IN SILICO IDENTIFICATION OF POTENTIAL INHIBITORS OF *Ostrinia furnacalis* β-*N*-ACETYL-D-HEXOSAMINIDASE IN THE ZINC NATURAL PRODUCTS DATABASE

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ABSTRACT

Chemical databases of natural products are extensively utilized for the initial screening of compounds with agrochemical properties. In this study, a virtual screening pipeline was developed to identify lead insecticidal compounds from the ZINC Natural Products database and to evaluate the pupation enzyme, β-*N*-acetyl-D-hexosaminidase as molecular target for development of insecticide specific for the Asian corn borer, *Ostrinia furnacalis*. The QEPest algorithm was utilized as primary filter to screen for ZINC compounds with high quantitative estimates of insecticide-likeness (QEI). ZINC compounds identified by the primary filter were further evaluated for inhibition of the pupation enzyme by virtual docking with Autodock Vina. The pharmacological properties of β-*N*-acetyl-D-hexosaminidase was determined *in silico* with iGemDock software.

Based on QEPest calculation, 8,000 ZINC molecules were identified to have QEI values ranging from 0.50 to 0.97. The high QEPest scoring compounds have a MW of 200-350, LogP of 2-6, HBA of 1-3, HBD of 0, RB of 3-8 and 0-1 arR. Virtual docking revealed that the top 1% of the 8,000 ZINC molecules had very tight binding affinities ranging from -10.5 kcal/mol (from 12 ZINC molecules) to -11.8 kcal/mol (ZINC04026248). Inspection of the binding interface showed that the tight binding of ZINC04026248 is attributed mainly to hydrophobic interactions with six hydrophobic amino acid residues in the binding pocket of 3NSN. The pharmacophore model derived from the binding of ZINC04026248 consisted of two hydrophobic, one aromatic, and one hydrogen acceptor. The pharmacological properties determined by iGemDock suggests that 3NSN is a good candidate as target for development of insecticide specific for control of *O. furnacalis*.

Key words: β-*N*-acetyl-D-hexosaminidase, *in silico* identification, inhibitors, *Ostrinia furnacalis,* Zinc natural products

INTRODUCTION

Natural products have been a rich source of compounds for agrochemicals. Pesticides such as herbicides, fungicides, and insecticides were originally discovered from natural products from various sources. However, due to the rise of pest resistance, there is a need to discover new agrochemicals for pest control (Gerwick & Sparks, 2014; Sparks et al., 2017). Because of technical barriers in high throughput screening of chemical compounds, there has been a shift to screen natural products using computational methods with chemical libraries. High throughput molecular docking approaches and virtual screening of chemical databases have emerged as the approach for discovering new bioactive compounds (Ma et al., 2011; Perola et al., 2000). The computational method utilizes computers to dock molecules or chemicals into the active site of target enzymes to identify potential candidates based on their binding affinities (Perola et al., 2000). Thousands to millions of compounds can be analyzed in a span of a few hours or days. However, there are concerns about the efficiency of screening thousands of compounds without prior pre-screening for molecules with the desired physiochemical properties that dictate their efficiency as agrochemical agents.

In this study, Quantitative Estimation of Pesticide-likeness (QEPest) was used prior to virtual docking to determine which compounds have potential for pesticide-like activity based on their molecular properties such as molecular weight, molecular hydrophobicity (LogP), number of hydrogen bond acceptors, hydrogen bond donors, rotatable bonds, and aromatic rings. QEPest offers an efficient way to estimate pesticide-likeness by scoring each pesticide class (herbicide, fungicide, or insecticide) by the corresponding quantitative estimate. QEPest's ability to rank compounds offers an efficient way to prioritize compounds with agrochemical properties. This is especially useful when analyzing vast chemical libraries for bioactive compounds useful in agriculture (Avram et al., 2014).

Using QEPest to prioritize compounds with high likelihood for pesticide activity for virtual docking, drastically reduced the number of compounds that needs to be analyzed in downstream applications such as protein-ligand interaction profiling. QEPest was initially tested using datasets of patented pesticides (Avram et al., 2014). In this study, QEPest was used to screen a chemical library of 83,215 natural products for potential insecticides. This will demonstrate if QEPest is able to correctly rank compounds with potential for insecticide activity.

Compounds with high quantitative estimates of insecticide-likeness (QEI) values have been docked with β-*N*-acetyl-D-hexosaminidase, OfHex1, an important enzyme in insect pupation (Yang et al., 2008; Liu et al., 2011; Chen et al., 2014). Protein-ligand interactions of compounds with high docking energies were also analyzed to determine if they interact with critical binding site residues that would lead to the inhibition of the target enzyme. Docking energies and

interactions of the compounds with essential enzyme residues will determine if QEPest is effective in identifying agronomically important bioactive compounds.

The objectives of this study are: 1) to develop a virtual screening pipeline to identify lead insecticidal compounds from the ZINC (=Zinc Is Not Commercial) Natural Products database, in particular, to estimate insecticidal potential by virtual inhibition of *O. furnacalis* β-*N*-acetyl-D-hexosaminidase; and 2) to evaluate the pupation enzyme, β-*N*-acetyl-D-hexosaminidase as molecular target for development of insecticide specific for the Asian corn borer, *Ostrinia furnacalis*.

MATERIALS AND METHODS

Natural products library

A subset (Natural Products library) of ZINC compounds comprising of ready to dock 83,215 molecules was downloaded from the ZINC database (http://www.zinc.docking.org) (Irwin & Shoichet, 2005).

Calculation of insecticide-likeness

The calculation of molecular descriptors was done using the ChemDes website (http://www.scbdd.com/chemdes/), a free web-based platform which integrates multiple packages for computing molecular descriptors and fingerprints. (Dong et al., 2015). In this study, the molecular descriptors for molecular weight (MW), molecular hydrophobicity (log of the octanol–water partition coefficient; LogP), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), rotatable bonds (RB), aromatic rings (arR) were calculated using RDKit (http://www.scbdd.com/rdk_desc/index). The molecular descriptors of 81,557 IBScreen ZINC molecules were further analyzed using QEPest to derive quantitative estimate of pesticide-likeness.

Quantitative estimation of pesticide-likeness

The QEPest software was used to determine which molecules in the dataset had the potential for pesticide-like activity (Avram et al., 2014). QEPest uses the enumerated molecular descriptors to provide quantitative estimates of herbicide- (QEH), insecticide- (QEI), fungicide- (QEF), and, finally, pesticide-likeness (QEP). In this study, the QEI was used to rank compounds with potential insecticidal activity, with QEI values close to 1.0 indicative of high potential (Avram et al., 2014).

Virtual screening

The target receptor, β-*N*-acetyl-D-hexosaminidase (Protein Data Bank ID: 3NSN) was downloaded from RCSB PDB (www.rcsb.org/pdb/). Heteroatoms

bound to the 3D structure of the receptor were removed using the Chimera software (Pettersen et al., 2004). The receptor was energy-minimized using ModRefiner (Xu & Zhang, 2011).

For targeted virtual docking, 8,000 compounds with quantitative estimates of insecticide-likeness (QEI) values ranging from 0.50-0.99 were docked to the binding site of 3NSN using Autodock Vina as implemented in MTiOpenScreen server (Labbé et al., 2015). Grid center coordinates were as follows: x=37, y=3, z=13. Grid dimension was set to 25 x 25 x 25 with a default resolution of 0.375Å. The top 1% high scoring compounds identified by virtual screening were further evaluated for specific pharmacological interactions with 3NSN binding site using iGemDock v2.1 software (Hsu et al., 2011). The 3NSN was docked with the ligands under docking accuracy settings (GA parameters) with binding site radius 8°A (X=8.3, Y=8.3, and Z=8.3°A each); population size: 200; solutions: 3; and number of generations: 70. The hydrophobic and electrostatic preference were set to 1.00. The empirical scoring function of iGEMDOCK was determined at: Fitness = van der Waal energy (vdW) + hydrogen bonding energy (Hbond) + electro statistic energy (Elec). The ZINC compounds were ranked based on pharmacological and energy-based scoring function built-in the software.

Pharmacophore generation

The best docked pose of each of the top scoring compounds was analyzed for the presence of pharmacophore using online version of Pharmit. Features supported by Pharmit include hydrogen bond acceptors and donors, negative and positive charges, aromatics, and hydrophobic features (Sunseri & Koes, 2016).

RESULTS AND DISCUSSION

Screening of natural products database

The Asian corn borer, *O. furnacalis* is the major insect pest of corn in the Philippines. Its larval stage causes damage by boring inside corn stalks, which eventually kills the plant. Yield loss resulting to *O. furnacalis* infestation could be as high as 80% (Wang et al., 2014). The adoption of transgenic Bt corn by farmers has been so far successful in controlling high *O. furnacalis* infestation but the onset of resistance development in certain *O. furnacalis* population warrants the need to search for alternative control measures to eliminate the buildup of insect resistance in the field.

A modern concept for the development of new insecticides is the target-based approach. This method consists of five major steps: target protein identification and subsequent validation, identification and optimization of chemical lead structures, and lastly, *in vitro* testing of the optimized lead structures. The

first step, target identification, very often involves bioinformatics searches for proteins that can serve as molecular points of attack for insecticidal compounds (Krasky et al., 2007). To support the lead identification and lead optimization process (steps 3 and 4 of the target-based approach), chemoinformatics methods like homology modeling and ligand docking can be used (Gasteiger, 2007). A combined bioinformatics and chemoinformatics approach was used in this study to search for lead insecticidal compounds. The QEPest software was used as *in silico* chemical filter to reduce and identify candidate insecticidal compounds from a selected subset of ZINC natural products database.

Scatter plot analysis (Figure 1) revealed that high QEPest scoring compounds have a MW of 200-350, LogP of 2-6, HBA of 1-3, HBD of 0, RB of 3-8, and 0-1 arR. A similar pattern had also been previously reported (Avram et al., 2014). Eight thousand out of 81,557 molecules from the subset of a ZINC natural products library were found to have high QEPest scores ranging from 0.50 to 0.9693 (data not shown).

The parameters used by QEI were important determinants of biological activity. The biological activity of a chemical compound depends on the ability to reach the specific target molecule and its subsequent capacity to interact with it. Hence, the three major types of interactions that the modeller must deal with are the hydrophobic, electronic, and steric (Voda et al., 2004). The said chemical characteristics (descriptors) represent a compound's ability to reach the target site (Dambolena et al., 2016). For example, the logP descriptor represents the lipophilicity of the compounds, which determines their ability to penetrate into the plasma membranes. However, a very high increase in lipophilicity leads to a decrease in activity. This is in agreement with Jang et al. (2005), who suggested that the hydrocarbon monoterpenes with high values are less bioactive than those with lower, because they can be accumulated in the cuticle of insects and, thus, inhibit their traffic to the target site (Yu, 2015).

In this study, two compounds (ZINC 03977934 and ZINC 03984308) had comparatively higher logP values with corresponding higher QEI scores, which might indicate that the observed higher logP values were optimal for these compounds. The number of rings is related to the steric aspect of the insecticidal activity of the compounds.

Previous study revealed a higher activity of compounds with fewer rings, which might indicate the importance of steric aspect in the interaction of molecules with the active sites of targets (Dambolena et al., 2016). The number of aromatic rings in the ZINC compounds was only in the range of 0-1. Electronic properties of the molecules are also important determinants of insecticidal activity. As the electron population or electron accessibility (rich) increased, toxicity also increased. This relationship might be due to the electrostatic interaction of these compounds to a receptor, and as electron accessibility for the insecticidal molecules increases, binding affinity also increases (Grodnitzky & Coats, 2002).

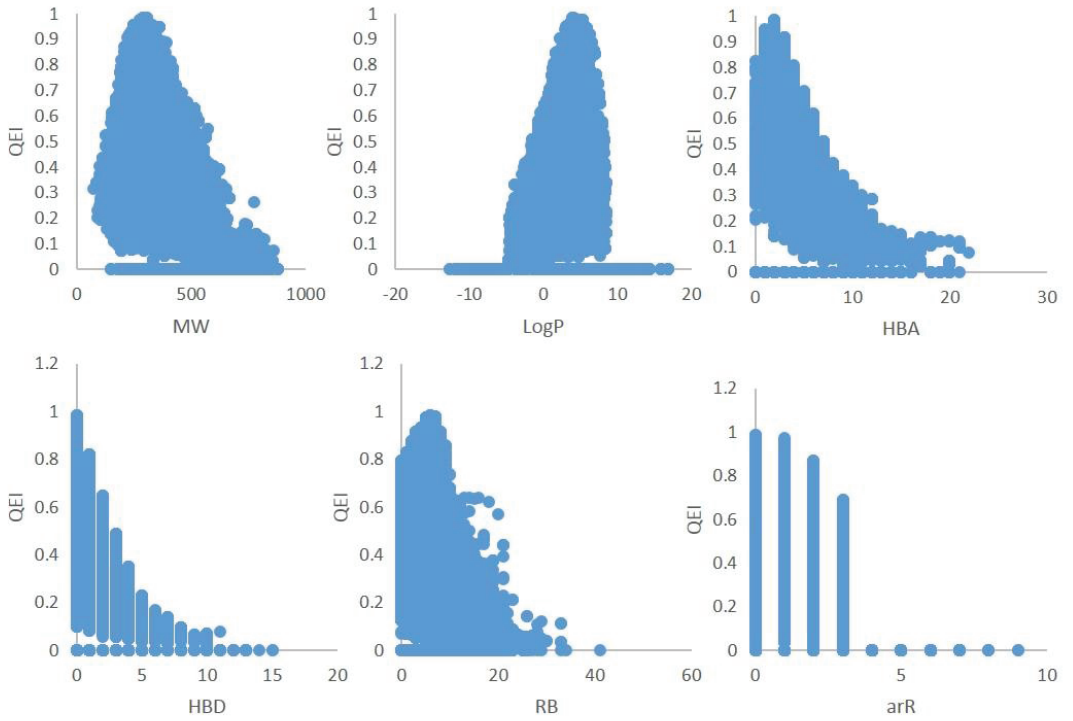


Figure 1. Comparison of QEI values with molecular descriptors of molecules from the IBScreen Natural Products library. Legend: MW (molecular weight), LogP (log of the octanol–water partition coefficient), HBA (number hydrogen bond acceptors), HBD (number hydrogen bond donors), RB (rotatable bonds), and arR (number of aromatic rings).

Virtual docking

Currently, there are more than 25 mode of action insecticides and 85% of the value of these MoAs are derived from insecticides that target the nerve-muscle system (Casida & Durkin, 2013). In contrast, insecticides targeting growth and development account for only a small portion of the total insecticide sales (Sparks & Nauen, 2015). Thus, the opportunity for discovery and development for natural products in agricultural applications is large (Galm & Sparks, 2016). β-*N*-Acetyl-D-hexosaminidase is considered a novel enzyme from *O. furnacalis*. The enzyme was revealed to be essential for normal pupation in *O. furnacalis* (Liu et al., 2011). Hence, the said enzyme could be used as target for development of a new insecticidal compound for control of *O. furnacalis*. Virtual (*in silico*) docking using Autodock Vina revealed the top 1% (out of 8,000 ZINC molecules) to 3NSN (Table 1). The predicted binding affinities of the ZINC molecules ranged from -10.5 kcal/mol (from 12 ZINC molecules) to -11.8 kcal/mol (ZINC04026248). Inspection of the binding interface showed that the tight binding ZINC04026248 is attributed mainly to hydrophobic interactions with six hydrophobic amino acid residues in the binding pocket of 3NSN (Figure 2). The top 1% ZINC compounds

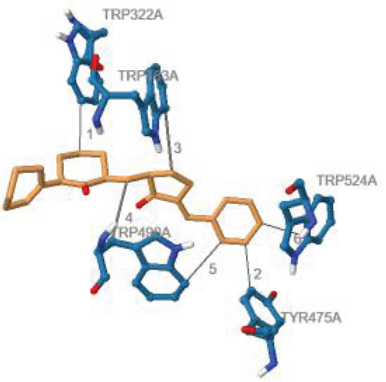
possessed desirable QEI scores ranging from 0.742-0.919 (Table 1). A four-point pharmacophore model of ZINC04026248 was derived from virtual docking. The pharmacophore model consisted of two hydrophobic, one aromatic, and one hydrogen acceptor (Figure 3). This model may also be used as alternative virtual screening tool in search for new inhibitors of 3NSN. The pharmacological properties shown in Table 2 suggests that 3NSN is a good candidate as target for development of insecticide specific for control of *O. furnacalis*. The six interacting residues shown in Table 2 were predicted to be involved in substrate binding. A previous site-directed mutagenesis study reported that Trp490 was highly essential for catalysis. Mutation of Trp490 to Ala resulted to a more than 2,277-fold decrease in sensitivity toward to its natural inhibitor, TMG-chitotriomycin as well as an 18-fold decrease in binding affinity for the substrate $(GlcNAc)_2$ (Liu et al., 2011). Valine 327 was found to be an important residue for substrate binding and catalysis. The residue, Glu328 is part of the subsite involved in binding the sugar unit of substrate in the active pocket of 3NSN (Liu et al., 2011). The predicted specific role of Trp328 is hydrophobic interaction with substrate inhibitor as exemplified by binding to ZINC04026248 (Figure 2). The specific mechanism of Asn 489 binding to substrate is unclear. However, all the aforementioned residues are considered as involved in pharmacological interactions because the value of Wj is greater than 0.40 (Table 3). These residues are also considered as hotspots (except Trp 483-H) because the consensus interaction ratio for each residue is ≥0.5, respectively (Hsu et al., 2011).

Table 1. Top 1% scoring compounds from ZINC natural products database predicted by Autodock Vina MTiScreen server.

Compound ID	Binding Affinity (kcal/mol)	QEI Score
ZINC04026248	-11.8	0.858
ZINC04026250	-11.7	0.858
ZINC18323127	-11.7	0.742
ZINC04027600	-11.6	0.821
ZINC03831193	-11.4	0.790
ZINC04084028	-11.4	0.788
ZINC04026249	-11.4	0.858
ZINC03881613	-11.4	0.790
ZINC04026251	-11.3	0.858
ZINC04026871	-11.3	0.790
ZINC03831191	-11.3	0.790
ZINC03881613	-11.3	0.790
ZINC04028254	-11.2	0.794

ZINC04083876	-11.2	0.798
ZINC04074054	-11.1	0.792
ZINC20111996	-11.1	0.784
ZINC03977934	-11	0.788
ZINC59408506	-11	0.784
ZINC02100488	-11	0.810
ZINC04082626	-10.9	0.825
ZINC02133746	-10.9	0.814
ZINC20111996	-10.9	0.784
ZINC04026871	-10.9	0.790
ZINC04025820	-10.9	0.784
ZINC04073849	-10.9	0.807
ZINC04027602	-10.9	0.821
ZINC38549364	-10.9	0.784
ZINC00519080	-10.8	0.802
ZINC04026132	-10.8	0.798
ZINC00490791	-10.8	0.823
ZINC04062098	-10.8	0.789
ZINC04026570	-10.8	0.801
ZINC04074042	-10.8	0.785
ZINC04587557	-10.8	0.866
ZINC03984167	-10.7	0.770
ZINC04428529	-10.7	0.825
ZINC00490950	-10.7	0.795
ZINC00518666	-10.7	0.847
ZINC01121205	-10.7	0.833
ZINC03881625	-10.7	0.789
ZINC00490134	-10.6	0.784
ZINC04083874	-10.6	0.797
ZINC13510922	-10.6	0.742
ZINC04028252	-10.6	0.794
ZINC04063548	-10.6	0.796
ZINC00519522	-10.6	0.826
ZINC01433937	-10.6	0.789
ZINC04083873	-10.6	0.797
ZINC04082451	-10.6	0.789
ZINC02087151	-10.6	0.919
ZINC04025807	-10.6	0.785

ZINC03984308	-10.6	0.867
ZINC12859899	-10.6	0.804
ZINC00002143	-10.6	0.823
ZINC04023363	-10.5	0.826
ZINC04023278	-10.5	0.799
ZINC01121071	-10.5	0.852
ZINC03860692	-10.5	0.810
ZINC05384812	-10.5	0.827
ZINC00487947	-10.5	0.865
ZINC03881923	-10.5	0.815
ZINC04024149	-10.5	0.817
ZINC04029444	-10.5	0.822
ZINC04024151	-10.5	0.817
ZINC04029446	-10.5	0.822
ZINC04074040	-10.5	0.785



Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	322A	TRP	3.52	17	2727
2	475A	TYR	3.63	2	4156
3	483A	TRP	3.75	10	4229
4	490A	TRP	3.68	14	4280
5	490A	TRP	3.90	3	4287
6	524A	TRP	3.61	1	4591

Figure 2. Binding interaction of ZINC04026248 (yellow orange stick) in binding pocket of 3NSN.

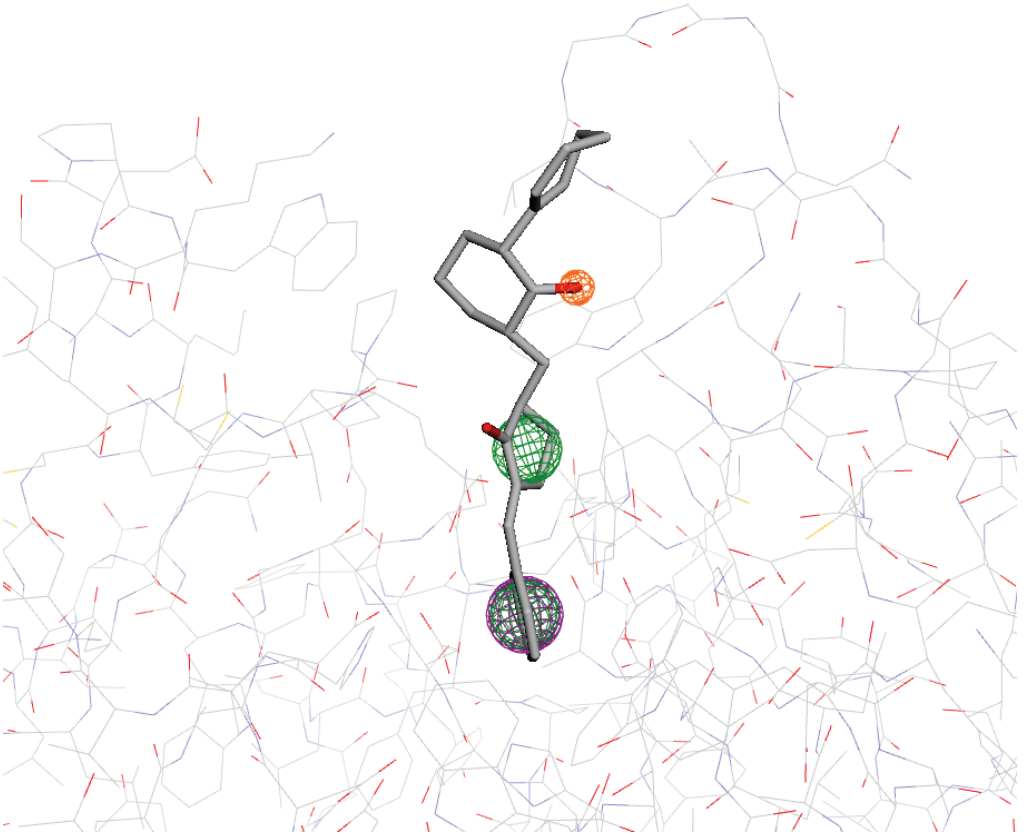


Figure 3. Pharmacophore hypothesis of ZINC04026248 in the binding pocket of 3NSN featuring hydrogen acceptor (orange sphere), hydrophobic (green), and aromatic (purple).

Table 2. Pharmacological properties of target receptor, 3NSN, estimated by iGemDock software.

Predicted pharmacological interaction	Pharmacological preference (W_j)	Z score	Consensus interaction ratio
Val327-V	0.59	8.87	0.96
Glu328-V	0.67	10.16	1.00
Trp483-H	1.00	7.76	0.41
Trp483-V	1.00	15.15	0.98
Val484-V	0.40	6.07	0.90
Asn489-V	0.44	6.67	0.98
Trp490-V	0.99	15.06	0.98

CONCLUSION

The ZINC natural products database contained inhibitors of the *Ostrinia furnacalis* pupation enzyme, β-*N*-acetyl-D-hexosaminidase. The assembled *in silico* pipeline consisting of QEPest for quantification of insecticide-likeness together with the virtual docking softwares, Autodock Vina and iGemDock which are both used for estimating binding affinity and pharmacological property, respectively, was effective in identifying compounds with potential insecticidal activity against *O. furnacalis* using a highly specific molecular target. The *in silico* method will be a useful tool in developing "green" insecticides for management of economically important insect pests. "Wet lab" enzyme inhibition assay is required to validate the results generated by the present study.

LITERATURE CITED

AVRAM S, FUNAR-TIMOFEI S, BOROTA A, CHENNAMANENI SR, MANCHALA AK & MURESAN S. 2014. Quantitative estimation of pesticide-likeness for agrochemical discovery. Journal of Cheminformatics 6(1): 1-9.

CASIDA J & DURKIN K. 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annual Review of Entomology 58: 99-117.

CHEN Q, GUO P, XU L, LIU T, QIAN X & YANG Q. 2014. Exploring unsymmetrical dyads as efficient inhibitors against the insect β-N-acetyl-d-hexosaminidase OfHex2. Biochimie 97: 152-162. doi.org/10.1016/j.biochi.2013.10.008.

DAMBOLENA J, ZUNINO M, HERRERA J, PIZZOLITTO R, ARECO V & ZYGADLO J. 2016. Terpenes: natural products for controlling insects of importance to human health-A structure-activity relationship study. Psyche 2016: Article ID 4595823, 17 pp. http://dx.doi.org/10.1155/2016/4595823.

DONG J, CAO D-S, MIAO H-Y, LIU S, DENG B-C, YUN Y-H, WANG N-N, LU A-P, ZENG W-B, CHEN AF. 2015. ChemDes: an integrated web-based platform for molecular descriptor and fingerprint computation. Journal of Cheminformatics 7: 60. https://doi.org/10.1186/s13321-015-0109-z.

GALM U & SPARKS T. 2016. Natural product derived insecticides: discovery and development of spinetoram. Journal of Industrial Microbiology & Biotechnology 43: 185-193.

GASTEIGER J. 2007. Chemoinformatics: a new field with a long tradition, Analytical & Bioanalytical Chemistry 384: 57–64.

GERWICK BC & SPARKS TC. 2014. Natural products for pest control: an analysis of their role, value and future. Pest Management Science 70: 1169-1185.

GRODNITZKY J & COATS J. 2002. QSAR evaluation of monoterpenoids' insecticidal activity. Journal of Agricultural & Food Chemistry 50: 4576-4580.

HSU K-C, CHEN Y-F, LIN S-R & YANG J-M. 2011. iGEMDOCK: a graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. BMC Bioinformatics 12(Suppl 1): S33. doi.org/10.1186/1471-2105-12-S1-S33

IRWIN JJ & SHOICHET BK. 2005. ZINC - a free database of commercially available compounds for virtual screening. Journal of Chemical Information and Modeling

45(1): 177-182. doi: 10.1021/ci049714+.

JANG Y-S, YANG Y-C, CHOI D-S & AHN Y-J. 2005. Vapor phase toxicity of marjoram oil compounds and their related monoterpenoids to *Blattella germanica* (Orthoptera: Blattellidae). Journal of Agricultural and Food Chemistry 53(20): 7892-7898.

KRASKY A, ROHWER J, SCHROEDER J & SELZER P. 2007. A combined bioinformatics and cheminformatics approach for the development of new antiparasitic drugs. Genomics 89: 36-43.

LABBÉ CM, REY J, LAGORCE D, VAVRUSA M, BECOT J, SPERANDIO O, VILLOUTREIX BO, TUFFÉRY P & MITEVA MA. 2015. MTiOpenScreen: a web server for structure-based virtual screening. Nucleic Acids Research 43(W1): W448-W454. doi: 10.1093/nar/gkv306.

LIU T, ZHANG HT, LIU FY, WU QY, SHEN X & YANG Q. 2011. Structural determinants of an insect β-*N*-acetyl-D-hexosaminidase specialized as a chitinolytic enzyme. Journal of Biological Chemistry 286(6): 4049–4058.

MA D-L, CHAN DS-H & LEUNG C-H. 2011. Molecular docking for virtual screening of natural product databases. Chemical Science 2: 1656-1665.

PEROLA E, XU K, KOLLMEYER TM, KAUFMANN SH, PRENDERGAST FG & PANG Y-P. 2000. Successful virtual screening of a chemical database for farnesyltransferase inhibitor leads. Journal of Medicinal Chemistry 43(3): 401-408. doi: 10.1021/jm990408a.

PETTERSEN EF, GODDARD TD, HUANG CC, COUCH GS, GREENBLATT DM, MENG EC & FERRIN TE. 2004. UCSF Chimera - a visualization system for exploratory research and analysis. Journal of Computational Chemistry 25(13): 1605-1612.

SPARKS TC & NAUEN R. 2015. IRAC: Mode of action classification and insecticide resistance management. Pesticide Biochemistry & Physiology 121: 122-128.

SPARKS TC, HAHN DR & GARIZI NV. 2017. Natural products, their derivatives, mimics and synthetic equivalents: role in agrochemical discovery. Pest Management Science 73(4): 700-715. doi:10.1002/ps.4458.

SUNSERI J & KOES D. 2016. Pharmit: interactive exploration of chemical space. Nucleic Acids Research 44: W442-W448.

VODA K, BOH B & VRTAČNIK M. 2004. A quantitative structure-antifungal activity relationship study of oxygenated aromatic essential oil compounds using data structuring and PLS regression analysis. Journal of Molecular Modeling 10: 76-84.

WANG H, DONG H, QIAN H & CONG B. 2014. Laboratory assessment of entomopathogenic nematode symbiotic bacteria to control maize pest, *Ostrinia furnacalis*, and fungi diseases, *Bipolaris maydis* and *Curvularia lunata*. Journal of Asia-Pacific Entomology 17(4): 823-827.

XU D & ZHANG Y. 2011. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. Biophysical Journal 101: 2525-2534.

YANG Q, LIU T, LIU F, QU M & QIAN X. 2008. A novel β-*N*-acetyl-d-hexosaminidase from the insect *Ostrinia furnacalis* (Guenée). The FEBS Journal 275(22): 5690-5702. doi:10.1111/j.1742-4658.2008.06695.x

YU SJ. 2015. The Toxicology and Biochemistry of Insecticides. 2nd ed. Boca Raton, Fla, USA: CRC Press & Taylor & Francis. xvii + 380 p.