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Larvicidal Activity of N^1,N^1,N^4,N^4-Tetramethylpiperazine-1,4-dicarboxamide Against *Aedes caspius* and *Culex pipiens* (Diptera: Culicidae)

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ABSTRACT

The present work describe a safe, mild, eco-friendly and versatile method for the synthesis of N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide. The prepared compound was screened against *Aedes caspius* (Pallas) and *Culex pipiens* (Linnaeus) larvae and compared to its parent piperazine moiety. Likewise, histological structure of gut of treated third instar larva was considerably disturbed. The results demonstrated that substituted urea (1,4-dicarboxamide) meddled with the development of the mosquitoes, and had its strongest poisonous impact at 200 ppm for 48 h. The third larval instar treated with the 1,4-dicarboxamide indicated cell devastation, vacuolization of epithelial cells and cell dispersing in little locales of the mid-gut. The 1,4-dicarboxamide appear to be eco-friendly larvicide to control *Ae. caspius* and *Cx. pipiens*.

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Authors' Contributions

AEF synthesized the compound, designed the chemical part of the study and collected x-ray data. AMAM determined the biological activities. Both authors wrote the article.

Key words

***Aedes caspinus*, histological changes, substituted urea, Piperazine.**

INTRODUCTION

More than a large portion of the world's populace lives under the danger of mosquiotes in light of the fact that they convey the causative specialists of infections, for example, intestinal sickness, West Nile fever, Chikungunya, dengue or lymphatic filariasis (Depoortere *et al.*, 2008). Roughly seventy five percent of all mosquito species happen in the muggy tropics and subtropics, mosquitoes are an issue not just in these districts. They additionally cause an extensive annoyance or can at times transmit pathogens to people in mild scopes. Mosquitoes are greatly effective life forms because of their capacity to adjust to an extensive variety of habitats (Becker *et al.*, 2010).

Culex pipiens (Pallas) complex members causes filariasis in humans in the tropics by transmitting the vector *Wuchereria bancrofti* (Cobbold) (Becker *et al.*, 2010). They also transmit WNV and SLE viruses in USA, and Rift Valley Fever virus in Egypt (Becker *et al.*, 2010). In the east of Saudi Arabia, *Aedes caspius* (Linnaeus) was the most abundant mosquito followed by *Cx. pipiens* (Ahmed *et al.*, 2010). *Ae. caspius* is highly distributed in Riyadh region (Al-Khreji, 2005), the eastern region (Büttiker, 1981; Mattingly and Knight, 1956) and southwestern regions (Abdullah and Merdan, 1995). In Saudi Arabia the bancroftian filariasis was transmitted by the vector *Cx. pipiens* (Omar, 1996). Serious outbreaks of vector-borne maladies have occurred in the South-Western districts of Saudi Arabia close to Yemeni outskirts (CDC, 2000; Empres, 2000; Rathor, 2009; WHO, 2004). The development of vector resistance to insecticides and high costs urged researchers to investigate alternative sources (Al-Mekhlafi *et al.*, 2013).

Substituted urea can be applied in numerous fields such as coloring of hair and cellulose fibers, cell reinforcements in fuel for cars, as added substances in cleansers and erosion inhibitors (Tyson and Shaw, 1952). The substituted urea is also used as herbicide, pesticide, tranquillizing and plant growth regulator (Vishnyakova *et al.*, 1985). Unsymmetrical substituted urea can inhibit the potent HIV-1 protease (Ryskiewicz and Silverstein, 1954; Silverstein *et al.*, 1955). Hossein *et al.* (2010) found that urea derivatives that contain piperazine rings were effective in blocking calcium ion channel activity.

We report here synthesis of N^1,N^1,N^4,N^4-

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tetramethylpiperazine-1,4-dicarboxamide, an bispiperazino urea and using it as a control agent against *Ae. caspius* and *Cx. pipiens*.

MATERIALS AND METHODS

Chemicals and equipment

The solvents utilized were of HPLC reagent grade. Piperazine and dimethyl carbamoyl chloride were purchased from Aldrich. The Mel-Temp apparatus was used to determine the melting point and are uncorrected. Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets was used to record the infrared (IR) spectra. At room temperature, 400 MHz Jeol spectrometer were used to record nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR spectra). Chemical shifts are reported in ppm and are referenced relative to residual solvent (*e.g.*, $CHCl_3$ at δ H 7.26 ppm for $CDCl_3$). Essential investigations were performed on Perkin-Elmer 2400 basic analyzer, and the values found were inside ±0.3% of the theoretical qualities. Follow-up of the reactions and checks of the virtue of the mixes was carried out by TLC on silica gel-ensured aluminum sheets (Type 60 GF254, Merck) and the spots were recognized by presentation to UV-light at λ 254 nm for a couple of seconds. The compounds were named using Chem. Draw Ultra version 11, Cambridge soft Corporation. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center and allocated with the deposition numbers: CCDC 1050448 for compound **3**. Copy of data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EW, UK (Fax: Int. code (1223) 336-033; E-mail: deposit@ccdc.cam.ac.uk).

Synthesis of N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide (# 3 in Fig. 1)

0.1 mole of piperazine 1 was dissolved in 100 ml dichloromethane (DCM) and 100 ml of 10% NaOH. The dimethyl carbamoyl chloride (# 2 in Fig. 1) (0.25 mole in 100 ml of DCM) was added in a period of 10 min. The reaction mixture was stirred at room temperature for 4h and afterward the organic layer was collected and NaOH layer was washed with 100 ml DCM. The consolidated DCM was washed with water (2x100 mL), dried ($MgSO_4$), filtered and the solvent was evacuated under vacuum to get white crystals yield 93%. Single crystal was obtained from the slow evaporation of dichloromethane-hexane (1:4).

N^1,N^1,N^4,N^4-Tetramethylpiperazine-1,4-dicarboxamide was obtained as white crystals, mp 92-93ºC [Lit. (Cao *et al*. (2007) 89-91ºC]; IR (KBr): 2922, 2861 (Sp^3 protons)), 1638 (CON-urea) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.78 (s, 12H, 2 $N(CH_3)_2$), 3.17(s, 8H, 2 NCH_2CH_2N). ^{13}C NMR ($CDCl_3$) δ (ppm): 38.3 (NCH_3), 46.5 NCH_2) , 164.6 (CO). Anal Calcd. for $C_{10}H_{20}N_4O_2$: C, 52.61; H, 8.83; N, 24.54; found: C, 52.71; H, 8.89; N, 24.63.

Larvicidal activity

Mosquito larval culture

Ae. caspius and *Cx. pipiens* larvae were obtained from a colony maintained in the laboratory of Department of Zoology, College of Science, King Saud University without exposure to any insecticide. They were reared in a plastic tray (24 x 35 x 5 cm) containing fish feed and were kept indoor at 27±2°C, 50±5% relative humidity, a 14:10 light: dark photo-period and fed daily until becoming pupae. The pupae were transferred from the plate to a glass containing faucet water and were kept in the insectary. They were then moved into a mosquito cage where the adults were sustained with a 10% glucose solution in a container with a cotton wick. A glass Petri dish lined with channel paper with 100 ml faucet water was kept inside the cage for oviposition.

Larvicidal bioassay

Different concentrations (50-200 µg/mL) of substituted urea and 100-400 µg/mL of piperazine 1 were prepared. Each test solution was placed in multi-well plates (12 wells) and left until dried. Later, it was dissolved in one ml of tap water and tested against 10 3rd instar larvae (*Ae. caspius* and *Cx. pipiens*). Every experiment was done in triplicate and faucet water was utilized as a negative control. The dead larvae were counted 24 h and 48 h after exposure.

Histology of gut

A histological assessment of the gut was performed utilizing third instar larvae (treated and control) settled in 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4) for 4 h. They were then dehydrated with expanding concentrations of ethanol (70-100%) in different concentrations of ethanol (70-100%). Next, they were implanted in Histores in JB4 and the 3-µm thick sections were cut. These sections were stained with hematoxylin-eosin (Bancroft and Stevens, 1996).

Statistical analysis

The experiment was designed as completed random design (CRD). The data were expressed as means ± standard error. Statistical analyses of mortality rate were performed by analysis of T-test (SPSS 13.0 for window 2004).

Table I.- Larvicidal activity (% mortality) of different concentrations of substituted piprazino urea 3 and piperazine against 3rd instar larvae of *Ae. caspius* and *Cx. pipiens*.

Species mosquito	Time	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	LD_{50} (µg/ml)	LD_{90} (µg/ml)
Piprazino urea 3							
Ae. caspius	24	1.62±2.23	10.57±3.41	40.25±4.72	77.21±2.07	159.29	237.28
	48	15.62±3.33	20.13±2.53	60.13±3.19	100±00	126.75	194.98
Cx. pipiens	24	-	5.57±5.67	15.91±3.67	45.27±3.47	219.90	320.65
	48	-	10.33±2.13	31.02±2.53	73.67±2.13	168.41	231.56
Piperazine							
Ae. caspius	24	1.67±2.47	33.33±1.27	90.21±2.26	261.48	397.72	
	48	10.54±1.53	56.67±3.13	100±00	213.28	352.84	
Cx. pipiens	24	1.67±0.71	32.67±3.67	91.73±4.29	259.97	393.52	
	48	5.54±5.67	51.23±4.27	100±00	225.94	357.22	

Control-Nil mortality

RESULTS

Product 3

The product **3** was prepared using a two phase system (DCM-H_2O) as shown in Figure 1.

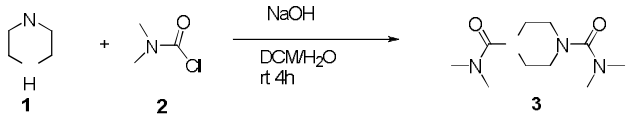

Fig. 1. Synthesis of N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide.

The IR spectra of 3 showed a characteristic peak at 1638 cm^{-1} related to the carboxamide (CON-). The NMR (^1H NMR and ^{13}C NMR) and X-Ray single crystal structure determination of compound 3 confirmed its structure.

Larvicidal activity

N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide **3** was very effective against the third instar larvae of *Ae. caspius*, promoting mortality of 100% of the larvae at a concentration of 200 µg/ml after 48 h. In case of *Cx. pipiens*, compound **3** caused about 73% mortality of the larvae at 200 µg/ml after 48 h. thus showing greater effectiveness on the mosquitoes (Table I). The LC_{50} and LC_{90} values for the compound **3** are summarized in Table I. Solution of compound **3** had toxicity against the larvae of *Ae. caspius* (LC_{50} = 180.11 µg/ml; LC_{90} = 241.34 µg/ml) and *Cx. pipiens* (LC_{50} = 219.91 µg/ml; LC_{90} = 281.14 µg/ml) after 24 h. Solution 3 had toxicity against the test larvae of *Ae. caspius* (LC_{50} = 148.77 µg/ml; LC_{90} = 193.21 µg/ml) and *Cx. pipiens* (LC_{50} = 184.99 µg/ml; LC_{90} = 244.99 µg/ml) after 48 h.

Table I also shows the effect of piperazine **1** against 3rd instar larvae of *Ae. caspius* and *Cx. pipiens*, 46% mortality of the larvae. A concentration of 200 µg/ml after 48 h, and 100% mortality of the larvae at 400 µg/ml after 48 h. Piperazine **1** caused 51% mortality when used against *Cx. pipiens* larvae at a concentration of 200 µg/ml after 48 h and 100% mortality of the larvae at 400 µg/ml after 48 h. Piperazine had toxicity against the test larvae of *Ae. caspius* (LC_{50} = 335.38 µg/ml; LC_{90} = 510.35 µg/ml) and *Cx. pipiens* (LC_{50} = 303.04 µg/ml; LC_{90} = 487.92 µg/ml) after 24 h, respectively, and the piperazine **1** had toxicity against the larvae of *Ae. caspius* (LC_{50} = 259.28 µg/ml; LC_{90} = 453.74 µg/ml) and *Cx. pipiens* (LC_{50} = 220.92 µg/ml; LC_{90} = 433.01 µg/ml) after 48 h.

Histology of gut

The gut of control mosquito larvae showed somewhat taller epithelial cells with thick brush border. This region contained a few cells with a more globose appearance, like caliciform cells, and a normal nucleus (Figs. 2, 3). The *Ae. caspius* and *Cx. pipiens* larvae treated with substituted ureas showed changes in the digestive tract with cell pulverization, vacuolization of epithelial cells, tissue complication with dispersing between the cells and some rupture points of muscle tissue. A few cells demonstrated an absence of cytoplasmic borders. There was an evident aggregation of granules in some parts of the cytoplasm, with weak and/or missing nucleus.

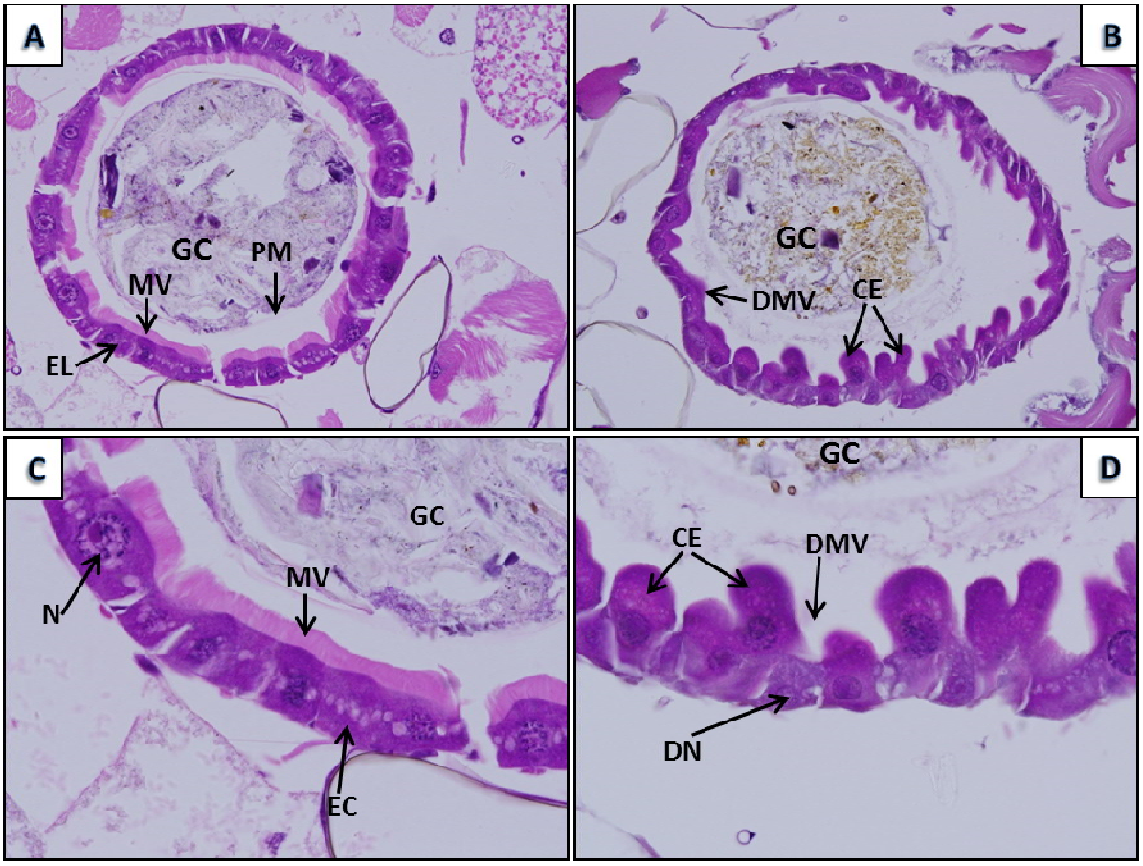

Fig. 2. Histopathological effect of substituted ureas on the midgut epithelial tissue of *Cx. pipiens* larvae, 24 h after treatment. A and C represent cross sections of midguts of untreated larvae, showing normal gut epithelial layer (EL), with healthy, normal epithelial cells (EC), peritrophic membrane (PM), microvellii (MV), nuclei (N), and nutritional gut contents (GC) filling the gut lumen. B and D represent cross sections in midguts of treated larvae, showing affected gut epithelial layer, with cytoplasmic extensions (CE), degraded microvellii (DMV), degenerating nuclei (DN).

DISCUSSION

In the present study, the target product **3** was prepared by modifying the technique illustrated in Figure 4 taking into account a variation of the Vilsmeier-Haack convention (Ali *et al.*, 2012; Ryskiewicz and Silverstein, 1954; Silverstein *et al*., 1955; Tanji *et al.*, 1993; Tyson and Shaw, 1952).

The recent modified method (DCM-H_2O) shown in Figure 1 is fast, easy, safe and eco-friendly gives the product in high yield and purity. Accordingly compound **3** was prepared by the reaction of dimethyl carbamoyl chloride 2 with piperazine **1** in ratio 2:1 using DCM as a solvent in the presence of 10% NaOH in water.

The IR and ^{1}H NMR of compound **3** was in a good agreement with its structure. The IR spectra showed the main peak for urea at 1638 cm^{-1} and the NMR spectra showed two singlets at δ 2.78 and 3.17 corresponding to the four methyl groups (2 NMe_2) and the two ethylene groups ($2NCH_2CH_2N$), respectively. The ^{13}C NMR showed the signals peaks at δ 38.3, 46.5, and 164.6 related to 4-NCH_3, NCH_2, and CO, respectively.

The X-Ray single crystal structure determination of compound **3** (Fig. 5) also confirmed its structure. The crystal structure of compound **3** (CCDC: 1050448) was dictated by X-beam crystallography and all bond lengths and points are in typical ranges (Allen *et al.*, 1987).

Mulla (1991) stated that, insect growth regulators were found to be highly active against mosquitoes. Most of them are groups of juvenoids, benzamides, carbamates and urea-type compounds. Insect growth regulators are selective insecticides and nontoxic to humans and other vertebrates (Graf, 1993; Mulla, 1995; Wilson, 2004). In a recent study, a series of thiourea and urea derivatives containing 1,2,4-triazole moieties were integrated and assessed for their antifungal and larvicidal action

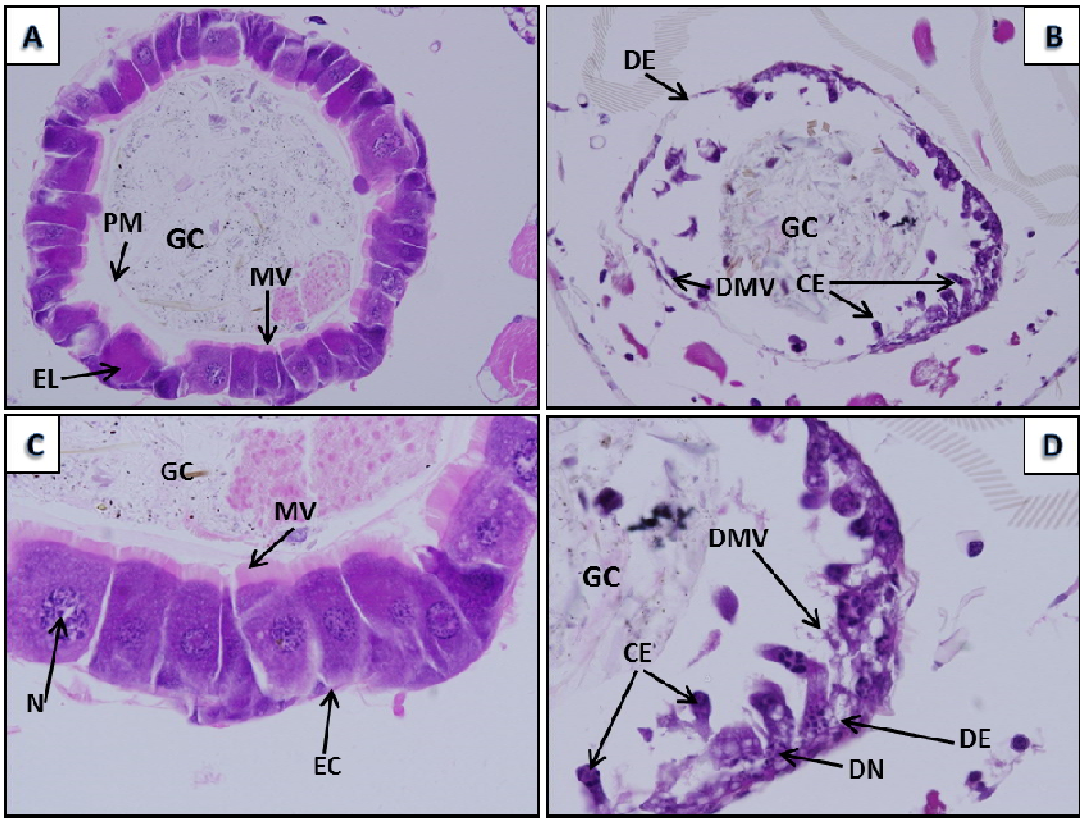

Fig. 3. Histopathological effect of substituted ureas on the midgut epithelial tissue of *Aedes caspius* larvae, 24 h after treatment. A and C represent cross sections of midguts of untreated larvae, showing normal gut epithelial layer (EL) with healthy, normal epithelial cells (EC), peritrophic membrane (PM), microvellii (MV), nuclei (N), and nutritional gut contents (GC) filling the gut lumen. B and D represent cross sections of midguts of treated larvae, showing affected gut epithelial layer, with cytoplasmic extensions (CE), degraded microvellii (DMV), degenerating epithelial cells (DE), degenerating nuclei (DN).

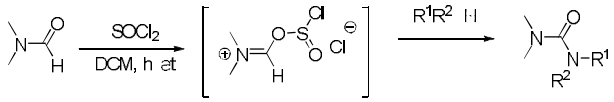

Fig. 4. Synthesis of Vilsmeier reagents.

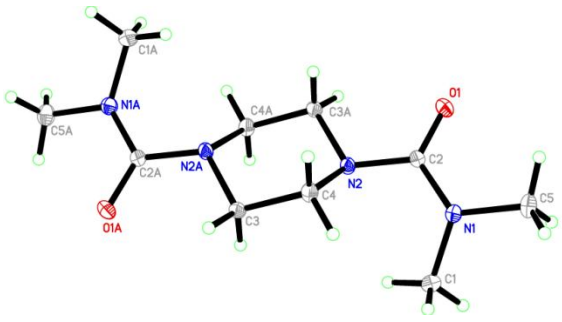

Fig. 5. ORTEP diagram of N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide 3 CCDC: 1050448.

(Kocyigit-Kaymakcioglu *et al.*, 2013).

In our study, it was observed that *Ae. caspius* and *Cx. pipiens* are susceptible to the substituted urea extracts tested. The mortalities expanded with expanding concentrations of the extracts tried in dose dependent manner. This affirms the report of Shadia *et al.* (2007) that there is a positive correlation between the rate of the larval mortality and concentration. Akira *et al.* (1988) stated that derivatives of unsymmetrical urea are known as pesticides and insecticides.

Larvae of *Ae. caspius* and *Cx. pipiens* treated with the substituted urea underwent a lethal disruption of the peritrophic membrane, cytoplasmic vacuolization and an extensive cellular microvillar disruption. Cytoplasmic vacuolization in these cell types may result from anosmotic imbalance. Abutaha *et al.* (2014) reperted the effects on the mid gut of *Ae. caspius* and *Cx. pipiens* by using *Aspergillus sydowi* (Bainier & Sartory) extract. Alves *et al.* (2010) studied the effect of many insecticides

that target the ion channels and found that cell vacuolization was a common response. Abed *et al*. (2007) reported that the mid-gut columnar cells of *Ae. aegypti* (Linnaeus) responded with intense cytoplasmic vacuolization following treatment with *Citrus reticulata* (Blanco) resin oil.

CONCLUSION

We report herein a simple, fast, eco-friendly method for the preparation of N^1,N^1,N^4,N^4-tetramethylpiperazine-1,4-dicarboxamide, which could be used as a larvicide.

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REFERENCES

Abdullah, M.A.R. and Merdan, A.I., 1995. Distribution and ecology of the mosquitofauna in the southwestern Saudi Arabia. *J. Egypt. Soc. Parasitol.*, **25**: 815-837.

Abed, R.A., Cavasin, G.M., Silva, H.H.G., Geris, R. and Silva, I.G., 2007. Alterações morfohistológicas em larvas de Aedes aegypti (Linnaeus, 1762) (Diptera, Culicidae) causadas pela atividade larvicida do óleo-resina da planta medicinal *Copaifera reticulate* Ducke (Leguminosae). *Rev. Patol. Trop.*, **36**: 75–86.

Abutaha, N., Al-Mekhlafi, F.A., Semlali, A., Baabbad, A. and Wadaan, M.A., 2014. *Aspergillus sydowi* metabolites efficacies against the mosquito larval (*Culex pipiens* and *Aedes caspius*) population and cytotoxicity after purification with column chromatography. *Res. J. Biotech.*, **9**: 84-89.

Ahmed, A.M., Shaalan, E.A., Aboul-Soud, M.A.M., Tripet, F. and Al-Khedhairy, A.A., 2010. Mosquito vectors survey in AL-Ahsaa district, eastern region, Kingdom of Saudi Arabia. *J. Insect Sci.*, **11**: 1-11.

Akira, K., Satoshi, O., Hitoshi, S., Satoshi, T., Tatsumi, H. and Akio, M., 1988. *Benzoylphenylurea derivative and its use as an insecticide.* US Patent 4734436.

Ali, T.E., Ibrahim, M.A., El-Amin, E.M. and El-Gendy, Z.M., 2012. *4,6-diacetylresorcinol in heterocyclic synthesis* Part I: *Vilsmeier-Haack reactions of 4,6 diacetylresorcinol and its Schiff bases and hydrazones to construct of new linearly and angularly substituted pyrano[3,2-g]chromenes*. The 16th international Electronic Conference on Synthetic Organic Chemistry, 1-30 November, 1-15.

Al-Khreji, M.A., 2005. *Survey and distribution of mosquito species (Diptera: Culicidae) and description of its habitat in Riyadh district, Kingdom of Saudi Arabia*. M.Sc. thesis, King Saud University, Kingdom of Saudi Arabia.

Allen, F.H., Kennard, O., Watson, D.G., Brammer, L., Orpen, A.G. and Taylor, R., 1987. Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds, *J. Chem. Soc., Perkin Trans.*, **2**: 1-19.

Al-Mekhlafi, F.A., Abu-Taha, N., Mashaly, A.M.A., and Wadaan, M.A., 2013. Larvicidal activity of selected xerophytic plants against *Culex pipiens* and *Aedes caspius* (Diptera: Culicidae). *Pakistan J. Zool*., **45**: 241-246.

Alves, S.N., Serrao, J.E. and Melo, A.L., 2010. Alterations in the fat body and midgut of *Culex quinquefasciatus* larvae following exposure to different insecticides, *Micron*, **41**: 592-597.

Bancroft, J.D. and Stevens, A., 1996. *Theory and practice of histological techniques*. 4nd ed. Churchill Livingstone, Edinburgh, pp. 766.

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C. and Kaiser, A., 2010. *Mosquitoes and their control*. Springer Verlag Berlin Heidelberg.

Büttiker, W., 1981. Observations on urban mosquitoes in Saudi Arabia. *Fauna of Saudi Arabia*, **3**: 472-479.

Cao, P., Huang, X., Ding, H., Ge, H., Ll, H., Ruan, B. and Zhu, H., 2007. Synthesis and cytotoxic evaluation of substituted urea derivatives as inhibitors of human-leukemia K562 cells. *Chem. Biodiv.*, **4:** 881-886

Centers for Disease Control and Prevention (CDC), 2000. Outbreak of Rift Valley fever--Saudi Arabia, August-October, 2000. *Morb. Mortal. Wkly. Rep*., **49**: 905-908.

Depoortere, E., Salmaso, S., Pompa, M., Guglielmetti, P. and Coulombier, D., 2008. Chikungunya in Europe. *The Lancet*, **371**: 723–723.

Empres, 2000. Update on RVF outbreaks in Saudi Arabia and Yemen. *Transbound. Anim. Dis. Bull*., **4**: 4-8.

Graf, J.F., 1993. The role of insect growth regulators in arthropod control. *Parasitol. Today*, **9**: 471-474.

Hossein, P., Snutch, D.Y. and Terrance, P., 2010. Urea derivatives as calcium channel blockers. US Patent.

Kocyigit-Kaymakcioglu, B., Celen, A.O., Tabanca, N., Ali, A., Khan, S.I., Khan, I.A. and Wedge, D.E., 2013. Synthesis and biological activity of substituted urea and thiourea derivatives containing 1,2,4-triazole moieties. *Molecules*, **18**: 3562-3576.

Mattingly, P.F. and Knight, K.L., 1956. The mosquito of Arabia I. *Bull. Br. Mus.*, (Natural History), **4**: 91-141.

Mulla, M.S., 1991. Insect growth regulators for the control of mosquito pests and disease vectors. *Chinese J. Ent. spec. Publ.*, **6**: 81-91.

Mulla, M.S., 1995. The future of insect growth regulators in vector control. *J. Am. Mosq. Contr. Assoc*., **11:** 269-273.

Omar, M.S., 1996, A survey of bancroftian filariasis among

South-East Asian expatriate workers in Saudi Arabia. *Trop. Med. Int. Hlth.*, **1**: 155-60.

Rathor, H.R., 2009. the role of vectors in emerging and re-emerging diseases in the Eastern Mediterranean Region. *Dengue Bulletin*; 2000; December, Volume 24, online(http://www.searo.who.int/en/Section10/Section332/Section522_2535.htm), last accessed, April, 20th.

Ryskiewicz, E.E. and Silverstein, R.M., 1954. N-Methyl-2-pyrrolealdehyde and N-Methyl-2-hydroxymethylpyrrole. *J. Am. Chem. Soc*., **76**: 5802-5803.

Shadia, E.A., Eisayed, A.O. and Aly, S.S., 2007. Chemical composition of cimumamericanum essential oils and biological effects against Agrotisipsilon (Lepidoptera: Noctuidae), *Res. J. Agric. biol. Sci*, **3**: 740–747.

Silverstein, R.M., Ryskiewicz, E.E., Willard, C. and Koehler, R.C., 1955. Improved synthesis of 2-pyrroleadehyde and of N-methyl-2-pyrrolealdehys further studies of pyrrole alcohols. *J. Org. Chem.*, **20:** 668-672.

Tanji, K., Sasahara, T., Suzuki, J. and Higashino, T., 1993. Pyrazolopyridines. I: Formylation and acylation of pyrazolo [1, 5-a] pyridines. *Heterocycles*, **35**: 915-924.

Tyson, F.T. and Shaw, J.T., 1952. A new approach to 3-Indolecarboxyaldehyde. *J. Am. Chem. Soc*., **74**: 2273-2274.

Vishnyakova, T.P., Golubeva, I.A. and Glebova, E.V., 1985. Substituted ureas. Methods of synthesis and applications *Russ. Chem. Rev. (Engl. Transl.)*, **54**: 249.

Wilson, T.G., 2004. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. *J. Insect Physiol*., **50**: 111-121.

World Health Organization (WHO), 2004. *Integrated vector management: Strategic framework for the Eastern Mediterranean Region 2004–2010*. The WHO Regional Office for the Eastern Mediterranean, Cairo, pp**.** 16.