, 2015).

A promising strategy in order to counteract insecticide resistance is the inactivation of selected P450s, either through inhibitors acting as synergists or by gene silencing via transgenic plants (Ye et al. 2022). Synergists can reduce the required insecticide doses, thus limiting the undesired effects on non-target species and delaying the emergence of insecticide resistance (Taillebois and Thany 2022). However, conventional synergists, such as the monooxygenase inhibitor piperonyl butoxide (PBO) and its analogs, exhibit limitations in efficiency and selectivity, as well as toxicity for non-target organisms (Shelley et al. 2012; Tomizawa and Casida 2005; Takahashi et al. 1997). Some low-risk active substances and putative synergists, including naturally derived green chemical insecticides (e.g., plant extracts, essential oils, etc.), have been introduced to the market or are under development, though their efficacy, alone or in combination with widely-used insecticides, requires further evaluation (Taillebois and Thany 2022; Cui et al. 2022; Roy et al. 2015; Liu et al. 2016, 2014).

In this work, the synergistic potential of a series of natural products, mostly polyphenols and synthetic curcuminoids was evaluated against a highly resistant whitefly *B. tabaci* population, which overexpress the cytochrome *Bt*CYP6CM1, using both in vitro and in vivo functional approaches.

Materials and methods

Materials

Chemicals

Oxidized glutathione, reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), 7-ethoxycoumarin and glutathione reductase were obtained by Sigma-Aldrich, USA (Merck). Analytical grade salts as well as resveratrol, gallic acid, safranal, naringenin, polydatin, taxifolin hydrate, curcumin, quercetin, epigallocatechin gallate and ellagic acid were also purchased from Sigma-Aldrich, USA (Merck). Ethanol and dimethyl sulfoxide (DMSO) were purchased from Scharlau (Spain). The insecticide imidacloprid (Confidor 200SC) was obtained from Bayer Crop Science.

Curcuminoids and curcumin derivatives

The synthesis of curcuminoids and curcumin derivatives was performed as previously described (Pantiora et al. 2022).

Bemisia tabaci strain

A population of *B. tabaci* collected from a greenhouse melon crop in the Tympaki region of Crete in 2021, with high levels of previously characterized neonicotinoid resistance (Stavrakaki et al 2023) associated with the overexpression of the *Bt*CYP6CM1 was used in this study. The population was stored in insect-proof rearing cages containing clean cotton plants (*Gossypium hirsutum* L.) under controlled environmental conditions, as previously described (Stavrakaki et al 2023).

Methods

Functional expression of the recombinant *Bt*CYP6CM1 in *E. coli* cells

The expression of recombinant *Bt*CYP6CM1 in *E. coli* BL21(DE3) STAR cells was performed following the methodology described by Karunker et al. (2009). Briefly, to functionally express CYP6CM1vQ, *E. coli* BL21(DE3) STAR cells were co-transformed with two plasmids: PcwOmpA-*Bt*CYP6CM1 and pACYC-pelB-*Ag*CPR. The latter plasmid enables the expression of cytochrome P450 reductase (CPR) from *Anopheles gambiae* (*Ag*CPR). Transformed cells were grown in Terrific Broth supplemented with ampicillin and chloramphenicol until OD_{595} exceeded approximately 0.8–0.9, and then induction was initiated by adding δ-aminolevulinic acid and isopropyl-β-d-thiogalactopyranoside (IPTG), each to a final concentration of 1 mM. After 24 h of expression, cells were harvested, and spheroplasts were generated by treating the cell pellet with lysozyme in TSE buffer (0.1 M Tris–acetate, pH 7.6, 0.5 M sucrose, 0.5 mM EDTA) for 1 h at 4 °C. The mixture was centrifuged at 2800 × g for 30 min, and the resulting pellet was resuspended in potassium phosphate buffer (0.1 M, pH 7.6) containing magnesium acetate, glycerol, dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), aprotinin, and leupeptin. The suspension was sonicated, and membranes were isolated by ultracentrifugation at 180,000 × g for 1 h. These membrane fractions were diluted in TSE buffer and stored at − 80 °C for later analysis. P450 content was determined by CO-difference spectra (Omura and Sato 1964), while *Ag*CPR activity was evaluated by measuring NADPH-dependent cytochrome c reduction at 550 nm (Pritchard et al. 2006). Both the cytochrome P450 reductase and cytochrome b_5 used in this study were derived from *Anopheles gambiae*. The *Ag*CPR sequence (GenBank accession no. AY183375) has been widely validated in heterologous co-expression systems and was chosen due to its demonstrated compatibility with a broad range of insect P450 enzymes, including *Bt*CYP6CM1 (Karunker et al. 2009). Similarly, the *Anopheles gambiae* cytochrome b_5 (GenBank accession no. AY183376) was selected as a cofactor to support efficient electron transfer in the reconstituted P450 system. Using redox partners from the same insect source ensures functional compatibility and has been shown to improve the catalytic performance of insect P450s in recombinant expression systems. Recombinant *A. gambiae* cytochrome b5, prepared according to the procedure by Holmans et al. (1994), was included in some assays. Total protein concentration was quantified using the Bradford assay (Bradford 1976) with bovine serum albumin as the standard.

Enzyme assays and inhibition studies

Screening of natural compounds and curcumin derivatives as potential inhibitors of *Bt*CYP6CM1 Enzyme activity of *Bt*CYP6CM1 was measured using 7-ethoxycoumarin as substrate, monitored on LS-45 Fluorescence Spectrometer (Perkin Elmer). Inhibition potency was evaluated for a range of natural compounds and curcumin derivatives. Ethanol was the solvent of the natural compounds, except for ellagic acid, which was dissolved in 1 M NaOH. DMSO was used to dissolve the curcumin derivatives. The screening was per-

formed with 2–5 pmol *Bt*CYP6CM1/CPR and 20–50 pmol b5 in 1:10 ratio. Each reaction mixture contained 0.83 mM of NADPH, which is added last, and 0.83 mM 7-ethoxycoumarin in 0.1 M sodium phosphate buffer, pH 7.2. The test compounds were added to the cuvette prior to the addition of NADPH, at final concentrations ranging from 5 to 50 µM. The enzyme reactions were incubated for 30 min at 30 ℃ in 500 µL final volume. Afterward, NADPH fluorescence was quenched by adding 83 µL of a solution containing 15 mM oxidized glutathione (GSSG) and 0.125 units of glutathione reductase. After a 10 min incubation at room temperature, the reactions were terminated by the addition of a stop solution [1:1 (v/v) ratio of 140 mM Tris–HCl, pH 8.5, and 15% (v/v) acetonitrile] up to 1,800 µL final volume. Fluorescence was measured with excitation at 390 nm and emission at 465 nm, which corresponds to the maximal fluorescing wavelength of 7-hydroxycoumarin (Aitio 1978). Inhibition was determined in triplicate with control reactions including P450s incubated without NADPH and cuvettes containing only buffer. Data analysis, including the determination of IC_{50} values and graph generation, was performed using GraphPad Prism version 8 (GraphPad Prism Software, Inc.).

Molecular modeling and docking

Generation of *Bt*CYP6CM1 model. A model of *Bt*CYP6CM1 was created using Alphafold2 (Jumper et al. 2021). The heme group was introduced into the model's inner cavity using the structural alignment between the PDB structure of human CYP3A4 with PDB code 4D6Z and the model. Interestingly, CYP3A4 showed the best sequence alignment with *Bt*CYP6CM1 (Kaur et al. 2016). The stereochemical quality of the generated protein model was evaluated using PROCHECK (Laskowski et al. 1993) and ERRAT (Colovos and Yeates 1993), both available at SAVES v6.0 server (https://saves.mbi.ucla.edu).

Protein–ligand dockings. Protein–ligand docking calculations were performed on the ten natural products and 13 curcumin derivatives using GOLD (Jones et al. 1997) with the ChemScore scoring function and using the custom parameters for cytochrome P450s provided in this software. Default parameters were used for each compound, and 50 possible docking solutions were generated.

Bioassays based evaluation of imidacloprid-curcumin analog combinations

Bioassays were performed as previously described (Stavrakaki et al 2023). Briefly, treatments were performed using leaf-dip bioassays on adults according to the IRAC method protocol 015 (www.irac-online.org). Cotton leaf discs (55 mm) were dipped in serial concentrations of imidacloprid (2–600 ppm) containing 0.2 g L^{-1} Triton X-100 as a non-ionic wetting agent. The discs were allowed to dry and then placed abaxial side up on petri dishes laid on a thin sterile agar bed (15 g L^{-1}). For each treatment three replications were performed with 30 female *B. tabaci* per replication. Mortality was assessed at 72 h.

To determine sub-lethal concentration of DM96, we used two different approaches. In the direct exposure approach (leaf-dip bioassay), DM96 was dissolved in a tank mix solution of dimethyl sulfoxide (DMSO) and emulsifier (EM) (3:1), and doses of 0.01%, 0.1%, and 0.3% (w/v) were tested using the IRAC method 015, as previously described (Roditakis et al. 2005). In the pre-exposure approach (tarsal contact), following the methodology as outlined in Stavrakaki et al. (2022), adults were exposed to 300 µL acetonic solution with DM96 at doses of 0.01%, 0.1%, and 0.3% (w/v) in coated glass vials (30 mL volume). The vials were then placed horizontally on a vial roller for 1 h to ensure uniform coating. The vials were allowed to dry for an additional hour, after which the adults were placed in each vial and exposed to the curcuminoid for 2 h. Subsequently, the DM96-exposed insects were used in leaf-dip bioassays, as previously described. Bioassays were conducted to evaluate the potential synergistic action of DM96 using both aforementioned approaches at a concentration of 0.3% (w/v, corresponds to 3,000 ppm) for DM96 which caused low mortality and 600 ppm imidacloprid, the concentration resulting in approximately 30% mortality in preliminary experiments, a sub-lethal mortality level appropriate for investigating synergistic effects. All treatments were conducted under controlled conditions (26 ± 1 °C, 50–60% RH, 16L:8D photoperiod).

One way ANOVA was used for the statistical analysis of individual mortality level. Detailed ANOVA statistics (F, df, and P values) are provided in the corresponding figure legends. The synergistic ratio for the mixture was determined by dividing the effect of the total mixture by the additive efficacy of the individual substances, taking into account the basic efficacy of the individual agents (Ali et al. 2017; Bliss 1939).

Results

Heterologous expression of BtCYP6CM1 in E. coli and identification of novel inhibitors

Recombinant *Bt*CYP6CM1 was heterologously expressed in *E. coli* BL21(DE3) STAR cells, and successful expression was confirmed by western blot analysis. The results are presented in Figure S1. Carbon monoxide difference spectroscopy was performed to assess the proper folding and catalytic competence of the expressed P450. As shown

in Table S1, the membrane preparations exhibited a characteristic Soret peak at 450 nm, confirming the presence of correctly folded holoenzyme. The absence of a major peak at 420 nm indicates minimal formation of inactive P420 species. In addition, membrane-specific content and CPR activity were quantified, supporting the presence of a functionally reconstituted CYP–CPR system. Taken together, these results confirm the successful and functional expression of *Bt*CYP6CM1 in the heterologous system.

The initial screening of potential inhibitors against *Bt*CYP6CM1 included a range of natural products, primarily polyhydroxy compounds, tested at final concentration of 50 µM. The results listed in Table 1**,** indicate curcumin, quercetin and ellagic acid as the most potent inhibitors, since they completely inhibit the activity of *Bt*CYP6CM1 (Fig. 1). Epigallocatechin gallate also exhibited substantial inhibition at 76.53 ± 0.73% (Inhibition % ± SD), while resveratrol, taxifolin hydrate, safranal and naringenin resulted in moderate inhibition, ranging from 31.36 to 43.27%. The lowest inhibition was determined for polydatin (15.35 ± 0.21%) and gallic acid (13.99 ± 3.13%). Consequently, the activity of the most potent inhibitors (curcumin, quercetin and ellagic acid) was further investigated at a tenfold dilution (concentration 5 µM), as shown in Table 1. The results indicate that curcumin demonstrated the highest inhibitory activity among the selected natural compounds, achieving 46.86 ± 2.44%, followed closely by quercetin at 45.00 ± 3.69%. In contrast, ellagic acid exhibited substantially lower inhibitory activity, with a value of 16.44 ± 0.71%.

The most potent inhibitor, curcumin, was selected for further investigation with dose–response studies. IC_{50} of curcumin toward *Bt*CYP6CM1 was determined at 6.82 ± 0.54 µM (Fig. 2). A series of curcumin derivatives, including curcuminoids and monocarbonyl curcumins (Table 2), previously synthesized by our group (Pantiora et al. 2022), were also evaluated as inhibitors of *Bt*CYP6CM1. As shown in Table 2, DM96 displays significantly improved inhibition potency (71.02 ± 0.15%), compared to curcumin (46.86 ± 2.44%). DM57, DM94 and MS238 exhibited the lowest inhibition ranging from 1.29 to 27.02%. Moderate inhibition was determined for the remaining compounds, ranging from 32.88 to 50.89%. The most potent inhibitor of *Bt*CYP6CM1, DM96, was selected for further dose–response studies and IC_{50} determination. The IC_{50} of DM96 toward *Bt*CYP6CM1 was determined at 2.41 ± 0.24 µM (Fig. 2).

Curcumin has been reported to act as a competitive inhibitor of human CYP3A4 (Appiah-Opong et al. 2007), a close homologue of *Bt*CYP6CM1. In line with this, and based on the spectroscopic stability of curcumin derivatives during our in vitro assays, we did not observe any evidence of metabolism of curcumin or its analogs by *Bt*CYP6CM1. These findings suggest that, for *Bt*CYP6CM1, curcumin derivatives functions as a reversible competitive inhibitor, with no indication of substrate turnover or irreversible (mechanism-based) inhibition.

Molecular modeling and docking

Structural assessment of the *Bt*CYP6CM1 model

The structural quality of the generated model was assessed using the PROCHECK (Laskowski et al. 1993) and ERRAT (Colovos and Yeates 1993). ERRAT score is 98.9384 (Figure S2), while Ramachandran plot indicates 92.3% of residues in the "most favored regions," 7.3% in "allowed regions," 0.2% in "generously allowed regions," and 0.2% in "disallowed regions" (Figure S2). Altogether, this means that the model generated is of very high quality.

Docking of natural compounds

Similar to most metabolizing P450s, the size of the *Bt*CYP6CM1 catalytic cavity is quite large and substrate-adaptive; a known challenge for modeling. Within the context of a protein–ligand docking framework, this means that the dimension of the search space should avoid being too large (to prevent solvent-exposed binding solutions) or too narrow (whose dimensions could be smaller than the chemical). Therefore, we performed a benchmark with four different radii of the search space; 10, 17, 25, and 30 Å, considering the sulfur atom of the heme-coordinating cysteine as a sphere center. A radius of 10 Å proved insufficient for accommodating certain molecules (e.g., Epigallocatechin gallate), while results obtained with radii of 17, 25 and 30 Å showed similar trends. However, radii of 25 and 30 Å do show poses on the protein surface and for these reasons, the following part of the study focuses on docking performed with a sphere 17 Å radius.

In general, all-natural products (Table 1) demonstrated favorable docking scores, ranging from 41.4 to 24.3 ChemScore units (Table 3). This shows that calculations agree with the experimental results regarding their potential inhibitory effect on *Bt*CYP6CM1. In agreement with the experimental data, curcumin was the compound with the best results (mean score of 35.6 and best solution at 41.4 ChemScore Units), indicating its potential as a promising template for inhibitor design. Docking solutions for all other compounds show lower scoring values with a narrow range of values (ca. 10 units). Interestingly, the Chem score units for quercetin ranged between 32.8 for best value and 31.8 for mean value, which are significantly lower than the curcumin performance, despite the fact that their experimental inhibitory activity was very close. The computational results in combination with the experimental data prompted us to exploit the curcumin skeleton further for the development

Table 1 Screening of inhibition potency (%) of selected natural products against *Bt*CYP6CM1 [Inhibition (%) ± SD, N = 3]. Each compound was tested at 50 µM final concentration. The compounds with the highest inhibition potency (ellagic acid, curcumin and quercetin) were also tested at 5 µM final concentration. Compounds were classified as strong inhibitors (> 70% inhibition), moderate inhibitors (30–50% inhibition), or low inhibitors (< 30% inhibition)

Compound	Enzyme Inhibition (%)
Resveratrol	33.7 ± 4.2
Taxifolin hydrate	35.5 ± 3.8
Safranal	31.36 ± 2.5
Ellagic acid	100 16.4 ± 0.7*
Polydatin	15.4 ± 0.2
Curcumin	100 46.9 ± 2.4*
Naringenin	43.27 ± 1.2
Epigallocatechin gallate	76.53 ± 0.7
Quercetin	99.63 ± 1.1 45.0 ± 3.7*
Gallic acid	13.99 ± 3.1

* The inhibition potency (%) determined at 5 µM final concentration

of new such inhibitors. With such values, direct correlation between predicted and experimental binding affinities is not achievable and stands out of the model. Discrepancies could come from a mechanistic aspect not considered with docking calculations like the possible transit of the molecule from a solvent-exposed region to the cavity site or the need to consider major dynamic effects related to induced fitting of the ligand into the protein cavity. Noteworthy, these ChemScore values should not be interpreted explicitly as measures of binding energy or binding affinity (Ferrara et al. 2004). Yet, this part of the study sustains a very good complementarity of curcumin with the catalytic center of *Bt*CYP6CM1 hence representing an excellent scaffold for inhibitor designs.

Docking of curcumin derivative DM96

The interaction of the strongest inhibitor DM96 with *Bt*CYP6CM1 was performed to understand further the critical elements that govern the interactions between

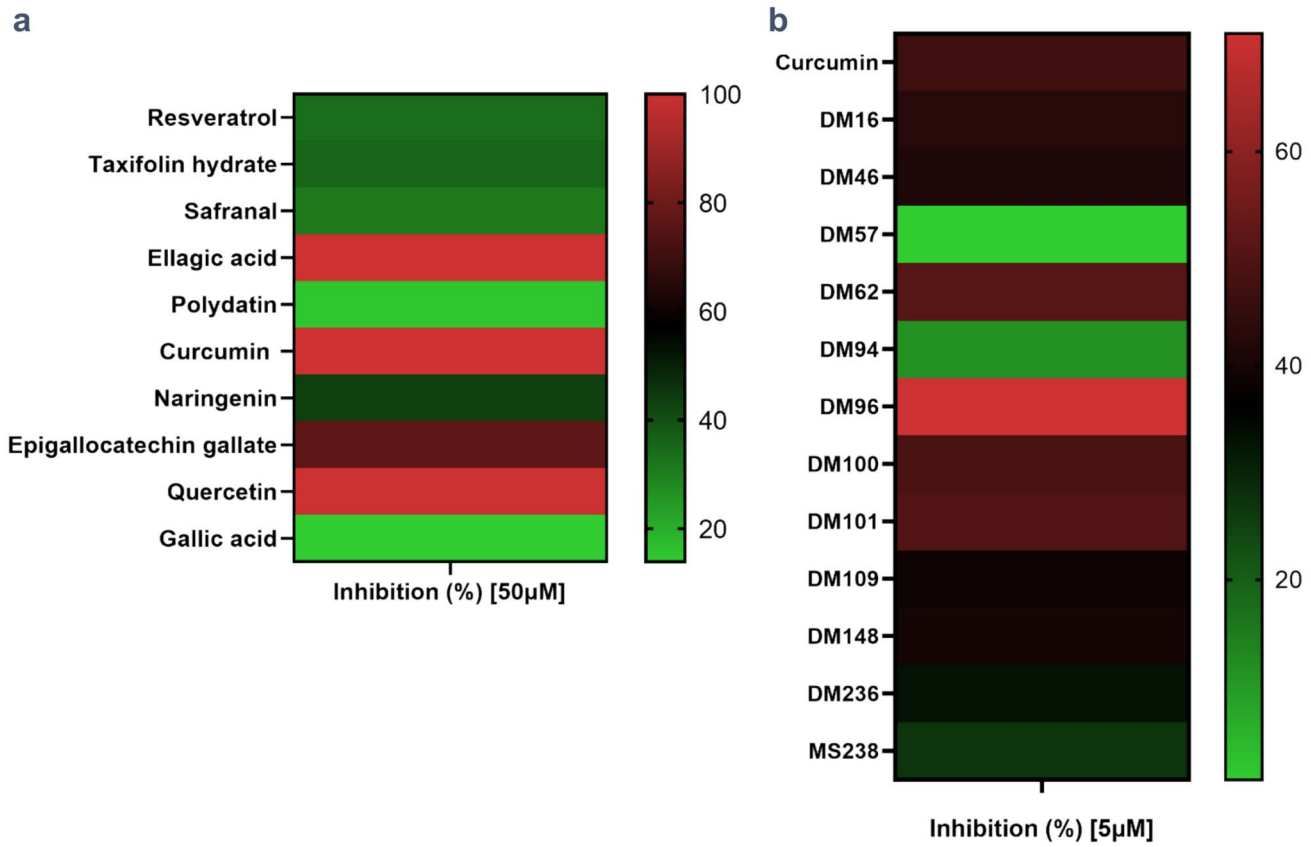


Fig. 1 Heat map depiction of the inhibition (%) against *Bt*CYP6CM1 of natural products at 50 μM final concentration (a) and curcumin derivatives at 5 μM final concentration (b)

curcumin and its derivative with *Bt*CYP6CM1 (Fig. 3). Two regions of interactions can be identified and correspond to each aromatic end of the molecules. One of the catecholic/phenolic moieties stands close to the heme, while the second stands in distant regions of the binding site. One aromatic moiety of curcumin interacts with the heme, Phe99, Ser357 and Thr472 through polar and hydrophobic interactions, a geometry very close to the one obtained for DM96, where an additional interaction appears between the iron and the hydroxyl group of the compound. The second aromatic end of the molecules present wider discrepancies between curcumin and DM96. In the best predicted poses, curcumin is stabilized through hydrophobicity with two phenylalanine residues, Phe193 and Phe471, and polar interactions of the hydroxyl and methoxy substituents with Glu294, Ser470. This is not the case, however, for the interaction of DM96 (Fig. 3), which seems to occur via Ser95 and Thr100 and is further stabilized through hydrophobic interactions with Phe99. One of the carbonyl groups of curcumin is interacting with Ser287 while the second one is stabilized by Ser290, an amino acid that similarly interacts with the carbonyl group of DM96, as well. Since flexibility and dynamic effects appear to be critical factors underlying the differences in binding properties of natural and synthetic chemicals, we deepened our analysis by comparing the full set of dockings solutions for curcumin and DM96. It was observed that the position of the distal end of curcumin is quite variable and occupies different subsites of the binding site. This clearly shows that the presence of sp^3 carbon inbetween the two carbonyl groups of curcumin's core leads to a reasonable degree of variability. On the contrary, this structural flexibility almost absent in the monocarboxylic skeleton of the synthestic derivatives, regardless of the substitution pattern of the six-membered rings.

Bioassays using imidacloprid and DM96, alone or in combination

The bioassays across a range of concentrations from 2 to 600 ppm of imidacloprid showed low mortality percentages ranging from 12.5% to 31.3%, indicating an exceptionally resistant *B. tabaci* strain (Fig. 4). Survival bioassays using DM96 were also conducted and the results are illustrated in Fig. 5. The mean percentage mortality of *Bemisia tabaci*, using a range of concentrations from 0.01 to 0.3 (w/v),

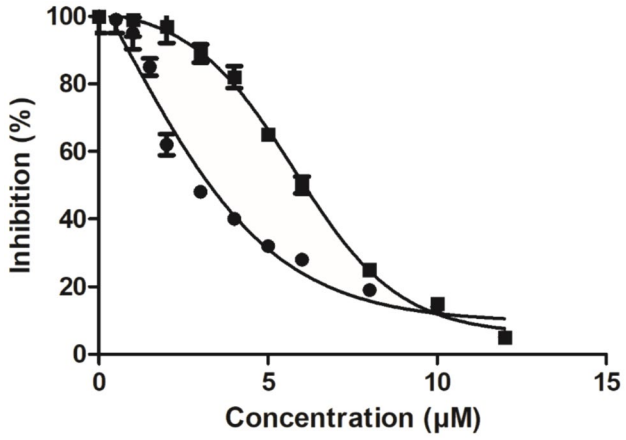


Fig. 2 Dose–response inhibition curves for the determination of the IC_{50} value of curcumin (■) and DM96 (●) against *Bt*CYP6CM1. The measurements represent the mean ± SD

was found to be at the low to moderate level, ranging from 19%—32.5% and 21%—23.8% in the leaf-dip and tarsal contact bioassays, respectively. Notably, both direct exposure of adults, and pre-exposure to DM96 solution (0.3% w/v) when combined with sub-lethal concentrations of imidacloprid (600 ppm), led to a dramatic increase in mortality leading to the complete elimination of the population (100% mortality). The synergist ratio (SR) values of 2.1 and 2, for direct exposure of adults and pre-exposure to DM96, respectively, indicate a synergistic effect between DM96 and the insecticide imidacloprid. Specifically, mortality increased significantly by 3- and 4- fold following direct exposure of adults and pre-exposure to DM96, respectively, as shown in Fig. 6. These promising results support the potential of DM96 as a non-toxic natural product in pest management.

Discussion

The present work underscores the outstanding performance of both natural products and curcumin derivatives as inhibitors of *Bt*CYP6CM1, an enzyme identified as a key determinant of neonicotinoid resistance in the whitefly *Bemisia tabaci*.

The successful heterologous expression of *Bt*CYP6CM1 in *E. coli* BL21(DE3) STAR cells enabled the functional characterization of the enzyme and the identification of novel inhibitors. The carbon monoxide difference spectroscopy confirmed the proper folding of the recombinant protein, as indicated by the characteristic Soret peak at 450 nm and the minimal formation of the inactive P420 form. These results collectively verify the establishment of a catalytically competent *Bt*CYP6CM1-CPR system suitable for inhibitor screening.

Table 2 Inhibition potency screening of curcumin derivatives against *Bt*CYP6CM1 [Inhibition (%) ± SD, N = 3]. Each curcumin derivative was tested at 5 μM final concentration

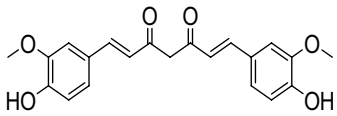
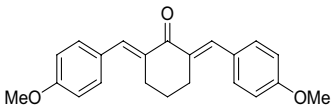
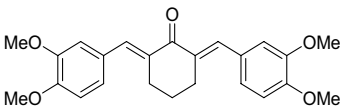
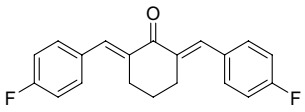
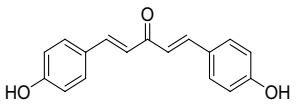
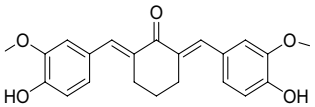
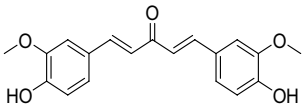
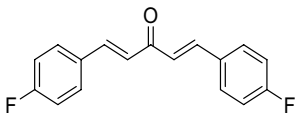
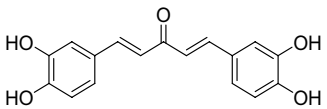
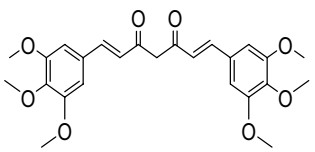
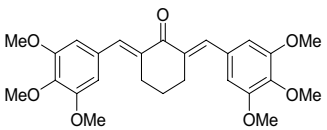
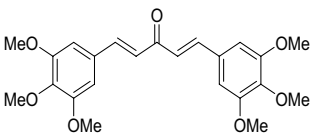
Curcumin derivative	Compound code	Enzyme Inhibition (%)
	**Curcumin**	46.9 ± 2.4
	**DM46**	41.1 ± 2.4
	**DM57**	1.3 ± 0.3
	**DM62**	50.9 ± 1.1
	**DM96**	71.0 ± 0.1
	**DM100**	48.7 ± 0.7
	**DM101**	50.0 ± 2.9
	**DM109**	38.6 ± 0.6
	**DM148**	39.7 ± 0.4
	**MS238**	27.0 ± 0.7
	**DM16**	42.6 ± 0.3
	**DM94**	11.32 ± 2.8

Table 3 Highest ChemScore units from the 50 solutions obtained for each compound. Best and mean ChemScore units are shown for each compound

Ligand	Best ChemScore units	Mean ChemScore units
Curcumin	41.4	35.6
Resveratrol	33.2	32.1
Quercetin	32.8	31.8
Naringenin	32.7	29.6
Taxifolin hydrate	30.9	29.2
Safranal	28.6	28.5
Epigallocatechin gallate	35.0	28.4
Polydatin	32.6	27.5
Ellagic Acid	26.5	25.1
Gallic Acid	24.3	22.8

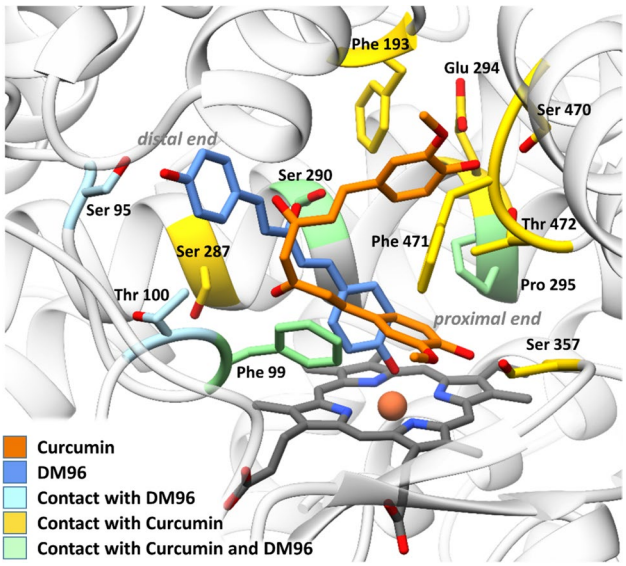


Fig. 3 Binding site of *Bt*CYP6CM1 with DM96 (dark blue) and curcumin (orange) best docking solutions. Residues at a distance lower than 3 Å from only DM96 are showed in light blue. Residues at a distance lower than 3 Å from only curcumin are showed in yellow. Residues at a distance lower than 3 Å from both DM96 and curcumin are shown in light green

The initial screening of natural compounds revealed that several polyphenols, including curcumin, quercetin and ellagic acid, strongly inhibited *Bt*CYP6CM1 activity (Table 1, Fig. 1). Among them, curcumin emerged as the most potent inhibitor, displaying an IC_{50} value of 6.82 ± 0.54 µM (Fig. 2). Further evaluation of curcumin derivatives demonstrated that structural modifications can significantly influence inhibitory potency (Table 2). Notably, the monocarbonyl derivative DM96 exhibited markedly enhanced inhibition, with an IC_{50} of 2.41 ± 0.24 µM. In contrast, several derivatives displayed limited inhibition, highlighting the importance of specific structural features, such as conjugation length and substitution pattern, in determining inhibitory efficiency. Multiple studies have determined curcumin and some of its analogs as inhibitors of P450 enzymes (Mashayekhi-Sardoo et al. 2021; Castaño et al. 2019; Appiah-Opong et al. 2007, 2008; Thapliyal and Maru 2001).

The molecular modeling component of this study provides deeper insights into the molecular mechanisms underlying the inhibitory profile of curcumin and its derivatives against *Bt*CYP6CM1. All tested compounds exhibited favorable docking scores (Table 3), supporting their experimentally observed inhibitory potential. The modest discrepancies between predicted (Table 3) and experimental data (Table 1) likely arise from dynamic protein–ligand interactions or conformational adaptations not captured in the static docking framework.

Curcumin displayed the highest ChemScore values (Table 3), consistent with its strong inhibition activity and confirming its structural compatibility with the *Bt*CYP6CM1 active site. Strong hydrophobic and polar complementarities are observed in all cases, with the intrinsic flexibility of the ligand identified as a distinguishing factor between curcumin and DM96 (Fig. 3). A more thorough evaluation of the impact of this flexibility on the binding properties by more extensive modeling approaches, e.g., molecular dynamics or ligand migration, would potentially aid in identifying novel derivatives with enhanced inhibitory effects. However, such analyses fall beyond the scope of the present study. Quercetin exhibited strong inhibitory activity comparable to that of the curcumin (Table 1). Quercetin also achieved high docking scores, although slightly lower than curcumin, reflecting its comparable but somewhat less optimal binding orientation.

Overall, these findings validate the docking approach as a complementary tool to experimental assays and highlight curcumin as a structurally promising scaffold for the rational design of potent *Bt*CYP6CM1 inhibitors.

The bioassay results clearly demonstrate that the *Bemisia tabaci* strain tested exhibits a high level of resistance to imidacloprid, as reflected by the consistently low mortality rates observed even at high insecticide concentrations (up to 600 ppm) (Fig. 4). Exposure of *Bemisia tabaci* to DM96 alone produced only moderate mortality levels, suggesting limited direct toxicity of this compound under the tested conditions (Fig. 5). However, when DM96 was applied in combination with imidacloprid, either through direct co-exposure or pre-exposure, the mortality rates increased dramatically. This pronounced synergistic effect, supported by synergist ratio (SR) values of 2.1 and 2.0 for direct and pre-exposure treatments, respectively, highlights the ability of DM96 to potentiate imidacloprid

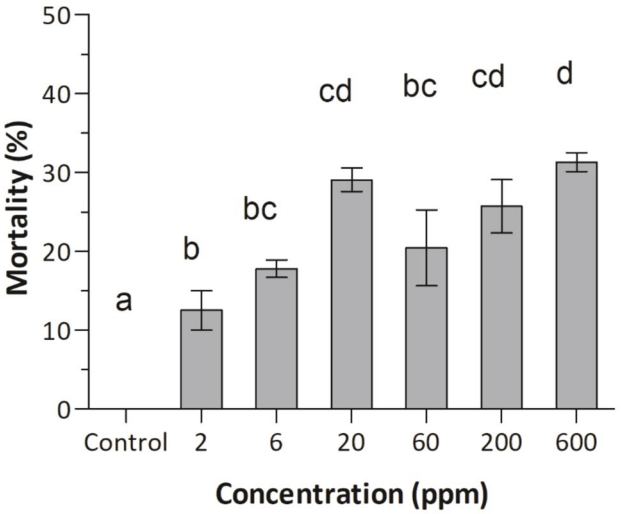


Fig. 4 Effect of imidacloprid concentration on *Bemisia tabaci* mortality (%) as determined using the leaf-dip bioassays on adult insects. Control: treatment with Triton X-100. Different Latin letters indicate statistically significant differences between the treatments (ANOVA, F: 17.977, df:20, $p < 0.001$)

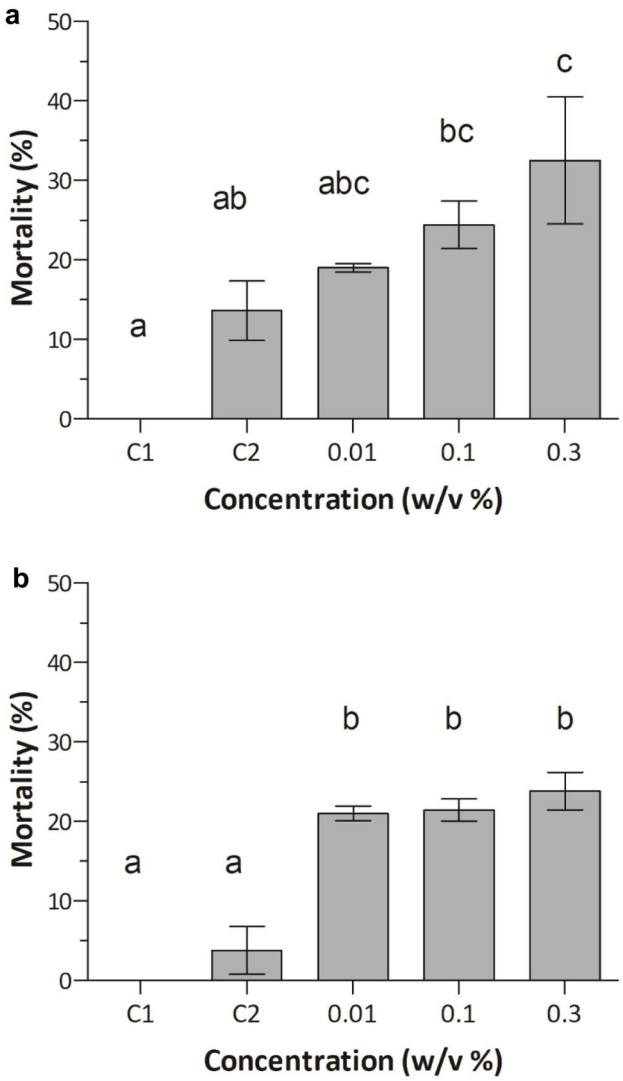


Fig. 5 **a** Mean percentage mortality of *Bemisia tabaci* in leaf-dip bioassays with tank mix DM95 dissolved in dimethyl sulfoxide (DMSO)/emulsifier (EM) (3:1). Different Latin characters indicate statistically significant differences between the treatments (ANOVA, F: 8.840, df:14, $p < 0.005$). **b** Mean percentage mortality of *Bemisia tabaci* in tarsal contact bioassays with DM95 dissolved in acetone. C1: control treatment with Triton X-100; C2: control treatment with acetone. Different Latin characters indicate statistically significant differences between the treatments (ANOVA, F: 36.760, df:14, $p < 0.001$)

efficacy. The observed 3- to fourfold enhancement in mortality strongly suggests that DM96 may act as a metabolic inhibitor, likely through the suppression of cytochrome P450-mediated detoxification pathways responsible for neonicotinoid resistance.

It is well established that curcumin entails insecticidal and acaricidal potential, primarily due to growth inhibition in various pests (Liu et al. 2016; Veeran et al. 2017, 2019; Chowdhury et al. 2000). Furthermore, curcumin has been reported to induce mortality in the early developmental stages of mosquitoes through the inhibition of acetylcholinesterase I (AChE) (Rao et al. 2021), and it has demonstrated larvicidal activity against *Aedes aegypti* (Subahar et al. 2022). A study by Cui et al. (2022) showed that curcumin can enhance the management of *Spodoptera litura* when used as a synergist with avermectin, primarily by promoting increased cellular apoptosis. Synergistic effects of insecticide mixtures can reduce the required dosage of insecticides, thereby minimizing the undesirable impacts on non-target organisms and delaying the pressures that lead to the emergence of resistant pests (Taillebois and Thany 2022).

Published studies have explored curcumin derivatives as alternatives to natural curcumin. For instance, early work by Sagnou et al. (2012) showed the efficacy of curcumin and its bis-demethyl derivative as potential larvicidal agents against *Culex Pipiens*. The authors found that curcumin and di-O-demethylcurcumin showed potent activity, with the latter being the most effective. In a more recent study, Matiadis et al. (2021) demonstrated that curcuminoids and curcumin derivatives exhibit larvicidal activity against *Culex pipiens* and *Aedes albopictus*.

Our results (Table 1) indicate that quercetin also acts as a strong inhibitor toward *Bt*CYP6CM1. Quercetin, has been reported to influence insect survival and development across Hemiptera, Diptera, and Lepidoptera while largely sparing Coleoptera, pollinators, and natural enemies (Riddick 2021). Mechanistically, quercetin exhibits species-specific interactions with cytochrome P450s: it can inhibit particular isoforms in *Tribolium castaneum* to enhance insecticide

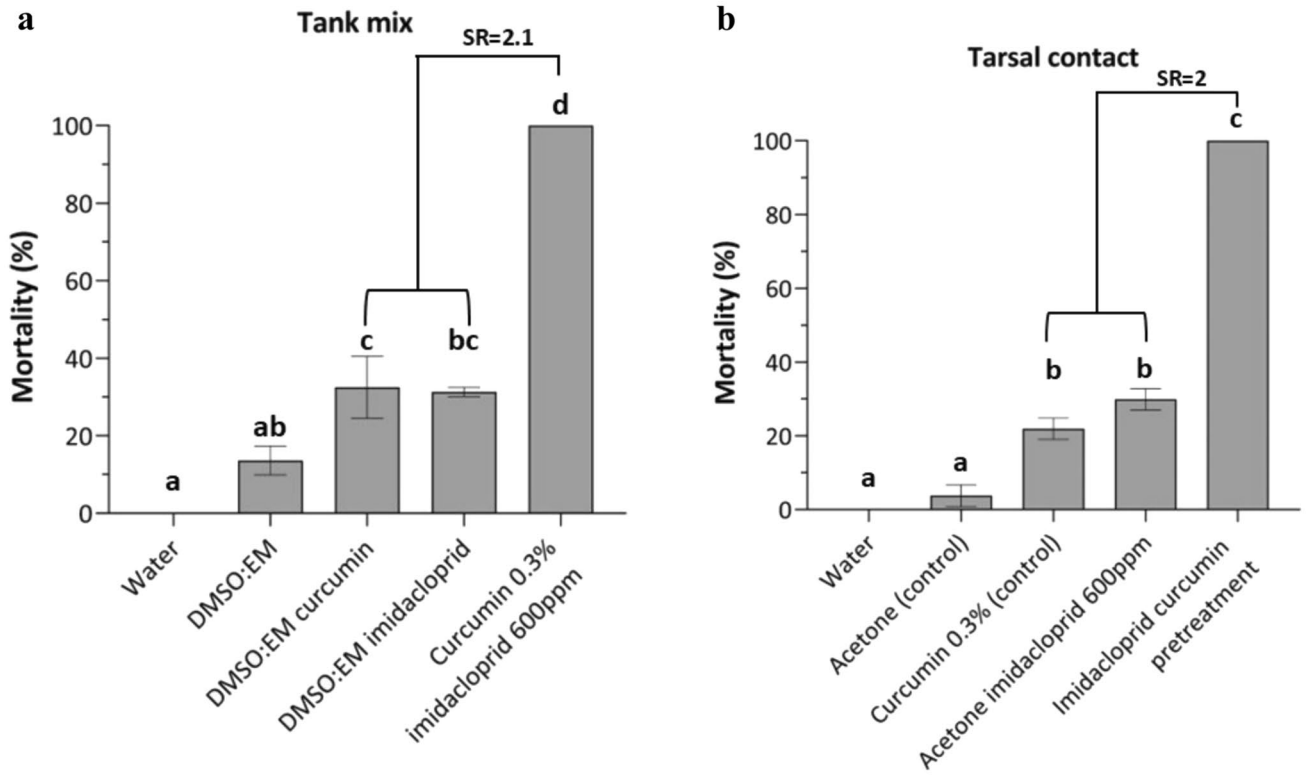


Fig. 6 **a** Mean percentage mortality of *Bemisia tabaci* in leaf-dip bioassays with tank mix DM95 dissolved in dimethyl sulfoxide (DMSO)/emulsifier (EM) (3:1) and the insecticide imidacloprid. The synergistic effect is expressed as the Synergism Ratio (SR) based on the observed increase in mortality in the combination treatment. Different Latin characters indicate statistically significant differences between the treatments (ANOVA, F: 93.061, df:14, $p < 0.001$). **b** Mean percentage mortality of *Bemisia tabaci* in tarsal contact bioassays with DM95 dissolved in acetone and the insecticide imidacloprid. The synergistic effect is expressed as the Synergism Ratio (SR) based on the observed increase in mortality in the combination treatment. Different Latin characters indicate statistically significant differences between the treatments (ANOVA, F: 315.825, df:14, $p < 0.001$)

efficacy (Ghaffar et al. 2020), yet it is also metabolized by bee P450s required for pollinator health (Mao et al. 2013). Together, these findings position both curcuminoids and quercetin as complementary tools within environmentally conscious pest management strategies.

In comparison with synergists such as piperonyl butoxide (PBO), curcumin derivatives offer several noteworthy advantages. PBO has been classified by the U.S. Environmental Protection Agency as a possible human carcinogen (Vardavas et al. 2025) and is generally described as a broad P450 inhibitor, without clear enzymatic evidence for direct inhibition of specific targets (Wang et al. 2012; Zimmer et al. 2017). In contrast, curcumin and its derivatives are considered safe for human use, with curcuminoids granted "Generally Recognized As Safe" (GRAS) status by the U.S. Food and Drug Administration (Gupta et al. 2013). Indeed, clinical studies have shown that curcumin can be administered at doses as high as 12 g/day with only minor side effects (Hewlings & Kalman 2017), while quercetin, has also demonstrated safety at daily doses up to 2,000 mg (Michala and Pritsa 2022). Furthermore, the synthesis of curcumin derivatives such as DM96 is straightforward and economical, requiring only a single high-yield Claisen–Schmidt condensation, in contrast to the multi-step preparation of PBO (Wang et al. 2012). Beyond synthetic accessibility, DM96 has also been identified as a natural product in *Cercis chinensis* (Li et al. 2022). Most importantly, our study provides the first direct enzymatic evidence of specific inhibition of *Bt*CYP6CM1 by DM96, thereby combining mechanistic specificity with a favorable safety and production profile that may offer significant advantages over conventional synergists such as PBO.

Conclusions

This study highlights the potential of natural compounds and synthetic curcumin analogs as inhibitors of cytochrome P450s involved in pesticide detoxification, such as the *Bt*CYP6CM1, a key factor in neonicotinoid resistance in *Bemisia tabaci*. The results demonstrated that both curcumin and its monocarbonyl analog, DM96, possess substantial in vitro

inhibitory potency, with DM96 proving to be the most effective inhibitor. The bioassays show that the tested *Bemisia tabaci* strain is highly resistant to imidacloprid, while DM96 alone yields only moderate mortality, but their combination (co- or pre-exposure) produces dramatic, synergistic increases in mortality (SR ≈ 2.1 and 2.0). These findings indicate that DM96 likely acts as a metabolic inhibitor, suppressing cytochrome P450–mediated detoxification, and thereby restores imidacloprid efficacy by roughly three- to fourfold. The findings could support the development of eco-friendly biopesticide synergists, contributing to lower-toxicity, more effective insecticide formulations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-025-01974-3.

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Author contributions EI, Collected the data, Performed the analysis, Wrote the paper; MS, Collected the data, Performed the analysis, Wrote the paper; MAC, Collected the data, Performed the analysis, Wrote the paper; J-DM, Collected the data, Performed the analysis, Wrote the paper; PP, Collected the data, Performed the analysis, Wrote the paper; NG, Collected the data, Performed the analysis, Wrote the paper; DT, Collected the data, Performed the analysis; DM, Collected the data, Performed the analysis, MS, Collected the data, Performed the analysis, Wrote the paper; ER, Wrote the paper; JV, Conceived and designed the analysis, Wrote the paper; NEL, Conceived and designed the analysis, Wrote the paper. All authors reviewed the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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