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1 **Predicting the insecticide-driven mutations in a crop pest insect: evidence for**  
2 **multiple polymorphisms of acetylcholinesterase gene with potential relevance**  
3 **for resistance to chemicals**

4

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22

23 **Abstract**

24 The silverleaf whitefly *Bemisia tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) is a serious  
25 invasive herbivorous insect pest worldwide. The ~~misuse and~~ excessive use of pesticides has ~~ve~~  
26 progressively selected *B. tabaci* specimens, ~~progressively~~ reducing the effectiveness of the  
27 treatments, and ultimately ending in the selection of pesticide-resistant ~~ce~~ strains. The management  
28 of this crop pest has thus become challenging owing to the ~~high~~ levels of resistance to all major classes  
29 of recommended insecticides, ~~including organophosphates (OPs)~~. Here, we used *in silico* techniques  
30 for detecting molecular mutations in *ace1* gene from naturally occurring *B. tabaci* variants, and  
31 monitor the presence and frequency of the detected mutations from 30 populations of the silver  
32 whitefly from Egypt and Pakistan. We found several point mutations in *ace1*-type acetylcholinesterase  
33 (*ace1*) in the studied *B. tabaci*-~~B~~ variants (~~MEAM1~~) naturally occurring in the field. By comparing *ace1*  
34 sequence data ~~and point mutations with those reported~~ from ~~on an~~ organophosphate-susceptible ~~and~~  
35 ~~an organophosphate-resistant~~ strains of *B. tabaci* (~~SUD-S~~), ~~and to~~ *ace1* ~~sequence data retrieved from~~  
36 ~~GenBank~~ for that species ~~and to~~ nucleotide polymorphisms from other arthropods, we identified novel  
37 mutations that could potentially influence insecticide resistance. ~~Seven Egyptian field strains had~~  
38 ~~100% homology with the fragment of the Israeli B. tabaci susceptible allele (SUD-SUD), revealing a~~  
39 ~~high conservation of the sequence/nucleotide, and/or possible missense mutations. We also report~~  
40 ~~novel amino acid substitutions (S369W, C316W, P317G, and P317F) as likely candidates conferring~~  
41 ~~resistance to organophosphates. Indeed, the location of these mutations, which we visualized on the~~  
42 ~~3D crystal structure of acetylcholinesterase, occur close to the access of the active site gorge of the~~  
43 ~~enzyme. The phylogenetic analysis revealed that these novel gene sequences appeared at the top of~~  
44 ~~the clade in the tree, suggesting that the reported novel point mutations have evolved~~  
45 ~~recently~~ Homology modeling and molecular docking analyses were performed to determine if the  
46 ~~mutation-induced changes in form 1 acetylcholinesterase (AChE1) structure could confer resistance~~  
47 ~~to carbamate and organophosphate insecticides. Mutations had small effects on binding energy ( $\Delta G_b$ )~~  
48 ~~interactions between mutant AChE1 and insecticides; they altered the conformation of the peripheral~~  
49 ~~anionic site of AChE1, and modified the enzyme surface, and these changes have potential effects on~~  
50 ~~the target-site sensitivity.~~ Altogether, the results from this study provides information on genic  
51 variants of *B. tabaci ace1* for future monitoring insecticide resistance development and report a  
52 potential case of environmentally driven gene variations.

53

54 **Keywords:** Acetylcholinesterase, environmental toxicants, resistance, putative mutations, ~~target site~~  
55 ~~insensitivity~~ ~~homology modeling, molecular docking~~

56 **Introduction**

57 Significant changes in farming procedures have become a vital necessity when human population  
58 numbers started to grow significantly, and this has favored the development and spread of land-  
59 extensive and intensive agriculture methods (Raven and Wagner 2021). Nowadays, about 11% of land  
60 surface is planted with crops (Raven and Wagner 2021) whose productivity is being continuously  
61 improved by novel technologies. For instance, genetic engineering has been promoted in the past  
62 decades to ensure subsistence and sufficiency in food production, and maintain competitiveness and  
63 economic reliability in the long term (Jordan 2002; Babar et al. 2020). To maximize agricultural  
64 productivity, application of soil amendments and synthetic fertilizers are additional techniques  
65 commonly employed for maintaining soil fertility in association with the spray of pesticides that limit  
66 crop losses (Hepperly et al. 2009; Gianessi 2013; Popp et al. 2013; Bonanomi et al. 2020). Yet, in the  
67 long-term, the frequent application of fertilizers and pesticides have impacts on the  
68 environment, including in the surrounding fields (Tscharntke et al. 2005), and the long-lasting effects after  
69 agricultural abandonment are alarming (Isbell et al. 2019). Worst, the misuse and excessive use of  
70 pesticides have progressively selected specimens of the chemically targeted species, progressively  
71 reducing the effectiveness of the treatments, and ultimately ending in the selection of pesticide-  
72 resistant strains (Vontas et al. 2011; Voudouris et al. 2011; Engell Dahl et al. 2021; Renault and Colinet  
73 2021). The phenomenon has been so important and widespread for insects that a committee  
74 dedicated to the management of insecticide resistance has been created in 1984: Insecticide  
75 Resistance Action Committee (IRAC, <https://irac-online.org/>) (Sparks et al. 2021).

76 The development of mechanisms of insecticide resistance occurs at any stage of the mode of  
77 action of the chemical on the insect, and can belong to one out of four categories: (i) behavioral resistance,  
78 whereby the insect avoids the insecticide behaviorally (Zalucki and Furlong 2017), (ii) increased  
79 resistance to penetration due to modifications in cuticle structure which reduce (or slow down) the  
80 insecticide's absorption (Lilly et al. 2016), (iii) increased metabolic resistance implying improvement  
81 in the detoxification capacities of the insect (Ahmad et al. 2006), and (iv) target site insensitivity and/or  
82 alteration of pharmacokinetic processes, whereby the sensitivity to the chemical is reduced by  
83 mutation-derived changes in the structure of the site(s) targeted by the insecticide (Soderlund and  
84 Knipple 2003). Importantly, different combinations of these categories have been reported from  
85 resistant populations of several insect species, offering a multi-layered protection against chemicals  
86 and an extremely high level of insecticide resistance (Ahmad et al. 2006).

87 Nowadays, increased metabolic resistance, target site insensitivity and/or modifications of  
88 pharmacokinetic processes represent the main known mechanisms of insecticide resistance  
89

90 (Soderlund and Knipple, 2003; Hemingway et al. 2004; Li et al. 2007; Panini et al. 2016). Metabolic  
91 resistance mainly relies on the action of the following enzymes: glutathione-S-transferases (Pavlidis et  
92 al. 2018), cytochrome P450 monooxygenases (Liu et al. 2015), and non-specific esterases (Li et al.  
93 2007). Target site insensitivity, developed as the resistance mechanism to carbamate and  
94 organophosphate insecticides, is associated with point mutations of the key enzyme in  
95 neurotransmission encoded by *ace* genes, acetylcholinesterase (AChE, acetylcholine acetylhydrolase,  
96 E.C. 3.1.1.7) (AChE, EC3.1.1.7) (Hemingway et al. 2004). With the exception of some species of Diptera,  
97 insects possess two distinct acetylcholinesterase (*ace*) gene clusters (Huchard et al. 2006). Meanwhile,  
98 it is assumed that among one of these two genes, only *ace1* which encodes acetylcholinesterase 1  
99 (AChE1), plays a role in insecticide-resistance (Bourguet et al. 1996; Benting and Nauen 2004; Kim et  
100 al. 2006). Mutations making AChE1 less sensitive to the organophosphate- and carbamate-synthesized  
101 insecticides have been reported from multiple insect species, including the silverleaf whitefly *Bemisia*  
102 *tabaci* (Gennadius, 1889) (Homoptera: Aleyrodidae) (Alon et al. 2008; Jiang et al. 2009; Zhang et al.  
103 2012; Hopkinson et al. 2020).

104 Historically, mutations and/or genic variations in insecticide resistance-related genes, as for  
105 instance *ace1*, have long been assumed to be somehow limited (Ffrench-Constant et al. 1998).  
106 However, this view has been refuted by several studies who reported that adaptation to man-made  
107 contaminants can occur rapidly, as found with the mutations at the *Agce* locus which occurred during  
108 three independent mutations, producing multiple simple and complex resistant-*ace* alleles in the fruit  
109 fly *Drosophila melanogaster* (Karasov et al. 2010). Yet, mutations and/or variation in nucleotides  
110 coding for amino acids that may confer resistance to organophosphates are now well-characterized  
111 for *ace* (Menozzi et al. 2004; Temeyer et al. 2013; Bendele et al. 2015; Aguilar et al. 2018; Nagar et al.  
112 2018). For the nicotinic acetylcholine receptor (nAChR: the neonicotinoid target site), Wang et al.  
113 (2017) identified 23 single-nucleotide polymorphisms leading to the replacement of 19 amino acids in  
114 insecticide resistant strains of *B. tabaci*.

115 The silverleaf whitefly *B. tabaci* is a key severe agricultural crop pest, causing considerable damage  
116 worldwide by sucking the phloem sap of more than 60 crop plants, in addition to transmitting  
117 hundreds of economically important plant viruses (Ex.: *Geminiviridae*) (Navas-Castillo et al. 2011; Leke  
118 et al. 2015). When feeding, the sugar droplets made by *B. tabaci* contaminate the cotton plant,  
119 severely affecting the cotton industry by making cotton fibers sticky (van der Sluijs 2019), and by  
120 favoring the development of microorganisms (Oliveira et al. 2011). Control of *B. tabaci* populations  
121 has been long depended on conventional insecticides such as organophosphates, carbamates and  
122 pyrethroids (Palumbo et al. 2001). By the late 1980s, these broad-spectrum insecticides have  
123 progressively failed in controlling this crop pest (Ahmad et al. 2010; Horowitz et al. 2020; Shaurub et

124 al. 2021), and management of *B. tabaci* populations became highly challenging as they developed high  
125 levels of resistance to recommended insecticides (Denholm et al. 1998; Horowitz et al. 2020).

126 Control strategies aiming at containing populations of the silverleaf whitefly are also further  
127 complicated by the fact that *B. tabaci* encompasses several cryptic species (at least 39 genetically  
128 divergent variants), and this complex of different genetic groups (populations) is distributed  
129 worldwide in tropical and sub-tropical regions (de Moya et al. 2019). Among these variants, the B and  
130 Q variants, also known as Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) genotypes  
131 (de Barro et al. 2011), respectively, have established outside their endemic range (de Moya et al.  
132 2019), and have developed strong resistance to all major insecticide classes (Horowitz et al. 2020).  
133 The B variant (MEAM1) causes extreme damages to field crops, in particular in irrigated  
134 agroecosystems in Egypt (Shaurub et al. 2021), Pakistan (Shah et al. 2020; Paredes-Montero et al.  
135 2021), and more largely in the Indian subcontinent, Sudan, and in Central and South America (Azab et  
136 al. 1971; Brown and Bird 1992; Horowitz et al. 2020).

137 ~~In Egypt and Pakistan,~~ the management of *B. tabaci* in Egypt and Pakistan has largely relied on  
138 the application of organophosphates (Abdallah 1991; Sobeiha et al. 1997; Ahmad et al. 2002,  
139 ~~2008~~2010; El Kady and Devine 2003; Shaurub et al. 2021), and farmers are increasingly complaining  
140 about the reduced effectiveness of the recommended chemicals. Hence, the extensive use of ~~these~~  
141 ~~chemicals~~organophosphates in addition to carbamates over the past decades has certainly  
142 contributed to the selection of naturally occurring genetic variants whose insecticide resistance is  
143 driven by distinct mutations in *ace1*-type AChE. In this work, we analyzed molecular sequence data  
144 from resistant strains of *B. tabaci* to determine if changes in the sites targeted by organophosphate  
145 insecticides have occurred and could confer insecticide resistance. In this study, we used *in silico*  
146 techniques for detecting molecular mutations in *ace1* gene from naturally occurring *B. tabaci* variants,  
147 and monitor the presence and frequency of the detected mutations from 30 populations of the silver  
148 whitefly from Egypt and Pakistan. We first examined if similar point mutations as those reported by  
149 Alon et al. (2008) from ~~an~~ organophosphate-resistant strain of *B. tabaci* (B-ref) could be observed.  
150 Second, to assess newly identified mutations that would potentially influence insecticide resistance,  
151 we detailed amino acid substitutions/indels and their positions in the protein sequence to infer the  
152 potential changes this would have on structural and chemical properties of the enzyme. The  
153 relatedness and differences of *ace1* gene among *B. tabaci* populations, alongside with other insect  
154 species, was examined; this phylogenetic analysis helped in determining if *B. tabaci* variants resistant  
155 to organophosphates have a common ancestor, and if some point mutations in *ace1* gene could be  
156 shared among species. Finally, computational methods were used to predict protein structure and  
157 protein-ligand interactions. A 3D structure of the susceptible and resistant *B. tabaci* AChE1 deduced

158 [amino acid sequence was built through homology modeling. Then, molecular docking was performed](#)  
159 [to determine if the structural modifications in form 1 acetylcholinesterase \(AChE1\) due to point](#)  
160 [mutations could alter the protein-ligand binding affinity, and confer resistance to carbamate and](#)  
161 [organophosphate insecticides.](#)

## 163 Material and Methods

### 164 Entrez searches and sequence alignment

165 The silverleaf whitefly *Bemisia tabaci* [sequence genes](#) encoding acetylcholinesterases (*ace1*) were  
166 downloaded from the National Center for Biotechnology Information nucleotide database (NCBI's  
167 GenBank, <https://www.ncbi.nlm.nih.gov/nucleotide/>) by using the Entrez search and retrieval system  
168 EDirect ([Kans, 2022](#)). Two mRNA sequence sets (LC199300–LC199317 [Egypt] and KU307116.1–  
169 KU307130.1 [Pakistan]) of the *B. tabaci* B variant (MEAM1) were retrieved. As the mutations in *ace1*  
170 mRNA sequences from *B. tabaci* populations from Australia, China and Israel were already analyzed  
171 ([Hopkinson et al. 2020](#); ~~[Jiang et al. 2009](#)~~[Zhang et al. 2012](#); [Alon et al. 2008](#), respectively), these  
172 datasets were not considered in our study.

173 The *esearch* function was used to scrutinize the NCBI database with the following queries:  
174 'acetylcholinesterase or AChE', 'ace1, ace 1 or ace-1', '*Bemisia tabaci*', 'B biotype', 'B variant', 'MEAM  
175 1', 'Egypt', 'Pakistan', and 'Middle East Asia Minor'. The *ace1* sequences were sorted out by length with  
176 the function *efilter* so fragments in the range 450-650 bp could be retained. The filtered nucleotide  
177 sequences were downloaded in FASTA format using the *efetch* function. Sequences were aligned using  
178 MUSCLE v3.8.31 ([Edgar 2004](#)) implemented with the "Align Multiple Sequences" tool available in  
179 Mesquite v3.70 ([Maddison and Maddison 2021](#)). They were additionally trimmed to remove poorly  
180 aligned sequence ends using trimAl v2.0 ([Capella-Gutiérrez et al. 2009](#)), based on [automatically](#)  
181 [selected](#) thresholds. These steps yielded a total of 16 *ace-1* sequences [for Egyptian variants](#)  
182 [\(Supplementary Information S1 Table 1\)](#), and 14 *ace-1* sequences for Pakistani variants.

183 Finally, the dataset was enriched by adding two reference sequences described by [Alon et al.](#)  
184 [\(2008\)](#) from ~~an~~ organophosphate-susceptible strain of *B. tabaci* (SUD-S)<sub>2</sub> and from one  
185 organophosphate-resistant strain (B-ref) from Israel. The verified sequences and documented  
186 mutations were obtained from GenBank. The *B. tabaci* resistant alleles (accession no. ABV45412) are  
187 characterized by the substitution of F392 with W392 (F392W), while the F392 is conserved in the  
188 alleles ~~(accession no. ABV45413)~~ from the susceptible variant [\(accession no. ABV45413\)](#). These  
189 documented sequences were used as reference sequences for performing comparisons with the 16  
190 *ace-1* sequences we ~~downloaded~~[uploaded](#) from GenBank.

192 **Sequence comparisons of *ace1*-type acetylcholinesterase**

193 Egyptian *Bemisia tabaci* variants

194 Sequence comparisons of the *ace1*-type acetylcholinesterase were realized in order to characterize  
195 and predict those point mutations inducing amino acid substitutions that could be associated with  
196 insecticide resistance. Multiple sequence alignment was realized with MultAlin (Corpet 1988; available  
197 at: <http://multalin.toulouse.inra.fr/multalin/>), and CLC Genomics (Qiagen, Netherlands), to compare  
198 alleles from reference (susceptible, resistant) *B. tabaci* variants with those from supposedly resistant  
199 Egyptian strains. The lengths of the nucleotide sequences, and their positions on the sequenced *ace1*  
200 fragments, and thus deduced protein sequences (AChE1), markedly differed between the Egyptian  
201 and Pakistan *B. tabaci* variants. Hence the screening for mutations, either nucleotide- or protein-  
202 based, had to be performed separately.

203 Specific changes in the amino acid sequences of *ace1* resulting in the alteration of the targeted  
204 site are commonly numbered according to the modeled protein structure. For consistency with former  
205 works, the conventional numbering system based on *Torpedo (=Tetronarce) californica* (Pacific electric  
206 ray) for AChE ~~proteingenes\_~~ (CAA27169.1) was used (Mutero et al. 1994), and this also aligns with the  
207 recommendations made by Hopkinson (et al. 2020) (Supplementary Information S2 Fig. 1A). ~~Then, we~~  
208 ~~used the three dimensional (3D) crystal structure of AChE obtained from *T. californica* AChE (Dvir et al.~~  
209 ~~2010) to make inference on the substrate binding domain, the amino acid residues forming the~~  
210 ~~catalytic triad (active site), and putative binding of AChE in the active site in *B. tabaci* AChE (Fig. 1B,C).~~

211 A molecular phylogenetic tree of *B. tabaci* AChE1 alongside with other arthropod species was  
212 constructed by neighbor-joining criteria using Archaeopteryx v.0.9920 beta (Han and Zmasek 2009)  
213 (based on forester 1.045), and by considering the JTT matrix-based model (Jones et al. 1992). This  
214 procedure conveniently describes the evolutionary distances among species (Roy et al. 2014). The 24  
215 species considered in the tree, and accession numbers, were: *Pediculus humanus corporis* (Body louse,  
216 ncbi:BAF46104), *Rhopalosiphum padi* (Bird cherry-oat aphid, ncbi:AAT76530), *Aphis citricidus* (Brown  
217 citrus aphid, ncbi:ALS35348), *Nilaparvata lugens* (Brown planthopper, ncbi:AFC88123), *Sogatella*  
218 *furcifera* (White-backed planthopper, ncbi:AGN31550), *Liposcelis paeta* (Grain psocid,  
219 ncbi:ADA60183), *Locusta migratoria manilensis* (Oriental migratory locust, ncbi:ABY75631),  
220 *Periplaneta americana* (American cockroach, ncbi:ALJ10969), *Blattella germanica* (German cockroach,  
221 ncbi:ABB89946), *Bombyx mori* (Silk moth, refseq:NP\_001037380), *Spodoptera exigua* (Beet  
222 armyworm, ncbi:AGO59308), *Helicoverpa armigera* (Cotton bollworm, ncbi:AAY59530), *Cydia*  
223 *pomonella* (Codling moth) ncbi:ABB76667, *Chilo suppressalis* (Asiatic rice borer moth,  
224 ncbi:ABO38111), *Plutella xylostella* (Diamondback moth, ncbi:AAY34743), *Leptinotarsa decemlineata*  
225 (Colorado potato beetle, ncbi:AEI70751), *Apis mellifera* (Honeybee, ncbi:ANT80563), *Harpegnathos*



226 *saltator* (Jerdon's jumping ant, ncbi:EFN89510, *Tremex fuscicornis* (Tremex wasp, ncbi:AKM70918),  
227 *Culex quinquefasciatus* (Southern house mosquito, ncbi:AFR68411), *Culex pipiens pallens* (Mosquito  
228 ncbi:AAV28503), *Aedes aegypti* (Yellow fever mosquito, ncbi:ABN09910), *Anopheles gambiae* (African  
229 malaria mosquito, ncbi:AJZ73939), and *Ctenocephalides felis* (Cat flea, ncbi:CBI832507). Bootstrap  
230 (10000 replicates) values are indicated for each root. The tick *R. microplus* AChE1 (JAC59086) was used  
231 as the out-group. The tree was drawn to scale, with branch lengths measured in the number of  
232 substitutions per site. The analysis involved 45 amino acid sequences (226 total sites, 29 conserved  
233 sites). All positions containing gaps and missing data were removed.

234

235 Pakistani *Bemisia tabaci* variants

236 The analysis of the different Pakistani *B. tabaci* variants characterized by *ace1* mutations was  
237 performed by collecting available information from GenBank from 14 Pakistan *B. tabaci* variants, *i.e.*,  
238 GenBank nucleotide sequences KU307116.1–KU307130.1; the corresponding deduced proteins are  
239 AMO25775.1–AMO25789.1. Note that the sequences KU307116–KU307121 belong to the B variant of  
240 *B. tabaci* while the nucleotide sequence KU307122–KU307130 belong to the Asia II I variant. The  
241 sequences were then compared with those of the Israeli resistant and susceptible variants of *B. tabaci*  
242 AChE1 (Alon et al. 2008) in order to examine if similar patterns of mutations were shared among *B.*  
243 *tabaci* strains. Malik et al. (2016) generated the sequence distance matrix for this dataset with SDT  
244 v1.2. They showed that *ace1* sequences were 100% conserved among the Pakistan-endemic *B. tabaci*  
245 strains.

246

#### 247 Predicting the nature of mutations through ESTHER database

248 To examine if point mutations are shared among species, or have resulted in different classes of  
249 AChE1, [BLAST-based searches against the ESTHER Database \(ESTerases and alpha/beta-Hydrolase](#)  
250 [Enzymes and Relatives\)](#) were conducted. This analysis further supported our predictions of the  
251 potential links between point mutations and insecticide resistance in the *B. tabaci* variants considered  
252 in the present work. To that aim, protein sequences were aligned, and compared to experimentally  
253 verified insecticide resistance-associated mutations in the AChE1-type acetylcholinesterase  
254 sequences from different insects, ticks, and mites (information obtained from ESTHER database,  
255 <https://www.re3data.org/repository/r3d100010542>). Specifically, we included the sequences from  
256 *Anopheles gambiae* (CAD32684), *Camponotus floridanus* (EFN61766), *Chilo suppressalis* (AKL78859),  
257 *Cyrtorhinus lividipennis* (AHW29553), *Lygus hesperus* (JAG37539), *Nasonia vitripennis* (K71U37),  
258 *Pediculus h. capitis* (BAF46106), *Phlebotomus papatasi* (AFP20868), *Rhipicephalus microplus*  
259 (AJA71270), and *Tetranychus urticae* (ADK12685). Sequence homology was analyzed with the program

260 SeqMan v.7.0.0 (DNASTAR Lasergene, Madison, WI, USA), and the translated AChE1 protein sequences  
261 were run against the ESTHER database. The sixteen deduced *B. tabaci* AChE1 amino acid sequences  
262 obtained from GenBank for Egypt (BAW19578-BAW19595; **Table 1**), the two reference sequences of  
263 *B. tabaci* AChE1 from Israel (ABV45412 and ABV45413, for resistant and susceptible alleles,  
264 respectively; **Supplementary Information S1Table 1**), and forty-two AChE1 sequences from various  
265 insect species and orders (the species and the GenBank accessions numbers are displayed on the  
266 phylogenetic tree) were aligned with MUSCLE.

267

### 268 **Three-dimensional structure modeling and molecular docking**

269 All-atom three-dimensional (3D) structure prediction of the sequence ABV45413.1 of the *B. tabaci*  
270 AChE1 protein was modeled with the SWISS-MODEL webserver (Biasini et al. 2014). The model quality  
271 assessment of the protein structure was evaluated with the SWISS-MODEL and Molprobit webserver  
272 (Williams et al. 2018). Then, molecular dynamics simulations were carried out for 100 ns with the  
273 GROMACS software (Van Der Spoel et al. 2005). For the simulations, the protein was solvated using  
274 the TIP4P water model, and ionized with 0.154 M NaCl at 37 °C and 1 atm pressure (Harrach and  
275 Drossel 2014). CHARMM27, a popular force field for biomolecular simulations, was run using periodic  
276 boundary conditions at normal volume and temperature (NVT) ensemble (Huang and MacKerell  
277 2013). Trajectory clustering was carried out with the University of California San Francisco (UCSF)  
278 Chimera 1.14 software (Pettersen et al. 2004), and allowed to identify four groups of trajectories  
279 (clusters) whose representative conformations (four representative structural models, i.e., one 3D  
280 representative structure for each group of trajectory) were further analyzed.

281 Next, the mutations (Egyptian strains: F392W, S369W, L370V, C316W, P317G, and P317F;  
282 Pakistani strains: G251D, N252R, P253S, C329S, and L330T) were introduced to each representative  
283 structural model, and subsequently minimized with the WinCoot 0.9.2 software (Emsley and Cowtan  
284 2004). The structure of acetylcholine (ACh), and of 20 organophosphate and 20 carbamate pesticides,  
285 were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in the 3D structure data  
286 file (SDF) format (Kimet al. 2020). Insecticide selection was based on information available at  
287 <https://irac-online.org/pests/bemisia-tabaci/>. For organophosphates, the list included: azinphos-  
288 methyl, bromophos ethyl, chlorfenvinphos, chlorpyrifos, cyanofenphos, dialifos, diazinon, EPN,  
289 isoxathion, leptophos, phosalone, phosmet, phoxim, pirimiphos-methyl, profenofos, prothiofos,  
290 quinalphos, sulprofos, tetrachlorvinphos, and triazophos. For carbamates, the list included: aldicarb,  
291 aminocarb, bendiocarb, butocarboxim, carbaryl, carbofuran, carbosulfan, ethiofencarb, fenobucarb,  
292 fenoxycarb, formetanate, formparanate, methiocarb, methomyl, metolcarb, mexacarbate, pirimicarb,  
293 promecarb, propoxur, and thiofanox.

Code de champ modifié

294 Molecular docking was performed on the four different conformations for each mutated protein  
295 (eleven) and the wild type. AutoDock Vina software was used with a flexible ligand in a flexible active  
296 site protocol (Trott and Olson 2010). The active site was retrieved by superimposing the *B. tabaci*  
297 AChE1 to the solved structure (PDB ID: 6ARX) (Cheung et al. 2018). Molecular dynamics simulations  
298 were run for 877 ns, as it allowed to reach one of the equilibration stages of the protein. A cartoon  
299 representation of the superimposition of the 3D structures was subsequently drawn after 877 ns of  
300 molecular dynamics simulation.

301 A comparison of the four representative structures of each mutated protein to the original 3D  
302 AChE1 conformation highlighted one active site triad (S262, E388 and H501; see results below). The  
303 effect of the mutations on the AChE1 pKa values was predicted with the PropKa online webserver (Li  
304 et al. 2005). Changes in active site pKa values (a lower pKa would reveal a greater ability in the release  
305 of the proton) in mutant AChE1 of *B. tabaci* in comparison to its wild type relative could yield an  
306 efficient mutant enzyme.

## 307

### 308 Results

#### 309 AChE1 homology and molecular phylogenetic tree

310 The homology matrix of amino acid sequences of AChE of *B. tabaci* from Egyptian field strains (see  
311 Supplementary Information S1Table 1 for strain designations) is presented in Figure 1. The partial  
312 predicted amino acid sequences of the eleven *B. tabaci* field strains (Mbs, Mas, Qbn, Mym, Myi, Gkt,  
313 Fas, Fib, Dbq, Beb, and Res (B variant [MEAM1])) had 96-100% homology; these sequences were  
314 homologue at 97.6-100% with the fragment of the *B. tabaci* resistant allele (B-ref variant) (Alon et al.  
315 2008). The analysis revealed that variations in specific amino acid residues did exist, suggesting the  
316 occurrence of nonsynonymous mutations. Seven field strains (HS-S, Dmg, Bwn, Fsn, Gzf, Qqb, and  
317 Mys) had 100% homology, including with the fragment of the *B. tabaci* susceptible allele (SUD-SUD),  
318 revealing a high conservation of the sequence/nucleotide, and/or possible missense mutations.

319 Phylogenetic analysis based on *B. tabaci* AChE1 sequences showed the existence of two groups:  
320 (i) with some few exceptions, one cluster grouped susceptible strains, and (ii) the second group  
321 encompassed resistant strains (Supplementary Information S3Fig-2). *Bemisia tabaci* formed a cluster  
322 with AChE1 from different insect species, including planthoppers, locusts, and cockroaches, suggesting  
323 the existence of similar forces driving evolutionary changes in AChE1s among species.

#### 324

#### 325 Sequence comparisons of *ace1*-type acetylcholinesterase from Egyptian *B. tabaci* variants

326 *Detection of ace1-type AChE mutations*

327 Eighteen cDNAs containing putatively partial open reading frames (ORFs) of *B. tabaci ace1* genes were  
328 obtained from GenBank under the accession numbers LC199300-LC199317 (Egypt) ([Supplementary](#)  
329 [Information S1Table 1](#)). Those sequences had a total length of approximately 500 bp. Point mutations  
330 that occurred in the coding region of *ace1* gene were investigated by comparing nucleic acid and  
331 deduced amino acid sequences ([Table 12](#) and [Fig. 2A,B](#)). The data revealed the existence of:

- 332 (a) four silent (synonymous; a mutation of a single DNA nucleotide that is not altering the amino  
333 acid sequence of the encoded protein but may alter the splicing process) mutations: (i) I298I  
334 (ATC→ATI) in the Dbq variant, alongside with three other silent mutations, (ii) G307G  
335 (GGC→GGI), (iii) V354V (GTC→GTG), and (iv) G389G (GGC→GGG) occurred in the eleven  
336 strains Egy-B Lab, Beb, Gkt, Qbn, Dbq, Mym, Mas, Fas, Myi, Mbs, and Fib.
- 337 (b) six non-silent (non-synonymous, includes missense mutations corresponding to the  
338 replacement of one amino acid by another amino acid in the protein; nonsense mutations  
339 corresponding to the substitution of an amino acid by a stop codon; mutations in splice sites;  
340 or finally insertions or deletions) mutations:
- 341 i) substitution of the amino acid F392W which was found in the 10 Egyptian field strains (Beb,  
342 Dbq, Fas, Fib, Gkt, Qbn, Mym, Myi, Mbs, and Mas) and in the laboratory strain Egy-B). This  
343 substitution has been reported to confer resistance to OPs in populations of the B variant of  
344 *B. tabaci* (Alon et al. 2008).
- 345 (II, III) two point mutations, the substitution of the amino acids S369W (TCC→TGG) and  
346 Leu370Val (CTG→GTG) were reported from four variants (Fas, Myi, Mbs, Fib).
- 347 (IV, V) two point mutations, the substitution of the amino acids C316W (TGC→TGG) and  
348 P317G (CCT→GGT) observed in two variants (Mym and Mas)
- 349 (VI) one point mutation, with the replacement of the amino acid P317F (CCT→TTT) in one  
350 variant (Fib) ([Table 12](#)).

351

#### *Conformational changes induced by the detected ace1-type AChE mutations*

353 ~~Mutations are likely to confer conformational changes to the encoded protein structure, thereby~~  
354 ~~reducing, or even preventing, the action of the insecticide. The detected substitutions of the amino~~  
355 ~~acids S369W, C316W, and P317F reported above are located at the acyl pocket of the protein, which~~  
356 ~~is the cavity corresponding to the active site of the enzyme and where the ligands bind (Fig. 1B,C). In~~  
357 ~~the four strains (Fas, Myi, Mbs, Fib) characterized by the substitution of the amino acid S369W, the~~  
358 ~~mutation resulted in a narrower and more hydrophobic acyl pocket, reducing its accessibility to alkoxy~~  
359 ~~group or inhibitors.~~

360

### 361 Sequence comparisons of *ace1*-type acetylcholinesterase from Egyptian-Pakistani *B. tabaci* variants

362 Our analysis of the data ~~yet~~ revealed the occurrence of five missense mutations among the fourteen  
363 *B. tabaci* strains (*ace1* sequence variants) from Pakistan (Fig. 3A,B). As exemplified with AMO25775.1,  
364 the N-terminal sequence of the Pakistan strain is characterized by the replacement of three amino  
365 acids: G251D (GGG→GAT), N252R (AAC→CGA), and P253S (CCG→ICG). Moreover, our analysis also  
366 revealed the substitution of two additional amino acids at the C-terminal sequence, namely C329S  
367 (TGC→TCG) and L330T (CTC→ACC) (Fig. 3A).

### 369 Searching for candidate mutations potentially linked to insecticide resistance

370 To search for common residues involved in insecticides' resistance and evaluate the frequency of  
371 mutations and variations in amino acid residues in two specific segments of our *B. tabaci* AChE1  
372 dataset from Egypt, the sequences were compared with other AChE1-type acetylcholinesterases from  
373 different arthropods (insects, ticks, and mites) using the ESTHER database (Marchot and Chatonnet  
374 2012; Lenfant et al. 2013) (Supplementary Information S4Fig-5). The sites where point mutations  
375 have occurred and conferred resistance to organophosphates and/or to carbamates show a high level  
376 of similarity. Specifically, AChE1 sequences from the different analyzed insect species exhibited 65-  
377 97% identity (79-98% similarity). Two different point mutations, P317G (BAW19591.1) and P317F  
378 (BAW19586.1), seem to be involved in the resistance of *B. tabaci* to organophosphates. By combining  
379 the available information, the analysis also suggested that the other mutations may not be related to  
380 insecticide resistance.

### 382 Three-dimensional structure models

383 The 3D protein structure prediction<sub>s</sub> of *B. tabaci* AChE1 were plotted using the SWISS-MODEL  
384 webserver. The global model quality estimation (GMQE), and the qualitative model energy analysis  
385 (QMEAN) scores were 0.77 and 0.87±0.05, respectively. The protein RMSD trajectory reached  
386 equilibrium after five ns, with an RMSD value of 2.1 Å, before it increased to 2.7 Å after 70 ns (Figure  
387 4A). The radius of gyration (RoG) and solvent accessible surface area (SASA) values were stable during  
388 the entire simulation, with values of 23 Å and 22500 Å<sup>2</sup>, respectively. The total number of formed H-  
389 bonds (780) was highly stable. The Ramachandran plot of the models, which was run to ensure the  
390 structure reliability, revealed that the proportions of residues in most favored, allowed, and  
391 disallowed regions were 99.4, 94.4 %, and 0.6 %, respectively (up-right panel of Figure 4B).

392 The root-mean-square fluctuations per residue (in Å) averaged over the duration of the simulation  
393 (100 ns) is presented in Figure 4B. Protein structure is shown in a cartoon mode, with red (L400-D450),  
394 magenta (S490-W496), and yellow (L545-S553) coloring indicating regions with high RMSF values (>

395 2.5 Å). The random coil-like conformation revealed the presence of regions of disorders which may  
396 potentially be involved in the binding of a ligand. Residues of active sites are labeled in black (S262,  
397 E388, and H501), and have RMSF values < 2 Å. [AChE1 mutations detected in Egyptian and Pakistani](#)  
398 [populations of \*B. tabaci\* lie apart from the three highly fluctuating regions \(RMSF < 2 Å\).](#)

#### 400 Molecular docking

401 The four 3D models were superimposed on each other, and the [residues of active sites](#) ~~residues~~ were  
402 represented by black dots (**Figure 5A**). [This procedure allowed us to identify the active site gorge of](#)  
403 [AChE1 and to characterize residues that may be part of gorge lining \(Fig. 5B,C\).](#)

404 Mutations detected in Egyptian and Pakistani populations (F392W, S369W, L370V, C316W, P317G,  
405 P317F, G251D, N252R, P253S, C329S, and L330T) were [then](#) introduced to the four conformations of  
406 the protein, and the mutated proteins were subjected to geometry optimization using the universal  
407 force field (UFF) ([Emsley and Cowtan, 2004; Rappé et al. 1992](#)). [In all mutants but one, F392W whose](#)  
408 [mutation which is was located in the choline-binding domain of AChE1, mutations were located](#)  
409 [outside the active site gorge. By focusing on the structural motifs of the active site gorge,](#) it appeared  
410 that the [three disulfide bonds \(C130-C157, C316-C329, and C463-C585; red colored sticks in Fig. 6A\)](#)  
411 [which are typical from wild type \*B. tabaci\* AChE1 were likely disrupted by point mutations C316W and](#)  
412 [C329S. These mutations are located at the peripheral anionic binding site \(black sticks in the dotted](#)  
413 [region of Figure 6A\) of AChE1. Three other mutation points, P317G, P317F, and L330T, are also located](#)  
414 [at this site, and altered the aromatic content at the gorge entrance. Point mutations S369W and L370V](#)  
415 [are located at the surface of the enzyme \(Fig. 6A, top-left panel; mutations are represented by black](#)  
416 [sticks\), and should thus affect binding with neighboring residues, and the structure of the protein at](#)  
417 [the bottom of the gorge \(Figure 6B\). Last, point mutations G251D, N252R, and P253S \(Fig. 6C;](#)  
418 [mutations are represented by black sticks\) are surface-accessible residues, with potential effects on](#)  
419 [protein recognition by other cellular factors.](#)

420 The average binding energies (in kcal/mol) of the 20 organophosphate and the 20 carbamate  
421 insecticides against the active site residues WT, F392W, S369W, L370V, C316W, P317G, P317F, G251D,  
422 N252R, P253S, C329S, and L330T of AChE1 protein are shown in [Supplementary Information](#)  
423 [Material S5A and S5B](#). The docking study showed that the average binding energies of the 40  
424 insecticides ranged from [-4.8](#) kcal/mol to [-8.55](#) kcal/mol, revealing weak to moderately weak  
425 interaction energies (**Figure 6A,B**). In general, the natural substrate molecule ACh had higher values,  
426 around [-4.6](#) kcal/mol, thus characterizing relatively weak interaction energies (see **Supplementary**  
427 **Information S5A and S5B** for the detailed average scores for each model). No significant change was  
428 reported in the binding affinity with ACh or with the pesticides between the WT and the mutated

429 protein isoforms (Figure 6A,B). Three insecticide organophosphates, methomyl, aldicarb, and  
430 butocarbaxim, and methomyl were characterized by average binding energies in the range of those  
431 reported for AChE1 (Figure 6A; Supplementary Information S5A).

#### 432 **pKa predictions of wild-type and mutant AChE1**

433 As pKa values of amino acid side chains play a crucial role in determining the pH-dependent  
434 characteristics of an enzyme, pKa values of wild-type and mutant AChE1 models of *B. tabaci* were  
435 predicted with the PropKa (<https://www.ddl.unimi.it/vegao/propka.htm>). E388 was the only  
436 ionizable residue from the active site triad, and calculations of averaged pKa values were thus  
437 restricted to E388 for each AChE1 variant (WT and 11 mutated AChE1), and for the four 3D models of  
438 the protein. One point mutation, F392W, altered average pKa value (Mutant:  $5.605 \pm 1.568$ ; Wild type:  
439  $pKa = 5.575 \pm 1.568$ ). This change echoed the slightly higher binding energy (-4.5 kcal/mol) of mutant  
440 F392W AChE1 as compared with the one reported for the wild type (-4.6 kcal/mol) (Supplementary  
441 Information 5A,B).

#### 442 **Discussion**

443  
444 The misuse and repeated application of insecticides has contributed to the resurgence of  
445 several insect pests, and to the selection of insecticide-resistant strains (Binu 2019; Li et al. 2019;  
446 Umina et al. 2019). The silverleaf whitefly *B. tabaci* is no exception (Horowitz et al. 2019) and has  
447 developed high resistance to several recommended insecticides (Elbert and Nauen, 2000; Zhang et al.  
448 2015; Naveen et al. 2017), in particular to organophosphates that target AChE (Elbert and Nauen,  
449 2000; Zhang et al. 2015; Naveen et al. 2017; Horowitz et al. 2019,20). This serine hydrolase is widely  
450 known for its role in hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses, in  
451 addition to being involved in the development, mutation, and maintenance of vertebrate and  
452 invertebrate nervous system (Grisaru et al. 1999; Ranson et al. 2002). In the resistant insects, the  
453 alteration of AChE structure prevents the action of OPs (Siegfried and Scharf, 2001). In this work, we  
454 analyzed molecular sequence data that could be candidates of the changes of the sites targeted by  
455 organophosphate insecticides in resistant strains of *B. tabaci*.

456  
457 Site mutations of AChE conferring insensitivity to organophosphates have been identified in  
458 insects as the primary mechanism of resistance to these insecticides (Walsh et al. 2001; Vontas et al.  
459 2002; Nabeshima et al. 2003; Cassanelli et al. 2006). In *B. tabaci*, the F392W mutation of the *ace1*  
460 gene confers resistance to organophosphates, and this mutation has been reported from several  
461 strains (Alon et al. 2008; Jiang et al. 2009; Vassiliou et al. 2011; Gauthier et al. 2014; Hopkinson et al.  
462 2020). In the original AChE model established from *Torpedo-T. californica*, this mutation corresponds

463 to F331W. We also report here the existence of three new novel putative point mutations C316W,  
464 P317F, and S369W from *B. tabaci ace* gene, and likely representing novel paralogous genes. The  
465 existence of *ace*-paralogous genes is frequent in insect pests, as found by Gao et al. (2002) in the green  
466 bug *Schizaphis graminum*, or by Weill et al. (2002, 2004) who formerly reported *ace*-paralogous gene  
467 from fifteen mosquito species. In the mosquitoes *Culex pipiens* and *Anopheles gambiae*, the  
468 insensitivity of AChE to insecticides was also resulting from the substitution of a single amino acid  
469 G119S (Weill et al. 2002, 2004).

470 A single amino acid substitution is sufficient for conferring insecticide resistance as reported by  
471 Nabeshima et al. (2003, 2004) in a pirimicarb-resistant strain of *Myzus persicae* (S431F) and in an  
472 organophosphate-resistant strain of *Culex tritaeniorhynchus* (F455W). In addition to the mutation  
473 point, the nature of the substitution, *i.e.* the type of amino acid, has a strong incidence on the  
474 subsequent activity of the enzyme. Specifically, the replacement of the amino acid C418 by tryptophan  
475 in the  $\alpha$  subunit M4 domain of the enzyme in *T. californica* nAChR resulted in a 26-fold increase in the  
476 ion channel response to acetylcholine. In contrast, a phenylalanine substitution in that position only  
477 produced a 3-fold increase in the open time constant (Ortiz-Miranda et al. 1997). Finally, it is worth  
478 noting that in some cases, more than one mutation in the target protein can act harmoniously  
479 together to result in much stronger resistance as seen in *Drosophila* and houseflies (Menozzi et al.  
480 2004; Wang et al. 2012).

481 Exposure to anthropogenic-related activities, in particular ~~to~~ repeated application of insecticides,  
482 greatly contribute to the selection of resistance in insects, as demonstrated in mosquitoes (Vonbas et  
483 al. 2018), in the diamondback moth *Plutella xylostella* (Trocicka et al. 2017). The silverleaf whitefly *B.*  
484 *tabaci* is no exception, with median lethal concentrations of pymetrozine being increased  
485 approximately 11 times after 18 generations (Wang et al. 2021). The recent results provided by Khalid  
486 et al. (2021) are even more striking, as these authors reported a very high increase of the resistance  
487 level to buprofezin (127-fold) and imidacloprid (86-fold) after five generations only. A masterpiece  
488 case of rapid adaptation was conducted by Karasov et al. (2010) in *Drosophila* demonstrating that  
489 organophosphate treatments quickly induced *ace* point mutations, resulting in multiple simple and  
490 complex resistant-*ace* alleles in the investigated fly subpopulations. In addition to being rapid, the  
491 selection of insecticide resistant specimens can quickly spread throughout population and strains. For  
492 instance, we found that four strains of *B. tabaci* shared the same non-silent mutations. In *D.*  
493 *melanogaster*, several strains can similarly share the same mutations in *ace1* (I161V, G265A, D330W  
494 and G368A) supporting the idea that mutations conferring insecticide resistance can be selected  
495 rapidly and become widespread in a given species (Menozzi et al. 2004).



496 When studying point mutations, gene sequence comparisons are most often performed by the  
497 analysis of single nucleotide polymorphisms (SNPs), as SNPs are often characterized by a high level of  
498 genetic variation (Zwick et al. 2003; Cingolani et al. 2012; Aguilar et al. 2018). Mutations occurring on  
499 SNPs are synonymous, meaning that they change the nucleotide sequence without altering the amino  
500 acid sequence – or change one single amino acid due to wobble rules in the genetic code (Zwick et al.  
501 2003). We found four synonymous mutations in the studied *B. tabaci* strains, and silent mutations  
502 have long been assumed to have no effect on insecticide resistance. However, the strong correlation  
503 in the distribution of non-synonymous and synonymous mutations in mosquitoes owing to their  
504 resistance to permethrin suggest that both mutation types could be major drivers of insecticide  
505 resistance and mutation inheritance (Xu et al. 2012, and also see the discussion in Liu 2015). The  
506 effects of co-occurring synonymous and non-synonymous mutations on insecticide resistance have  
507 never been investigated in *B. tabaci*, even though ~~this~~-these co-occurring mutations have been also  
508 seen in other variants (Hopkinson et al. 2020). We thus suggest that this could represent an  
509 interesting research avenue for future works. Also, even if synonymous mutations on SNPs are not  
510 resulting in divergence between genomes, multi-nucleotide polymorphisms of 2 or 3 nucleotides  
511 (signaled as double and triple nucleotide polymorphisms) can be potential sources of genetic  
512 variation. These nucleotide changes would affect multiple positions in a codon that can change the  
513 amino acid residue(s) at two adjacent positions (Rosenfeld et al. 2010). Similar changes were observed  
514 in Egyptian and Pakistani *B. tabaci* variants. Specifically, the Egyptian strains Fas, Myi, Mbs, and Fib  
515 were characterized by the same two adjacent point mutations (S369W (TCC-TGG) and L370V (CTG-  
516 >GTG)) corresponding to double nucleotide polymorphisms. Similarly, the strains Mym and Mas  
517 exhibited replacements of two adjacent amino acids (C316W (TGC->TGG) and P317G (CCT->GGT)). For  
518 Pakistan, the analyzed sequences revealed triple nucleotide polymorphism (G251D (GGG->GAT),  
519 N252R (AAC->CGA), and P253S (CCG->ICG)). Moreover, our analysis also revealed the substitution of  
520 two additional amino acids (double nucleotide polymorphism) at the C-terminal sequence (C329S  
521 (TGC->TCG) and L330T (CTC->ACC).

522 The phylogenetic relationship of the AChE1 protein sequences from Egypt with other AChE1 from  
523 different insect orders was inferred using the Neighbor-neighbor-joining method to determine the  
524 closest associations among these AChE1. Interestingly, sequences from *B. tabaci* appeared at the top  
525 of the clad in the phylogenetic tree, suggesting that the reported point mutations have evolved  
526 recently as compared with the other analyzed insect species. AChE1 from higher insect orders, in  
527 particular Hymenoptera, Diptera (mainly mosquitoes), and Lepidoptera were grouped together  
528 forming a large clade, excluding homopterans (including aphids and whiteflies) and orthopteroids,  
529 which are continuously in contact with agricultural insecticides, group together under different

530 branches in another successive clade. This analysis reveals that the most frequent mutations of  
531 acetylcholinesterase associated with insecticide resistance in insects are heterogeneous, as formerly  
532 reported by [Menozi et al. \(2004\)](#) from several populations of *D. melanogaster*. Yet even if mutations  
533 can occur in combination, or isolated, it seems that frequent exposures to organophosphates ~~has~~have  
534 a tendency to group insect variants with mutated AChE proteins.

535 When some amino acid substitutions disturb the space and hydrophobicity of the acyl pocket of  
536 the protein, the enzymatic properties of AChE can be modified, as a consequence of altered specificity  
537 with the ligand. More generally, and as mentioned above, a single amino acid substitution is sufficient  
538 for conferring insecticide resistance (Nabeshima et al. 2003, 2004). To predict the functional effects  
539 of amino acid mutations on AChE1-insecticide interactions, homology models were built. Mutations  
540 detected in Egyptian and Pakistani populations (F392W, S369W, L370V, C316W, P317G, P317F,  
541 G251D, N252R, P253S, C329S, and L330T) were introduced to the four conformations of the protein,  
542 and the mutated proteins were subjected to geometry optimization using the universal force field  
543 (UFF). By constructing 3D model of the AChE1 enzyme, we have been able to visualize the locations of  
544 amino acid mutations detected in Egyptian and Pakistani populations of *B. tabaci*. The highest RMSF  
545 values were found in three regions of the enzyme, and subsequent docking calculations revealed that  
546 those mutations have slightly affected AChE1-insecticide binding affinities (values ranging from -6.0  
547 to -8.5 kcal/mol). However, some insecticides, in particular aldicarb, butocarboxim, and methomyl  
548 were characterized by binding affinities (> -5.5 kcal/mol) to AChE1 in the range of those reported for  
549 the positive control ACh (-4.6 kcal/mol). These results suggest that mutations may have little effects  
550 on the protein-pesticide interaction. They may rather induce inadequate steric interaction that  
551 prevents the completion of the phosphorylation of the enzyme, as formerly proposed by Reyes-  
552 Espinosa et al. (2019). This assumption was further confirmed by calculations of pKa values of the  
553 active site of the wild-type and mutant AChE1 of *B. tabaci*, and evidenced the alteration of the AChE1-  
554 Acetylcholine interaction induced by the mutation F392W. The mutation of the protein altered the  
555 acidity of the active site, and lowered its affinity with the ligand (acetylcholine). This result suggested  
556 that the slight increase in the pKa value of the active site E388 may contribute to explain the slight  
557 modification of the binding energy (lower binding affinity).

558 By analyzing the models and the active site gorge of AChE1, it has been possible to make  
559 inferences on the effects of each point mutation on the structure and function of the AChE1 enzyme.  
560 Point mutations C316W and C329S most probably disrupted the disulfide bonds of the enzyme. As  
561 these bonds are essential for the molecular architecture of proteins (Siadat et al. 2006; Feige et al.  
562 2018; ~~Betz 1993; Wedemeyer et al. 2000~~), these mutations may have resulted in structural  
563 modifications at the entrance of the active site gorge. In previous studies, two out of the three regions

564 containing disulfide bonds (C313-326 and C332-340) were suggested to be part of the peripheral  
565 anionic binding site of AChE1 (Dvir et al. 2010). Here, we provided supporting evidence that mutations  
566 C316W and C329S, as well as P317G, P317F, and L330T affected the peripheral anionic binding site of  
567 the protein by changing the aromatic content at the gorge entrance. Point mutations S369W and  
568 L370V may confer structural modifications near the bottom of the gorge, and these neighboring  
569 residues of the binding site should affect ligand binding owing to the changes of electrostatic  
570 interactions they induce. Consistently, point mutation S369W added new bonds with the residue E364  
571 (red lines in Fig. 6B) which lost the interaction with the neighboring residue P468. As a consequence,  
572 the distance between residues P468 and L370 was 3.7 Å in the wild type enzyme, and has been  
573 increased by 1 Å in the mutated enzyme (interaction with the residue V370). Mutations G251D,  
574 N252R, and P253S will change the surface charge of the protein. Mutation G251D will add a -1 charge  
575 to the enzyme surface, while the mutation N252R will add a +1 charge. The P253S mutation will affect  
576 hinge flexibility as proline will be replaced by serine, which is a flexible and water-soluble residue as  
577 compared with proline which confers an exceptional conformational rigidity.

578  
579 Future work should investigate the dose-responses and resistance ratios to organophosphates in  
580 the Egyptian and Pakistani variants we studied to ~~get a~~ better understanding of the incidence of the  
581 AChE protein mutations we found. If the resistance is confirmed by bioassay data, the methodology  
582 developed in this study could be valuably used for getting predictions of the level of resistance of  
583 different *B. tabaci* variants, and assess the frequency of this resistance among field populations. More  
584 generally, we encourage the implementation of more studies on the genetic structure of *B. tabaci*  
585 population, and on the identification of resistance genes, as it would allow a better understanding of  
586 the evolution of resistance. Finally, the target insensitivity or inheritance patterns of organophosphate  
587 resistance in *B. tabaci* will ~~also~~ require additional research to confirm the resistance and to establish  
588 more efficient management strategies for *B. tabaci* worldwide.

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601

#### 602 **Authorship contribution**

603 AM and DR conceived and designed the study. AM, AE, and DR analyzed the data. AM, AE, and DR  
604 interpreted the results and prepared the figures and the tables. AM and DR wrote the first version of  
605 the manuscript. AM, and DR reviewed the drafts of the manuscript.

606

#### 607 **Availability of data and materials**

608 All data analyzed during this study are included in this published article.

609

#### 610 **Declarations**

611 **Ethics approval and consent to participate** Not applicable.

612 **Consent for publication** Not applicable.

613 **Competing interests** The authors declare no competing interests

614

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979 **Figure legends**

980

981 **Fig 1.** The amino acid sequence homology matrix of *B. tabaci* AChE from Egyptian field strains (see  
982 Table 1 for strain designations), and from the Israeli resistant (R, ABV45412) and susceptible (SUD,  
983 ABV45413) *B. tabaci* variants from Alon et al. (2008).

984

985 **Fig 2.** Sequence alignment using ClustalX. **A.** Alignment of amino acid sequences of the different partial  
986 AChEs from Egyptian field-strains of *B. tabaci* and resistant (accession no. ABV45412.1) and  
987 susceptible (accession no. ABV45413.1) AChE1 strains from Alon et al. (2008). The numbers above the  
988 sequences denote the position of the amino acids. Dots represent identity in amino acid residues. **B.**  
989 Multiple sequence alignments of the partial *ace1* nucleotide sequences of the Egyptian field-strains of  
990 *B. tabaci* and susceptible strain (accession no. EF675188.1) from Alon et al. (2008). Nucleotides'  
991 position numbers indicated above sequences, while dots indicate the identical nucleotide.

992

993 **Fig 3. A.** Visualization of putative spread of *ace1* mutation in *B. tabaci* variants from different  
994 geographic regions (Egypt and Pakistan), based on deduced AChE1 sequences, in comparison with the  
995 resistant and sensitive strains described by Alon et al. (2008). The amino acid residues identical to  
996 ABV45413 are indicated by dots. The numbers above the lines indicate the amino acid residues  
997 according to accession no. ABV45413.1 and ABV45412.1 (Alon et al. 2008). Shadowed amino acids  
998 represent five missense mutations (nonsynonymous substitution or polymorphisms) in form of DNPs  
999 and TNPs. **B.** Visualization of *ace-1* mutation occurrence in *B. tabaci* strains from different localities of  
1000 Pakistan. The nucleotide positions identical to accession EF675187.1 (S allele) EF675188.1 (R allele)  
1001 are indicated by dots.

1002 In both figures, the differences in amino acid or nucleotide are shown by the appropriate letters, and  
1003 '-' indicate unidentified gaps.

1004

1005 **Fig. 4.** Molecular dynamics simulation of *Bemisia tabaci* AChE1. **(A)** Root-mean-square deviation  
1006 (RMSD) in Å (blue), radius of gyration (RoG) in Å (green), surface accessible surface area (SASA) in Å<sup>2</sup>  
1007 (gray), and total number of formed H-bonds (yellow) as a function of the simulation time (in  
1008 nanoseconds). **(B)** Root-mean-square fluctuations (RMSF) per residue (in Å) over the duration of the  
1009 simulation. The three regions with high RMSF values (high flexibility of amino acid residues, large  
1010 fluctuations of amino acid residues during the simulation) are highlighted in red, magenta, and yellow,  
1011 and the cartoon diagram of the protein structure was constructed with the same color codes. The  
1012 three amino acids forming the active site triad, Glutamic acid in position 388 (E388), Serine in  
1013 position 262 (S262) and Histidine in position 501 (H501) are presented. The Ramachandran plot of  
1014 the generated model is presented at the top right of the figure.

1015

1016 **Fig. 5. (A)** Superimposition of the representative structures of the four different clusters of wild type  
1017 *Bemisia tabaci* AChE1. (B) Cartoon representation of the active site gorge of *Bemisia tabaci* AChE1  
1018 after 877 ns of simulation, and showing the locations of the acyl pocket, peripheral anionic site,  
1019 acetylcholine, choline binding site, residues of active site triad, and oxyanion hole. The residues lining  
1020 the active site gorge of wild type AChE1 enzyme are depicted in colored cartoons according to the  
1021 color code presented at the bottom of the figure. (C) Zoom-in on the active site gorge of *Bemisia tabaci*  
1022 AChE1 after 877 ns of simulation, alongside with residues that may be part of the gorge lining.

1023

1024 **Fig. 6.** The structural motifs of the active site gorge of *Bemisia tabaci* AChE1. (A) Cartoon  
1025 representation of AChE1 active site gorge; the position of each mutation is represented in black.  
1026 Disulfide bonds are red-colored; Peripheral anionic site (PAS) are represented in cyan and the region  
1027 reported to be part of the peripheral anionic site is represented by several dark dots. The blue surface  
1028 highlights the location of the active site triad (S262, E388, and H501), and green sticks show the ligand

1029 ACh. (B) Superposition of the wild type (WT, black), and variants S369W (magenta) and L370V (yellow)  
1030 of *Bemisia tabaci* AChE1 showing the structural modifications of the mutated enzymes. For each  
1031 mutation, the interacting residues are shown by red lines. (C) Superposition of the wild type (WT, black  
1032 sticks), and variants G251D (purple lines), N252R (gray lines), and P253S (red lines) of *Bemisia tabaci*  
1033 AChE1. The enzyme surface charges are modified by the mutation for the variants G251D and N252R.  
1034  
1035 **Fig. 7.** The average binding energies of the different organophosphate **(A)** and carbamate **(B)**  
1036 insecticides in the wild type (WT) and mutated (F392W, S369W, L370V, C316W, P317G, P317F, G251D,  
1037 N252R, P253S, C329S, and L330T) AChE1 enzyme. Acetylcholine (ACh) is used as a positive control.

|            |    | Percent Identity |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |            |                  |                    |
|------------|----|------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------|------------------|--------------------|
|            |    | 1                | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   |      |            |                  |                    |
| Divergence | 1  | ■                | 98.4 | 98.4 | 100  | 98.4 | 98.4 | 100  | 97.6 | 97.6 | 100  | 100  | 100  | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 1    | ABV45412.1 | R B <sup>1</sup> |                    |
|            | 2  | 1.6              | ■    | 96.8 | 98.4 | 96.8 | 100  | 98.4 | 99.2 | 99.2 | 98.4 | 98.4 | 98.4 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 2          | BAW19594.1       | R Mbs              |
|            | 3  | 1.6              | 3.3  | ■    | 98.4 | 100  | 96.8 | 98.4 | 96.0 | 96.8 | 98.4 | 98.4 | 98.4 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 3          | BAW19595.1       | R Mas              |
|            | 4  | 0                | 1.6  | 1.6  | ■    | 98.4 | 98.4 | 100  | 97.6 | 97.6 | 100  | 100  | 100  | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 4          | BAW19590.1       | R Qbn              |
|            | 5  | 1.6              | 3.3  | 0    | 1.6  | ■    | 96.8 | 98.4 | 96.0 | 96.8 | 98.4 | 98.4 | 98.4 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 5          | BAW19591.1       | R Mym              |
|            | 6  | 1.6              | 0    | 3.3  | 1.6  | 3.3  | ■    | 98.4 | 99.2 | 99.2 | 98.4 | 98.4 | 98.4 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 6          | BAW19592.1       | R Myi              |
|            | 7  | 0                | 1.6  | 1.6  | 0    | 1.6  | 1.6  | ■    | 97.6 | 97.6 | 100  | 100  | 100  | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 7          | BAW19588.1       | R Gkt              |
|            | 8  | 2.5              | 0.8  | 4.1  | 2.5  | 4.1  | 0.8  | 2.5  | ■    | 98.4 | 97.6 | 97.6 | 97.6 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 8          | BAW19585.1       | R Fas              |
|            | 9  | 2.5              | 0.8  | 3.3  | 2.5  | 3.3  | 0.8  | 2.5  | 1.6  | ■    | 97.6 | 97.6 | 97.6 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 96.8 | 9          | BAW19586.1       | R Fib              |
|            | 10 | 0                | 1.6  | 1.6  | 0    | 1.6  | 1.6  | 0    | 2.5  | 2.5  | ■    | 100  | 100  | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 10         | BAW19583.1       | R Dbq              |
|            | 11 | 0                | 1.6  | 1.6  | 0    | 1.6  | 1.6  | 0    | 2.5  | 2.5  | 0    | ■    | 100  | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 11         | BAW19580.1       | R Beb              |
|            | 12 | 0                | 1.6  | 1.6  | 0    | 1.6  | 1.6  | 0    | 2.5  | 2.5  | 0    | 0    | ■    | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 99.2 | 12         | BAW19578.1       | R B                |
|            | 13 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | ■    | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 13         | ABV45413.1       | S SUD <sup>2</sup> |
|            | 14 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | ■    | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 14         | BAW19579.1       | S HS-S             |
|            | 15 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | ■    | 100  | 100  | 100  | 100  | 100  | 100  | 15         | BAW19582.1       | S Dmg              |
|            | 16 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | 0    | ■    | 100  | 100  | 100  | 100  | 100  | 16         | BAW19581.1       | S Bwn              |
|            | 17 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | 0    | 0    | ■    | 100  | 100  | 100  | 100  | 17         | BAW19584.1       | S Fsn              |
|            | 18 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | 0    | 0    | 0    | ■    | 100  | 100  | 100  | 18         | BAW19587.1       | S Gzf              |
|            | 19 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | 0    | 0    | 0    | 0    | ■    | 100  | 100  | 19         | BAW19589.1       | S Qqb              |
|            | 20 | 0.8              | 2.5  | 2.5  | 0.8  | 2.5  | 2.5  | 0.8  | 3.3  | 3.3  | 0.8  | 0.8  | 0.8  | 0    | 0    | 0    | 0    | 0    | 0    | 0    | ■    | 100  | 20         | BAW19593.1       | S Mys              |
|            | 1  | 2                | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   |      |      |            |                  |                    |

Fig. 1



Fig. 2A



```

841                                     966
EF675188.1 TTC AGC CAG GCG ATC ATG GAG TCC GGG TCG GCG ACG GCG CCG TGG GCC ATC ATC TCC CGC CAG GAG TCC ATC ATC CCG GGC CTC CGC CTC GCC GAG GGT GTC GGC TGC CCT CAC ACG CGE GCG CAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

967                                     1092
EF675188.1 ATC CCT GAG GCT ATC GAG TCC CTC CGA AAG GTC AAC GCG AGC GTG CTC GTG GAG AAC GAG AGC GGG ACC CTA GGG ATC TGC GAC TTC CCG TTC GTC CCG GTC GTG GAC GGC TCC TTC CTG GAC GAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

1093                                     1212
EF675188.1 ATG CCG TCC AAG TCC CTG GCG ACG AAG AAC TTC AAG AAG ACC AAC ATC CTC ATG GGG AGC AAC ACG GAG GAG GGC AAC TAC TTC ATC ATG TAC TAC CTG ACG GAC CTC TTC CCG AAG GAG
LC199315.1 .....
LC199311.1 .....
LC199301.1 .....
LC199309.1 .....
LC199303.1 .....
LC199304.1 .....
LC199306.1 .....
EF675187.1 .....
LC199300.1 .....
LC199310.1 .....
LC199302.1 .....
LC199312.1 .....
LC199305.1 .....
LC199317.1 .....
LC199313.1 .....
LC199316.1 .....
LC199314.1 .....
LC199307.1 .....
LC199308.1 .....

```

Fig. 2B



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242                                     321
ABV45413.1 IRNNIHAFGGNPHNITLFGESAGAVSVMHLLSPLSRNLFSSQAIMESGSATAPWAIISRQESIIRGLRLAEAVGCPHTRA
ABV45412.1 .....
BAW19595.1 .....WG.....
AMO25775.1 -----DRS.....

322                                     401
ABV45413.1 QIPEAIECLRKVNASVLVENESGTLGICDFPFVFPVVDGSFLDEMPSKSLATKNFKKTNILMGSNTEEGNYFIMYYLTDLF
ABV45412.1 .....W.....
BAW19595.1 .....W.....
AMO25775.1 .....ST-----

```

**Fig. 3A**

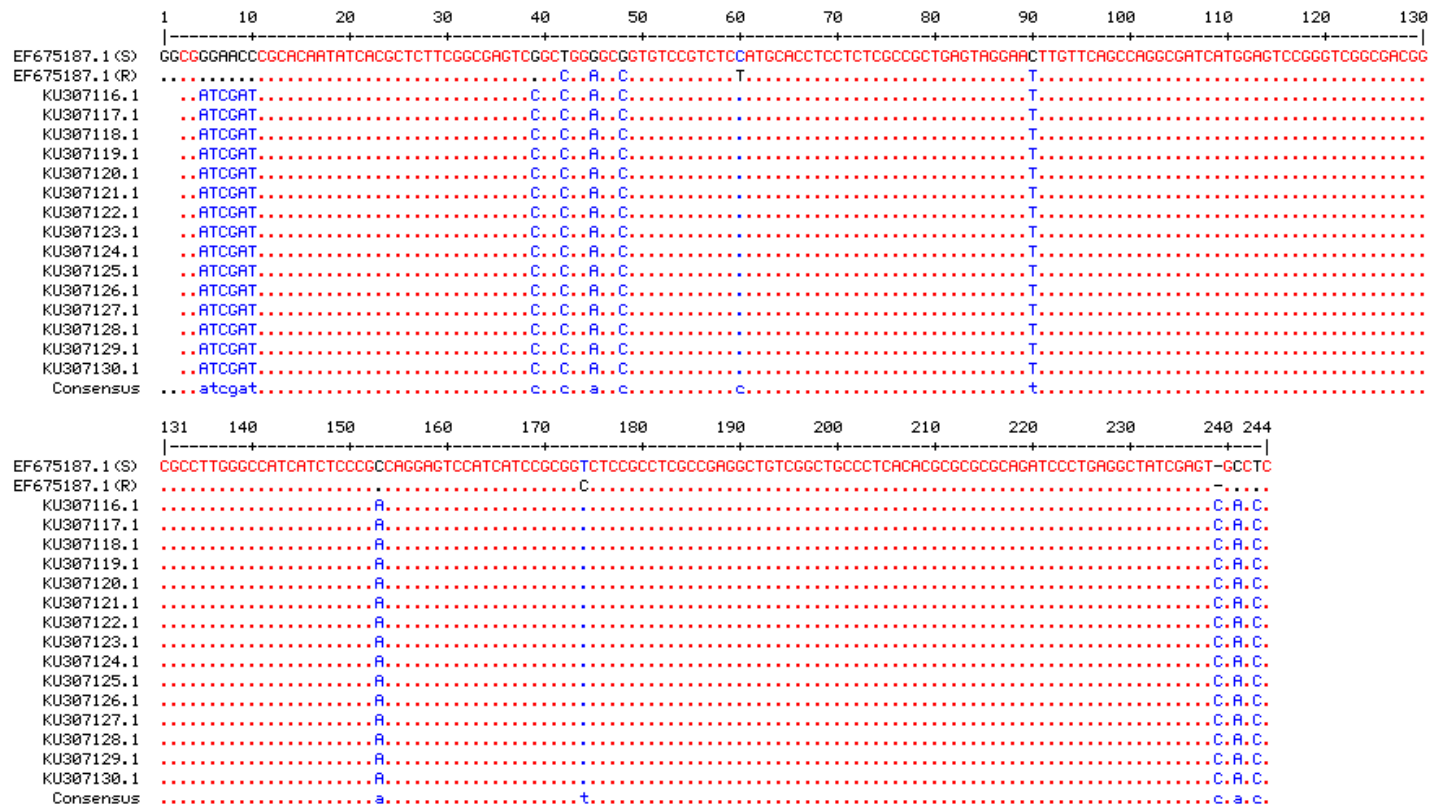
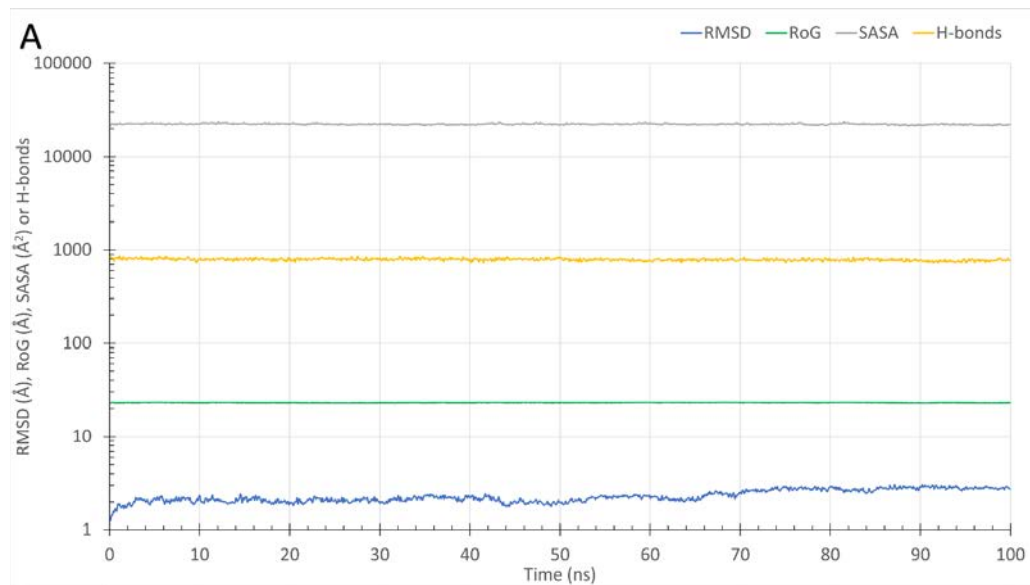


Fig. 3B



**Fig. 4A**

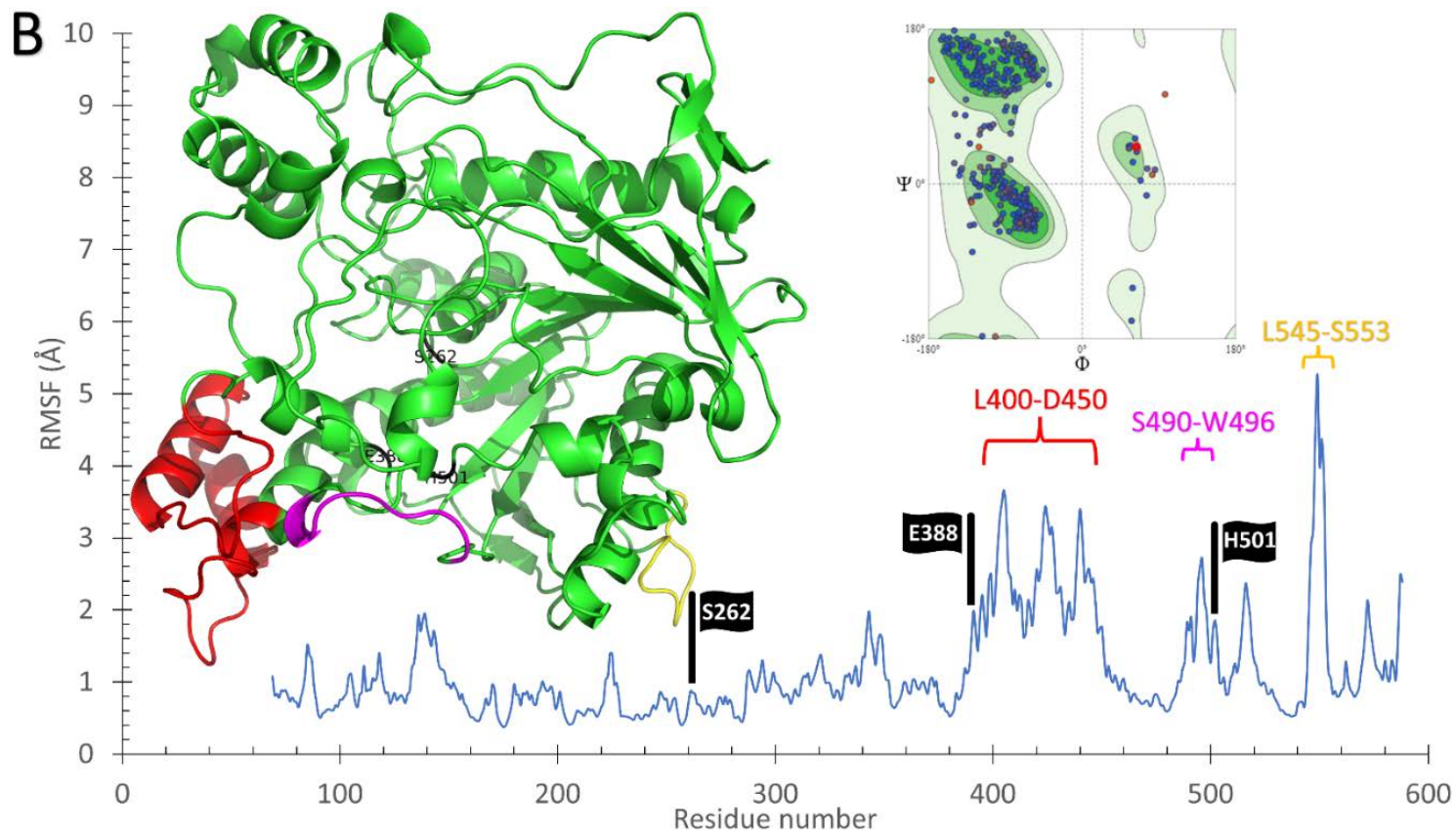
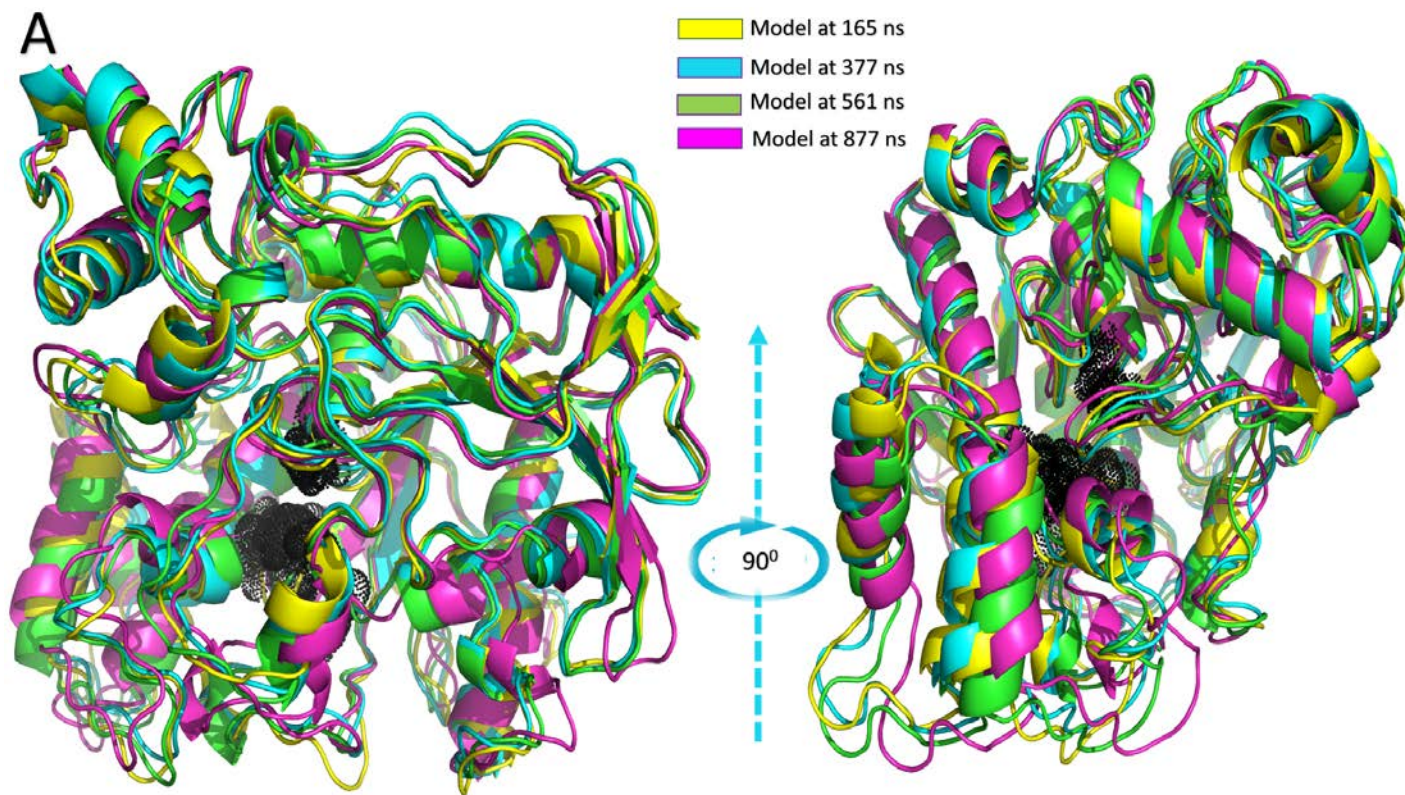


Fig. 4B



**Fig. 5A**



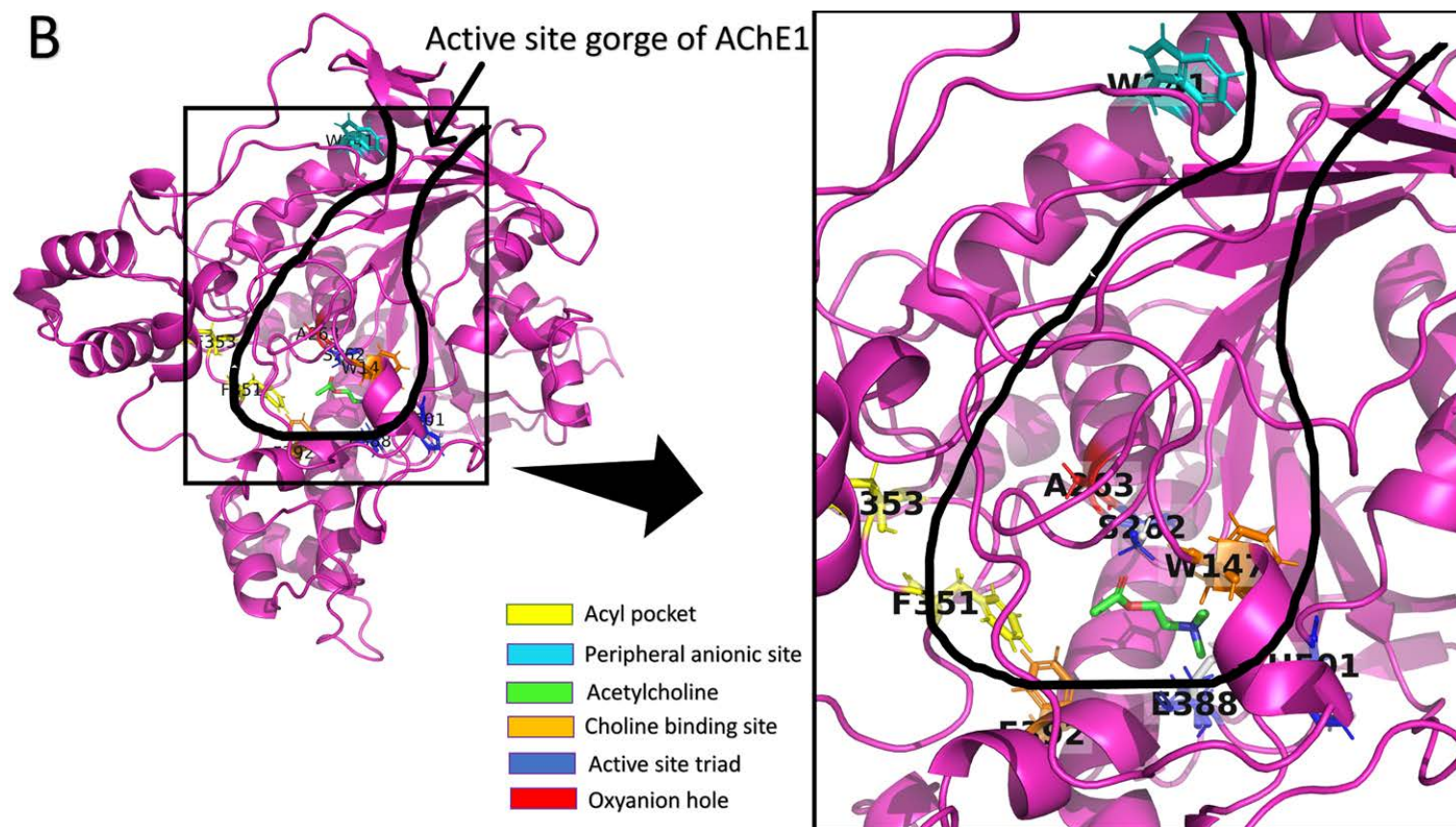
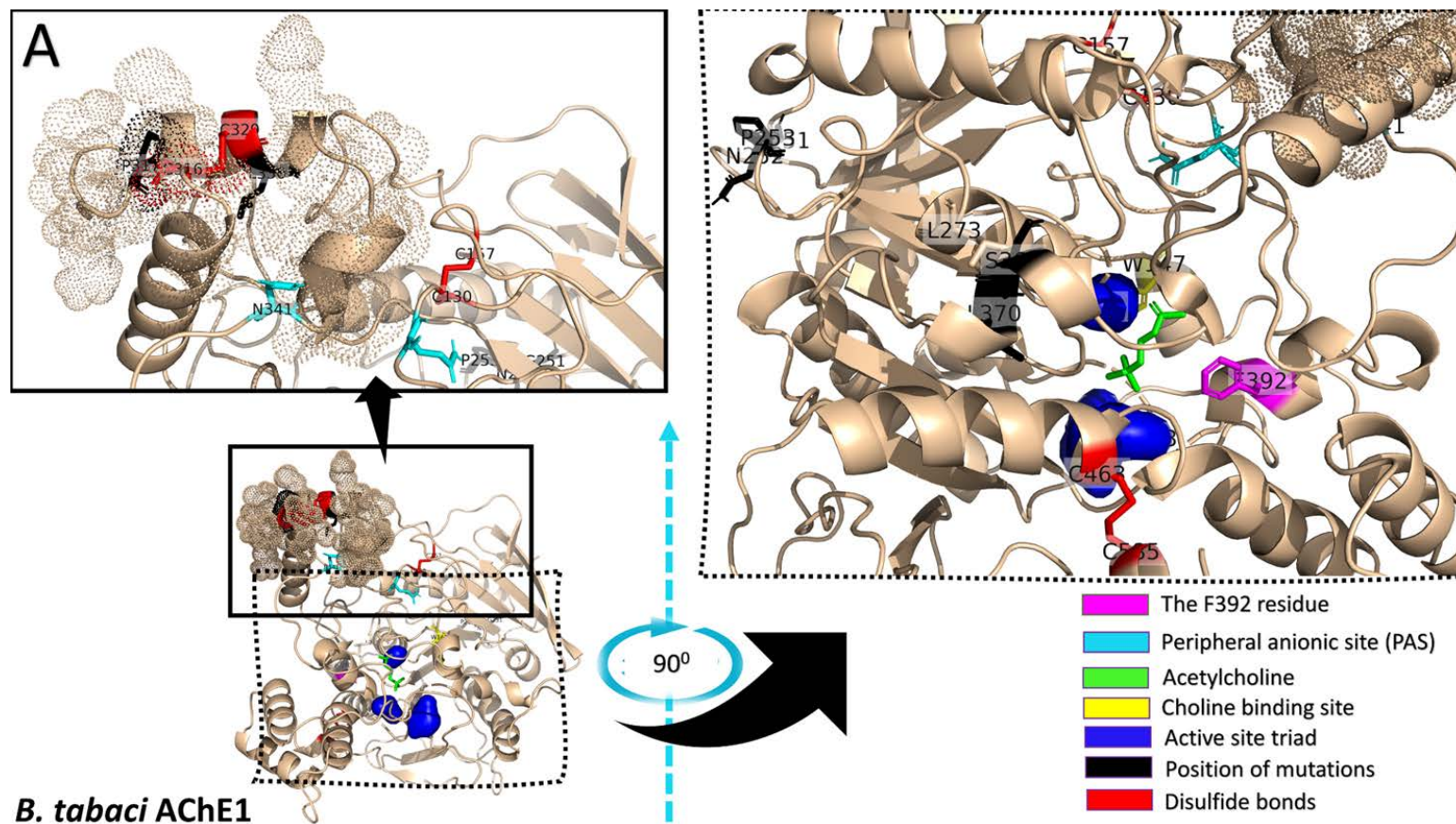


Fig. 5B



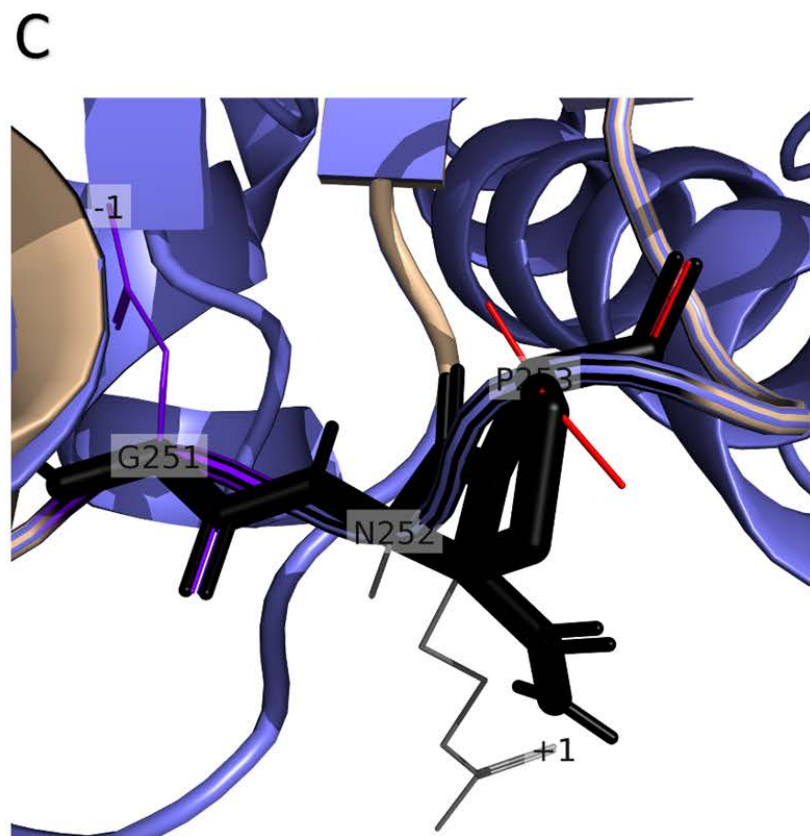
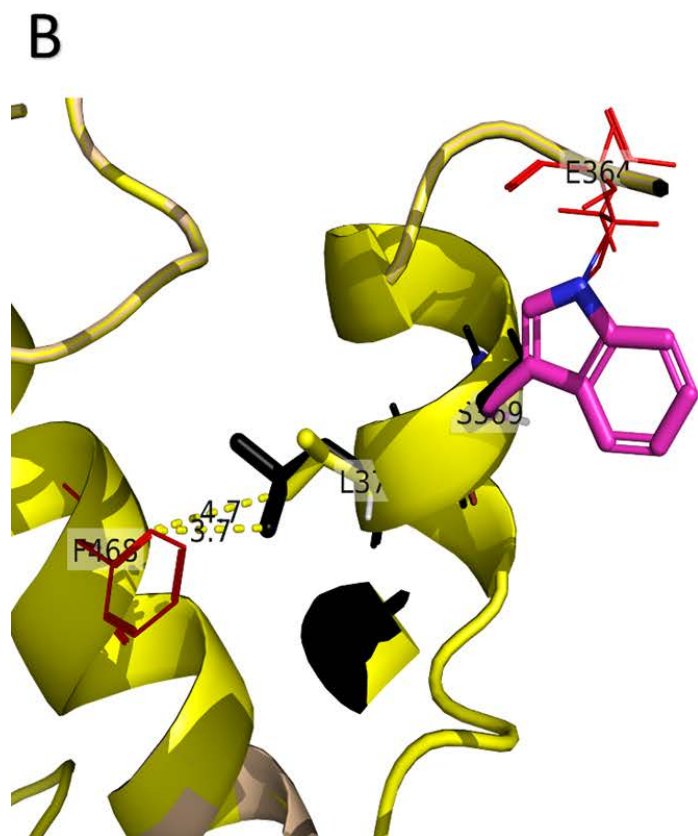


Fig. 6B, 6C



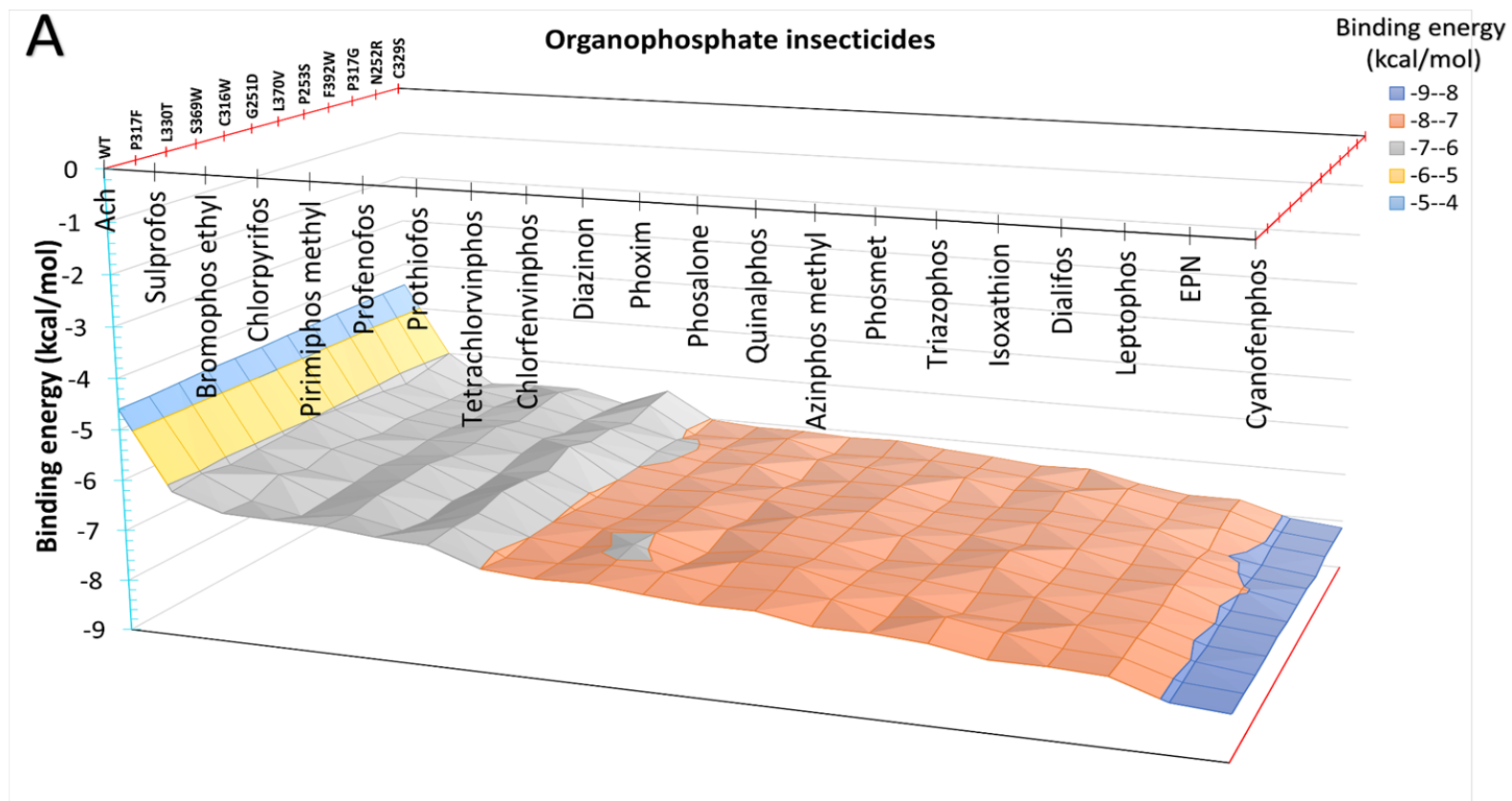


Fig. 7A

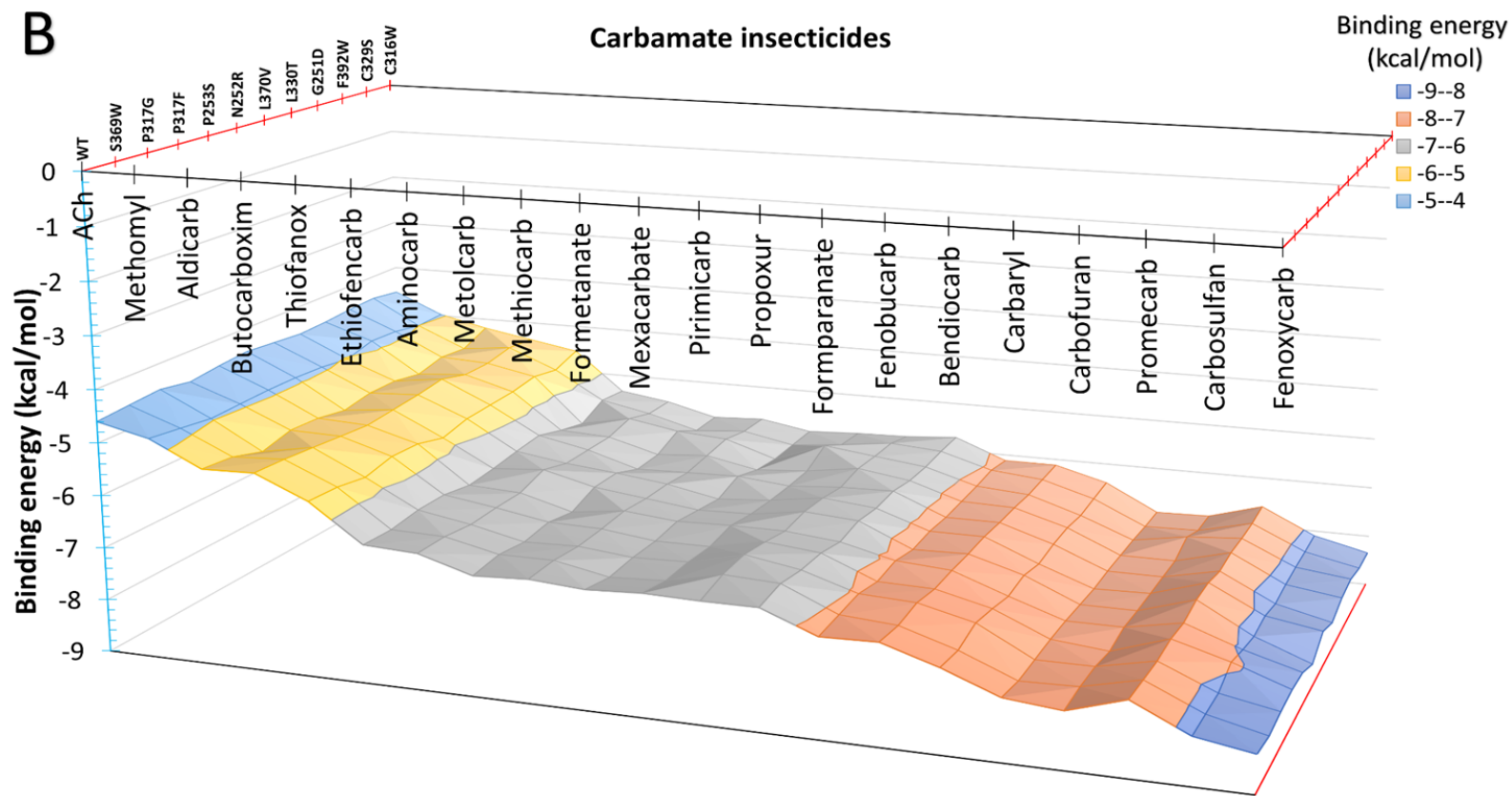


Fig. 7B

**Table 1.** Amino acid polymorphism and sequence variation (alleles) among the analyzed sequences of *B. tabaci ace1*-type acetylcholinesterase from different Egyptian populations

| Position n(aa)*            | AA side chain chemistry change          | Resistant group       |     |     |     |            |            |            |            |            |            |            | Susceptible group    |     |     |     |     |     |     |
|----------------------------|---|-----------------------|-----|-----|-----|------------|------------|------------|------------|------------|------------|------------|----------------------|-----|-----|-----|-----|-----|-----|
|                            |   | Egy-B <sub>Lab.</sub> | Beb | Gkt | Qbn | Dbq        | Mym        | Mas        | Fas        | Myi        | Mbs        | Fib        | HS-S <sub>Lab.</sub> | Bwn | Dmg | Fsn | Mys | Gzf | Qqb |
| *[S]892(298)<br>(ATC->ATT) | Neutral hydrophobic →<br>None           | I<br>(ATC)            | ... | ... | ... | I<br>(ATT) | ...        | ...        | ...        | ...        | ...        | ...        | I<br>(ATC)           | ... | ... | ... | ... | ... | ... |
| [S]919(307)<br>(GGC->GGT)  | Neutral hydrophobic →<br>None           | G<br>(GGT)            | ... | ... | ... | ...        | ...        | ...        | ...        | ...        | ...        | ...        | G<br>(GGC)           | ... | ... | ... | ... | ... | ... |
| 946(316)<br>(TGC->TGG)     | Neutral polar → Aromatic hydrophobic    | C<br>(TGC)            | ... | ... | ... | ...        | W<br>(TGG) | W<br>(TGG) | ...        | ...        | ...        | ...        | C<br>(TGC)           | ... | ... | ... | ... | ... | ... |
| 949(317)<br>(CCT->GGT)     | Neutral hydrophobic →<br>None           | P<br>(CCT)            | ... | ... | ... | ...        | G<br>(GGT) | G<br>(GGT) | ...        | ...        | ...        | F<br>(TTT) | P<br>(CCT)           | ... | ... | ... | ... | ... | ... |
| 958(320)<br>(CGC->CCG)     | Basic →<br>Neutral hydrophobic          | R<br>(CGC)            | ... | ... | ... | ...        | ...        | ...        | P<br>(CCG) | ...        | ...        | ...        | R<br>(CGC)           | ... | ... | ... | ... | ... | ... |
| [S]1060(354)<br>(GTC->GTG) | Neutral hydrophobic →<br>None           | V<br>(GTG)            | ... | ... | ... | ...        | ...        | ...        | ...        | ...        | ...        | ...        | V<br>(GTC)           | ... | ... | ... | ... | ... | ... |
| 1105(369)<br>(TCC->TGG)    | Neutral polar →<br>Aromatic hydrophobic | S<br>(TCC)            | ... | ... | ... | ...        | ...        | ...        | W<br>(TGG) | W<br>(TGG) | W<br>(TGG) | W<br>(TGG) | S<br>(TCC)           | ... | ... | ... | ... | ... | ... |
| 1108(370)<br>(CTG->GTG)    | Neutral hydrophobic →<br>None           | L<br>(CTG)            | ... | ... | ... | ...        | ...        | ...        | V<br>(GTG) | V<br>(GTG) | V<br>(GTG) | V<br>(GTG) | L<br>(CTG)           | ... | ... | ... | ... | ... | ... |
| [S]1165(389)<br>(GGC->GGG) | Neutral hydrophobic →<br>None           | G<br>(GGG)            | ... | ... | ... | ...        | ...        | ...        | ...        | ...        | ...        | ...        | G<br>(GGC)           | ... | ... | ... | ... | ... | ... |
| 1174(392)<br>(TTC->TGG)    | Aromatic hydrophobic →<br>None          | W<br>(TGG)            | ... | ... | ... | ...        | ...        | ...        | ...        | ...        | ...        | ...        | F<br>(TTC)           | ... | ... | ... | ... | ... | ... |

\*Unbracketed numbers indicate the nucleotides position, while bracketed numbers denote amino acids position.

\*Silent mutation; mutations that do not change the organism's phenotype. Although occur in nucleotides of a coding region but result in the same amino acid.

The conserved residues are shown with dots. The polymorphic codons between the resistant and susceptible strains are highlighted with gray color. The numerical designations indicating the positions of amino acids and nucleotides are based on the two *B. tabaci ace1*, obtained from GenBank, mentioned below.

Comparison of the nucleotide and deduced amino acid sequences of *B. tabaci ace1* (reference strains: S, susceptible; R, resistant, GenBank accession no. EF675188 and EF675187, respectively) and eighteen different strains from diverse Egyptian localities. The Egyptian strains were divided into two groups based on their sequence homology with the aforementioned S and R Alon et al. (2008) alleles (see Supplementary [Information Table S1](#)); the first group includes eleven strains (Egy-B, Beb, Gkt, Qbn, Dpq, Mym, Mas, Fas, Mai, Mbs, and Fib) which showed F392 substituted with W392 residue (F392W), based on the resistant *B. tabaci* AChE deduced amino acid sequence (GenBank accession no. ABV45412.1). The second group of sequences is expressed to be susceptible (HS-S, Bwn, Dmg, Fsn, Mys, Gzf, and Qqb), where the F392 is conserved among all the seven strains compared to the susceptible *B. tabaci* AChE deduced amino acid sequence (GenBank accession no. ABV45413.1).