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Research Article

Synthesis, Polymorphism, and Insecticidal Activity of Methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-Carboxylate Against *Anopheles arabiensis* Mosquito

Katharigatta N. Venugopala[1,*], Susanta K. Nayak[2], Raquel M. Gleiser[3,4], Mariela E. Sanchez-Borzone[5], Daniel A. Garcia[5] and Bharti Odhav[1,*]

[1]Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, Durban 4001, South Africa
[2]Department of Chemistry, Visvesvaraya National Institute of Technology, Nagpur, Maharashtra 440010, India
[3]CREAN-IMBIV (CONICET-UNC), Universidad Nacional de Córdoba, Av. Valparaíso s.n., Córdoba 5000, Argentina
[4]FCEFyN, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, Córdoba 5000, Argentina
[5]Instituto de Investigaciones Biológicas y Tecnológicas (IIBYT), CONICET-Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, Córdoba 5016, Argentina
**Corresponding authors: Katharigatta N. Venugopala, katharigattav@dut.ac.za*; Bharti Odhav, *odhavb@dut.ac.za*

Mosquitoes are the major vectors of pathogens and parasites including those causing malaria, the most deadly vector-borne disease. The negative environmental effects of most synthetic compounds combined with widespread development of insecticide resistance encourage an interest in finding and developing alternative products against mosquitoes. In this study, pyrimido[2,1-*b*]quinazoline derivative DHPM3 has been synthesized by three-step chemical reaction and screened for larvicide, adulticide, and repellent properties against *Anopheles arabiensis*, one of the dominant vectors of malaria in Africa. The title compound emerged as potential larvicide agent for further research and development, because it exerted 100% mortality, while adulticide activity was considered moderate.

Key words: antimosquito properties, crystallography, polymorphism, pyrimido[2,1-*b*]quinazoline, synthesis

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Mosquitoes are the major vectors of parasites and pathogens that cause malaria, filariasis, dengue fever, yellow fever, Japanese encephalitis, and other diseases (1). Although malaria control programs have seen a tremendous expansion over the last decade, according to the 2012 World Malaria Report 219 million cases (range 154–289 million) and 660 000 deaths (range 490 000–836 000) still occur every year (2). Vector control programs mainly use four classes of chemical insecticides: organochlorines, organophosphates, carbamates, and pyrethroids. Bacterial insecticides and insect growth regulators have also become more widely used in recent years. However, the use of chemicals on a vast and increasing scale has led to the widespread development of resistance as a result of selection for certain genes (3), and some species have even become resistant to multiple insecticides (4). Mosquito resistance to at least one insecticide used for malaria control has been identified in 64 countries (4). Besides, many synthetic organic insecticides, such as those currently used to control mosquitoes, affect non-target organisms and result in negative environmental effects (5). Thus, there is an interest in the finding and development of alternative antimosquito products. In continuation of our recent findings on selection of South African indigenous plants (6) and synthesis of heterocyclic compounds for antimosquito properties against *Anopheles arabiensis* (7–9), in the present investigation, we decide to work on pyrimido[2,1-*b*]quinazoline system. The synthesis and polymorphism property of methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate has been the focus of our continuing interest because the presence of pharmacophore pyrimido[2,1-*b*]quinazoline in the title compound is responsible for diverse pharmacological activities. For example, the substituted dihydropyrimidine ring system exhibited numerous pharmacological activities such as antibacterial (10,11), anticancer (12,13), antifungal (13), antihypertensive (14), antiinflammatory (15), and antitubercular properties (16). Some of the substituted dihydropyrimidine derivatives have been used as dipeptidyl peptidase-IV (DPP-4) inhibitors (17) and non-nucleoside HIV-1 reverse transcriptase inhibitors (18). Among the dihydropyrimidine pharmacophores, wide spectrum of biological activities include larvicide actions against mosquitoes (9,19).

On the other hand, some of the quinazolinone derivatives have been found to show anticancer (20), anticonvulsant

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(21), antifungal (22), antiinflammatory (23), antimicrobial properties (24). In addition, quinazolinone ring has been the core structural skeleton in various alkaloids and a variety of natural products, such as luotonin A (25), oxoglyantrypine (26), and schizocommunin (27). Imidazo- and pyrimido[2,1-*b*]quinazolines are reported for antihypertensive and antidepressant activities (28). Most importantly, quinoline moieties incorporated into the compounds have previously shown activity against insects (29), and quinoline compounds gave good inhibitory activity for acetylcholinesterase (AChE) (30). The introduction of a pyrimidine system to quinazolinone ring is predicted to influence the significant antimosquito properties. The title compound methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate has been achieved by three-step chemical reactions starting from Biginelli reaction (31) and characterized by IR, NMR, LC-MS, elemental analysis, and single crystal X-ray studies and evaluated for antimosquito properties against *A. arabiensis* for larvicide, adulticide, and repellency properties. For adulticide properties, the test compounds were sprayed onto ceramic tiles and screened using the cone bioassay method. The larvicide activity was tested by monitoring larval mortality daily and up to 3 days of exposure. Repellency properties were tested in a feeding-probe assay using unfed female *A. arabiensis*. Previously, we reported synthesis and polymorphic forms of heterocyclic compounds (32,33). Polymorphism is known to lead to changes in various physical and chemical properties of the compound such as melting point, particle size, stability, tableting, dissolution rates, bioavailability, and pharmacological activity (29). In some cases, it is also observed that the polymorphic forms exhibit nearly similar properties; for example, the polymorphic forms atovaquone, antimalarial drug results in a similar binding feature with cytochrome bc1 (30). It is interesting to note that the structures in solution state have been also observed to be similar to those determined by X-ray crystallography or other spectroscopic methods in solution state (31).

Methods and Materials

General chemistry

All the chemicals were obtained from Aldrich and Merck chemical company. Reactions were monitored by thin-layer chromatography (TLC). TLC was performed on Merck (Sandton, South Africa) 60 F-254 silica gel plates with ethyl acetate and *n*-hexane (6:4) as solvent system and visualization with UV-light. Melting points were determined on a Büchi melting point B-545 apparatus. The IR spectra were recorded on a Nicolet 6700 FT-IR spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 MHz instruments in DMSO as a solvent. Chemical shifts (δ) were indicated in parts per million downfield from tetramethylsilane, and the coupling constants (J) are recorded in Hertz. Splitting pattern is abbreviated as follows: s, singlet; d, doublet; m, multiplet. Mass spectra were recorded using LC–MS-Agilent 1100 series with MSD (Ion trap) using 0.1% aqueous trifluoroacetic acid in acetonitrile system on C18-BDS column. Elemental analysis was performed on Thermo Finnigan FLASH EA 1112 CHN analyzer. Single crystal X-ray diffraction study was accomplished using Bruker KAPPA APEX II DUO diffractometer using graphite monochromator, Mo-Kα radiation (λ = 0.71073 Å.

Synthesis of methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (DHPM1)

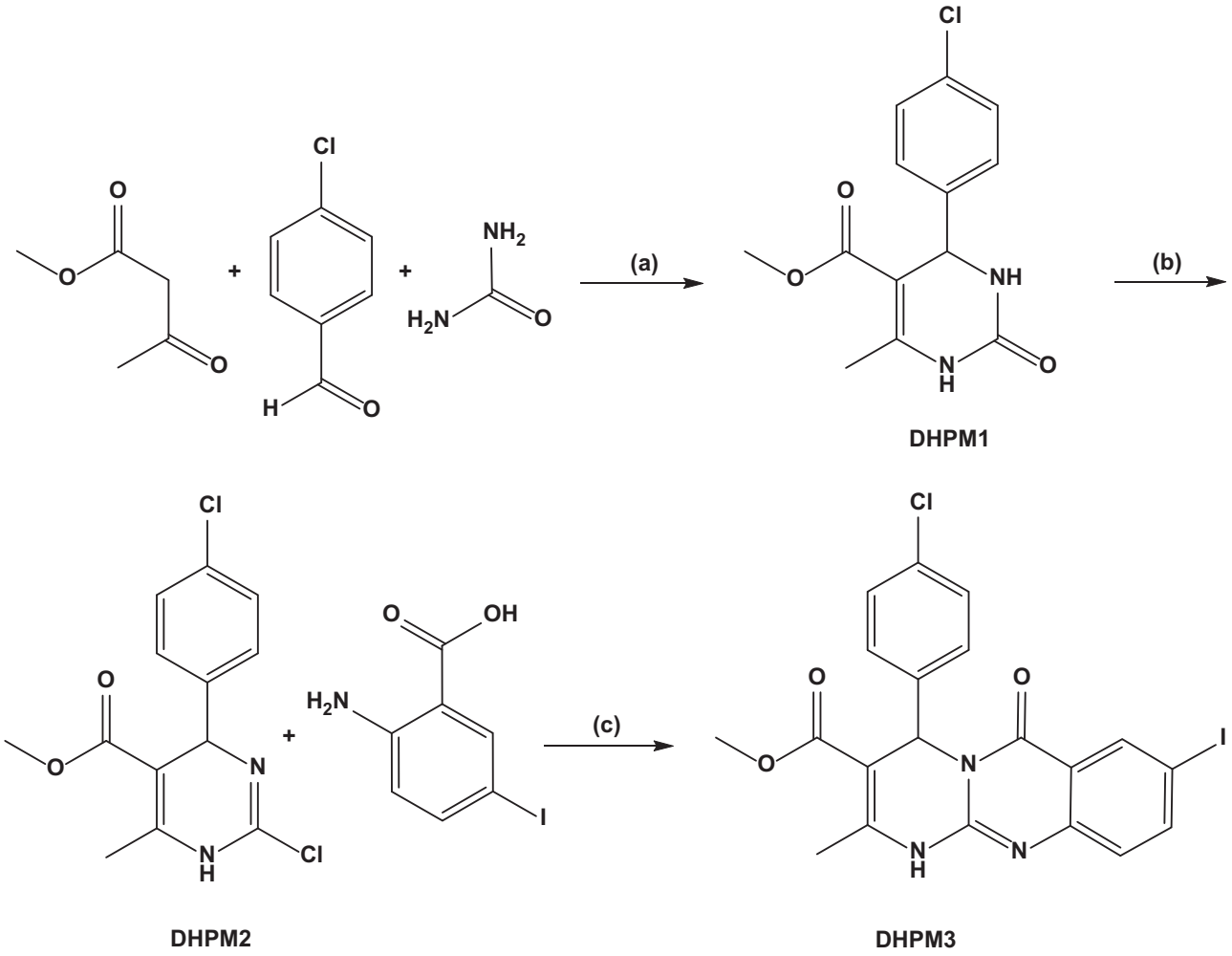
A solution of methyl acetoacetate (0.12 mol), 4-chlorobenzaldehyde (0.1 mol), and urea (0.1 mol) was refluxed in the presence of concentrated hydrochloric acid (0.05 mol) for 8 h in 50 mL of ethanol (Scheme 1). Completion of reaction was monitored on TLC. The reaction mixture was then cooled to room temperature, and the pure precipitate was collected by filtration. The solid obtained was washed with cold Et-OH, dried, and recrystallized using Et-OH solvent. Appearance: yellow solid; yield 66%. m.p. 205–206 °C. IR (KBr) ν cm^{-1} 3240, 3110, 1724, 1704, 1649, 1490, 1461, 781; ^{1}H-NMR (400 MHz, DMSO-*d*6) δ 2.30 (s, 3H), 3.92 (s, 3H), 5.43 (s, 1H), 7.13 (d, 2H, J = 9.05 Hz), 7.51 (s, 1H), 7.86 (d, 2H, J = 9.05 Hz), 9.01 (s, 1H). ^{13}C-NMR (400 MHz, DMSO-*d*6) δ 18.66, 52.05, 54.35, 109.58, 113.20, 128.23, 136.26, 148.25, 153.40, 159.18, 167.76. MS: m/z 281 [M + H]$^{+}$.

Synthesis of methyl 2-chloro-4-(4-chlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate (DHPM2)

A solution of compound **DHPM1** (1 mol) in $POCl_3$ (5 mol) was refluxed for 16 h (Scheme 1). The reaction was monitored by TLC. Unreacted $POCl_3$ was evaporated completely at room temperature, and the remaining residue was taken in ethyl acetate and washed with 10% sodium bicarbonate solution followed by water and finally brine solution. The ethyl acetate layer was dried over sodium sulfate and evaporated to obtain a solid which was recrystallized using ethanol solvent. Appearance: brown solid; yield 72%. m.p. 218–219 °C. IR (KBr) ν cm^{-1} 3244, 3108, 1708, 1641, 1491, 1466, 786. ^{1}H-NMR (400 MHz, DMSO-*d*6) δ 2.32 (s, 3H), 3.91 (s, 3H), 5.44 (s, 1H), 7.13–7.86 (m, 4H), 9.02 (s, 1H). ^{13}C-NMR (400 MHz, DMSO *d*6) δ 18.72, 53.67, 54.35, 105.41, 113.20, 128.54, 135.29, 141.20, 145.67, 158.39, 167.76. MS m/z 299 [M + H]$^{+}$.

Synthesis of methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4H-pyrimido[2,1-b] quinazoline-3-carboxylate (DHPM3)

A mixture of methyl-2-chloro-4-(4-chlorophenyl)-6-methyl-1,4- dihydropyrimidine-5-carboxylate **DHPM2** (1 mmol), 2-amino-5-iodobenzoic acid (1 mmol), and methanamine (1 mmol) in 2-propanol (15 mL) was refluxed for 12 h. The reaction completion was monitored by TLC. The reaction



Scheme 1: Synthetic routes to dihydropyrimidine analogue **DHPM3**: Reagents and conditions; (a) Ethanol, HCl, 8 h, reflux; (b) $POCl_3$, 16 h, reflux; (c) Methanamine, 2-propanol, 12 h, reflux.

medium was cooled to room temperature, and the product was filtered, washed with cold 2-propanol, and dried to obtain the crude product. The product was purified by recrystallization using ethanol. Appearance: yellow crystalline compound; yield 69%. m.p. 194 °C. IR (KBr): ν cm^{-1} 3356, 3193, 1674, 1588, 1527, 1492, 838. ^1H NMR (400 MHz, DMSO-*d*6): δ 2.38 (s, CH_3, 3H), 3.60 (s, CH_3, 3H), 5.39 (s, CH, 1H), 7.32–7.83 (m, Ar-H, 7H), 10.77 (s, NH, 1H), ^{13}C NMR (400 MHz, DMSO-*d*6): δ 18.14, 51.82, 55.82, 98.97, 102.01, 104.77, 116.39, 128.92, 129.17, 130.16, 133.20, 141.01, 145.78, 150.20, 158.54, 161.16, 165.27. LCMS (m/z): 507 (M^+). Anal. Calcd for $C_{20}H_{15}ClIN_3O_3$: C, 47.31; H, 2.98; N, 8.28%. Found C, 47.39; H, 3.01; N, 8.31%.

Crystallography

Crystals of polymorphic form DHPM3a was obtained from mixture of methanol and tetrahydrofuran at 1:1 ratio as plates by slow evaporation method at room temperature. However, polymorphic form DHPM3b was obtained by similar way employing a mixture of acetonitrile and tetrahydrofuran at 1:1 ratio as plates by slow evaporation method at room temperature. Structural confirmation of polymorphs **DHPM3a** and **DHPM3b** was achieved by single crystals X-ray diffraction study using Bruker KAPPA APEX II DUO diffractometer using graphite monochromator, Mo-Kα radiation (λ = 0.71073 Å; Table 1). Data collections were carried out at 173 (2) K temperature using liquid Nitrogen (N_2) cryo-system attached with Oxford cryostat. Cell refinement and data reduction were performed using the program SAINT. The crystal structure solution was worked out by full matrix least-squares method using SHELXL97 and absorption correction performed using SADABS (34). All the non-hydrogen atoms and some of hydrogen atoms were located in difference Fourier maps and were refined anisotropically, and the remaining hydrogen atoms were fixed geometrically and refined isotropically. Graphical presentations were drawn using Ortep-3 and Mercury (35,36). The crystal structure solution

Table 1: Crystal data and measurement details for polymorphs **DHPM3a** and **DHPM3b**

Crystal data	**DHPM3a**	**DHPM3b**
Formula	$C_{20}H_{15}ClIN_3O_3$	$C_{20}H_{15}ClIN_3O_3$
CCDC Number	920295	1415679
Formula weight	506.98	506.98
Crystal morphology	Plate	Plate
Crystal size(mm)	0.25 × 0.14 × 0.12	0.16 × 0.10 × 0.04
Temperature/K	173 (2)	173 (2)
Radiation	Mo K_α	Mo K_α
Wavelength (Å)	0.71073	0.71073
Crystal system	Triclinic	Triclinic
Space group	*P* -1	*P* -1
a (Å)	7.3443 (15)	7.2435 (4)
b (Å)	10.847 (2)	9.8117 (5)
c (Å)	12.474 (3)	14.3351 (7)
α (°)	106.657 (3)	72.132 (1)
β (°)	103.527 (3)	81.315 (1)
γ (°)	92.791 (3)	83.968 (9)
Volume ($Å^3$)	918.47 (1)	956.68 (9)
Z	2	2
Density (gm/cm^3)	1.917	1.76
μ (1/mm)	1.91	1.84
F (000)	500	500
ϑ (min, max)	2.9, 26.0	1.5, 26.0
Total number of refl^n	7109	13 403
No. Unique refl^n	3605	3744
No. of parameters	255	259
*R*_ obs, wR_2_obs	0.024, 0.059	0.019, 0.050
$\Delta\rho_{min}(eÅ^{-3})$, $\Delta\rho_{max}(eÅ^{-3})$	−0.518, 0.888	−0.267, 0.447
GooF	1.085	1.073

was worked out by full matrix least-squares method using SHELXL97 and absorption correction performed using SADABS. All the non-hydrogen atoms and some hydrogen atoms were located in difference Fourier maps and were refined anisotropically, and other hydrogen atoms were fixed geometrically and refined isotropically.

Antimosquito properties

Larvicide activity

The *A. arabiensis* used were from a colonized strain from Zimbabwe reared according to the WHO (1975) guidelines (37) in an insectary reproducing the temperature (27.5 °C), humidity (70%), and lighting (12/12) of a malaria endemic environment. One milliliter of test compound (0.125–1 mg/mL in acetone) was added to distilled water (249 mL) producing a final concentration of 0.5–4 μg/mL. Groups of thirty 3rd instar larvae were exposed to test concentrations of 0 (control), 0.5, 1.0, 2.0, and 4.0 μg/mL of the test compound in distilled water. The negative controls (0 μg/mL) were set up using a solvent (acetone) and distilled water, and a positive control included temephos (Mostop; Agrivo), an effective emulsifiable organo-phosphate larvicide used by the malarial control program. Bioassays were triplicate to ensure validity of results. Each container was monitored for larval mortality at 24-h intervals for a period of 2 days, and the percentage mortality was calculated relative to the initial number of exposed larvae. Throughout the experiment, larvae were fed specially made cat food with reduced oil/fat content at regular intervals.

Adulticide activity

Adulticide activity was assessed by exposing susceptible adult mosquitoes to a treated surface, in accordance with WHO protocol (1975) (1), and determining the knockdown rate, which was based on temporary paralysis of the mosquitoes during a 60-min exposure period, and mortality 24-h postexposure. One mL of test compound solution (1, 1.5, or 2 mg/mL in acetone) was sprayed onto a clean, dry, non-porous ceramic tile using a precalibrated Potter's Tower apparatus (38) that was then air-dried. The assay began within 24 h of spraying, by fixing a cone over the sprayed tile and introducing thirty non-blood-fed, 2 to 5-day-old susceptible adult *A. arabiensis* mosquitoes into the cone. Acetone and water were used as a negative controls and deltamethrin (15 g/L; K-Othrine®) as a positive control. All bioassays were triplicate. Lethal adulticide concentration was estimated based on the following concentration gradient: 0 (control), 0.5, 1.0, and 2.0 μg/mL of the test compound in acetone.

Repellence assays

Repellent activity was assessed by topical application of the compound to skin of a rodent and subsequent exposure of the treated skin to unfed female mosquitoes, as described in Venugopala *et al.* (8). Ethical approval for the use of *Mastomys coucha* in these trials was approved from the Medical Research Council's Ethics Committee for Research on Animals. Adult *Mastomys* were anesthetized by injecting intraperitoneally with sodium pentobarbital (concentration in accordance with the weight of the animal). The anesthetized rodents were then shaved on the ventral surface, and a test compound in acetone (1 or 1.5 mL) was applied to each rodent's abdomens. Acetone was used as a solvent for the preparation of stock solution (1 mg/mL). Laboratory grade DEET (IUPAC: *N,N*-Diethyl-3-methylbenzamide) was used as the positive control, and plane acetone was used as negative control. Thirty unfed 4-day-old *A. arabiensis* females were introduced into paper cups (500 mL) whose base was replaced with mosquito netting and held in contact with the treated ventral surface of each rodent. Mosquito activity was observed through transparent plastic film closing the mouth of the cup. After a period of 2 min, the numbers of mosquitoes probing were recorded. The cups holding the mosquitoes were removed, and mosquitoes were further observed for 24 h. All tests were triplicate. The rodent was then returned to the animal facility and allowed to recover from anesthetic. No adverse reactions to the applied components were observed on any of the *Mastomys* rodents during the 3 days they were monitored. Repellence of the

test compounds was calculated as the percentage of mosquitoes not biting from the total exposed to the rodent.

Statistical analysis

General linear mixed models[a] were used to determine differences between treatments registered in larval mortality (larvicide assays) and adulticide effects. LSD Fisher test was used for post hoc analyses. In all cases, a value of $p < 0.05$ was considered statistically significant. Throughout the text, the results are presented as the mean plus/minus the standard error. Dependent variables were *A. arabiensis* knockdown or mortality. Fixed effects were test compound (quinazoline, water, acetone, temephos, or K-Othrine) and observation period (24 and 48 h for larvicide assays; 30, 60 min, and 24 h for adulticide assays). Random effects were mosquito groups (i.e. container in larvicide tests; cup in adulticide). For repellent effects, general linear models were used to determine differences between treatments. LSD Fisher test was used for post hoc analyses. Dependent variables were *A. arabiensis* repellence and knockdown. Fixed effects were test compound (quinazoline, water, acetone, DEET). Data gathered from the larvicide and adulticide assays were used to determine the concentration of each of the compounds needed for 50% mortality. The concentration of compound inducing 50% mortality (LC50) was calculated using GraphPad Prism version 6.00 for Windows[b].

Results and Discussion

Chemistry

Design of the molecule is achieved based on the pharmacophore available in dihydropyrimidine larvicide compounds (9). The synthetic strategy employed to produce **DHPM3** is illustrated in Scheme 1. Intermediates **DHPM1** and **DHPM2** are achieved at 66% and 72% yield, respectively. Title compound **DHPM3** exhibited –NH- and -CO- stretching at 3356 and 1674 cm^{-1}, respectively. Molecular ion peak M + 1 was in agreement with calculated molecular mass of the compound 507 (m/z). Carbonyl carbon in **DHPM3** compound was found at δ 165.27 in ^{13}C NMR spectra and δ 5.39 for –CH- in ^{1}H NMR spectra. Elemental analysis result was according to the calculated values of the proposed title compound **DHPM3**. The IR, NMR (^{1}H & ^{13}C), and elemental analysis data are discussed in detail under experimental section.

Crystallography

From X-ray crystallography, the **DHPM3** shows polymorphism properties with differences in their preference of hydrogen and/or halogen bonding intermolecular interactions. The crystallographic details (Table 1) of **DHPM3a** and **DHPM3b** prove clearly the existence of two different crystalline form of **DHPM3**. The structural details of **DHPM3a** form is only reported previously by us (39); however, for the comparison, we report both **DHPM3a** and **DHPM3b** form. In **DHPM3a** form, molecule is stabilized by two intermolecular C—H···O type interactions (methyl and aromatic hydrogen atoms), whereas in **DHPM3b**, the molecule is stabilized by methyl hydrogen C—H···O interaction. It is interesting to note that DHPM3a and DHPM3b are structural isomers obtained from crystallization method using combination of different solvents. In solution state, the structural preferences of DHPM3 could be either one of them due to their preferred non-covalent interactions.

The dihedral angle between the planes of the 4-chlorophenyl and iodophenyl groups is 80.3 (2)° and 89.9 (2)° for **DHPM3a** and **DHPM3b,** respectively (Figure 1). The crystal structure of both forms is stabilized by N—H···O infinite

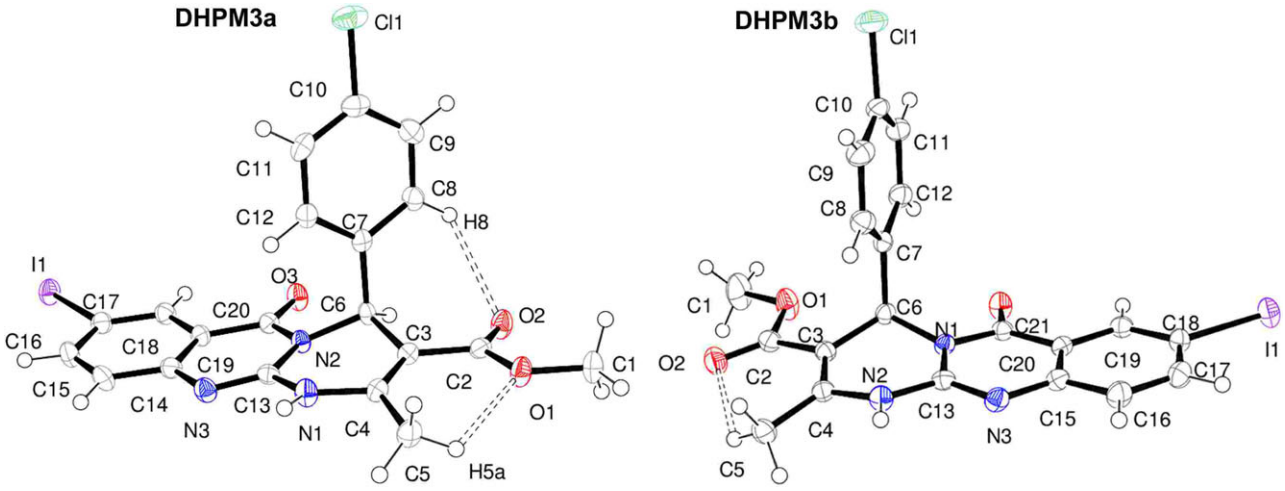


Figure 1: The molecular structure with ellipsoids for non-H atoms drawn at the 50% probability level for **DHPM3a** and **DHPM3b** forms with intramolecular C—H···O interactions with dashed lines.

hydrogen bond chains. It is worthy to mention that the halogen-involving short contacts I···Cl [3.427 (2) Å, $\theta 1$ = 166.1 (2)°; $\theta 2$ = 90.5 (2)°, Type II (40) are present in **DHPM3a** form and absent in **DHPM3b** form which confirm the existence of packing polymorphs of the compound **DHPM3** (Figure 2).

Antimosquito properties

Larvicide activity

There were significant effects of treatment ($p < 0.0001$), exposure time ($p = 0.002$), and treatment exposure interaction ($p = 0.02$) on larval mortality (Table 2). The interaction was due to a mortality increase in larvae exposed to the positive control temephos. The higher concentrations of **DHPM3** (2 and 4 μg/mL) exerted significantly higher mortality (both 100%) than the positive controls (71.4% and 73.9% at 24 and 48 h, respectively). At the lowest concentration tested (0.5 μg/mL), mortalities were not significantly higher than the negative controls. The LC50 was estimated to be 1.08 μg/mL (95% confidence intervals are 0.99–1.19).

Adulticide activity

On adulticide assays, there were also significant effects of the compound ($p < 0.0001$), of exposure time ($p < 0.0001$), and their interaction ($p < 0.0001$) on mosquito knockdown/mortality. The results of the adulticide assays are shown in Table 3. The positive control K-Othrine showed 100% knockdown/mortality from the first 30 min of exposure, while quinazoline killed 70% of the mosquitoes after 24 h of exposure to the highest concentration (2 μg/mL). Still, mortality from exposure to quinazoline at all concentrations was significantly higher than the negative controls, and increased with time. The LC50 was estimated to be 1.33 μg/mL (95% confidence intervals are 1.21–1.46 μg/mL).

Repellence assays

Treatment had a significant effect on mosquito repellence ($p < 0.0001$). The results of repellent and knockdown activities of the quinazoline compared to controls are shown in Table 4. Although **DHPM3** exerted a statistically higher repellent activity than the negative controls, it was only 3.3% repellent (only at 1 μg/mL) and significantly lower than the positive control DEET (100%) ($p < 0.05$). On the other hand, mosquito knockdown (96.7% and 98.9% for 1 and 1.5 μg/mL, respectively) was significantly higher than the negative controls ($p < 0.05$), and equal to or slightly lower than the positive control (100%).

Table 2: Mortality of *Anopheles arabiensis* larvae exposed to methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate (**DHPM3**) at 0.5, 1, 2, and 4 μg/mL, and their negative (acetone, water) and positive (Temephos) controls

	Mortality	
Compound	24 h	48 h
DHPM3 (4 μg/mL)	100.0 ± 8.8^{A}	100.0 ± 8.8^{A}
DHPM3 (2 μg/mL)	100.0 ± 8.8^{A}	100.0 ± 8.8^{A}
DHPM3 (1 μg/mL)	33.3 ± 8.8^{B}	37.8 ± 8.8^{B}
DHPM3 (0.5 μg/mL)	15.6 ± 8.8^{BC}	16.7 ± 8.8^{bc}
Temephos	71.4 ± 4.4^{D}	73.9 ± 4.4^{E}
Acetone	0.0 ± 4.4^{C}	0.0 ± 4.4^{C}
Water	0.0 ± 4.4^{C}	0.0 ± 4.4^{C}

Compounds not sharing a letter differ significantly ($p < 0.05$).

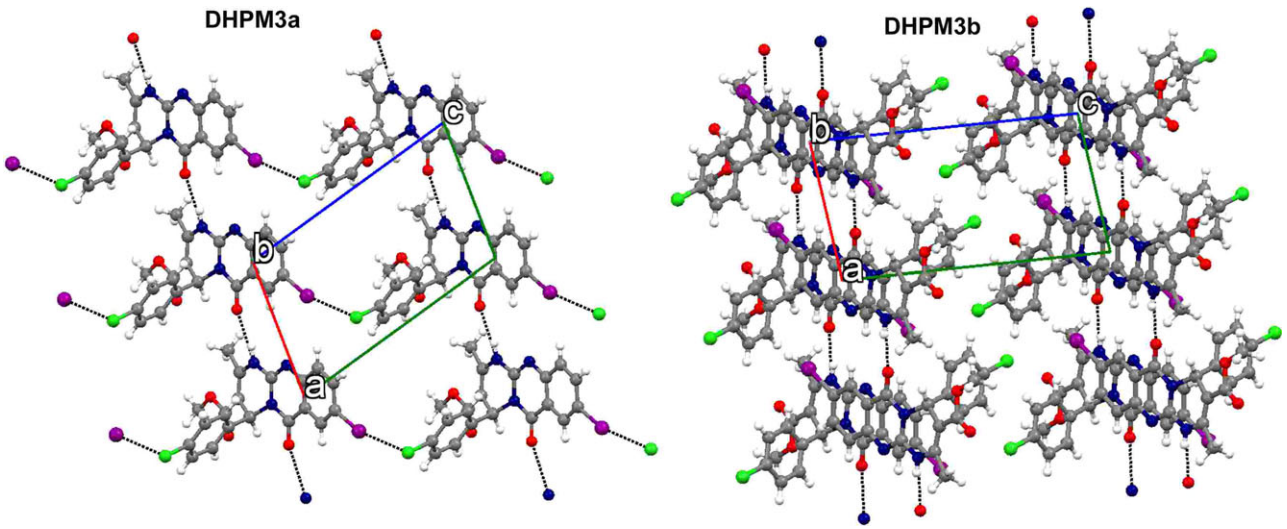


Figure 2: Intermolecular N—H···O hydrogen bonds for both forms (**DHPM3a** and **DHPM3b**) and **DHPM3a** form shows additional I···Cl short contacts.

Table 3: Mortality of *Anopheles arabiensis* adults exposed to methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate (**DHPM3**) at 1, 1.5, 2 μg/mL, and their negative (acetone, water) and positive (K-Othrine) controls

Compound	30 min	60 min	24 h
DHPM3 (2 μg/mL)	53.3 ± 1.4^{A}	63.3 ± 1.4^{B}	70.0 ± 1.4^{C}
DHPM3 (1.5 μg/mL)	45.6 ± 1.4^{D}	51.1 ± 1.4^{A}	52.2 ± 1.4^{A}
DHPM3 (1 μg/mL)	35.6 ± 1.4^{E}	38.9 ± 1.4^{F}	38.9 ± 1.4^{F}
K-Othrine	100.0 ± 0.8^{G}	100.0 ± 0.8^{G}	100.0 ± 0.8^{G}
Acetone	0.0 ± 0.8^{H}	0.0 ± 0.8^{H}	0.0 ± 0.8^{H}
Water	0.0 ± 0.8^{H}	0.0 ± 0.8^{H}	0.0 ± 0.8^{H}

Compounds not sharing a letter differ significantly ($p < 0.05$).

Table 4: Adult *Anopheles arabiensis* repellent and knockdown activity after 24 h of a 2 min exposure to methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate (**DHPM3**) at 1, 1.5 μg/mL, and their negative (acetone, water) and positive (DEET) controls during repellence assays

	Repellent assay	
Compound	Repellence	Knockdown
DHPM3 (1.5 μg/mL)	0.0 ± 0.6^{A}	98.9 ± 0.7^{A}
DHPM3 (1 μg/mL)	3.33 ± 0.6^{B}	96.7 ± 0.7^{B}
DEET	100.0 ± 0.4^{C}	100.0 ± 0.5^{A}
Acetone	0.0 ± 0.4^{A}	0.0 ± 0.5^{C}
Water	0.0 ± 0.4^{A}	0.0 ± 0.5^{C}

Within each column, compounds not sharing a letter differ significantly ($p < 0.05$).

No adverse reactions to the treatments applied were observed on any of the *Mastomys* rodents during the 3 days they were monitored.

Quinazoline derivatives have been widely studied for their antibacterial, antifungal, and anti-HIV among other bioactivities (41). Fenazaquin (4-[[4-(1, 1-dimethylethyl)phenyl) ethoxy]quinazoline), a quinazoline, has shown miticidal activity, controlling all stages of mites including eggs. It is a metabolic inhibitor that interrupts mitochondrial electron transport at Site 1^{C}. To our best knowledge, very few other quinazolines have been researched for their insecticidal activity. A series of 3-[(2- chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones evidenced larvicide activity against an aquatic midge (*Chironomus tentans* Fabricius, Chironomidae) (42). Consistently, the pyrimido[2,1-*b*]quinazoline derivative **DHPM3** showed larvicide activity against the mosquito *A. arabiensis,* killing 100% larvae after 24-h exposure to 2 μg/mL of this product. The estimated LC50 of 1.08 μg/mL was much lower than those for 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones against aquatic midge larvae (60–80 μg/mL) (42), indicating a more promising potential of our compound as larvicide to control mosquitoes.

On the other hand, adulticide activity was moderate, which could be due to stage-related differences in susceptibility or to differences in actual exposure of the insect to the compounds (larvicide is delivered to the water where the larvae dwells; exposure of adult to compounds could be due to either through surface contact or through vapors). Although significantly higher than controls, it only achieved 70% mortality after 48 h with the highest concentration tested (2 μg/mL). Repellent activity was negligible; however, exposure to rodent skin treated with the compounds knocked down a high proportion of mosquitoes (close to 99%).

Conclusion

We designed and synthesized the title compound **DHPM3** in good yield and developed two polymorphs such as **DHPM3a** and **DHPM3b**. Confirmation and characterization of the polymorphs were carried out by single crystal X-ray studies, and antimosquito results indicate that the pyrimido[2,1-*b*]quinazoline derivative **DHPM3** shows larvicide potential against the malaria vector *A. arabiensis* that merits further research and development. Our future work will further examine to understand the antimosquito activities of both forms of **DHPM3**.

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Conflict of Interest

The authors declare no conflicts of interest.

References

1. Service M.W. (1983) Management of vectors. In: Youdeowei & M W Service, editor. Pest and Vectors Management in Tropics. London: Longman; p. 265–280.
2. WHO (2012) World Malaria Report. 195.
3. Baleta A. (2009) Insecticide resistance threatens malaria control in Africa. Lancet;374:1581–1582.
4. WHOPES (2006) Pesticides and their application for the control of vectors and pests of public health importance. WHO Pesticide Evaluation Scheme (WHOPES), 6th edn. Geneva: WHOPES; p. 114.

5. Pimentel D., Lehman H. (1993) The Pesticide Question: Environment, Economics and Ethics. London: Chapman and Hall; p. 281.
6. Chalannavar R.K., Hurinanthan V., Singh A., Venugopala K.N., Gleiser R.M., Baijnath H., Odhav B. (2013) The antimosquito properties of extracts from flowering plants in South Africa. Trop Biomed;30:559–569.
7. Narayanaswamy V.K., Gleiser R.M., Kasumbwe K., Aldhubiab B.E., Attimarad M.V., Odhav B. (2014) Evaluation of halogenated coumarins for antimosquito properties. Sci World J;2014:6.
8. Venugopala K.N., Krishnappa M., Nayak S.K., Subrahmanya B.K., Vaderapura J.P., Chalannavar R.K., Gleiser R.M., Odhav B. (2013) Synthesis and antimosquito properties of 2,6-substituted benzo[d]thiazole and 2,4-substituted benzo[d]thiazole analogues against *Anopheles arabiensis*. Eur J Med Chem;65:295–303.
9. Venugopala K.N., Gleiser M.R., Chalannavar R.K., Odhav B. (2014) Antimosquito property of 2-substitutedphenyl/benzylamino-6-(4-chlorophenyl)-5-methoxycarbonyl-4-methyl-3,6-dihydropyrimidin-1-ium chlorides against the *Anopheles arabiensis* mosquito. Med Chem;10:211–219.
10. Gossnitzer E., Feierl G., Wagner U. (2002) Synthesis, structure investigations, and antimicrobial activity of selected s-trans-6-aryl-4-isopropyl-2-[2-[(E)-1-phenylalkylidene]-(E)-hydrazino]-1,4-dihyd ropyrimidine hydrochlorides. Eur J Pharm Sci;15:49–61.
11. Sedaghati B., Fassihi A., Arbabi S., Ranjbar M., Memarian H., Saghaie L., Omidi A., Sardari A., Jalali M., Abedi D. (2012) Synthesis and antimicrobial activity of novel derivatives of Biginelli pyrimidines. Med Chem Res;21:3973–3983.
12. Venugopala K.N., Govender R., Khedr M.A., Venugopala R., Aldhubiab B.E., Harsha S., Odhav B. (2015) Design, synthesis, and computational studies on dihydropyrimidine scaffolds as potential lipoxygenase inhibitors and cancer chemopreventive agents. Drug Des Devel Ther;9:911–921.
13. Hussein M.A., Moty S.G.A., Aziz S.A.A., Salim M.A.A. (2011) Synthesis and antimicrobial activity of new substituted dihydropyrimidine derivatives. Bull Pharm Sci;34:37–52.
14. Chikhale R.V., Bhole R.P., Khedekar P.B., Bhusari K.P. (2009) Synthesis and pharmacological investigation of 3-(substituted 1-phenylethanone)-4-(substituted phenyl)-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates. Eur J Med Chem;44:3645–3653.
15. Devi K., Venugopala K.N., Rao G.K. (2009) Synthesis of substituted 3, 4-dihydropyrimidine-2(1H)-thiones and their biological activity. Ind J Heterocycl Chem;18:305–306.
16. Venugopala K.N., Susanta K.N., Pillay M., Renuka P., Yacoob M.C., Odhav B. (2013) Synthesis and antitubercular activity of 2-(substituted phenyl/benzyl-amino)-6-(4-chlorophenyl)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chlorides. Chem Biol Drug Des;81:219–227.
17. Zhang Z., Wallace M.B., Feng J., Stafford J.A., Skene R.J., Shi L., Lee B., Aertgeerts K., Jennings A., Xu R., Kassel D.B., Kaldor S.W., Navre M., Webb D.R., Gwaltney S.L. (2011) Design and synthesis of pyrimidinone and pyrimidinedione inhibitors of dipeptidyl peptidase IV. J Med Chem;54:510–524.
18. Zhang J., Zhan P., Wu J., Li Z., Jiang Y., Ge W., Pannecouque C., De Clercq E., Liu X. (2011) Synthesis and biological evaluation of novel 5-alkyl-2-arylthio-6-((3,4-dihydroquinolin-1(2H)-yl)methyl)pyrimidin-4(3H)-ones as potent non-nucleoside HIV-1 reverse transcriptase inhibitors. Biorg Med Chem;19:4366–4376.
19. Rajanarendar E., Reddy M.N., Murthy K.R., Reddy K.G., Raju S., Srinivas M., Praveen B., Rao M.S. (2010) Synthesis, antimicrobial, and mosquito larvicidal activity of 1-aryl-4-methyl-3,6-bis-(5-methylisoxazol-3-yl)-2-thioxo-2,3,6,10b-tetrahydro-1H- pyrimido[5,4-c] quinolin-5-ones. Bioorg Med Chem Lett;20:6052–6055.
20. Chen K., Al Aowad A.F., Adelstein S.J., Kassis A.I. (2007) Molecular-docking-guided design, synthesis, and biologic evaluation of radioiodinated quinazolinone prodrugs. J Med Chem;50:663–673.
21. Ugale V.G., Bari S.B. (2014) Quinazolines: new horizons in anticonvulsant therapy. Eur J Med Chem;80:447–501.
22. Guillon R., Pagniez F., Picot C., Hédou D., Tonnerre A., Chosson E., Duflos M., Besson T., Logé C., Le Pape P. (2013) Discovery of a novel broad-spectrum antifungal agent derived from Albaconazole. ACS Med Chem Lett;4:288–292.
23. Manivannan E., Chaturvedi S.C. (2012) Analogue-based design, synthesis and docking of non-steroidal anti-inflammatory agents. Part 2: methyl sulfanyl/methyl sulfonyl substituted 2,3-diaryl-2,3-dihydro-1*H*-quinazolin-4-ones. Biorg Med Chem;20:7119–7127.
24. Shi L.P., Jiang K.M., Jiang J.J., Jin Y., Tao Y.H., Li K., Wang X.H., Lin J. (2013) Synthesis and antimicrobial activity of polyhalobenzonitrile quinazolin-4(3H)-one derivatives. Bioorg Med Chem Lett;23:5958–5963.
25. Ma Z., Hano Y., Nomura T., Chen Y.J. (1997) Two new pyrroloquinazolinoquinoline alkaloids from *Peganum nigellastrum*. Heterocycles;46:541–546.
26. Peng J., Lin T., Wang W., Xin Z., Zhu T., Gu Q., Li D. (2013) Antiviral alkaloids produced by the mangrove-derived fungus *Cladosporium* sp. PJX-41. J Nat Prod;76:1133–1140.
27. Uehata K., Kimura N., Hasegawa K., Arai S., Nishida M., Hosoe T., Kawai K., Nishida A. (2013) Total synthesis of schizocommunin and revision of its structure. J Nat Prod;76:2034–2039.
28. Yamamoto M., Morooka S., Koshiba M., Inaba S., Yamamoto H. (1979) Imidazo- and pyrimido[2,1-b]quinazolines and preparation thereof. United States Patent US4179560 A:

29. Chi-Hoon L., Ju-Hyun J., Sang-Guei L., Hoi-Seon L. (2010) Insecticidal properties of Euphorbiaceae: *Sebastiania corniculata*-derived 8-hydroxyquinoline and its derivatives against three planthopper species (Hemiptera: Delphacidae). J Korean Soc Appl Biol Chem;53:464–469.
30. Khorana N., Changwichit K., Ingkaninan K., Utsintong M. (2012) Prospective acetylcholinesterase inhibitory activity of indole and its analogs. Bioorg Med Chem Lett;22:2885–2888.
31. Puripat M., Ramozzi R., Hatanaka M., Parasuk W., Parasuk V., Morokuma K. (2015) The Biginelli reaction is a urea-catalyzed organocatalytic multicomponent reaction. J Org Chem;80:6959–6967.
32. Parthapratim M., Venugopala K.N., Jayashree B.S., Row T.N.G. (2004) Concomitant polymorphism in 3-acetylcoumarin: role of weak C-HâââO and C-Hâââð Interactions. Cryst Growth Des;4:1105–1107.
33. Panini P., Venugopala K.N., Odhav B., Chopra D. (2014) Polymorphism in two biologically active dihydropyrimidinium hydrochloride derivatives: quantitative inputs towards the energetics associated with crystal packing. Acta Crystallogr Sect B;70:681–696.
34. Sheldrick G. (2008) A short history of SHELX. AcCrA;64:112–122.
35. Macrae C.F., Bruno I.J., Chisholm J.A., Edgington P.R., McCabe P., Pidcock E., Rodriguez-Monge L., Taylor R., van de Streek J., Wood P.A. (2008) Mercury CSD 2.0 – new features for the visualization and investigation of crystal structures. J Appl Crystallogr;41:466–470.
36. Farrugia L. (1997) ORTEP-3 for windows – a version of ORTEP-III with a Graphical User Interface (GUI). J Appl Crystallogr;30:565.
37. WHO (1975) Manual on Practical Entomology. Geneva: WHO.
38. Potter C. (1952) An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. Ann Appl Biol;39:1–28.
39. Nayak S.K., Venugopala K.N., Odhav B. (2013) Methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4H-pyrimido-[2,1-b]quinazoline-3-carboxylate. Acta Cryst E;69:123–124.
40. Nayak S.K., Reddy M.K., Guru Row T.N., Chopra D. (2011) Role of hetero-halogen (F···X, X = Cl, Br, and I) or homo-halogen (X···X, X = F, Cl, Br, and I) interactions in substituted benzanilides. Cryst Growth Des;11:1578–1596.
41. Wang D., Gao F. (2013) Quinazoline derivatives: synthesis and bioactivities. Chem Cent J;7:1–15.
42. Roopan S.M., Khan F.R., Jin J.S. (2013) 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3H)-ones as potential larvicidal agents. Pak J Pharm Sci;26:747–750.

Notes

[a]Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2014) InfoStat versión 2014. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available online at http://wwwinfostatcomar (visited August 12, 2015).
[b]GraphPad Prism version 6.00 for Windows, GraphPad Software. 2015. La Jolla California USA, wwwgraphpadcom Accessed July 14, 2015.
[c]Ware GW (2002) An introduction to insecticides (3rd edition). University of Minnesota;http://www.bio-nica.info/biblioteca/Ware2002IntroductionInsecticides.pdf (visited Septmeber 03, 2015).