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New Route for Synthesis and Characterization of Halogen Pyrimidine Derivatives with Promising Antimicrobial Activities

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Abstract

The synthesis of novel 10,11 dihydro-3-methoxy-9-methyl-12- (*p*-halophenyl)- 12 *H*-naphtho-[2,1-*b*]pyrano[2,3-d]pyrimidine-11-one derivatives has been reported. The key intermediate 2-amino-4-(*p*-halophenyl)- 7-methoxy-4*H*-naphtho[2,1-*b*] pyrano -3-carbonitrile (**3**) was obtained by treating 4-halobenzylidenmalononitriles **(2a–c)** and ethyl 4-halobenzylidenmalonates **(2d–f)** with 6-methoxy-2-naphthol **(1)** in ethanolic piperidine solution. Antimicrobial activity was shown for most of the synthesized compounds.

Keywords: 4-Halobenzylidenmalononitriles; ethyl 4-halobenzylidenmalonates; 6-methoxy-2-naphthol; naphtho[2,1-*b*] pyrano ; naphtho[2,1-*b*]pyrano[2,3-*d*]pyrimidine; antimicrobial activity.

1. INTRODUCTION

Pyran and fused 4*H*-pyran derivatives have attracted a great deal of interest owing to their antimicrobial activity (Ashraf et al. 2003; Fathy et al., 2004; El-Agrody et al., 2001; Bedair et al., 2000; El-Agrody et al., 2000), inhibition of influenza, virus sialidases (Taylor et al., 1998), mutagenic activity (Hirmoto et al. 1997), activity as antiviral (Martinez et al. 1997) and antiproliferaction agents (Dell et al., 1993), sex-pheromones (Bianchi et al., 1987), antitumor (Eiden et al., 1991) and anti-inflammatory agents (Shishoo et al., 1981). Moreover, pyrane derivatives are well known for their antihistaminic activity (Noda et al., 1977).

In continuation of the previous works (El-Agrody et al., 1997), it seemed interesting to synthesize new 2-amino-4-(*p*-halophenyl) -6- methoxy-4*H*-naphtho[2,1-b]pyran derivatives and 12-(*p*-halophenyl)-12H-naphtho[2,1-*b*]-pyrano[2,3-*d*]pyrimidine derivatives.

by using 4 - halobenzylidenmalononitriles; ethyl 4 - halobenzylidenmalonates; 7 - me thoxy-2-naphthol; as starting material. These derivatives might be active against some Gram-positive and Gram-negative organisms.

2. EXPERIMENTAL

Melting points were measured using the melting point apparatus (Stuart Scientific Co., UK) and remained uncorrected. The IR spectra were recorded on a Shimadzu IR 440 spectrophotometer (Shimadzu, Japan) in KBr. 1H NMR spectra were measured on a Varian Mercury (300 MHz) spectrometer (Varian, UK), using tetramethylsilane (TMS) as the internal standard and (DMSO-d6) as solvent. Microanalytical data (Table 1) were obtained from the Microanalytical Unit of the Cairo University (Egypt).

2.1. Synthesis of 4H-pyrane derivatives (3a-f). General procedure

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A mixture of substituted 4-halobenzylidenmalononitriles (**2a-c**) (0.01 mol) and/or ethyl 4-halobenzylidenmalonates; (**2d-f**) (0.01 mol), 6-methoxy-2-naphthol (1) (0.01 mol) and piperidine (0.5 mL) in absolute ethanol (50 mL) was heated until precipitation was completed. The precipitate was collected by filtration and recrystallized from the suitable solvent.

*2-Amino-4-(p-halophenyl)-7-methoxy-4H-naphtho[2,1-b]pyran-3-carbonnitrile (***3a-c***) and ethyl 2-amino-4-(p-halophenyl)-7-methoxy-4H-naphtho[2,1-b]pyran-3-carboxylate* (**3d-f**) afforded colourless needles from dioxane and ethanol, respectively.

*Synthesis of 2-acetylamino-7-methoxy-4-(p-halopheny)-4H-naphtho[2,1-b]pyran--3-carbonitrile (***4a-c***)*

A solution of **3a-c** (0.01 mol) in Ac2O (20 mL) was heated under reflux for 30 min. The solid product formed was filtered off and washed with cold EtOH to give **4a-c** as pale yellow crystals (from ethanol).

*Synthesis of 10,11-dihydro-3-methoxy-9-methyl-12-(p-halophenyl)-12H-naphtho-[2,1-b]pyrano[2,3-d]pyrimidine-11-one (***5a-c***)*

Method A. – A solution of **3a-c** (0.01 mol) in Ac2O (20 mL) was heated under reflux for 3 h. The precipitate was filtered off, washed with cold EtOH to give **5a-c** as colourless crystals from DMF.

Method B. – Gaseous dry HCl was bubbled through the mixture of **3d-f** (0.01 mol) and CH3CN (30 mL) for 4-6 h. The reaction mixture was poured into ice water and alkalized with 10% aqueous ammonium hydroxide to give **5a-c**.

*Synthesis of 10 , 11 - dihydro - 3 - me thoxy - 9 - phenyl - 12 - (p-halophenyl) - 12H - naphtho [2,1-b] - pyrano [2,3-d] pyrimidine - 11 - one (***6a-c***)*

A solution of **3a-c** (0.01 mol) in benzoyl chloride (20 mL) was heated under reflux for 6 h. The excess of benzoyl chloride was removed under reduced pressure and the residue was poured into cold water. The precipitate was collected by filtration, washed with carbon tetrachloride (10 mL) to remove the formed benzoic acid and the residue was dried to give **6a-c** as colourless crystals from DMF.

*Synthesis of 11-amino-3-methoxy-12-(p-halophenyl)-12H-naphtho[2,1-b] pyrano[2,3-d]pyrimidine (***7a-c***)*

Method B. – A solution of **3a-c** (0.01 mol) in formamide (20 mL) was heated under reflux for 6 h to give **7a-c**.

Method A. – Gaseous NH3 was bubbled through **8a-c** (0.01 mol) in methanol for 1 h. The solid formed was collected to give **7a-c** as colourless needles from benzene.

Synthesis of 2-ethoxymethyleneamino-7-methoxy-4-(p-halophenyl)-4H-naphtho-[2,1-b] pyran-3-carbonitrile **(8a-c)**

A mixture of **3a-c** (0.01 mol) and triethyl orthoformate (2 mL) in acetic anhydride (10 mL) was refluxed for 2 h. After cooling, the precipitated product was filtered off and washed several times with cold ethanol to give **8a-c** as colourless crystals from benzene.

2.2. Synthesis of pyranopyrimidine derivatives* (9-11). *General procedure

A mixture of **8a-c** (0.01 mol), hydrazine hydrate (5 mL, 99%) or methylamine (0.01 mol) in absolute ethanol (50 mL) was stirred for 1 h at room temperature to give **9a-c**. 10-Amino-10,11-dihydro-11-imino-3-methoxy-12-(p-halophenyl)-12H-naphtho[2,1-b] pyrano[2,3-d]pyrimidine **(9a-c)** and 10,11-dihydro-11-imino-3-methoxy-10-methyl-12-(p-halo-phenyl)-12H-naphtho[2,1-b] pyrano[2,3-d]pyrimidine **(10a-c)** were obtained as colourless crystals from dioxane.

Synthesis of 7 - methoxy - 2 - (N , N - dimethylaminomethylene) - 4 - (*p*-halophenyl) -4H - naphtho [2,1-b] pyran - 3 - carbo nitrile **(11a-c)** A mixture of **8a-c** (0.01 mol) and dimethylamine (5 mL) in ethanol was stirred for 1 h. The white solid formed was

filtered, washed with cold ethanol to give **11a-c** as colourless crystals from benzene.

Synthesis of 10,11-dihydro-3-methoxy-12-(p-halophenyl)-12H-naphtho-[2,1-b] pyrano[2,3-d]-pyrimidine-11-thion **(12a-c)**

H_2S was bubbled through a solution of **8a-c** (0.01 mol) in absolute ethanol (50 mL) at room temperature for 1 h. The reaction mixture was stirred for an additional hour to give **12a-c** as yellow crystals from dioxane.

3. ANTIBACTERIAL ACTIVITY

The new synthesized compounds were screened for their antimicrobial activity in vitro against two species of Gram-positive bacteria, Staphylococcus aureus (NCTC-7447), Bacillus cereus (ATCC-14579) and two Gram-negative bacteria, Escherichia coli (NCTC-10410), Serratia marcescens (IMRU-70) and using the paper disc diffusion method (Hewitt W and Vincent S,1989). Filter paper discs manufactured by Bristol-Myers Squibb (Egypt) were used.

The tested compounds were dissolved in N,N-dimethylformamide (DMF) to get a solution of 1 mg mL-1. The inhibition zones were measured in millimeters at the end of an incubation period of 48 h at 28 °C. DMF showed no inhibition zones. The ampicillin (30 µg) standard was used as a reference.

4. ANTIFUNGAL ACTIVITY

The new synthesized compounds were screened for their antimicrobial activity in vitro for their antifungal activity against two species of fungi, Aspergillus fumigatus (MTCC-3008) and Candida albicans (MTCC-227) using the paper disk diffusion method (Cremer A, 1980). The tested compounds were dissolved in DMF to get a 1 mg mL-1 solution. The inhibition zones were measured in millimeters at the end of an incubation period of 48 h at 28oC. A standard of Calforan (30 µg) was used as a reference and the results are shown in Table 3.

5. RESULTS AND DISCUSSION

Condensation of 6-methoxy-2-naphthol (1) with 4-halobenzylidenmalononitriles (**2a-c**) and/or ethyl- 4-halobenzylidenmalonates; (**2d-f**) afforded the corresponding 2-amino-4(-p-halophenyl)-7-methoxy-4H-naphtho[2,1-b]pyrane-3-carbonitrile (**3a-c**) and ethyl-3-carboxylate (**3d-f**), respectively (Elagamey et al. 1990, 18).

Treatment of **3a-c** with Ac_2O gave two products dependening on the reaction time; one product was identified as 2-acetyl-amino - 7 - methoxy - 4 - (*p*-halo-phenyl) - 4H -naphtho[2,1-b]pyrane-3-carbonitrile (**4a-c**) (30 min), while the other was identified as 10,11-dihydro-3-methoxy-9-methyl-12-(p-halo-phenyl)-12H-naphtho[2,1-b] pyrano[2,3-d]pyrimidine- 11-one (**5a-c**, 3 h). Structure **4a-c** was established on the basis of IR, which showed the presence of CN at 2217 cm^{-1} and CO of the acetyl group at 1732 cm^{-1} and for **5a-c** the absence of CN and the presence of amide carbonyl at 1660 cm1. support for structure **5a-c** was obtained by the independent synthesis of **5a-c** by the reaction of **3d-f** with CH3CN in the presence of dry HCl gas (Dave et al., 1980) (Scheme 2).

Treatement of **3a-c** with benzoyl chloride gave the pyranopyrimidin-11-one derivatives **6a-c**, while with formamide afford 11-amino-3-methoxy-12-(p-halo-phenyl)-12H-naphtho-[2,1-b]pyrano[2,3-d]pyrimidine **7a-c**. Structures **7a-c** is also supported by independent synthesis of the same product by ammonolysis of **8a-c** in methanol at room temperature (m.p and mix. m.p.) (Scheme 3).

Reaction of **3a-c** with triethyl orthoformate gave the corresponding 2-ethoxymethyleneamino derivative **8a-c**. Hydrazinolysis of **8a-c** in ethanol at room temperature yielded 10 - - amino -1 0 , 11 - dihydro - 11- imino - 3 - methoxy - 12-(*p*-halo-phenyl) - 12H-naphtho [2,1-b] pyrano [2,3-d]pyrimidine **9a-c**. Aminolysis of **8a-c**

Table 1. Elemental analyses of the new compounds

Compd. No.	Yield (%)	M.p. (°C)	Mol. formula (Mr)	Found/calcd. (%) C	H	N
3a	88	261	$C_{21}H_{15}BrN_2O_2$ **407.26**	61.90 61.93	3.69 3.71	6.84 6.88
3b	90	247	$C_{21}H_{15}ClN_2O_2$ **362.80**	69.50 69.52	4.15 4.17	7.70 7.72
3c	86	256	$C_{21}H_{15}FN_2O_2$ **346.35**	72.80 72.82	4.35 4.37	7.99 8.09
3d	79	168	$C_{23}H_{20}BrNO_4$ **454.31**	60.80 60.81	4.41 4.44	3.02 3.08
3e	82	173	$C_{23}H_{20}ClNO_4$ **409.86**	67.38 67.40	4.90 4.92	3.39 3.42
3f	77	187	$C_{23}H_{20}FNO_4$ **393.40**	70.20 70.22	5.10 5.12	3.52 3.56
4a	85	197	$C_{23}H_{17}BrN_2O_3$ **449.29**	61.46 61.48	3.78 3.81	6.20 6.23
4b	89	176	$C_{23}H_{17}ClN_2O_3$ **404.84**	68.20 68.23	4.19 4.23	6.90 6.92
4c	83	181	$C_{23}H_{17}FN_2O_3$ **388.39**	71.09 71.13	4.39 4.41	7.20 7.21
5a	82	301	$C_{23}H_{17}BrN_2O_3$ **449.29**	61.46 61.48	3.78 3.81	6.20 6.23
5b	85	291	$C_{23}H_{17}ClN_2O_3$ **404.84**	68.20 68.23	4.19 4.23	6.90 6.92
5c	79	286	$C_{23}H_{17}FN_2O_3$ **388.39**	71.09 71.13	4.39 4.41	7.20 7.21
6a	78	> 360	$C_{28}H_{19}BrN_2O_3$ **511.36**	65.70 65.76	3.71 3.75	5.45 5.48
6b	80	> 360	$C_{28}H_{19}ClN_2O_3$ **466.91**	72.01 72.03	4.00 4.10	5.89 6.00
6c	73	> 360	$C_{28}H_{19}FN_2O_3$ **450.46**	74.60 74.66	4.21 4.24	6.19 6.22
7a	70	331	$C_{22}H_{16}BrN_3O_2$ **434.28**	60.81 60.84	3.70 3.71	9.64 9.68
7b	75	318	$C_{22}H_{16}ClN_3O_2$ **389.83**	67.72 67.78	4.10 4.14	10.74 10.78
7c	71	294	$C_{22}H_{16}FN_3O_2$ **373.38**	70.74 70.77	4.30 4.32	11.21 11.25
8a	74	196	$C_{24}H_{19}BrN_2O_3$ **463.32**	62.20 62.22	4.10 4.13	6.00 6.05
8b	77	211	$C_{24}H_{19}ClN_2O_3$ **418.87**	68.80 68.82	4.51 4.57	6.62 6.69
8c	72	188	$C_{24}H_{19}FN_2O_3$ **402.41**	71.60 71.63	4.73 4.76	6.92 6.96
9a	76	273	$C_{22}H_{17}BrN_4O_2$ **449.30**	58.80 58.81	3.79 3.81	12.43 12.47
9b	81	256	$C_{22}H_{17}ClN_4O_2$ **404.84**	65.25 65.27	4.20 4.23	13.82 13.84
9c	73	243	$C_{22}H_{17}FN_4O_2$ **388.39**	68.00 68.03	4.39 4.41	14.40 14.43
10a	75	262	$C_{23}H_{18}BrN_3O_2$ **448.31**	61.60 61.62	4.00 4.05	9.33 9.37

10b	80	257	$C_{23}H_{18}ClN_3O_2$ **403.86**	68.20 68.40	4.41 4.49	10.38 10.40
10c	74	268	$C_{23}H_{18}FN_3O_2$ **387.40**	71.29 71.31	4.63 4.68	10.82 10.85
11a	76	204	$C_{25}H_{23}BrN_3O_2$ **477.37**	62.87 62.90	4.80 4.86	8.78 8.80
11b	79	218	$C_{25}H_{23}ClN_3O_2$ **432.92**	69.31 69.36	5.30 5.35	9.68 9.71
11c	73	197	$C_{25}H_{23}FN_3O_2$ **416.46**	72.00 72.10	5.52 5.57	10.02 10.09
12a	83	272	$C_{22}H_{15}BrN_2O_2S$ **451.35**	58,30 58.55	3.01 3.35	6.00 6.21
12b	85	287	$C_{22}H_{15}ClN_2O_2S$ **406.88**	64.45 64.94	3.42 3.72	6.25 6.88
12c	86	258	$C_{22}H_{15}FN_2O_2S$ **390.43**	67.30 67.68	3.54 3.87	7.01 7.18

Table 2. Spectral data of the prepared compounds

Compd. No.	**IR (v, cm^{-1})**	**^{1}H NMR (d, ppm) (DMSO-d_6)**
3a	3430, 3331 (NH_2), 3015,2963, 2924, 2850 (stretching CH), 2210 (CN), 1690(C=C)	3.80 (s, 3H, OCH_3), 5.30 (s, 1H, pyrane CH),6.90 (br, 2H, NH_2, exchangeable), 7.04-7.83 (m, 9H, Ar-H)
3b	3410, 3329 (NH_2), 3058,2965, 2926 (stretching CH)2195 (CN), 1660 (C=C)	3.79 (s, 3H, OCH_3), 5.20 (s, 1H, pyrane CH),6.91(br, 2H, NH_2, exchangeable), 7.03-7.82 (m, 9H, Ar-H)
3c	3436, 3335 (NH_2), 3017,2925, 2847 (stretching CH),2210 (CN), 1690 (C=C)	3.80 (s, 3H, OCH_3), 5. 20 (s, 1H, pyrane CH),6.90 (br, 2H, NH_2, exchangeable), 7.01-7.83 (m, 9H, Ar-H)
3d	3438, 3317 (NH_2), 2992,2922, 2831(stretching CH), 1675 (CO)	1.38 (t, 3H, CH_3, J = 7.1Hz), 380 (s, 3H,OCH_3), 4.23 (q, 2H, CH_2, J = 7.1Hz), 5.30 (s, 1H, pyrane CH), 6.40 (br, 2H, NH_2, exchangeable), 6.89-7.88 (m, 9H, Ar-H)
3e	3435, 3322 (NH_2), 2925,2869 (stretching CH),1671 (CO)	1.37 (t, 3H, CH_3, J = 7.1Hz), 384 (s, 3H,OCH_3), 4.30 (q, 2H, CH_2, J = 7.1Hz), 5.30 (s, 1H, pyrane CH), 6.42 (br, 2H, NH_2, exchangeable), 6.90-7.88 (m, 9H, Ar-H)
3f	3412, 3314 (NH_2), 2991,2932, 2873 (stretching CH), 1667 (CO)	1.38 (t, 3H, CH_3, J = 7.1Hz), 380 (s, 3H,OCH_3), 4.23 (q, 2H, CH_2, J = 7.1Hz), 5.31 (s, 1H, pyrane CH), 6.42 (br, 2H, NH_2, exchangeable), 6.90-7.90 (m, 9H, Ar-H)
4a	3356 (NH), 3039,2953 (stretching CH), 1726(CO)	2.42 (s, 3H, $COCH_3$), 3.78 (s, 3H, OCH_3),5.76 (s, 1H, pyrane CH), 7.11-7.90 (m, 9H, Ar-H), 10.60 (br,1H, NH, exchangeable)
4b	3356 (NH), 3039, 2953,2922 (stretching CH), 2216(CN), 1726 (CO)	2.40 (s, 3H, $COCH_3$), 3.76 (s, 3H, OCH_3), 5.77 (s, 1H, pyrane CH), 7.10-7.90 (m, 9H, Ar-H), 10.60 (br,1H, NH, exchangeable)
4c	3356 (NH), 3039, 2953,2922 (stretching CH), 2216(CN), 1726 (CO)	2.40 (s, 3H, $COCH_3$), 3.77 (s, 3H, OCH_3),5.78 (s, 1H, pyrane CH), 7.11-7.90 (m, 9H, Ar-H), 10.60 (br,1H, NH, exchangeable)
5a	3433 (NH), 3011, 2985, 2924 (stretching CH), 1664 (CO)	2.29 (s, 3H, CH_3), 3.76 (s, 3H, O CH_3), 5.70 (s, 1H, pyrane CH), 7.40-8.70 (m, 10H, Ar-H + NH)

5b	3325(NH), 3004, 2961 (stretching CH) 1665 (CO)	2.29 (s, 3H, CH_3), 3.77(s, 3H, O CH_3), 5.70 (s, 1H, pyrane CH), 7.39-8.70 (m, 10H, Ar-H + NH)
5c	3325 (NH), 3004, 2961 (stretching CH) 1665 (CO)	2.30 (s, 3H, CH_3), 3.76 (s, 3H, O CH_3), 5.71 (s, 1H, pyrane CH), 7.40-8.70 (m, 10H, Ar-H + NH)
6a	3343 (NH), 3010, 2961 (stretching CH) 1660 (CO)	3.77 (s, 3H, OCH_3), 5.75 (s, 1H, pyrane CH), 7.40-8.20 (m, 15H, Ar-H + NH)
6b	3343 (NH), 3010, 2961 (stretching CH) 1660 (CO)	3.79 (s, 3H, OCH_3), 5. 76 (s, 1H, pyrane CH), 7.40-8.20 (m, 15H, Ar-H + NH)
6c	3343 (NH), 3010, 2961 (stretching CH) 1660 (CO)	3.79 (s, 3H, OCH_3), 5.77(s, 1H, pyrane CH), 7.40-8.20 (m, 15H, Ar-H + NH)
7a	3476, 3370 (NH_2), 3002, 2993, 2924 (stretching CH), 1615 (C=N), 1583 (C=C)	3.79 (s, 3H, OCH_3), 4.85 (br, 2H, NH_2, exchangeable),5.70 (s, 1H, pyrane CH), 6.90-7.98 (m, 9H, Ar-H),8.40 (s, 1H, pyrimidine CH)
7b	3470, 3371 (NH_2), 3001, 2993, 2923 (stretching CH), 1615 (C=N), 1582 (C=C)	3.79 (s, 3H, OCH_3), 4.85 (br, 2H, NH_2, exchangeable),5.70 (s, 1H, pyrane CH), 6.90-7.99 (m, 9H, Ar-H),8.40 (s, 1H, pyrimidine CH)
7c	3476, 3371 (NH_2), 3002, 2992, 2925 (stretching CH), 1614 (C=N), 1582 (C=C)	3.79 (s, 3H, OCH_3), 4.85 (br, 2H, NH_2, exchangeable), 5.70 (s, 1H, pyrane CH), 6.90-7.99 (m, 9H, Ar-H),8.40 (s, 1H, pyrimidine CH)
8a	3042, 2986, 2950 (stretching CH), 2200 (CN), 1649 (C=N)	1.41 (t, 3H, CH_3, J = 7.1Hz), 3.88 (s, 3H, OCH_3), 4.45 (q, 2H, CH_2, J = 7.1Hz), 5.28 (s, 1H, pyrane CH),7.09-7.18 (m, 9H, Ar-H), 8.42 (s, 1H, N=CH)
8b	3042, 2986, 2950 (stretching CH), 2203 (CN), 1650 (C=N)	1.40 (t, 3H, CH_3, J = 7.1Hz), 3.85 (s, 3H, OCH_3), 4.44 (q, 2H, CH_2, J = 7.1Hz), 5.30 (s, 1H, pyrane CH), 7.10-7.20 (m, 9H, Ar-H), 8.40 (s, 1H, N=CH)
8c	3040, 2983, 2952 (stretching CH), 2203 (CN), 1650 (C=N)	1.42 (t, 3H, CH_3, J = 7.1Hz), 3.86 (s, 3H, OCH_3), 4.45 (q, 2H, CH_2, J = 7.1Hz), 5.30 (s, 1H, pyrane CH),7.10-7.19 (m, 9H, Ar-H), 8.40 s, 1H, N=CH)
9a	3316, 3270(NH_2), 3210 (NH), 2949, 2898(stretching CH), 1647 (C=N)	3.80 (s, 3H, OCH_3), 5.65(s, 1H, pyrane CH), 5.82 (br, 2H, NH_2, exchangeable), 6.90-7.83 (m, 10H, Ar-H+NH), 8.17 (s, 1H, pyrimidine)
9b	3316, 3270 (NH_2), 3209 (NH), 2948, 2899 (stretching CH), 1647 (C=N)	3.80 (s, 3H, OCH_3), 5.66(s, 1H, pyrane CH), 5.82 (br, 2H, NH_2, exchangeable), 6.90-7.84(m, 10H, Ar-H+NH), 8.16 (s, 1H, pyrimidine)
9c	3314, 3266 (NH_2), 3207 (NH), 2948, 2897 (stretching CH), 1647 (C=N)	3.80 (s, 3H, OCH_3), 5.66 (s, 1H, pyrane CH), 5.80 (br, 2H, NH_2, exchangeable), 6.90-7.82 (m, 10H, Ar-H+NH), 8.16 (s, 1H, pyrimidine)
10a	3377 (NH), 3006, 2980, 2830 (stretching CH), 1620 (C=N)	3.37 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 6.01 (s, 1H, pyrane CH), 7.02-7.94 (m, 9H, Ar-H), 8.30 (s, 1H, pyrimidine CH)
10b	3376 (NH), 3006, 2982, 2830 (stretching CH), 1620 (C=N)	3.37 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 6.00 (s, 1H, pyrane CH), 7.02-7.93 (m, 9H, Ar-H), 8.31 (s, 1H, pyrimidine CH)
10c	3376 (NH), 3006, 2981, 2831 (stretching CH), 1621(C=N)	3.36 (s, 3H, NCH_3), 3.84 (s, 3H, OCH_3), 6.01 (s, 1H, pyrane CH), 7.03-7.95 (m, 9H, Ar-H), 8.31(s, 1H, pyrimidine CH)
11a	2983, 2924 (stretching CH), 2204 (CN), 1620 (C=N)	3.10 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 5.60 (s, 1H, pyrane CH), 7.01-7.99 (m, 9H, Ar-H), 8.53 (s, 1H, N=CH)
11b	2984, 2925 (stretching CH), 2204(CN), 1620 (C=N)	3.10 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 5.61 (s, 1H, pyrane CH), 7.01-7.98 (m, 9H, Ar-H), 8.54 (s, 1H,N=CH)

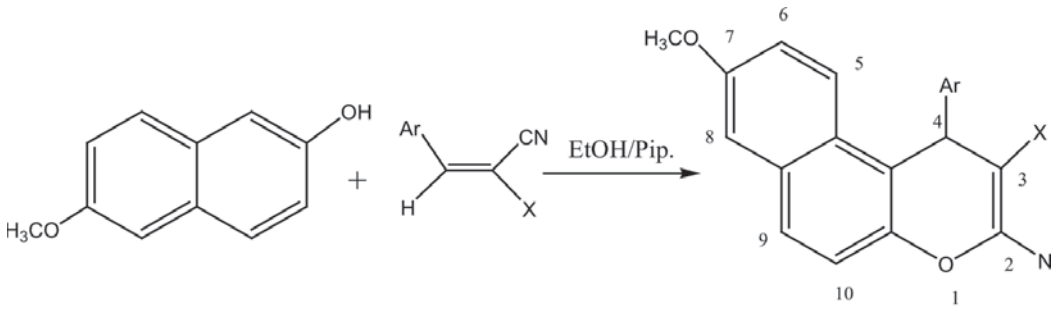
11c	2983, 2924 (stretching CH), 2205 (CN), 1620(C=N)	3.10 (s, 3H, NCH_3), 3.30 (s, 3H, NCH_3), 5.61 (s, 1H, pyrane CH), 7.01-7.98 (m, 9H, Ar-H), 8.54 (s, 1H,N=CH)
12a	3329(NH), 2948, 2899 (stretching CH), 1647 (C=N), 1043 (C=S)	3.83 (s, 3H, OCH_3), 6.02 (s, 1H, pyrane CH),6.9-7.88 (m, 10H, Ar-H+NH), 8.33 (s, 1H, pyrimidine CH)
12b	3327(NH), 2948, 2899 (stretching CH), 1647 (C=N), 1042 (C=S)	3.80 (s, 3H, OCH_3), 6.02 (s, 1H, pyrane CH),7.0-7.88 (m, 10H, Ar-H+NH), 8.30 (s, 1H, pyrimidine CH)
12c	3329(NH), 2947, 2896 (stretching CH), 1647(C=N), 1040 (C=S)	3.80 (s, 3H, OCH_3), 6.03 (s, 1H, pyrane CH),7,0-7.88 (m, 10H, Ar-H+NH), 8.33 (s, 1H, pyrimidine CH)

Table 3. Antimicrobial activity of the new compounds

	Inhibition zone diameter in mm					
Compd. No.[a]	**Staphylococcusaureus (NCTC-7447)**	**Bacillus cereus (ATCC-14579**	**Escherichia coli (NCTC-10410)**	**Serratia marcescens (IMRU-70)**	**Aspergillus fumigatus (MTCC-3008)**	**Candida albicans (MTCC-227)**
3a	20	20	21	22	9	11
3b	22	21	22	22	10	13
3c	20	22	20	21	9	10
3d	15	14	12	10	-	-
3e	17	15	-	15	-	-
3f	-	11	10	10	-	-
4a	-	16	14	17	12	10
4b	18	15	13	15	11	10
4c	-	10	16	-	10	9
5a	26	27	28	26	16	18
5b	27	28	28	26	17	17
5c	26	28	27	28	15	14
6a	25	26	25	27	18	15
6b	28	27	28	27	15	17
6c	26	28	25	27	17	16
7a	27	28	26	27	15	17
7b	26	27	27	26	14	18
7c	28	26	28	27	17	17
8a	15	-	14	-	-	-
8b	14	15	16	-	-	-
8c	-	-	12	11	-	-
9a	22	22	22	20	13	16
9b	22	26	20	23	12	15
9c	22	20	20	21	17	18

10a	20	21	22	21	14	15
10b	21	22	23	20	16	18
10c	20	20	22	20	15	17
11a	16	--	-	14	-	-
11b	15	17	-	18	-	-
11c	16	-	-	15	-	-
12a	28	25	27	25	15	13
12b	26	27	24	26	16	14
12c	28	26	24	25	14	16
Ampicillin Calforan	22	22	22	22	- 20	- 20

[a] c = 1 mg mL^{-1} of new compounds in DMF.



1 **2a-f** **3-a-f**

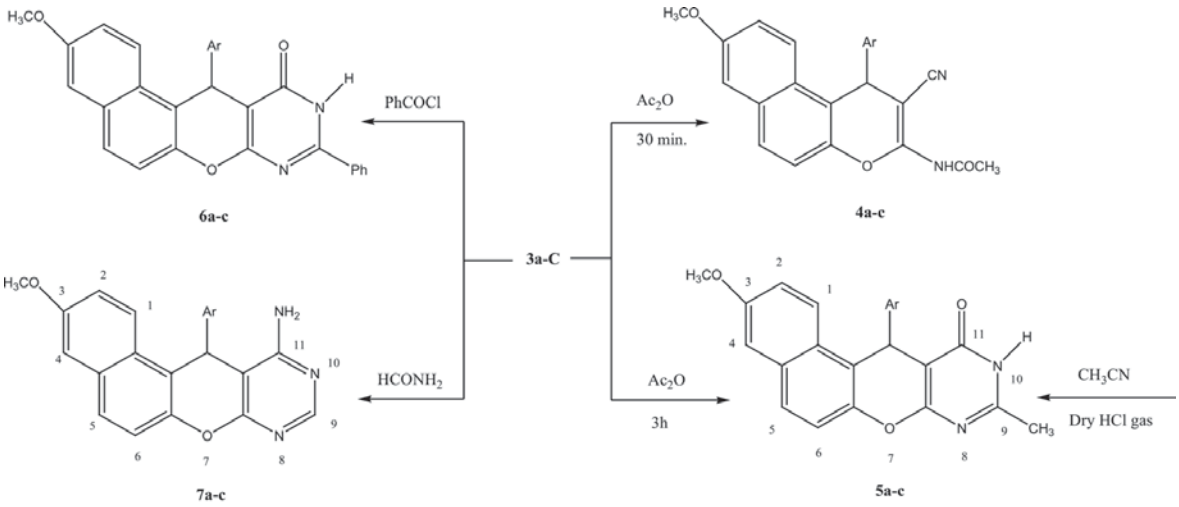
a, Ar = *p*-Br-C_6H_4 X = CN
b, Ar = *p*-Cl-C_6H_4 X = CN
c, Ar = *p*-F-C_6H_4 X = CN
d, Ar = *p*-Br-C_6H_4 X =COOEt
e, Ar = *p*-Cl-C_6H_4 X = COOEt
f, Ar = *p*-F-C_6H_4 X = COOEt

(Scheme 1)

with methylamine gave the corresponding 10-methyl-pyranopyrimidine derivative **10a-c**, and with dimethylamine it gave 2-N,N-dimethylaminomethylene derivatives **11a-c**.

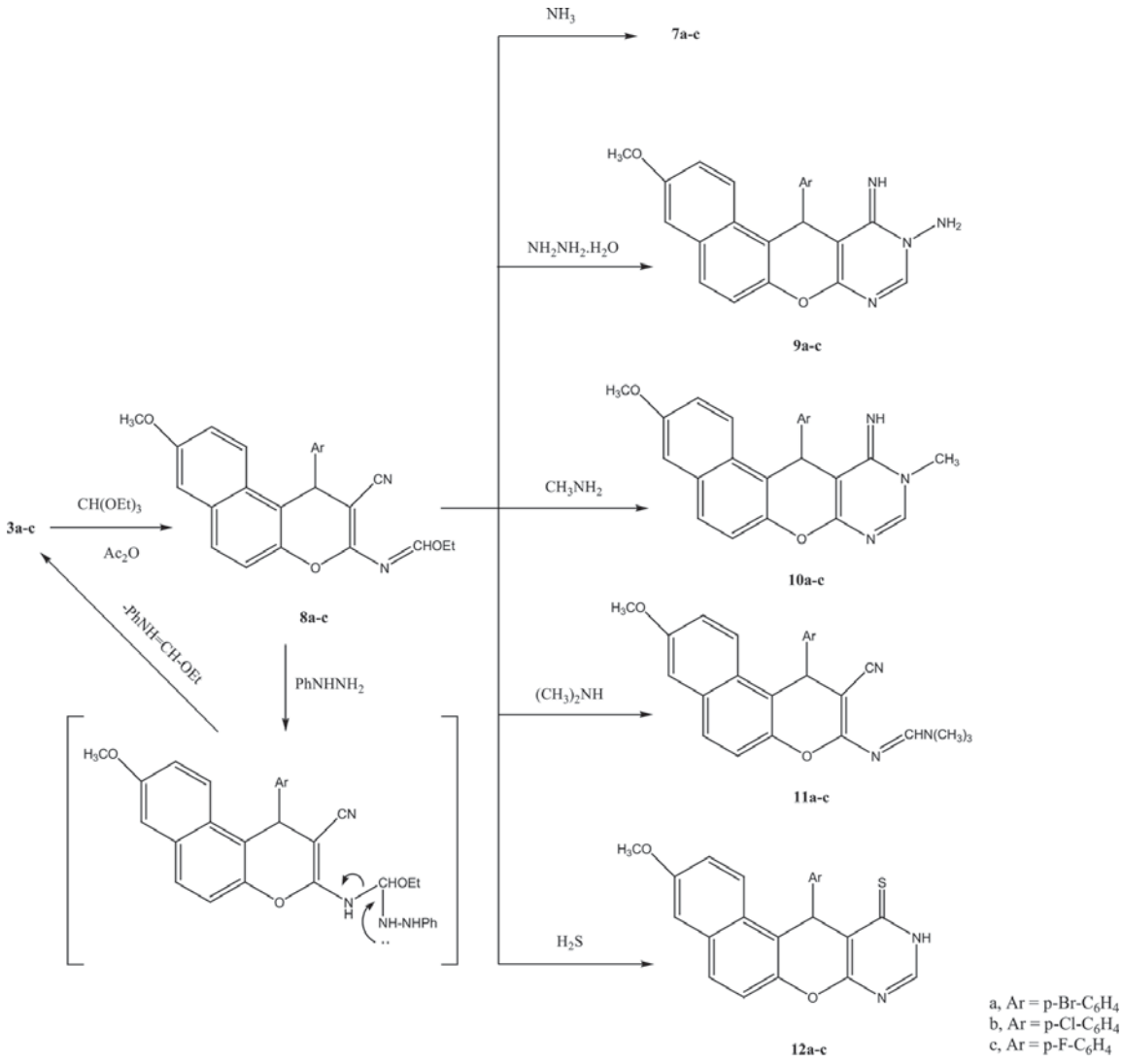
Condensation of **8a-c** with H_2S yielded a product that was identified as 10,11-dihydro-3-methoxy-12-(*p*-halophenyl)-12*H*-naphtho-[2,1-*b*]pyrano[2,3-*d*]pyrimidine-11-thione (**12a-c**). When **8a-c** was treated with phenylhydrazine in ethanol at room temperature, an addition product formed, from which elimination of ethyl formate phenylhydrazone gave the enaminonitrile **3a-c** (Scheme 3).

It was found that compounds **5a-c, 6a-c ,7a-c, 10a-c and 12a-c** possess a pronounced antimicrobial activity against all tested microorganisms, namely, markedly stronger antimicrobial activity against both Gram-positive and Gram-negative bacteria than ampicillin. Compounds **3b** and **9a** exhibited equal activities as ampicillin towards *Staphylococcus aureus* and *Escherichia coli,* compounds **3c**, **9a** and **10b** the same activities



a, Ar = p-Br-C_6H_4
b, Ar = p-Cl-C_6H_4
c, Ar = p-F-C_6H_4

(Scheme 2)



a, Ar = p-Br-C_6H_4
b, Ar = p-Cl-C_6H_4
c, Ar = p-F-C_6H_4

(Scheme 3)

towards Bacillus cereus, compounds **3a** and **3b** also the same effect against towards *Serratia marcescens* while compound **10a** and **10c** similar activities towards *Escherichia coli*, compared with ampicillin.

Compounds **5a-c, 6a-c ,7a-c, 10a-c and 12a-c** showd moderately inhibition to Claforan towards *Aspergillus fumigatus and Candida albicans* .

The remaining compounds differed in their ability to inhibit the growth of microorganisms in dependence on their chemical structure and are all less effective than ampicillin.

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تخليق مشتقات الهالوجين نافثو 2,1-b - بيران ونافثو 2,1-b بيرانو 2,3-d - بيريميدين لها نشاط بيولوجي.

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المُلخّص

تخليق مشتقات جديدة من ونافثو١٠و ١١-ثنائي الهيدرو-٣- ميثوكسي-٩-ميثل-١٢-(بار-هالوجين فينيل)-H12 - نافثو[2,1-b] بيرانو [2,3-d] -[2,3-d] بيريميدين باستخدام المركب ٢- أمينو-٤-(بار-هالوفينل)-٧-ميثوكسي - 4H- نافثو بيران -٣- كربونيتريل (٣) بمعالجة ٤- هالوبيزيليدين مالونو نيتريل (2a–c) أو إيثيل-٤- هالوبيزيليدين مالونو نيتريل (2d–f) مع ٦- ميثوكسي -٢-نافثول(١) في موجودة الأيثانول و بيبريدين .ثم دراسة النشاط بيولوجي لبعض هذة المركبات.

كلمات مفتاحيه : ٤- هالوبيزيليدين مالونو نيتريل أو إيثيل-٤- هالوبيزيليدين مالونو نيتريل ، ٦- ميثوكسي -٢-نافثول ، نافثو[2,1-b] بيران ، نافثو[2,1-b] بيرانو [2,3-d] -[2,3-d] بيريميدين ، النشاط بيولوجي.

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Pathology of the Aquatic Fungus Coelomomyces Stegomyiae in Adult Female Aedes Aegypti

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Abstract

Fungi of the genus Coelomomyces infect adult female mosquitoes Aedes aegypti moving from infected fourth instar larvae and develop within the adult hemocoel. Adults usually survive until they take a blood meal. To examine this pathology, adults A. aegypti infected with C. stegomyiae were chemically fixed, embedded in epoxy and sectioned for light microscopy. While C. stegomyiae directly invade fat body cells, hyphae were also developed within muscles, Malpighian tubules, tracheoles, hemopoietic organs, and ovaries. Hyphae were ramified throughout the fat body in the lymphatic spaces between trophocytes leading to cell lyses and depletion of the fat body, then they gradually develop and severely effect the other tissues. For these reasons, Coelomomyces species and in particular C. stegomyiae remains a candidate for use in classical biological control.

Keywords: Biological control, Aedes aegypti, Coelomomyces stegomyiae

1. INTRODUCTION

The fungus *Coelomomyces* (subdivision Mastigomycotina, class Chytridiomycetes, order Blastocladiales) is an obligate parasite that alternates its sporophytic stages between mosquitoes and copepods or ostracods, respectively (Couch et al., 1985, Lucarotti et al., 1995, Shoulkamy 1996, Whisler 1985). *Coelomomyces* zygotes preferentially encys on the intersegmental membranes of the host mosquito larvae (Travland 1979, Zebold et al., 1979). Larvae with patent *Coelomomyces* infection will die in the final instar, however, larvae with light infections may pupate and eclose to produce infected adults. Infected adult males contain resting sporangia in the hemocoel, which get dispersed when the infected male dies and decomposes in another breeding site (Lucarotti 1987). The infection of adult females is mostly localized in the ovaries (Lucarotti, 1987, Shoulkamy, 1996), but the hyphae in the ovaries will only ma ture to resting spo rangia following a blood meal (Lucarotti et al., 1988). In infected female *Aedes aegypti* pupae and adults, fungal hyphae were observed in the tracheal and cuticular epithelial cells in addition to fat body (Lucarotti, 1992, Shoulkamy et al., 2000). Possibly because of the belief that *Coelomomyces* develops by absorbing nutrients while floating in the hemocoel (Couch et al., 1963). Several studies on the development of *Coelomomyces* in the adult have been focused on hyphal growth and maturation to resting sporangia (Federici et al., 1986, Madelin et al., 1972, Martin 1969, Lucartotti 1992, Shoulkamy 1996) and not on its actual pathology in the adult. In the present study, the pathology of *Coelomomyces stegomyiae* in adult female Aedes aegypti was investigated.

2. MATERIALS AND METHODS

Maintenance of the colony *Aedes aegypti* (L.) (Diptra, Culicidae), *Phyllognathopus*

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viguieri (Maupas), (Copepoda, Harpacticoidae), and *Coelomomyces stegomyiae* Keilin (Chytridiomycetes, Blastocladiales) and their infection with C. *stegomyiae* has been described (Shoulkamy et al., 1997). Adults A. aegypti were held in 0.03 m3 insect cages (American Biological Supply, Baltimore, MD) and fed warmed bovine blood (approximately 37oC) supplemented with 5% Isoleucine and 5% adenosine triphosphate through a parafilm membrane stretched over an artificial feeder. Fresh split raisins were also provided as an additional source of nutrition. Specimens were dissected in 2.5% glutaraldehyde and then fixed for 3h in 2.5% glutaraldehyde made in 0.1 M sucrose and 0.05 M sodium cacodylate buffer, pH 7.4, at 20oC according to the method of Lucarotti et al., (1988). Samples were rinsed in 0.1 M sucrose, 0.05 M sucrose, and 0.025 M sucrose made in 0.05 M sodium cacodylate, pH 7.4 and finally in buffer alone, 15 min each. Afterwards, samples were post-fixed for 3h, in the dark at 20oC, in 1% osmium tetroxide in 0.05 M sodium cacodylate, pH 7.4. Subsequently, samples were rinsed in two changes of 0.05 M buffer, 15 min. each, followed by a 15 min. rinsed in distilled water and then stained en bloc, overnight at 4oC, in 2% aqueous uranyl acetate. Afterwards, samples were dehydrated in an acetone series (30 to 100%) and embedded in Epon-Araldite (Mollenhauer 1964). Sections, 1 µm thick for light microscopy, were cut using a Diatome Histo knife on an RCM MT-7 ultramicrotome. Sections were then transferred to acid- cleaned slides (Angerer and Angerer 1989) using a thin metal loop and dried onto the slides overnight at 80oC under vacuum (-80 KPa). Sections were stained in warm 0.1% toluidine made in 2.5% aqueous sodium carbonate, pH 11.1 (Trump et al., 1961), rinsed in tap and distilled water, dried, and mounted in Permounttm(Fisher Scientific, Ottawa, Ontario). Photomicrographs were taken on Ilford XP-2 film (Mobberley, United Kingdom) using a Carl Zeiss West Germany photomicroscope.

3. RESULTS

The pathology of *C. stegomyiae* in the adult female mosquito *Aedes aegypti* was classified into three stages, early, intermediate, and late, according to the development of the fungal hyphae in resting sporangia and depending on whether blood feeding had occurred.

4. EARLY STAGE

Hyphagen and hyphae are wall-less coenocytic and contain many nuclei (Plate I, Figs. 1 and 2). Hyphae were observed just under the cuticle (Plate I, Fig.1), then between the fat body (Plate I, Figs. 2 and 3), in the hemocoel (Plate I, Fig. 4), around some organs such as the hemopoietic organ, Malpighian tubules, and muscles (Plate I, Figs. 4, 5, and 6).

Gradually, hyphae ramify between the fat body causing signs of lysis (Plate II, Figs. 1, 3, and 5) leading to a depletion of the fat body cells. Similarly, hyphae were distributed around Malpighian tubules (Plate II, Fig. 2), muscles (Plate II, Fig. 3), hemopoietic organ (Plate II, Fig. 4) and tracheole (Plate II, Fig. 6).

Intermediate stage. Body fat was almost absent from some parts of the peripheral area, while others were invaded by hyphae and severely degraded (Plate III, Figs. 1 and 2). Hyphae were concentrated, to some degree, around some organs such as tracheoles (Plate III, Figs. 3 and 4). Moreover, they caused some degradation of the muscle myofibrils (Plate III, Fig. 4). In the hemocoel, however, hyphae started to differentiate into resting sporangia (Plate III, Figs. 5 and 6).

Late stage. Hyphae and resting sporangia

were found at various degrees of maturation displacing much of the fat body and hemocoel (Plate IV, Figs. 1 and 2). In this stage, hyphae were also appressed to the adult female ovary (Plate IV. Fig. 1). As in the case with egg production in healthy adult females, development and maturation of fungal resting sporangia were dependent on the female taking at least one blood meal (Plate IV, Figs. 3-6). Cleavage furrows, meiozoospores, dehiscence slit and gelatinous plugs were first observed in thin-walled resting sporangia at or near the "go" stage in the ovaries (Plate IV, Fig. 6). There also appeared to be some fungus related damage to follicles following the blood meal (Plate IV, Figs. 3-6).

5. DISCUSSION

Early taxonomical studies on the genus *Coelomomyces* were limited with many more species being described after 1940 (Couch et al., 1985). Currently, there are more than 50 described species of Coelomomyces from more than 22 genera and at least 135 species of mosquitoes (*Anopheles, Culex, Psorophora, and Uranotaenia*) and two species of *chironomids* (Couch et al.,1985).

The information on the actual pathology of *Coelomomyces* in mosquito adults was limited. As previously reported the fungal hyphae and hyphagens of *Coelomomyces* species migrate through the penetration tube, infect the cuticular epithelial cells, and then enter the hemocoel (Travland 1979 and Lucarotti 1992).

Hyphagens that first enter the hemocoel originate from hyphae, which migrate during the pupal and young adult stages. Then, they occupy the interstitial spaces of the ovaries (Lucarotti 1992, Lucarotti et al., 2000, Shoulkamy et al., 1997). Previously, *Coelomomyces* has been described as developing in the hemocoel at the expense of the fat body (Couch 1968). The means by which fungal hyphae accomplish this has not been made clear. The sense given in a number or reports was that Coelomomyces hyphae in the hemocoel absorb nutrients directly from the hemocoel and indirectly from the fat body (Couch 1968, Shoulkamy 1997, Shoulkamy et al., 2000). In the present study, it is evident that infection by *C. stegomyiae* was aggressive with hyphae directly invading fat body in addition to muscles, Malpighian tubules, tracheoles, hemopoietic organs, and ovaries.

Federici et al., (1986) and Lucarotti aet al., (1984) reported that both the diploid and haploid hyphae of *C. dodgei* are wall-less, but they are covered by a thin fibrous coat. The absence of rigid wall could allow hyphae to migrate between cells in the affected tissues and to squeeze through openings created by the fungus in the basal lamina and other connective tissues. Consequently, hyphae of *C. stegomyiae* enter and aggregate in the interstitial spaces of the ovaries, but they do not directly infect follicle or germaria cells (Lucarotti 1992, Lucarotti et al.,1988, Lucarotti et al., 2000). Similarly, in larval *A. aegypti C. stegomyiae* hyphae penetrate the basal lamina of a number of tissues, but they do not enter the individual cells of these tissues. These tissues, however, show signs of sever deterioration over the course of infection (Shoulkamy et al., 2000).

Hyphae are transferred to the ovaries at least by tracheole epithelial cells, which harbor the infection (Lucarotti 1992, Shoulkamy 1996). Hyphae migrate into the ovaries; the adult produces tracheoles to meet the increased demand for gaseous exchange during maturation of the ovaries (Wigglesworth 1954, 1959, and 1991). Within 72 hours after eclosion of infected adult females, the ovaries are distended and full of hyphae (Lucarotti et al., 1988, Shoulkamy 1996). These hyphae mature only to rest-

ing sporangia following blood meals in response to 20-hydroxyecdysone (Lucarotti 1987, Lucarotti 1992, Lucarotti et al., 1988, Shoulkamy 1996). In order to fully mature all of the hyphae in the infected ovaries, the females must take more than one blood meal (Lucarotti 1987, Lucarotti et al., 1988, Shoulkamy et al., 2000). Lucarotti (1992), Lucarotti et al., (1988), Shoulkamy (1996), and Shoulkamy et al., (2000) assured the fact that fungal hyphae do not damage the ovarian follicles particularly the follicular epithelium. As this happens, it would disrupt the production of 20 hydroxyecdysone effectively interrupting the hormonal feed back system that controls reproductive behavior in adult female mosquitoes (Hagedorn 1986).

Coelomomyces is part of a complex of biotic and abiotic factors that affects the natural dynamics of mosquito populations. *Coelomomyces* has widely been reported to cause epizootics in mosquito larval populations and it can cycle in the environment. Additionally, infection of adult female mosquitoes with *C. stegomyiae* allows it to be dispersed to new habitats (Lucarotti et al., 1995, Shoulkamy et al., 2000). The fact that *Coelolmomyces* species require alternate hosts to complete their life cycle and that they cannot be cultured in vitro negate their eligibility as microbial adulticides such as Bacillus *thringiensis* var. *isrealensis* (Federici 1993). *Coelomomyces* spp. have been widely reported to cause epizootics environments. For these reasons, *Coelomomyces* species and in particular *C. stegomyiae* remains a candidate for use in classical biological control.

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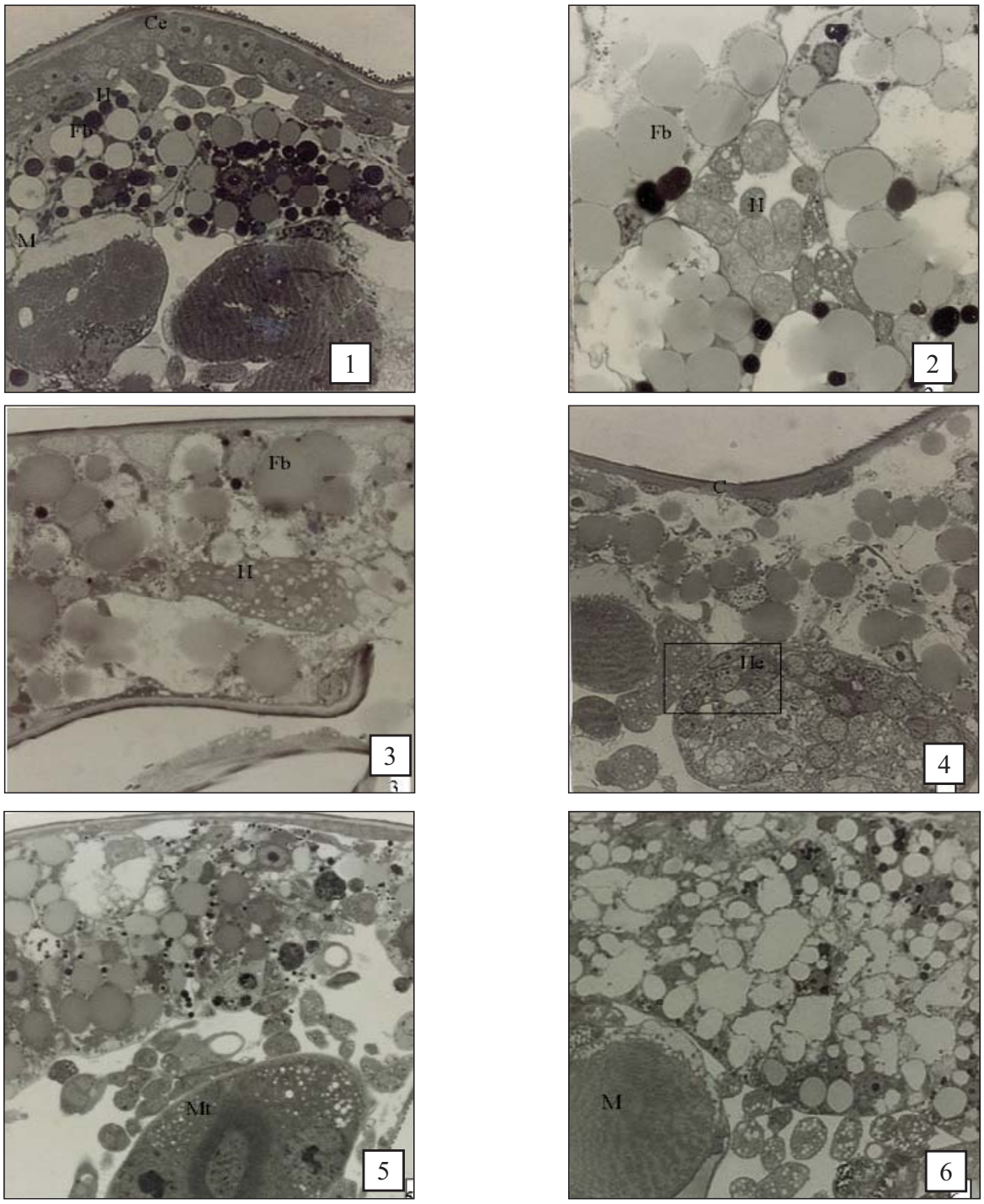

Plate I. *Coelomomyces stegomyiae* and adult female *Aedes aegypti* tissues: mass of hyphae (H) between cuticular epithelium (Ce) and fat body (Fb) (Fig.1, x640); mass of hyphae (H) between lobes of fat body (Fb) in the abdomen of infected adult (Fig. 2, x800); hyphae (H) within the fat body (Fb) (Fig. 3, x800); Hyphae (H)between hemopoietic organs and muscle (Fig. 4, x640); round hyphae (H) floating freely in the hemocoel and around Malpighian tubules (Mt) (Fig. 5, x640); round hyphgen (H) floating freely in the hemocoel between fat body (Fb) and muscles (M) (Fig. 6, x640).

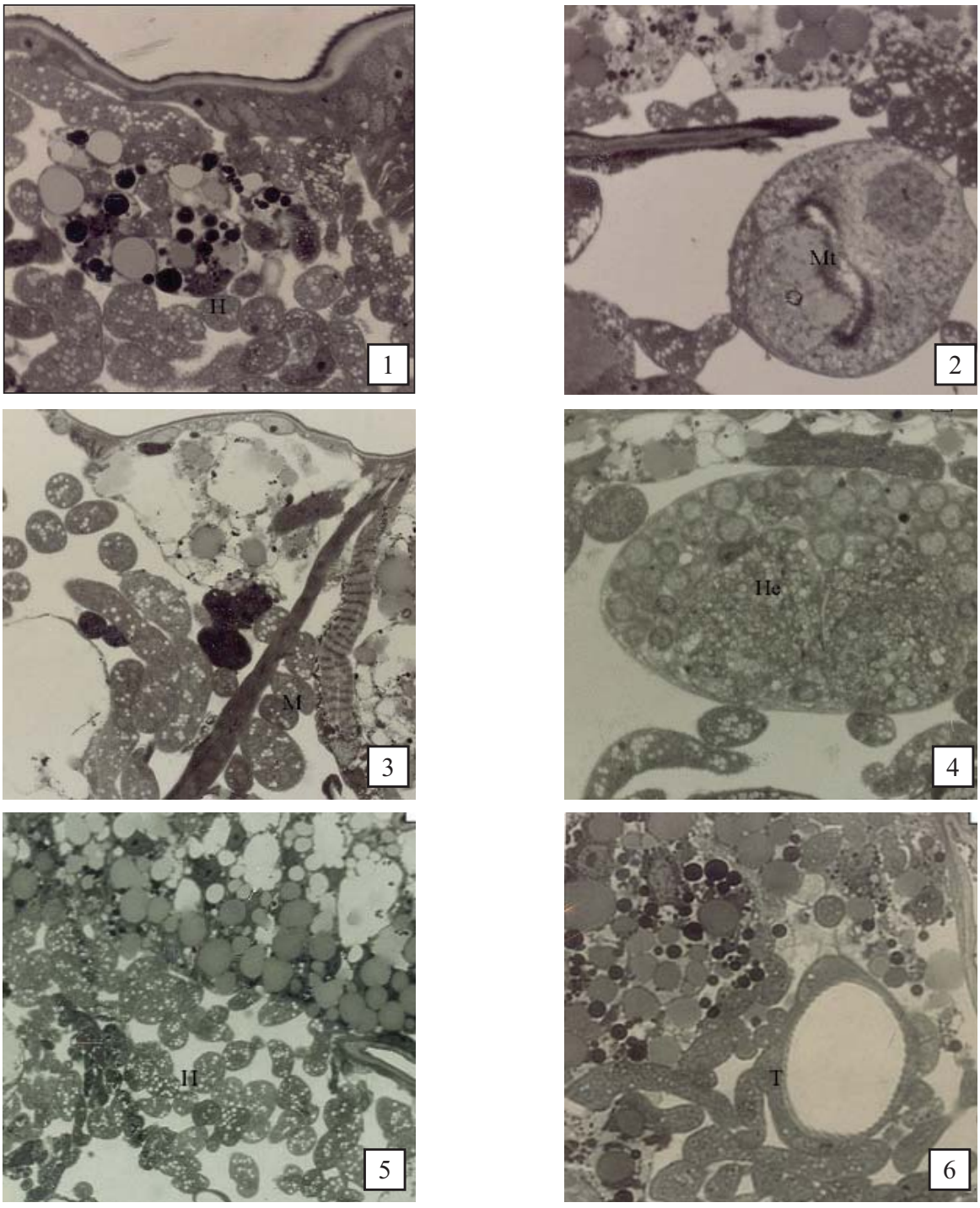

Plate II. *Coelomomyces stegomyiae* and adult female *Aedes aegypti* tissues: elongate hyphae lie within the fat body(Fb) below the cuticle (C) of the adult (Fig. 1); round hyphae (H) floating freely in the hemocoel of the adult and around the Mlpighian tubules (Mt) (Fig. 2); hyphae (H) in the fat body (Fb) and adjacent to the muscle bundles(M) (Fig. 3); elongate hyphae (H) surround the hemopoietic organ (Ho) (Fig. 4); round hyphae (H) floating in the hemocoel and between the tracheole (T) (Fig. 5); elongate hyphae (H) surround the tracheole (T) (Fig. 6). All Figs.X640.

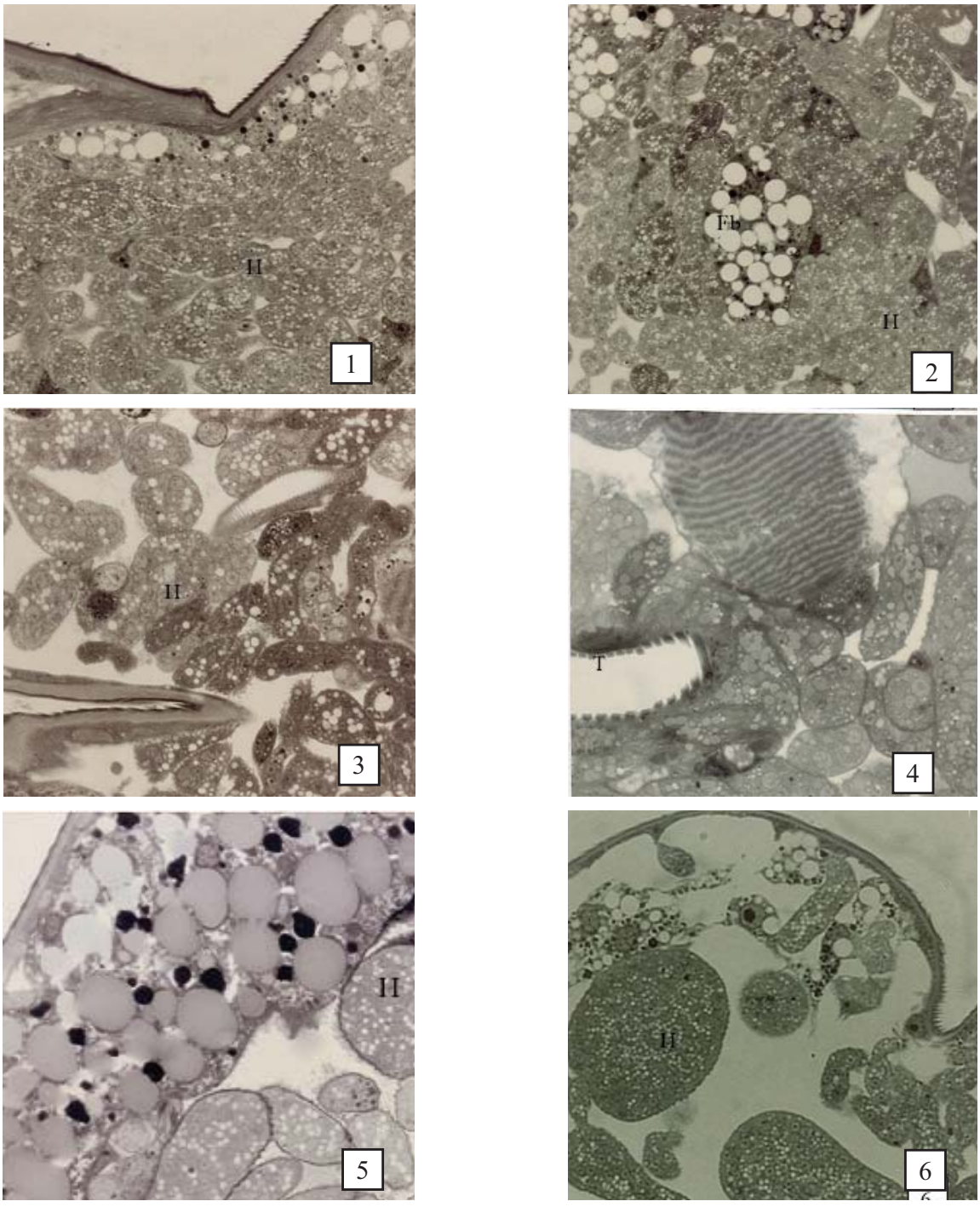

Plate III. *Coelomomyces stegomyiae* and adult female *Aedes aegypti* tissues: hyphae (H) can be seen appressed against the fat body (Fb) in the hemocoel and around the Malpighian tubules (Mt) (Fig. 1, x640); elongate hyphae (H) in the hemocoel surround lipid globules (Lg) from deteriorating fat body (Fig. 2, x640); some hyphae (H) floating in the hemocoel between the muscle (M) and tracheoles (T) (Fig. 3, and 4, x800 and x1000 respectively); hyphae (H) floating freely in the hemocoel and some of them have begun to differentiate to resting sporangia (Rs) (Fig. 5, and 6, x 640).

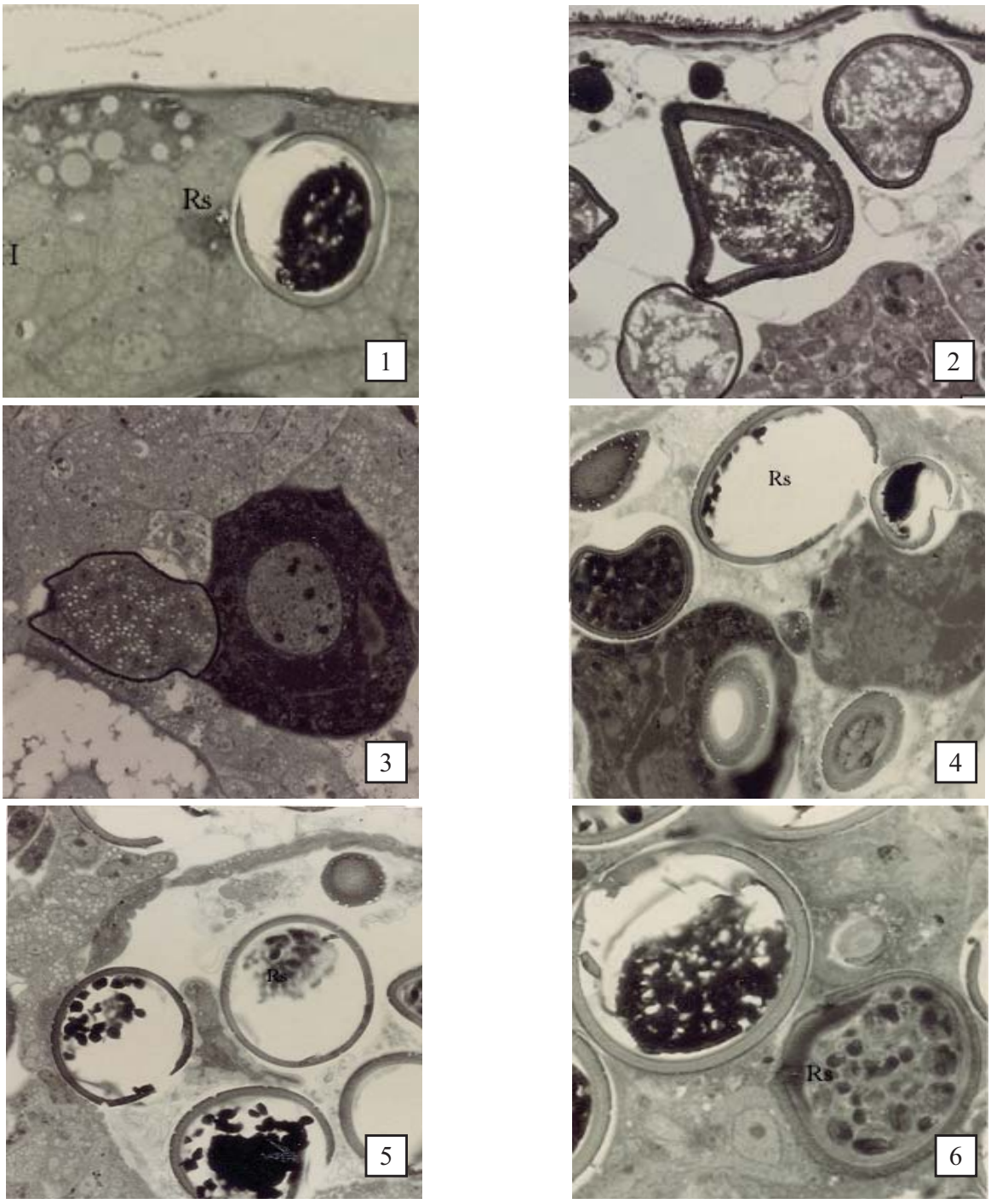

Plate IV. *Coelomomyces stegomyiae* and adult female *Aedes aegypti* tissues: where the fat body (Fb) was invaded by C. stegomyiae hyphae (H): the degree of fat body degradation could be limited or complete (Fig. 1, and 2, x500); ovaries (O) were filled with hyphae and some of un- matured resting sporangia (Rs) appeared attacking the follicle (F) (Fig. 3, x625); some hyphae (H) but not all have matured to resting sporangia (Rs); one of the resting sporangia (Rs) was found inside the follicle (Fig. 4, x625); o varies were filled with resting sporangia (Rs) (Fig. 5, x625); cleavage furrows and meiozoospores (Mz); dehiscence slit could be seen in thin -walled resting sporangia (Fig. 6, x625).

التأثير المرض لفطرة السيلومايسز في الطور اليافع لأنثى الأييدز اجيبتاي

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المُلخّص

تقوم بعض الفطريات من جنس السيلومايسز استيجومايا بإصابة أنثي البعوض من نوع الأييدز اجيبتاي حيث تبدأ الإصابة لطور اليرقة وهي في الطور الرابع ثم ينمو الفطرة داخل هيموسيل اليرقة وتتحول اليرقة المصابة إلي طور الحشرة اليافعة وهي مازالت مصابة بالفطرة وتسلك سلوكها الطبيعي وتأخذ وجبة الدم من العائل الخاص بها. ولفحص مراحل الإصابة في الحشرات اليافعة المصابة بفطرة السيلومايسز يتم تجهيز العينات للفحص تحت المجهر الضوئي و الالكتروني وجد من خلا ل المتابعة بالإضافة إلي إصابة هيفات الفطرة منطقة الجسم الدهني وجد أنها تصيب أيضا مناطق العضلات ، أنابيب ملبيجي والقصيبات والجسم المنتج لكرات الدم وكذلك المبايض . وقد وجد أن هيفات الفطرة تنتشر خلال الجسم الدهني والذي يؤدي في النهاية إلي استنفاذ خلايا الجسم الدهني. هذا يؤدي في النهاية إلي إصابة شديدة لهذه الأنسجة ولهذه الأسباب يتم استخدام فطرة السيلومايسز كأحد أنواع المقاومة البيولوجية لبعوضة الأييدز اجيبتاي

كلمات مفتاحية : المقاومة الحيوية – بعوضة الأييدز اجيبتاي – فطر السيلومومايسز

Some Tachinidae (Diptera: Calyptrata) from South-Western Saudi Arabia

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Abstract

During a survey of the insect fauna of Asir, south-western Saudi Arabia using mainly Malaise traps and sweep nets between 2001 and 2006, ten species of Tachinidae were identified and recorded in this study, eight of which are reported from Saudi Arabia for the first time. Thirteen species of Tachinidae are now known for Saudi Arabia. Comments on the taxonomy and geographical distribution of the species recorded in this study are given. The ten species collected represent tachinids of Palaearctic and Afrotropical affinities. A further new species is reported but a male is required before it can be formally described. The need for further field and laboratory work and surveillance is highlighted.

Keyword: Diptera, Tachinidae, Parasitoid, Biological control, Oestroidea, Calyptrate, Saudi Arabia

1. INTRODUCTION

Tachinidae are poorly known for most of the Middle East. Seven species have been reported from Iraq (Abdul-Rassoul, 1976; Al-Ali, 1977). Al-Houty (1989) reported two species of Tachinidae from Kuwait. Zeegers (2007) recorded 83 species of Tachinidae from Yemen belonging to 62 genera of which 16 species and two genera were newly described. Zeegers (2010) recorded 34 species from the United Arab Emirates. In contrast, Kugler (1979) listed 286 species of Tachinidae from Israel, the West Bank, the Golan Heights and Mt. Hermon, clearly indicating that this family is much more diverse than other studies have shown. The list of these territories has been updated by Cerretti & Freidberg (2009) and now there are 299 species listed in Israel. Al-Ahmadi & Salem (1999) listed only five species of Tachinidae in Saudi Arabia.

The family Tachinidae is a member of the superfamily Oestroidea which includes the families Sarcophagidae, Callphoridae, Oes tridae and Rhinophoridae and Mystacinobi -idae. Tachinidae is one of the largest families of Diptera with almost species 10,000 described species worldwide and many thousands of undescribed species (Irwin, et al., 2003; Stireman, et al., 2006; O'Hara, 2010). The species numbers are especially deceptive because only the faunas of the Palaearctic (Herting & Dely-Draskovits, 1993) and Nearctic regions (O'Hara & Wood, 2004) are relatively known. Tachinidae is an immense and taxonomically difficult family. The taxonomy of Tachinidae is largely based on the morphological characters of its adult flies, but also on reproductive habits and the immature stage. Members of the family Rhinophoridae are superficially very similar to some Tachinidae but they lack or have only a weakly swollen subscutellum. Apart from some members of the tribe Catharosiini, Tachinidae can be easily recognized by their convex subscutellum and row of meral (hypopleural) setae. In the field they are usually easily distinguished from other larger flies by their rapid gait and their outspread

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wings which are also kept outspread when at rest. Some species of Tachinidae are often bare (e.g., subfamily Phasiinae) while most species are often conspicuously bristly (e.g., subfamily Exoristinae). There are four subfamilies of Tachinidae which are recognized currently: Exoristinae, Tachininae, Phasiinae and Dexiinae.

Tachinidae are found in nearly all terrestrial environments and despite their abundance, diversity, and ecological importance, relatively little is known about the evolutionary history, ecology and behaviour of tachinids (Stireman, et al., 2006; O'Hara, 2010).

Tachinidae are classified as koinobiont because they do not kill their hosts immediately after parasitization (Askew & Shaw, 1986; Stireman, et al., 2006) (but there are exceptions: see English-Loeb, 1990). On the other hand, Dindo (2010) stated that Tachinidae do not fit well into the koinobiont/idiobiont dichotomy because only some species show a high degree of physiological adaptation to the host, whereas the larvae of other species grow quickly following their attack on the host and kill the host rapidly, thus behaving more as idiobionts. They parasitize larvae of Lepidoptera, larvae or adults of Coleoptera Others attack adults of Hemiptera (e.g., Phasiinae), larvae of Hymenoptera (sawflies and social Vespoidea) and Orthoptera. Tachinid parasitism of other Diptera is rare. Tachinidae do not parasitize the Isopoda (woodlice) but the related family Rhinophoridae does (Crosskey, 1977; Zeegers, 2008); no species of Tachanidae are known to attack pupal or egg stages of their hosts (Herting, 1960; Stireman, et al., 2006).

Though most tachinids belong to solitary parasitoids, gregarious forms are not rare. Some species of Tachinidae parasitize many species of insects in several orders, for example *Compsilura concinnata* Meigen attacks more than 200 species in 12 families of several insect orders. Many Tachinidae are parasitoids of many important pest insects as well as some millipedes, centipedes and spiders (Vincent, 1985; Wood, 1987, Williams, et al., 1990). Therefore, more than 100 species of tachinids have been used as biological control agents of crop and forest pests (e.g., *Bessaremota* Aldrich, 1925) against the coconut moth *Levuana iridescens* Bethune-Baker, 1906 (Zygaenidae) (for review see: Grenier, 1988; van Emden & Service, 2004; O'Hara, 2008). As many species of Tachinidae typically feed on pollen, they can be important pollinators of some plants, especially of higher elevations in mountains where bees decrease in numbers. Tachinid flies are however regarded as pests by silkworm rearers (e.g., Uzi fly *Exorista sorbillans* Wiedeman, 1830) or when attacking Lepidoptera and Coleoptera that are themselves useful biological agents of noxious weeds.

Egg morphology of tachinids is related to whether the female lays her eggs on the host (externally or internally) or lays her eggs away from the host. A more modern and very relevant introduction is given by O'Hara (2008), which summarized the existing knowledge of the biology and classification of Tachinidae.

The biology of Tachinidae is interesting and diverse especially in (1) the adaptations of the female reproductive system, (2) the morphology of the immature stages (3) in the relations between the larvae and their hosts (for details and more information see Ferrar 1987). However, our knowledge of the ecology of most tachinid species is at best rudimentary, hampered by practical issues such as inability to breed most species in the laboratory. The objective of the present paper is to report the Tachinidae collected in south-western Saudi Arabia, together with new records, world-wide distribution

of the species recorded, some taxonomic remarks and to summarise all the available literature about Tachinidae of Saudi Arabia.

2. MATERIAL AND METHODS

Malaise traps were set up by the author and were operated during time periods between 2001 and 2006 in Saudi Arabia in four localities in Asir (a province of southwest Saudi Arabia): (1) a farm located in the centre of Abha; (2) a farm located in Abha, Hay Al-Sed, Madenate Al-Ameer Sultan; (3) Al-Hudaithy fruit farm in Maraba and (4) Al Rakaba, Alhurytha, 90 km south of Abha. These sites were visited every week to collect the insects and replenish alcohol in the collecting containers. A collection of voucher specimens of all species collected were deposited in the National Museum of Wales, Cardiff (NMWC). Available specimens were also deposited in the Research Centre for Environmental Studies, Jazan University (RCES). Specimens of Tachinidae were identified to species level by Dr Theo Zeegers using van Emden (1945; 1947; 1960) and Mesnil (1944-1945). When necessary genitalia dissections were made and placed in microvials beneath specimens. The distribution sections and nomenclature of the species of Tachnidae reported in this study are based on Crosskey (1980) and Herting & Dely-Draskovits (1993). The Tachinidae classification to subfamilies is according to Herting & Dely-Draskovits (1993). The countries of species distribution are arranged alphabetically and according to their geographical zones. References to the original descriptions of the genera and species, together with details of synonymy will be found in Crosskey (1980) and Herting & Dely-Draskovits (1993) and are not repeated here.

3. RESULTS

Ten known species of Tachinidae were identified during this study, eight of which represent new country records for Saudi Arabia. The total number of species of Tachinidae known for Saudi Arabia is 13.

Subfamily Dexiinae

***Periscepsia carbonaria* (Panzer, 1798)**

Musca carbonaria (Panzer, 1798). - *Fauna Insectorum Germaniae* 54:15.

Five synonyms are listed in Herting & Dely-Draskovits (1993:379)

Specimens examined: Saudi Arabia: 2 ♀, Abha Farm Centre, 18o 50' N´ 42o 30' E, 2150m, Malaise trap, 3rd March.-3rd June, 2001, H.A. Dawah, NMWC, 1♀, RCES.

Distribution: This is the first record of this species from Saudi Arabia. It was described from Austria and further recorded from the following areas (Crosskey, 1980; Kugler ,1979; Herting & Dely-Draskovits, 1993; Zeegers, 2007): Palaearctic Region: Europe Israel. Afrotropical Region: widespread north East of Africa to southern Africa, Yemen. Oriental Region: India.

Remark: Members of the subfamily Dexiinae are exclusively parasitoids of Lepidoptera and Coleoptera.

***Stomina* sp.** (Plate 1)

Specimens examined: Saudi Arabia: 1♀, Abha Farm Centre, 18o 50' N´ 42o 30' E, 2150m, Malaise trap, 3rd March.-3rd June, 2001, H.A. Dawah, NMWC.

Remark: The female specimen of *Stomina* represents a new species, but a male is required before it can be formally described as a new species (T. Zeegers pers. comm.).

4. SUBFAMILY EXORISTINAE

***Gonia bimaculata* Wiedemann, 1819**

Gonia bimaculata Wiedemann, 1819. - *Zoologisches Magazin Kiel* 1(3): 25 Herting & Dely-Draskovits (1993:258) list three synonyms.

Specimens examined: Saudi Arabia: 1♀, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´ 42o 40' E, 2150m, Mal-

aise trap, 25 Feb.-25th May. 2002, H.A. Dawah, NMWC.

Distribution: This species was previously recorded from Saudi Arabia by Shalaby (1961). This species was originally described from South Africa and further recorded from the following areas: Palaearctic Region: Canary Islands, Southern Europe, China, Israel, Tunisia. Afrotropical Region widespread (excluding W. Africa): Yemen. Oriental Region: China (Kugler, 1979; Crosskey, 1980; Herting & Dely-Draskovits, 1993; Zeegers, 2007).

***Drino (Palexorista) parachrysops* (Bezzi, 1925)**

Sturmia (Palexorista) parachrysops Bezzi, 1925. - *Bulletin of Entomological Research* 16: 114

Specimens examined: Saudi Arabia: 1♀, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´42o 40' E, 2150m, Malaise trap, 25 Feb.-25th May. 2002, H.A. Dawah, NMWC.

Distribution: This is the first record for Saudi Arabia. It was described from Malaya and further recorded from the following areas (Crosskey, 1980; Zeegers, 2007): Afrotropical Region: Ghana, Kenya, Mali, Nigeria, Yemen, Senegal. Oriental Region: Sri Lanka, India.

***Dolichocolon paradoxum* Brauer & Bergenstamm, 1889**

Dolichocolon paradoxum Brauer & Bergenstamm 1889. - *Denkschriften der Kaiserlichen Akademie der Wissenschaften. Wien. Mathematisch- Naturwissenschaften Klasse* 56: 100 and 165.

One synonym is listed in Herting & Dely-Draskovits (1993:251)

Specimens examined: Saudi Arabia: 1♀, Abha Farm Centre, 18o 50' N´42o 30' E, 2150m, Malaise trap, 3rd March.-3rd June, 2001, H.A. Dawah, NMWC

Distribution: This is the first record of the genus for Saudi Arabia.

Remark: Dr P. Cerretti (per.comm.) has kindly drawn to my attention the fact that more than a single species exists in material identified by this name. Separation is possible only by the examination of males. Regrettably, the single specimen listed here is a female, and so the identification cannot be performed to species level with absolute certainty There are some specimens identified as *D. paradoxum* from Iraq in the Natural History Museum, London.

5. SUBFAMILY PHASIINAE

***Leucostoma obsidianum* (Wiedemann, 1830)**

Tachina obsidianum Wiedemann, 1830. - *Aussereuropäische zweiflügelige Insekten* 2 :341

One synonym is listed in Herting & Dely-Draskovits (1993: 420)

Specimens examined: Saudi Arabia: 2♀, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´42o 40' E, 2150m, Malaise trap, 25 Feb.-25th May. 2002, H.A. Dawah, 1♀, NMWC, 1♀, RCES.

Distribution: This is the first record of this species in Saudi Arabia. It was described from Sudan and further recorded from the following areas (Herting & Dely-Draskovits, 1993; Zeegers, 2007): Palaearctic Region: Europe, Israel. Afrotropical Region: Yemen.

Remark: Members of the subfamily Phasiinae are exclusively parasitoids of Hemiptera. In *Leucostoma* Meigen 1803 females have pincer-like projections on the apex of the abdomen which are part of the genital system (Plate 2). These can be diagnostic in structure. Unlike parasitic Hymenoptera, tachinids lack a primitive piercing ovipositor, with the exception of a few groups (most phasiinae, e.g., *L. obsidianum*), in which piercing structures have evolved from modified sternites.

***Besseria longicornis* Zeegers, 2007**

Besseria longicornis Zeegers, 2007. - *Fauna of Arabia* 23: 402.
Specimen examined: Saudi Arabia: 1♂, Aseer Al Rakaba Alhurytha, 90 km South of Abha, 17o 07' N´ 42o 39' E, 60m, Malaise trap, 3rd Feb. 2006, H.A. Dawah, NMWC
Distribution: This is the first record of this species from Saudi Arabia. It was described recently from Yemen by Zeegers (2007).

6. SUBFAMILY TACHININAE

***Fischeria bicolor* Robineau-Desvoidy, 1830**

Fischeria bicolor Robineau-Desvoidy, 1830. - *Essai Myod*: 101.
Four synonyms are listed in Herting & Dely-Draskovits (1993:344)
Specimens examined: Saudi Arabia: 1♂, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´ 42o 40' E, 2150m, Malaise trap, 25 Feb.-25th May. 2002, H.A. Dawah & M.A. Abdullah, NMWC.
Distribution: This is the first record for Saudi Arabia. It was described from France and further recorded from the following areas (Crosskey, 1980; Kugler, 1979): Palaearctic Region: Europe, Iran, Israel, Malta, and Italy.
Remarks: This species is a member of the Tachininae which are parasitoids of Lepidoptera and Coleoptera. *Fischeria* (Robineau-Desvoidy, 1830) is placed as a synonym of *Leskia* (Robineau-Desvoidy, 1830) in Crosskey (1980:844). However, it is recognized as a valid genus by Tschorsnig & Richter (1998). Kugler (1979) reported that he reared *F. bicolor* from pyralid moth larvae in Israel.

***Peleteria varia* (Fabricius, 1794)**

Musca varia Fabricius, 1794. – *Entomologia systematica emendate et aucta. Secandum classes, ordines, genera, species adjectis synonimis, locis, observationibus,* descriptionibus. Hafnine, 4: 327.
Three synonyms are listed in Herting & Dely-Draskovits (1993:344)
Specimens examined: Saudi Arabia: 1 ♂, Maraba, 60 Km South of Abha, 17o 54' N´ 42o 23' E, 80m, Malaise trap; 1st-31st December, 2004; H.A. Dawah, NMWC
Distribution: This is the first record of this species in Saudi Arabia. It was described from "India Orientalis" and further recorded from the following areas (Herting & Dely-Draskovits, 1993): Palaearctic Region: Europe, North Africa (Algeria),

***Mintho praeceps* (Scopoli, 1763)**

Musca praeceps Scopoli, 1763. - *Methodo Linnaeana*: 333.
Five synonyms are listed in Crosskey (1980)
Specimens examined: Saudi Arabia: 1♂, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´ 42o 40' E, 2150m, Malaise trap, 25 Feb.-25th May. 2002, H.A. Dawah , NMWC.
Distributions: This is the first record for Saudi Arabia. It was described from Spain and further recorded from the following areas (Kugler, 1979; Herting and Dely-Draskovits, 1993; Zeeger, 2007): Palaearctic Region: Algeria, Canary Islands, Egypt, Europe, Israel, Morocco, North Africa, Yemen.

***Microphthalma europaea* Egger, 1860**

Microphthalma evropaea Egger, 1860. - *Verhandlungen des Zoologisch-Botanischen Vereins in Wien*, 10:801
Four synonyms are listed in Herting & Dely-Draskovits (1993: 351)
Specimen examined: Saudi Arabia: 10♀, Abha, Madinat al-Ameer Sultan, 15 Km E of Abha, 18o 50' N´ 42o 40' E, 2150m, Malaise trap, 25 Feb.-25th May. 2002, H.A. Dawah, 8♀, NMWC, 2♀, RCES.
Distribution: This is the first record of this species in Saudi Arabia. It was described from Austria and further recorded from the Palaearctic Region: Algeria, Europe, North Africa (Herting & Dely-Draskovits, 1993).
Discussion:

In this survey of the Tachinidae of south-western Saudi Arabia, ten species were found, eight of which represent new country records for Saudi Arabia. With these records, a total of 13 species of Tachinidae are currently known to occur in Saudi Arabia (Table 1). Interestingly, the ten species collected represent tachinid species of equal Palaearctic and Afrotropical affinities. More than 2,600 species of Tachinidae are known from the Palaearctic and Afrotropical regions (O'Hara, 2010), an indication that the potential of the number of species that occur in Saudi Arabia is much higher than currently known. For example the number of species of Tachinidae recorded from Israel or Yemen is much higher, and may reflect the actual diversity of these flies. It is expected that many species of the Tachinidae from the surrounding countries will eventually also be found in Saudi Arabia in suitable habitats where their hosts occur. The taxonomy of the Tachinidae is difficult, and the use of all approaches: external and internal morphological characters, molecular techniques (Vogler & Monaghan, 2007; Valentini, et al., 2009), mating behaviour and host preferences (Claridge, 2008) is needed to clarify the status of morphologically similar species or species complexes (Schlick-Steiner, et al., 2007).

Future studies on the Tachinidae (also see Stireman et al., 2006) should be focused on: (1) examining the genetic structure of tachinid populations and how this may vary according to geography and host use (Sanchez & Cardé, 1998) using molecular biology as this could provide a great deal of information on the evolutionary distribution and host relationships of Tachinidae; (2) evolutionary relationships of.tachinidae in order to understand the evolution of morphological behavioural and ecological traits in the family using phytogenetic analyses as well as basic taxonomic studies; (3) study of the tritrophic interactions involving tachinids, herbivorous hosts and plants in determining the ecological structure and dynamics of tachinid-host interactions and their larger roles in the structure and dynamics of natural ecosystems in order to understand how and why parasitism frequencies and richness of parasitoid species vary among host species (Lil, et al., 2002; Singer, et al., 2004); (4) mating systems, host preference and utilization and habitats requirements; (5) mechanisms by which most tachinids locate and select hosts; (6) study of the importance of adult resources such as nectar, salts, leaf exudates or potential sources of protein.

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Table 1 List of Tachinidae species recorded from Saudi Arabia:

(A: Afrotropical; P: Palaearctic; O: Oriental).

Species	References	Origin
Subfamily Exoristinae		
Drino imberbis Wiedemann	Martin 1972	A, P
Gonia capitata (de Geer)	Dabbour & Hamad 1982	A, P
Gonia bimaculata (Wiedemann)	Shalaby 1961: this study	A, P
Drino (Palexorista) parachrysops Bezzi	Dabbour 1979; this study	A, O
Dolichocolon paradoxum Br. & Ber.	This study	A, P
Subfamily Tachininae		
Tachina larvarum L.	Dabbour & Hamad 1982	A
Fischeria bicolor Robineau-Desvoidy	This study	P
Peleteria varia (Fabricius,)	This study	O, P
Mintho compressa (Fabricius)	This study	P
Microphthalma europaea Egger	This study	P
Subfamily Phasiinae		
Leucostoma obsidianum (Wiedemann)	This study	A, P
Besseria longicornis Zeegers	This study	A , P
Subfamily Dexiinae		
Periscepsia carbonaria (Panzer)	This study	A, P, O

List of plates:

Plate 1: shows the female of *Stomina* sp. (photograph, R. Esteves)

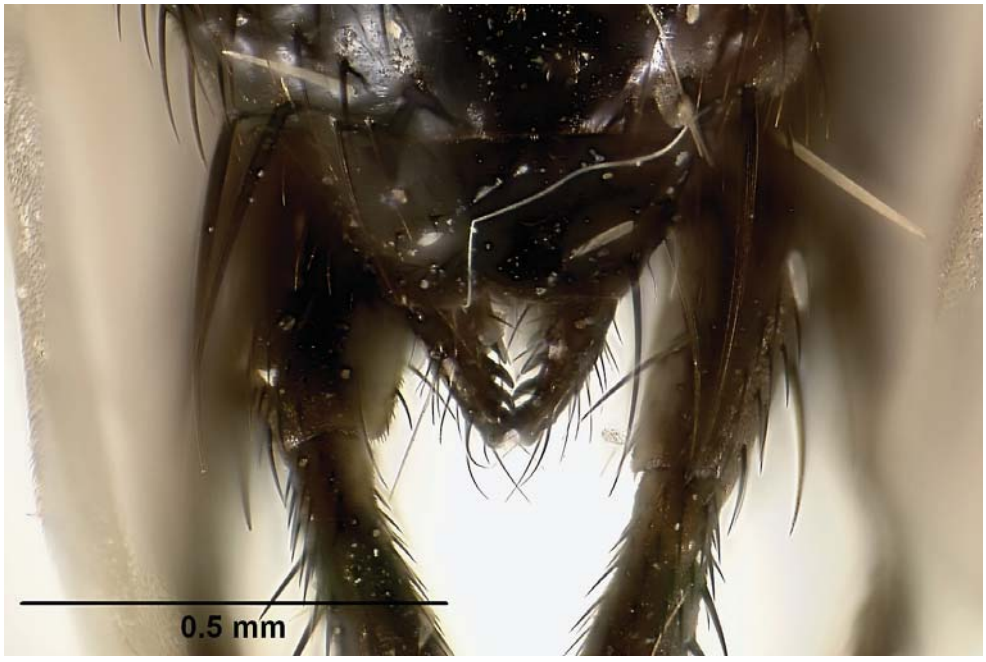

Plate 2: shows the pincer-like projections on the apex of the abdomen of female of *L. obsidianum* which are part of the genital system (photograph, J. Turner)

تسجيل لبعض أنواع التكانيدس Tachinidae التابعة لرتبة ثنائية الأجنحة من جنوب غرب المملكة العربية السعودية

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المُلخّص

تم جمع وتصنيف عشرة أنواع من الحشرات الطفيلية أثناء عملية مسح للحشرات في منطقة عسير جنوب غرب المملكة العربية السعودية تعود إلى عائلة **Tachinidae** ذات الأهمية في المكافحة الحيوية ، وبذلك يصبح عدد أنواع **Tachinidae** المسجلة في المملكة العربية السعودية ثلاثة عشر نوعاً . تضمن البحث معلومات حول التوزيع الجغرافي للأنواع المسجلة إضافة إلى بعض الملاحظات التصنيفية وتوصيات حول الدراسات المستقبلية لهذه العائلة. تبين هذه الدراسة أن عدد الأنواع ذات الأصول الإفريقية الاستوائية متقارب مع عدد الأنواع ذات الأصول القطبية القديمة. سجلت هذه الدراسة نوعاً جديداً للعلم ولكنه لم يوصف في هذا البحث لعدم الحصول على أفراد تمثل الذكور المهمة في عملية وصف النوع الجديد.

كلمات مفتاحية: ثنائية الأجنحة – مكافحة حيوية – التكانيدس - الحشرات الطفيلية - جنوب غرب المملكة.

Comparative Analysis of Withania Somnifera and Rhus Coriaria on Hyperglycemia and Insulin Sensitivity in Type 2 Diabetic Rats

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Abstract

We investigated and compared the effects of an aqueous extract of Withania somnifera (L.) Dunal and methanolic extract of Rhus coriaria Rhus Wriaria L. on hyperglycemia and insulin sensitivity in type 2 diabetic rats. Type 2 diabetes was induced by single intraperitoneal injection of streptozotocin (STZ, 100 mg/kg) to 2 days old rat pups. W. somnifera & R. coriaria (200 mg/kg and 400 mg/kg) was administered orally once a day for 5 weeks after the animals were confirmed diabetic (i.e., 90 days after STZ injection). A group of citrate control rats were also maintained which had received citrate buffer on the 2nd day after birth. Significant increases in blood glucose, glycosylated hemoglobin (HbA1c) and serum insulin levels were observed in type 2 diabetic control rats. Treatment with W. somnifera & R. coriaria reduced the elevated levels of blood glucose, HbA1c and insulin in the type 2 diabetic rats. An oral glucose tolerance test (OGTT) was also performed on the same groups, in which we found a significant improvement in glucose tolerance in the rats treated with W. somnifera & R. coriaria. The insulin sensitivity was assessed for both peripheral insulin resistance and hepatic insulin resistance. W. somnifera & R. coriaria treatment significantly improved the insulin sensitivity index (KITT) which was significantly decreased in type 2 diabetic control rats. There was a significant rise in homeostasis model assessment of insulin resistance (HOMA-R) in type 2 diabetic control rats whereas W. somnifera & R. coriaria treatment significantly prevented the rise in HOMA-R in type 2 diabetic treated rats. Based on our findings, it is possible to postulate that R. coriaria is more effective than W. somnifera in controlling hyperinsulinemia and glucose tolerance and improved insulin sensitivity thus normalizing hyperglycemia in type 2 diabetic rats.

Keywords: *Rhus coriaria*; *Withania somnifera* hyperglycemia; Streptozotocin; Insulin sensitivity; Diabetes mellitus

1. INTRODUCTION

Type 2 diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder which results from defects in insulin secretion (Kahn, 2001) on one side, and insulin resistance on the other (Polonsky et al., 1996). The progression of type 2 DM begins with an impairment of glucose tolerance (Zimmet et al., 2003) and is often associated with a state of insulin resistance, which means insulin that is secreted by the β-cells and bound to the liver, muscle and fat cells is subnormally efficacious in carrying out its metabolic actions (Robertson et al., 2006). In recent years there has been an upsurge in the clinical use of indigenous drugs. Management of type 2 DM without any side effects is still a challenge to the medical system. The conventional pharmacological treatments for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Kim et al., 2006). Medicinal plants

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with antidiabetic activities were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects (Halim et al., 2002). This leads to increasing demand for natural products which have antidiabetic activity with fewer side effects and are relatively economical as compared to oral hypoglycaemic agents. It is assumed that herbal medicine can only be effective as an alternative to oral hypoglycaemic agents in the treatment of type 2 DM, where pancreatic islets are not totally destroyed.

Withania somnifera Dunal (Family: Solanaceae), commonly known as ashwagandha is widely used in the Ayurvedic system of medicine in India. It is an official drug and is mentioned in the Indian Pharmacopoeia (1985). Several studies of this plant indicate that it possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hematopoietic and rejuvenating properties besides its positive influence on the endocrine, cardiopulmonary and central nervous system (Ghosal et al., 1989; Bhattacharya et al., 1995; Mishra et al., 2000). In a previous report (Anadulla et al., 2000), it was found that *Withania somnifera* reduces blood glucose level in mild type 2 diabetic subjects. An Ayurvedic herbal formulation (Tarsina) containing *Withania somnifera* as one of the ingredients have been found to attenuate STZ-induced hyperglycaemia and pancreatic islet superoxide dismutase activity in type 1 diabetic rats (Bhattacharya et al., 1997). Dried fruit extract of *Withania coagulans* (Stocks) Dunal (other species of Withania) has been shown to have hypoglycaemic activity in type 1 diabetic rats (Hemlatha et al., 2004).

Rhus coriaria L. (Family: Anacardiaceae), commonly known as sumac (also spelled as sumach) is a well-known spice in the Middle-East and grown in the central region of Turkey. Maulyanov et al., (1997) has reported that sumac contains flavonols, phenolic acids, hydrolysable tannins, anthocyans, and organic acids. Phytochemicals in *Rhus coriaria* are being used as antibacterial, antidiarrhoeal, antispasmodic, antiviral, astringent, candidicide, hepatoprotective, antigastric, anti-inflammatory, antioxidant, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitor due to their contents of ellagic acid, gallic acid, quercetin, isoquercitrin, myricetin and tannic acid (Duke et al., 2003). Recently, the hypoglycaemic efficacy of sumac (*R.coriaria* L.) has been investigated through inhibition of a glycoside hydrolase: alpha-amylase in the treatment and prevention of diabetes (Giancarlo et al., 2006). Methanolic extract (FROM THE water-soluble part) of *Rhus coriaria* was found to be an uncompetitive inhibitor of xanthine oxidase and scavenger of superoxide radicals (Candan, 2003).

Hence, the present study was undertaken to evaluate and compare the effects of *Withania somnifera* and *Rhus coriaria* on hyperinsulinemia, glucose intolerance and insulin sensitivity in type 2 diabetic rats.

2. MATERIALS AND METHODS

Animals

Healthy albino Wistar rats were kept for breeding. The animals were maintained under controlled conditions of illumination (12 hr light/12 hr darkness) and temperature between 20-25 (^{0}C). They were housed under ideal laboratory conditions, maintained on A standard pellet diet and given water ad libitum throughout the experimental period.

Preparation of plant extract

R.coriaria L. seeds were collected and washed with water and dried in shade. Dried *Rhus coriaria* seed was extracted with methanol at room temperature three times with 5 volumes of methanol (w/v). The solvent was evaporated at 35-40 oC under reduced pressure of -760 mmHg to give methanolic

extract, yielding approximately 10% (w/w). A dark semi-solid (greenish black) material was obtained. It was stored at 4 (^{0}C) until used. When needed the residual extract was suspended in distilled water and used in the study.

Drugs and Chemicals

Standardized powdered, aqueous root extract of *W.somnifera* (Batch No. *W. somnifera* /05002) was a gift sample by Natural Remedies, Bangalore, India. It was stored at 4 (^{0}C) until used. When needed the residual extract was dissolved in distilled water and used in the study. It contains total withanolides (3.9% w/w). Streptozotocin was procured from Sigma Aldrich, USA. The enzyme-linked immunosorbent assay (ELISA) kit for insulin assay was purchased from Mercodia, Sweden. All the other biochemicals and chemicals used for the experiment were of analytical grade.

Induction of diabetes

To induce type 2 diabetes, STZ (100 mg/kg) in citrate buffer (pH-4.5) was administered intraperitoneally to 2 days old rat pups (Shinde and Goyal, 2003). Another group of pups received only citrate buffer on the 2nd day of their birth. All the surviving pups were kept (mortality ≤ 30%) to adulthood. 90 days after STZ treatment, the development of diabetes was confirmed by measuring blood glucose level. Rats with fasting blood glucose levels of 200 mg/dl or higher were considered to be diabetic.

Experimental design

The rats were divided into eight groups consisting of six animals in each group as follows :

• Group I: Citrate control, received citrate buffer (0.1 ml/kg, i.p)
• Group II: Type 2 diabetic control, received STZ in a single dose (100 mg/kg, i.p)
• Group III: *W. somnifera* only treated rats, received *W. somnifera* (400 mg/kg, p.o)
• Group IV: Type 2 diabetic treated rats, received *W. somnifera* (200 mg/kg, p.o)
• Group V: Type 2 diabetic treated rats, received *W. somnifera* (400 mg/kg, p.o)
• Group VI: *R. coriaria* only treated rats, received *R. coriaria* (400 mg/kg, p.o)
• Group VII: Type 2 diabetic treated rats, received *R. coriaria* (200 mg/kg, p.o)
• Group VIII: Type 2 diabetic treated rats, received *R. coriaria* (400 mg/kg, p.o)

W. somnifera (200 mg/kg and 400 mg/kg; Visavadiya & Narasimhacharya 2007) was dissolved in water and given until the end of the study (5 weeks) to groups III, IV and V animals. *Rhus coriaria* (200 mg/kg and 400 mg/kg) was also dissolved in water and given until the end of the study (5 weeks) to groups VI, VII and VIII animals. On the last day of experiment, blood samples were collected for biochemical estimations.

Biochemical Parameters Studied

Glucose was estimated by glucose oxidase method (Braham et al., 1972), glycosylated haemoglobin was estimated by Bannon (1982). Plasma insulin level was assayed by ELISA kit (Morgan and Lazarow, 1963). Oral glucose tolerance test (OGTT) was measured according to the method of Pari and Saravanan (2002). Insulin sensitivity index (KITT) was measured for the determination of peripheral insulin resistance (Murali et al., 2002). Homeostasis model assessment of insulin resistance (HOMA-R) was calculated using fasting blood glucose (FBG) and fasting insulin (FI) level and was used for the determination of hepatic insulin resistance (Uno et al., 2005).

Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Karmer post hoc test was applied to identify significance among groups. $P< 0.05$ was considered to be statistically signifi-

cant.

3.RESULTS

Effect of *W. somnifera* and *R. coriaria* on hyperglycemia in type 2 diabetic rats

Table 1 shows the effect of *W. somnifera* and *R. coriaria* on blood glucose levels. Significant ($P < 0.001$) increase in blood glucose levels were observed in type 2 diabetic control rats when compared with citrate control rats. Oral administration of *W. somnifera* and *R. coriaria* at two doses (200 mg/kg and 400 mg/kg) reduced the blood glucose levels significantly ($P < 0.001$) in a dose-dependent manner when compared with type 2 diabetic control rats. *R. coriaria* was found to be more effective than *W. somnifera* in controlling hyperglycemia. *W. somnifera* and *R. coriaria* (400 mg/kg) per se effect did not produce any significant ($P < 0.001$) change in the blood glucose levels when compared with citrate control rats.

Effect of *W. somnifera* and *R. coriaria* on HbA1c levels in type 2 diabetic rats

Table 1 shows the effect of *W. somnifera* and *R. coriaria* on glycosylated haemoglobin levels. It was observed that type 2 diabetic control rats showed significant ($P < 0.001$) increase in HbA1c levels when compared with citrate control rats. Oral administration of *W. somnifera* and *Rhus coriaria* at two doses (200 mg/kg and 400 mg/kg) decreased the HbA1c levels significantly ($P < 0.01$) in a dose-dependent manner when compared with type 2 diabetic control rats. The effect of *Rhus coriaria* on HbA1c was greater than those of *W. somnifera*. There was no significant change in HbA1c levels of *W. somnifera* and *R. coriaria* (400 mg/kg) per se treated rats when compared with citrate control rats. The data are expressed in mean ± S.E.; n=6 in each group. $^{x}P<0.001$ compared with the corresponding value for citrate control rats (group II vs I). yP<0.001 compared with the corresponding value for type 2 diabetic control rats (group II vs IV, V, VII, VIII). *P<0.01 compared with the corresponding value between *W. somnifera* & *R. coriaria* (200 mg/kg; group IV vs VII). nsP>0.05 compared with the corresponding value between *W. somnifera* & *R. coriaria* (400 mg/kg; group V vs VIII)

Effect of *W. somnifera* and *R. coriaria* on OGTT in type 2 diabetic rats

Table 2 shows the blood glucose levels of citrate control, type 2 diabetic control, *W. somnifera* and *R. coriaria* treated rats after oral administration of glucose (2 gm/kg). In type 2 diabetic control rats the peak increase in blood glucose levels were observed after 1 h. The blood glucose levels remained high over the next one hour. *W. somnifera* and *R. coriaria* treated rats showed significant ($P < 0.01$) decrease in blood glucose levels at 1 and 2 h when compared with type 2 diabetic control rats. The effect was most pronounced at 2 h intervals. *R. coriaria* was found to be more effective in improving glucose tolerance than *W. somnifera*. *W. somnifera* and *R. coriaria* (400 mg/kg) per se treatment did not produce any significant change in the blood glucose levels at 1 and 2 h during OGTT when compared with citrate control rats.

The data are expressed in mean ± S.E.; n=6 in each group. xP<0.001 compared with the corresponding value for citrate control rats (group II vs I). yP<0.001 compared with the corresponding value for type 2 diabetic control rats (group II vs IV, V, VII, VIII).

Effect of *W. somnifera* and *R. coriaria* on insulin levels in type 2 diabetic rats

Table 3 shows the effect of *W. somnifera* and *R. coriaria* on insulin levels. Hyperinsulinemia was observed in type 2 diabetic control rats when compared with citrate control rats. *W. somnifera* and *R. coriaria* treatment significantly ($P < 0.01$) reduced the elevated levels of insulin when compared with type 2 diabetic control rats. *R. coriaria* was found to be more effective than *W. somnifera* in

preventing hyperinsulinemia. *W. somnifera* and *R. coriaria* (400 mg/kg) per se treatment did not induce any significant change in the levels of insulin.

Effect of *W. somnifera* and *R. coriaria* on insulin sensitivity in type 2 diabetic rats

Table 3 shows the levels of K^{ITT}, an index of insulin sensitivity and HOMA-R, an index of hepatic insulin resistance. Type 2 diabetic control rats showed significant decrease in K^{ITT} levels with a significant increase in HOMA-R levels when compared with citrate control rats. Treatment with *W. somnifera* and *R. coriaria* significantly ($P < 0.001$) increased the levels of K^{ITT} and prevented rises in HOMA-R levels when compared with type 2 diabetic control rats. The effect of *R. coriaria* on improving the insulin sensitivity was found to be more effective than those of *W. somnifera*. There was no significant change in the levels of K^{ITT} and HOMA-R in *W. somnifera* and *R. coriaria* (400 mg/kg) per se treated rats when compared with citrate control rats.

The data are expressed in mean ± S.E.; n=6 in each group. xP<0.001 compared with the corresponding value for citrate control rats (group II vs I). yP<0.001 compared with the corresponding value for type 2 diabetic control rats (group II vs IV, V, VII, VIII). *P<0.01 compared with the corresponding value between *W. somnifera* & *R. coriaria* (200 & 400 mg/kg; group IV vs VII & V vs VIII). **P<0.001 compared with the corresponding value between *W. somnifera* & *R. coriaria* (200 & 400 mg/kg; group IV vs VII & V vs VIII).

4. DISCUSSION

Type 2 diabetes mellitus results from a combination of tissue resistance (or insulin sensitivity) to insulin action and an adequate compensatory insulin secretory response (American Diabetes Association, 1999). Treatment that is inadequate or instituted too late predisposes the affected individual not only to the basic metabolic disturbances but also to a number of serious complications of diabetes. STZ is frequently used to induce DM in experimental animals (Szkudelski, 2001; Yamagishi et al., 2001). Although, it is generally accepted that the cytotoxicity produced by STZ depends on

Table 1: Effect of *W. somnifera* and *R. coriaria* on blood glucose and glycosylated haemoglobin levels in type 2 diabetic rats.

Groups	Treatment	Blood Glucose (mg/dl)	Glycosylated Haemoglobin (%)
I	Citrate Buffer (1 ml/kg, i.p)	97.18±3.02	5.70±0.265
II	STZ (100 mg/kg, i.p)	324.66±10.87^{x}	12.18±0.322^{x}
III	Only WS (400 mg/kg, p.o)	98.60±1.16	5.67±0.155
IV	Type 2 diabetic + WS (200 mg/kg, p.o)	151.01±4.08$^{y, *}$	9.30±0.118$^{y, *}$
V	Type 2 diabetic + WS (400 mg/kg, p.o)	121.28±1.80$^{y, ns}$	6.95±0.169$^{y, ns}$
VI	Only RC (400 mg/kg, p.o)	96.66±1.89	5.90±0.134
VII	Type 2 diabetic + RC (200 mg/kg, p.o)	139.65±1.89$^{y, *}$	8.87±0.109$^{y, *}$
VIII	Type 2 diabetic + RC (400 mg/kg, p.o)	117.08±1.87$^{y, ns}$	6.83±0.129$^{y, ns}$

DNA alkylation and subsequent activation of poly ADP-ribose synthetase that causes rapid and lethal depletion of NAD in pancreatic islets (Bennet et al., 1981; Bolzan et al., 2002), several lines of evidence indicate that the free radicals may play an essential role in the mechanism of β-cell damage and diabetogenic effect of STZ (Ohkuwa et al., 1995).

The method of type 2 diabetes induction was first described by Portha et al. (1974). At 8 – 10 weeks of age and thereafter rats neonatally treated with STZ manifest hyperglycaemia, an impaired response to the glucose tolerance test (Portha et al., 1979) and loss of β-cell sensitivity to glucose (Giroix et al., 1983). People who develop diabetes usually pass through the phases of excessive adipogenesis, nuclear peroxisome proliferator-activated receptor (PPAR) modulation, insulin resistance, hyperinsulinemia, pancreatic β-cell stress and impaired glucose postprandial and fasting levels (Porte et al., 2001; Hayden et al., 2002). Hyperinsulinemia has generally been considered as a marker of insulin resistance, i.e. a decrease in the effect of insulin to stimulate glucose uptake at a given serum insulin concentration ((Tenenbaum et al., 2003). Those considered as at risk for developing type 2 DM tend to exhibit a constellation of risk factors i.e, abdominal obesity, hypertension, dyslipidaemia, and insulin resistance (Reaven, 1988; Cordain et al., 2003). Hence in addition to glycemic control, management of hyperinsulinemia is also essential for controlling insulin resistance and thus limiting the complications of type 2 diabetes.

Type 2 diabetic control rats exhibited persistent hyperglycaemia. Recently

Table 2: Effect of *W. somnifera* and *R. coriaria* on oral glucose tolerance test in type 2 diabetic rats.

Groups	Treatment	Blood Glucose (mg/dl)				
		0 min	15 min	30 min	60 min	120 min
I	Citrate Buffer (0.1 ml/kg, i.p)	82.82± 1.67	112.20± 2.05	149.49± 2.92	124.14± 1.69	98.43± 1.28
II	STZ (100 mg/kg, i.p)	256.56± 5.84x	301.51± 7.72^{x}	328.78± 5.69^{x}	42.92± 9.78^{x}	318.18± 6.83^{x}
III	Only WS (400 mg/kg, p.o)	85.34± 2.69	120.20± 3.29	156.06± 3.51	127.77± 2.34	92.92± 3.51
IV	Type 2 diabetic + WS (200 mg/kg, p.o)	140.90± 1.67^{y}	166.77± 1.48^{y}	189.56± 2.58^{y}	166.77± 1.86^{y}	150.50± 1.39^{y}
V	Type 2 diabetic + WS (400 mg/kg, p.o)	119.26± 2.17^{y}	138.20± 2.53^{y}	173.88± 5.73^{y}	150.30± 1.76^{y}	128.18± 2.8^{y}
VI	Only RC (400 mg/kg, p.o)	83.14± 1.73	115.32± 2.91	157.69± 2.63	140.73± 2.92	96.08± 2.26
VII	Type 2 diabetic + RC (200 mg/kg, p.o)	137.32± 1.92^{y}	164.07± 1.81^{y}	184.30± 1.51^{y}	165.36± 1.52^{y}	148.90± 2.69^{y}
VIII	Type 2 diabetic + RC (400 mg/kg, p.o)	114.71± 1.83^{y}	130.31± 2.23^{y}	155.45± 2.41^{y}	137.79± 1.90^{y}	120.02± 1.61^{y}

Giancarlo et al. (2006) has shown that *R. coriaria* has hypoglycaemic activity. Previously dried fruit extract of W.coagulans has been shown to have hypoglycaemic activity in type 1 diabetic rats (Hemlatha et al., 2004). Treatment with *W. somnifera* and *R. coriaria* (200 mg/kg and 400 mg/kg) to type 2 diabetic rats reduced the elevated blood glucose levels thereby showing its antihyperglycaemic activity.

In diabetes, there is an increased glycosylation of a number of proteins including haemoglobin and β-crystalline of lens (Alberti et al., 1982). Measurement of glycosylated haemoglobin (HbA1c) has proven to be particularly useful in monitoring the effectiveness of therapy in diabetes (Goldstein, 1995). The levels of HbA1c were increased in type 2 diabetic control rats when compared with citrate control rats. Agents with antioxidant or free radical scavenging properties may inhibit oxidative reactions associated with protein glycation (Elgawish, 1996). Some recent studies have shown that *W. somnifera* has antioxidant properties and prevents lipid peroxidation (Chaudhary et al., 2003; Bhattacharya et al., 2001). A previous report has shown that methanolic extracts of *R. coriaria* fruits have considerable antioxidant activity against free radicals and lipid peroxidation (Candan et al., 2004). Administration of *W. somnifera* and *R. coriaria* to type 2 diabetic rats reduced the glycosylation of haemoglobin by virtue of its free radical scavenging properties and thus decreased the levels of HbA1c. A decrease in blood glucose levels might also contribute to decreased levels of glycated haemoglobin in *W. somnifera* and *R. coriaria* treated type 2 diabetic rats.

Hyperinsulinemia appears to be a compensatory mechanism that responds to increased levels of circulating glucose and is often associated with the progression to insulin resistance (Goldstein, 2002). The β-cells normally compensate insulin re-

Table 3: Effect of Withania somnifera and Rhus coriaria on insulin levels, K^{ITT} and HOMA-R in type 2 diabetic rats.

Groups	Treatment	Insulin Level (mU/L)	K_{ITT}	HOMA-R
I	Citrate Buffer (0.1 ml/kg, i.p)	13.13±0.245	10.15±0.162	3.14±0.037
II	STZ (100 mg/kg, i.p)	24.09±0.329^{x}	4.61±0.162^{x}	19.28±0.541^{x}
III	Only WS (400 mg/kg, p.o)	13.57±0.137	10.24±0.213	3.29±0.130
IV	Type 2 diabetic + WS (200 mg/kg, p.o)	20.01±0.283$^{y, **}$	6.10±0.180$^{y, **}$	7.38±0.097$^{y, **}$
V	Type 2 diabetic + WS (400 mg/kg, p.o)	16.95±0.228$^{y, **}$	8.72±0.135$^{y, **}$	5.07±0.085$^{y, *}$
VI	Only RC (400 mg/kg, p.o)	12.88±0.235	10.19±0.197	3.088±0.068
VII	Type 2 diabetic + RC (200 mg/kg, p.o)	19.09±0.222$^{y, **}$	6.66±0.115$^{y, **}$	6.57±0.060$^{y, **}$
VIII	Type 2 diabetic + RC (400 mg/kg, p.o)	15.89±0.200$^{y, **}$	9.18±0.164$^{y, **}$	4.59±0.056$^{y, *}$

sistance by secreting greater amounts of insulin to maintain glucose homeostasis. Bonora et al., (1983) has reported that hyperinsulinemia is associated with decreased hepatic insulin clearance and hypersecretion of β cells in mild glucose intolerance obese subjects. Results of the present study clearly showed that hyperinsulinemia (as evident by increased serum insulin level) was seen in type 2 diabetic control rats. Therefore, the hyperinsulinemia in type 2 diabetic rats could be either due to decreased hepatic clearance of insulin or by down-regulation of insulin receptors and desensitizing post-receptor pathways (Olefsky et al., 1985), resulting in decreased insulin binding and degradation. Even people with diabetes who take oral medication or require insulin injections to control their blood glucose levels can have higher than normal blood insulin levels due to insulin resistance. Despite high insulin levels (hyperinsulinemia), the glucose levels were greater in type 2 diabetic control rats than type 2 diabetic treated rats. However, *W. somnifera* and *R. coriaria* treatment was found to be effective in reducing insulin levels of type 2 diabetic rats thereby preventing hyperinsulinemia. It seems that both *W. somnifera* and *R. coriaria* exert antihyperglycaemic effect by attenuating hyperinsulinemia.

An insulin-resistan state is a key phase of the metabolic syndrome, constituting the major risk factor for the development of glucose intolerance and diabetes mellitus (Groop, 2000). Thus interventions to decrease insulin resistance may postpone the development of type 2 diabetes and its complications. When animals were subjected to OGTT, increased blood glucose levels were found with the increase in time and were maintained until 2 h in type 2 diabetic rats. Treatment with *W. somnifera* and *R. coriaria* significantly improved glucose tolerance, as indicated by reduction in peak blood glucose levels at 1 and 2 h in type 2 diabetic treated rats during OGTT. In the present investigation the rate of glucose disposal was found to be significantly decreased in type 2 diabetic control rats when compared with citrate control rats. *W. somnifera* and *R. coriaria* might enhance glucose utilization by peripheral tissues and increase the glycogen stores in the liver due to restoration of delayed insulin response because they significantly decreased the blood glucose levels in glucose loaded rats.

Our results showed that *W. somnifera* and *R. coriaria* decreased blood glucose levels, prevented hyperinsulinemia and improved glucose tolerance in type 2 diabetic rats. These results suggest that *W. somnifera* and *R. coriaria* can improve insulin sensitivity. Thus, K^{ITT} was determined to check peripheral insulin resistance (Murali et al., 2002) whereas HOMA-R was determined to check hepatic insulin resistance (Bonora et al., 2000). The results obtained clearly showed that K^{ITT} was significantly improved by *W. somnifera* and *R. coriaria* treatment in type 2 diabetic rats. Additionally, *W. somnifera* and *R. coriaria* treatment significantly prevented the rise in HOMA-R in type 2 diabetic rats. This suggests that *W. somnifera* and *R. coriaria* are pharmacologically effective in improving insulin sensitivity.

In the present study the treatment of *R. coriaria* showed most significant results in decreasing the levels of blood glucose and HbA1c and preventing hyperinsulinemia as compared to *W. somnifera* treatment. Similarly *R. coriaria* treatment showed the most significant results in improving glucose tolerance and insulin sensitivity as compared to *W. somnifera* treatment.

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دراسة تحليلية مقارنة عن تاثير عشبي سم الفراخ والسماق في ارتفاع السكر في الدم و مدي استجابته للانسلين في الجرذان المصابه بالنوع الثاني من مرض السكري

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المُلخّص

تم في هذه الدراسه اختبار فاعليه مستخلصات مائيه لنباتين مختلفين وهما (وثانيا سومنيفرا (*W. somnifera*) و (روس كوريايا (*R. coriaria*) ضد ظاهرتين لمرض السكري-٢ في جرذان ذات يومين من العمر، ألا وهما إرتفاع سكر الدم والاستجابه لهرمون الأنسولين. ولقد تم إحداث مرض السكري بحقن جرعه واحده (١٠٠مجم) من ماده (الأستربتوزوتوسين) داخل الغشاء البريتوني للحيوانات حديثه الولاده. عقب التأكد من إصابه الحيوانات بالسكري، تم إعطاء جرعه محدده من المستخلصات (٢٠٠مجم *W. somnifera*) و (٤٠٠مجم *R. coriaria*) يوميا لمده ٥ أسابيع. المجموعه الضابطه تلقت فقط الماده المذيبه لنفس المده.

عقب هذه المده لوحظ في الحيوانات المصابه بالسكري إرتفاعات مؤثره بالدم في نسبه الجلوكوز وماده جليكوزيلات الهيموجلوبين (HbA1c) ونسبه هرمون الأنسولين. المعالجه بكل من مستخلصات *W. somnifera* و *R. coriaria* أدت الي تخفيض كل هذه النسب المرتفعه. ولقد تم أيضا إجراء اختبار تحمل الجلوكوز (عن طريق الفم) في تلك المجموعات نفسها حيث تبين تحسن في القدره علي إستهلاك الجلوكوز في الحيوانات المعالجه بالمستخلصات *W. somnifera* و*R. coriaria*. وبقياس الإستجابه لهرمون الانسولين في الأطراف وفي الكبد تبين أيضا تحسن ملحوظ في تلك الإستجابه عقب إستخدام كل من هذه المستخلصات، هذا أيضا الي جانب تحسن في معدل ثبات الجلوكوز بالدم نتيجه لتقليل المقاومه لهرمون الأنسولين بهذين العقارين. ونخلص من هذه التجارب جميعا إلي أن عقار *R. coriaria* كان أكثر فاعليه من عقار *W. somnifera* في مقاومه مرض السكري وتداعياته علي نسبه جلوكوز الدم والإستجابه لعمل الأنسولين وذلك في نموذج حيواني لمرض السكري-٢.

كلمات مفتاحية : سم الفراخ – السماق – مرض السكري – الأنسولين – جليكوزيلات الهيموجلوبين – الأستربتوزوتوسين.

Sleep: Recent Advances in Humans & Animals: Types, Neurotransmitters and Disorders

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Abstract

This review deals with the recent developments in the pharmacological and neurophysiological developments in sleep and its disturbances. It details out the various neurotransmitters that control the stage of wakefulness starting with the hypothalamic polypeptide hypocretin/orexin and the others such as ACh (Acetylcholine), NA (noradrenaline) and histamine. Furthermore, the review dealt with the neurotransmitters involved in the two cycles of sleep: the slow-wave-sleep (SWS) and the rapid-eye-movement sleep (REM sleep). In case of SWS stress was given to gamma amino butyric acid (GABA), adenosine, serotonin, anandamide and melatonin whereas in case of REM sleep stress was given to NA, ACh and the polypeptide hypocretin/orexin. Discussion is also directed to the differences in animals in showing the two cycles of sleep. Man, cats and pigeons are the best examples that show the two well defined cycles SWS and REM sleep whereas reptiles show only SWS sleep. However, infants and babies show only REM sleep. Furthermore, stress is also given to the unihemspherical brain sleep that is shown by the marine animals and birds-characterized by one eye closure. Part of the review is devoted to the importance of sleep in animals that encompasses replenishment of brain energy store, consolidation of memory and protein synthesis. The consequences of sleep deprivation with the ultimate suppression of physical and mental developments is also outlined. The review also discussed the various parasomnias encountered during sleep such as somnambulism, terrors, talking, bruxism, enuresis and sleep apneas together with other sleep disturbances such as narcolepsy, sleepiness and insomnias. The onset time of dreaming during sleep whether during the REM sleep or other phases of sleep is also discussed.

Keywords: Sleep; GABA; REM Sleep; Neurotransmitters; Acetylcholine.

1. INTRODUCTION

The impetus for writing this mini-review is to present the readers with the recent developments regarding the basic pharmacological and neurophysiological developments in the fields of sleep and sleep disturbances or disorders.

Broadly speaking the daily 24 hours of almost all animals can be divided into two phases: wakefulness and sleep. This division never implicates that wakefulness is a full stage that comprises several hours and then it is followed by sleep during the com plementary hours of the day.

The cycle of wakefulness and sleep in animals is controlled by what is generally termed "the 24-hour circadian biological clock". This clock is sensitive to the cycle of light and dark. In mammals it is located in the hypothalamus in the suprachiasmatic nucleus. This nucleus receives direct inputs from various brain regions and the eyes (Earnest, 2009).

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Basically, wakefulness is controlled by the reticular activating system of the brain stem. Various neurotransmitters are implicated in the control of the state of wakefulness in mammals. The most recent of these is the polypeptide hypocretin/orexin in the hypothalamus. Its neurones project to cholinergic, noradrenergic and serotoninergic brain stem neurones, to the fore brain and even to the spinal cord (Baumann et al., 2005; Samson et al., 2005). The hypocretin / orexin system is affected by neuronal stimuli via the suprachiasmatic nucleus, the GABA(gamma amino butyric acid) ergic, NA (noradrenaline) ergic, serotoninergic and the cholinergic brain stem neurones as well as metabolically via glucose, leptin and ghrelin (Baumann et al., 2005; Samson et al., 2005). The importance of this system in wakefulness is clearly demonstrated by the precipitation of narcolepsy in those subjects who suffer from degeneration of hypocretin / orexin neurones or deficiency of release of the peptide (Wurtman, 2006). The other systems and neurotransmitters involved include:

a. The cholinergic neurones that are located in the pontomesen cephalic tegmentum and the basal forebrain which projects to the forebrain and the cerebral cortex (Jones, 2009).

b. The NA ergic and serotoninergic neurones with their cell bodies in the Locus coeruleus and Raphe nuclei of the brain stem, respectively (Jones, 2009; Aghajanian et al., 2009).

c. The 20 aminoacid – neuropeptide (Reinscheid, 2009).

d. Histamine (Passani, et al., 2004) and this is very clearly demonstrated by the well known and persistently observed sedation of the first generation of histamine H_1 receptor blockers that pass the blood brain barrier and by the recently introduced histamine H3 receptor activators that decrease brain neuronal histamine release (Passani, et al., 2004).

2. SLEEP

Sleep is a biological condition that is experienced by most species of the animal kingdom. In humans, it consumes 20 – 33% of life time whereas in some other animals it comprises 10 – 83% of life time (see below). It is experienced by mammals, birds, reptiles, insects and various invertebrates. Each of these species has its characteristic type of behaviour during its sleep. Generally, sleep can be defined using behavioural and electrophysiological criteria. The behavioural features can be summarized as:

a. Quietness with very minimal movements of the body.

b. Species – characteristic body posture.

c. Lack of awareness of the environment.

d. Weak response to external stimuli i.e. an elevated arousal threshold that can be reversed by an increase in the external arousing stimulus.

e. Movement of the eyes balls at certain stages and in certain species.

The electrical criteria involves changes in the brain neuronal activity as followed by recording of electroencephalogram (EEG) that was introduced by Eugene Aserinksy and his Ph.D supervisor professor Nathaniel Kleitman in 1953 (Aserinsky, et al., 1953). These EEGs revealed that human and other mammalian sleep comprises consecutive cycles each comprised of 5 stages labelled as stages I–IV and a rapid eye movement sleep stage (REM sleep stage) or de-synchronized sleep (D-sleep) or paradoxical sleep (Siegel, 1999). The first 4 stages are also known by the names synchronized sleep (S-sleep), slow – wave sleep or non REM sleep. Generally the two types of sleep alternate cyclically. In humans each cycle, the non-REM sleep lasts 65 – 70 minutes whereas the

REM sleep lasts 15 – 25 minutes. Thus, the single sleep cycle duration ranges from 75 – 95 minutes. This can be repeated multiple times (2 – 5 times) during a normal nocturnal sleep. The recorded electrical activity is generated by 3 brain areas namely the reticular formation of the brain stem, the thalamus in the midbrain and the cortex. Specifically, the EEG activity during REM sleep originates in the amygdala and the temporal cortex (Siegel, et al., 1997; Siegel, 2007; Zisapel, 2007).

In stage 1 of the non REM sleep, the alfa waves which characterize the wakefulness state which are of low-voltage (low amplitude) and high frequency 12 to 14 cycles per second start to decrease gradually for a period of 5 – 7 minutes to enter stage II that is characterized by the gradual re-appearance of alfa-waves with alternating negative and positive waves with a relatively higher voltage appearing at intervals of 0.75 second between each wave and the other. This stage can extend up to 40 minutes when stage 3 starts by the appearance of delta waves with a high voltage (high amplitude) and very low frequency of 2 waves per second accompanied by the presence of sleep spindles of the very very low amplitudes. This stage extends up to 7 minutes followed by stage 4 in which only slow delta waves with high voltage (high amplitude) and very low frequency (1 – 2 per second) appear. This stage extends up to 18 minutes. Thereafter starts the phase of the REM sleep that is characterized by low-voltage but high frequency, approaching or exceeding that of the wakefulness stage whereby the frequency may be up to 20 cycles per second. The duration of this phase is in the range of 15 – 25 minutes.

The above wave characteristics of the EEG during the two phases apply to all mammals except the platypus which shows the opposite i.e. waves of low voltage and high frequency in the non-REM sleep and waves of high voltage and low frequency in the REM sleep (Siegel, et al., 1997). In this connexion, it should be recalled that the platypus is a mammal that belongs to the Monotremes in which paradoxically offsprings hatch from eggs and then climb in the mother's pouch and get their milk nutrition from their mothers for several months. In this aspect they mimic the other monotreme the echidna, the ant-eaters (Siegel, et al., 1997).

Another exception of wave characteristics is observed in the infant placental mammals e.g. rats pups or human infants where the EEG through out sleep shows only activities similar to those observed usually in the REM sleep (Frank, et al., 1997). In the rat's pups the slow delta waves observed in Stages III & IV of the non REM sleep appear only at the time of weaning and thereafter.

3. SLEEP-MEDIATORS: NEUROTRANSMITTERS & OTHERS

3 A. Slow-wave sleep:

The site of the brain that acts to control non-REM sleep (or slow-wave sleep) is controlled by the basal forebrain in the area in front of the hypothalamus (Siegel, 1994). The major neurotransmitters involved in initiation and maintenance of this phase of sleep include:

a) γ-Amino butyric acid (GABA) is an inhibitory neurotransmitter released from the GABAergic neurones of the basal forebrain (Nitz, et al., 1996) acting via GABAA receptors activation of which inhibits brain neuronal activity via an increase in CL^- conductance.

b) Adenosine (Mc Carley, 2007; Basheer, et al., 2008). Adenosine is a brain depressant. It induces sleep. During wakefulness, brain metabolism and energy utilization in form of glucose and ATP is very high. The

breakdown of ATP to provide energy results in production of adenosine that accumulates to reach high concentrations leading to brain depression and initiation of sleep. In this process it is helped by release of neuronal GABA. Indeed, the hypnotic action of benzodiazepines involves both GABA and inhibition of adenosine metabolism by re-uptake back into cells (Phillis, et al., 1988) and barbiturates – induced hypnosis involves activation of GABA A receptors. Furthermore, caffeine and theophylline – induced insomnia is mainly due to blockade of adenosine A1 receptors in the brain (Mc Carley, 2007; Fredholm, et al., 1982).

c) Anandamide (Mechoulam, et al., 1997). This is an endogenous cannabinoid (endocannabinoid) characterized chemically as arachidonyl ethanolamide that induces sleep via activation of CB1 receptors. Indeed sleep has been induced by inhibiting the cellular uptake of anandamide by compound (Sz, 8z, 11z, 14z] N- (4 – hydroxy – 2 – methyl phenyl) 5, 8, 11, 14 – eicosa tetraenamide which acts to elevate brain levels of anandamide. The induced sleep was blocked by the CB1 receptor blocker SR 141716 A.

d) Serotonin (5 – HT) (Pujol, et al., 1971):

Serotonin implication in non REM sleep stemmed from the induction of insomnia in cats via administration of p-chlorophenyl alanine or the destruction of the Raphe nuclei, the cell bodies of brain serotonineric neurones. Furthermore, 5 – HT seemed to act to modulate the state of wakefulness. The serotonineric neurones of the brain stem are highly active during waking states (Aghajanian G, et al., 2009). However, it should also be noted that serotonin depletion did not induce insomnia in the rat (Rechtschaffen, et al., 1973).

e) Melatonin (Zisapel, 2007). Melatonin is a hormone that is released by the pineal gland. It is a serotonin metabolite. Its release is stimulated by darkness and suppressed by light. It induces sleep.

f) TNFα (Tumor necrosis factor alpha). This ploy peptide has been shown to enhance non REM sleep in both man and animals (Krueger, et al., 2009).

g) Growth Hormone (GH)

The anterior pituitary hormone GH and its hypothalamic factor GH – Releasing hormone enhanced slow-wave sleep by prolonging stage II (Frieboes, et al., 1995).

h) Other Endogenous Sleep Mediators:

Various endogenous substances have been implicated in induction of sleep. Some of these include: the delta sleep-inducing peptide characterized as Arginine vasotocin, the adrenal steroid Tertrahydro-deoxy corticosterone, the cytokines IL-1 and IL-2, the cis 9, 10 – octadecenoamide and PGD2 (Mc Ginty, et al., 1990).

3 B. Rapid – Eye Movement Sleep (REM Sleep) Mediators:

The area in the brain that acts to control REM sleep is located in the brain stem – pons area specifically in the dorsolateral pontine tegmentum. The motor activity of the extra-ocular muscles is triggered by activation of the para-median pontine reticular formation that controls the movement as well as the descending reticulospinal systems that induce the distal muscle twitches during REM sleep (Siegel, 1994; Chase, et al., 1983).

Various neurotransmitters and other mediators are believed to contribute to the behavioural and electrophysiological activities observed during REM sleep. These include:

a. Noradrenaline: The activity of the brain stem NAergic neurons originates in the Locus Coeruleus and is observed to be suppressed during REM sleep. This allows the atonia of the muscles observed in this

phase of sleep. It is opposite of the activity observed in wakefulness (Jones, 2009; Siegel, 1994; Mc Carley, 2007). In conscious rats, stimulation of NA release from the Locus Coeruleus resulted in suppression of REM sleep both of its frequency of generation and its duration via β and α adrenoceptors.

b. 5-Hydroxytryptamine (Serotonin, 5-HT). As in case of NA, the activity of the brain stem serotonineric neurons originating in the Raphe nuclei is greatly inhibited during REM sleep resulting in enhancement of the muscle atonia observed in this stage of sleep (Jones, 2009; Siegel, 1994; Mc Carley, 2007).

c. ACh: The cholinergic brain stem neurons are believed to activate the pontine neurons resulting in initiation of REM sleep (Siegel, 1994; Mc Carley, 2007; Monk, 1991; Frank, et al., 1997). Furthermore, the cholinergic neurons in the pontomesencephalic tegmentum and the basal forebrain that project to the cerebral cortex act to stimulate cortical activity during REM sleep (Jones, 2009).

d. Glycine: During REM sleep high activity has been observed in the brain stem glycinergic neurons (Siegel, 1994). These act to induce REM sleep-observed atonia.

e. Nitric Oxide [NO]. Release of NO from the pontine tegmentum neurons is shown to enhance REM sleep (Gautier, et al., 2005).

f. Hypocretin / Orexin. This hypothalamic neurotransmitter has been shown to contribute to the actions observed in REM sleep (Baumann et al., 2005).

g. Prolactin. This anterior pituitary hormone has been observed to enhance REM sleep (Krueger, et al., 2009).

4. INFLUENCE OF SLEEP ON BODY PHYSIOLOGICAL FUNCTIONS

4 A. Effect of slow-wave sleep (Non REM sleep):

Most of the normal physiological functions in mammals are greatly suppressed except the release of some hormones. Thus, we find that during non REM sleep there are significant decreases in respiration, the arterial blood pressure, heart rate, body temperature, cerebral activity and cerebral blood flow (Maquet, et al., 1997). However, the release of some hormones such as GH, prolactin and gonadotropins is not affected but that of the thyroid hormones is greatly inhibited.

4 B. Effect of REM sleep:

Contrary to what is observed in non REM sleep, during REM we always observe an increase in the excitability of the sensory motor cortex, the vestibular and bulbar reticular formation together with fine body movements of the throat, ankle, upper arm, shoulder and abdomen (Baldridge, et al., 1965). Characteristically we observe rapid eye movements with lids closed, skeletal muscles atonia (i.e. muscle relaxation of back, neck, arms and legs) together with dreams, penile erection (tumescence) and clitorial tumescences. In addition, one observes clear increases in the heart rate, blood pressure, respiratory rate and brain metabolism (Siegel, 1994; Kleitman, 1963). However, with regard to muscles activity during REM sleep it should be noted, although this effect is observed in most mammals, yet it is not observed in both of platypus and echidna. Also no changes in muscles tones were observed in lizards although they do show REM sleep (Tauber, et al., 1966; Tauber, et al., 1968).

5. TYPES of SLEEPS IN ANIMALS

If one followed the sleep of various animals via use of the EEG and observation of the animals' behavior, he can notice many differences regarding the animals' postures during sleep, their eyes statuses, their brains

Table 1. Types of Sleep in Animals

No.	Animal	Body Posture	Eyes› Status	% of Brian Involved	Duration of Sleep		
					Total Hours	Slow Wave	REM Hours
1.	Human	Laying on the back	Both closed	All 100%	5 – 8		1.9
2.	Giraffe	Kneeling and bending its long neck to rest its head in the bend (crook) of the hind knee	Both closed	All 100%	2 – 4		0.5
3.	Dolphin	Sleep while swimming in bouts of several seconds	One eye open	50%			0.25
4.	Bat	Upside down with head downwards	One eye open	50%	18–20		
5.	Opossum				20		7
6.	Armadillo		One eye open	50%	20		6
7.	Elephant	Standing	Both closed	100%	2 – 4		
8.	Horse	Standing sometimes laying down		100%	2.5 divided in 15 minutes bursts		
9.	Birds	Standing some types while flying Head is tucked beneath the wing.	One eye is open	50%	2.5 min per cycle		9 sec. per cycle
10.	Dog	Laying with ears & nose twigging and tail wagging	Both closed	100%	10 –11		
11.	Rat (Rodents) rats pups till weaning	Laying	Both closed	100%	13 (7–16.6) 16		0.8 – 3.4 16
12.	Seal	Sleep while swimming	One eye open	50% unihemispeherical sleep			0.25

13.	Cat	Laying down with quivering of paws & whiskers & twitching of tail	Two eyes closed	100%	10 - 16	3.2
14.	Platypus					7 - 8
15.	Human Babies (newly born) After 5 years	Laying on back	Both closed Both closed	100%	17 - 18 10-12	17 – 18 2-2.4
16.	Duck	Standing	One eye closed contralateral to the hemisphere	50%	10 - 12	
17.	Porpoises			50% hemispherically		
18.	Whales	Swimming	One eye open	50% unihemispherical		
19.	Cow	Standing	Both open	100%		0.7
20.	Pigeon	Standing	One eye closed	50%		
21.	Lizard	Flat	One eye is open	50%		
22.	Ferret		Both closed	100%		6

References: (36-40)

Table 2. Effect of some drugs on slow-wave sleep

No.	Drug(s)	Effect on Slow-Wave Sleep Non REM Sleep
1.	Barbiturates	Prolong stages I & II and suppress stages III & IV
2.	Benzodiazepines	Prolong stages I & II and suppress stages III & IV
3.	Zolpidem, Zaleplon, Eszopiclone	Prolong stages I & II and suppress stages III & IV
4.	Ethanol	Prolongs stages 1 & II and suppresses stages III & IV
5.	Tricyclic Anti-Depressants	No effect or may increase or decrease
6.	Cannabis (Tetra-Hydrocannabinol)	Prolongs Stage IV
7.	CGP36742 $GABA_{B \text{ and } C}$ Blocker	Suppressed all phases
8.	Amphetamine & Pemoline	Suppressed all phases

(**Reference:** Deschaux, et al., 2006)

activity, the duration of the total sleep or that of its two main stages: the slow-wave and the REM sleeps. Indeed one can observe that some animals do sleep with one part of the brain awake and the other suppressed whereas others sleep with one or both eyes open or even while swimming or flying!! Indeed, follow-up of sleep is highly interesting. Table 1 shows a concise summary of the above some of indicated parameters during sleep in various animals as reported in the literature.

From Table 1 it can be clearly seen that mammals don't show unihemispherical sleep. Both hemispheres of the brain are involved in sleep. Unihemispherical sleep is observed only in marine animals, birds and some reptiles. This God gift to some animals e.g. marine animals will allow them to continue rise to the top of water and take their normal breath while still asleep. In addition, it should be noted that some seals can hold their breath for 30 minutes during their sleep. Furthermore, it should be noted that the dolphins sleep is not a continuous process as in case of mammals but occurs in bouts of several seconds (Mukhametov, 1987; Mukhametov, 1995; Mukhametov, 1984). These bouts of sleep now and then remind one of what has been along time ago written about Leonardo da Vinci type of sleep which was short snoozes every 4 hours through out the day. This is because he believed that sleep is a waste of time!! In birds, the unihemispherical sleep with one eye open – usually the outward facing eye – helps them to get the benefit of sleep while watching their predators / enemies.

Furthermore, it should be noted that in case of marine animals the unihemispherical sleep is alternated between the two sides of the brain.

A further note about marine animals regards the vertebrate zebra fish known by the Latin name Danio ***rerio*** which is shown recently to experience behavioural, physiological and pharmacological characteristics of mammalian sleep (Zhdanova, 2006). Indeed, hypnotics can induce sleep in this fish.

Regarding Amphibians e.g. frogs and invertebrates e.g. insects such as bees and flies there are no EEG changes as observed in mammals but they do have a sleep-like rest as demonstrated by decrease in responsiveness and hemostasis. These animals don't have brain structures akin to those of mammals and thus they can not generate the same electrical pattern seen in the mammalian EEG during true sleep.

Another look to Table 1 also reveals that in human infants and rat offsprings (pups) till the time of weaning) the sleep is 100% of the REM sleep – almost 16 – 18 hours per day. Other animals with a high percentage of REM sleep 25 – 33% of their total sleep time (16 – 20 hours per day) are the platypus, opossum, armadillo and the ferret (Zeppelin, et al., 1974). In fact this group of animals together with the bat spend 80% of their life sleeping!! Marine animals show very short periods of time in REM sleep per day in the range of 15 minutes only and these are followed by the two mammals giraffe and cow 0.5 – 0.7 hour of REM sleep per day.

6. OCURRENCE OF REM SLEEP IN ANIMALS

Recording the EEG during sleep in various animals revealed that not all animal species show the two phases of sleep namely the slow-wave and the REM sleep. The groups of animals in which both phases are shown included: man (Aserinsky, et al., 1953), pigeons: ***Columbia*** livia (Fuchs, et al., 2006), Chicken, lizards (***Tenosaura pectina***) (Tauber, et al., 1968), cats, platypus (Duckbill) (Siegel, 1999), the giraffe the armadillo, the ferret and dolphins (Mukha-

metov, 1995). On the other hand, the animals that show only slow-wave sleep and no REM sleep phase included: Turtles and tortoises, reptiles (Admin, 2003) and the crocodile (***Caiman sclerops***) (Meglasson, et al., 1979).

7. FUNCTIONS OF SLEEP

Throughout the last five decades sleep functions have been discussed now and then. The opinions regarding the functions and benefits of sleep to man or other animals included:

a. Replenishment of brain energy stores that have been used during wakefulness. Thus, leading to restoration of the energy balance in the brain and other organs (Hartman,1973).

b. Consolidation of memory and enhancement of the learning process. It is believed that during slow-wave sleep stages III & IV there is abundant secretion of GH which stimulates uptake of amino acids by brain cells to enhance the synthesis of various types of proteins and other macromolecules. These proteins are then utilized to maintain the plasticity of the connections between the brain neurons that are involved in storing i.e. consolidation of memories and improvement of skills during REM sleep. (Zisapel, 2007; Kleitman, 1963; Hartman,1973; Dave, et al., 2000).

This is partly supported by the general observations that many people tend to remember things better if they sleep after learning them and that sleepless nights induce forgetfulness and loss of concentration. However, more recently, a paradoxical finding has been reported by Rasch et al., (Rasch, et al., 2008) whereby the administration of the selective serotonin re-uptake inhibitor fluvoxamine and the selective NA re-uptake inhibitor reboxetine which suppress REM sleep enhanced skill memory.

c. Stimulation of growth in non-adolescents due to the excessive release of GH.

8. CONSEQUENCES OF SLEEP DEPRIVATION

Studies in humans revealed that sleep deprivation and prolonged wakefulness lead to suppression of mental and physical functions together with an increase in irritability and confusion (Kleitman, 1963; Hartman,1973). Indeed such findings had been miss-used with various brain-washing procedures to impair the human brain function. These findings are also supported by experimental research in rats. REM sleep deprivation in rats resulted in alteration of various brain functions via disturbances in brain neurotransmitters and enzymes together with disturbances in memory consolidation (Siegel, 1999; Siegel, 1994; Dave, et al., 2000). Furthermore, REM sleep deprivation in rats for 17 days induced skin lesions, hypothermia and resulted in death of the animals when the deprivation continued for 37 days (Kushida, 1989).

9. EFFECTS OF DRUGS ON SLEEP

Many drugs are available that can affect generally the sleep process via either inducing sleep or insomnia. The group of sleep inducers generally acts to activate the central nervous system endogenous neurotransmitters involved in slow wave sleep. This group includes the barbiturates and their predecessor the benzodiazepines and the laterly introduced the non-benzodiazepines hypnotics such as Zolpidem, Zaleplon and Eszopiclone which activate benzodiazepine Bz_1 and GABA receptors. The barbiturate group e.g. phenobarbitone and pentobarbitone interact with the GABA A receptors to prolong the openning of the brain neuronal CL^- channel to induce a prolonged hyperpolarization, resulting in depression of brain activity and sleep. On the other hand, the benzodiazepines e.g. diazepam, nitrazepam

Table 3. Effect of drugs on REM sleep

No.	Drug	Effect on REM Sleep	References
1.	Zimelidine (Serotonin re-uptake inhibitor)	Reduction (in humans & pigeons)	(Fuchs, et al., 2006), (Frank, et al., 1997)
2.	CGP 36472 $GABA_{B\&C}$ Blocker	No effect	(Deschaux, et al., 2006)
3.	Cocaine	Suppression	
4.	Marijuana (Tetrahydrocannabinol)	Reduction	
5.	β-Adrenoceptor Blockers: Propranolol Atenolol; Delevalol; Pindolol; Celiprolol; Metoprolol	Decreased the duration	(Deschaux, et al., 2006)
6.	Fluvoxamine, (5-HT re-uptake inhibitor)	Reduction	(Rasch, et al., 2008)
7.	Reboxetine NA re-uptake inhibitor	Reduction	(Rasch, et al., 2008)
8.	Desipramine (NA re-uptake) inhibitor Tricyclic Anti-depressant	Reduction of duration (34%)	(Frank, et al., 1997), (Rijnbeek, et al., 2003)
9.	$5\text{-}HT_{1A}$ agonists e.g. Buspirone	Suppression	(Argyropoulos, et al., 2008)
10.	Fluoxetine (5-HT re-uptake) inhibitor	Suppression	(Bakalian, et al., 1990)
11.	Tryptophan Methylester	Suppression	(Bakalian, et al., 1990)
12.	Valine Methylester	No effect	(Bakalian, et al., 1990)
13.	Alanine Methyl ester	No effect	(Bakalian, et al., 1990)
14.	Benzodiazepines	Mild suppression (8.7%)	(Rijnbeek, et al., 2003)
15.	Caffeine	Reduction	
16.	Zolpidem Zaleplon Eszopiclone	Decrease	
17.	Donepezil (Anticholinesterase)	Increased duration	
18.	Nefazodone 5-HT re-uptake inhibitor	No effect	(Vogel, et al., 1998)
19.	Dexamphetamine	Reduction	
20.	Pemoline	Reduction	
21.	α-Methyl-p-Tyrosine (NA synthesis inhibitor)	Prolonged duration	
22.	P-Chlorophenyl alanine	Reduction	
23.	Physostigmine (Anti-Cholinesterase)	Prolongation	
24.	Arecoline Cholinergic Agonist	Prolongation	

25.	Scopolamine Anti-Cholinergic	Decreased duration
26.	Cannabis withdrawal	Increased
27.	Alcohol	Decreased
28.	Barbiturates	Decreased

and medazepam interact with their specific Bz_1 receptors in the brain that are linked with GABA α1 and α3 subunits to increase the frequency of opening of the CL^- channel with the consequent drowsiness and sleep (El Tahir, 2004). Furthermore, some volatile oils can also induce sleep (El Tahi, et al., 2008).

Central nervous system stimulants that induce insomnia act to inhibit directly or indirectly the sleep neurotransmitters. For instance amphetamine, cocaine, cathine and cathinone all act indirectly to increase the availability of both NA and DA that inhibit GABA release with the consequent enhancement of the central stimulants glutamic acid and aspartic acid release resulting in general brain activation and precipitation of insomnia (El Tahir, 2004).

Aside from these general actions detailed pharmacological studies revealed that many drugs can act to specifically affect the non-REM or the REM sleep phases. The spectrum of drugs tested is very broad but the most important results are hereby summarized in Tables 2 and 3 which depict the effects of some drugs on slow-wave and REM sleep, respectively.

As shown in Table 2, all drugs that act to enhance the endogenous sleep mediators involved in non REM sleep e.g. GABA and enandamide acted to prolong slow-wave sleep whereas GABA blockers shortened the duration of this phase of sleep.

As shown in Table 3, all drugs that have the ability to elevate the levels of NA, 5-HT or GABA or to antagonize ACh acted to suppress the duration of REM sleep. On the other hand, drugs that elevate the brain cholinergic activity prolonged the duration of this phase of the sleep. A similar effect was noted for drugs that decrease the availability of NA. The failure of the GABA B and GABA c receptor blocker CG P36742 to affect REM sleep points to the disinvolvement of GABA A and GABA C receptors – mediated action on REM sleep.

10. EFFECT OF DISEASES ON REM SLEEP

Both Autism and Alzheimer's disease that are associated with decreases in the brain cholinergic activity and decreased learning abilities have been shown to decrease the duration of REM sleep. This finding points to the involvement of the brain ACh in the initiation of REM sleep and also to the possibility that REM sleep is involved in the enhancement of the learning process.

11. BEHAVIOURAL DISTURBANCES DURING SLEEP (PARASOMNIAS)

Observation of people during sleep revealed that in some of them peculiar behaviours do accompany certain stages of their sleep. These include behaviours labelled as somnambulism, sleep talking, night terrors, sleep apnea, snoring, enuresis, bruxism and sleep kicking movements. Collectively these disturbances are coined the name parasomnias. In the following paragraphs brief definitions of the disturbances and their time of occurrence during the sleep stages are given.

a. Somnambulism: Somnambulism is a term given to the condition in which some people walk while they are sleeping. It usually occurs during non-REM sleep. In this condition the affected subject stands up from his bed with his eyes open, walk to different

places inside or even outside his home. The person may dress or undress, mumbles or speaks various words or moan or can perform a certain act and then returns to his bed and continue sleeping till the normal awakening. This episode can be short for seconds or may extend to up to 30 minutes. In the following morning the subject cannot recall and denies the acts!! (Mahowald, et al., 2009). It usually occurs following withdrawal from intake of benzodiazepines.

b. Sleep Terrors: These are also known by the names sleep night-mares or pavor ***nocturnus.*** They usually occur during non-REM sleep in stages III & IV. The inducing factor is mainly emotional stress but it may be associated with some psychiatric disorders. In this condition the subject is agitated, aroused and shows signs of stimulation of the sympathetic nervous system such as tachycardia, mydriasis, increases in the arterial blood pressure and the rate of respiration (tachypnea) and sweating. The subject screams loudly and some times cries but then returns to normal and continues the sleep. In the morning he cannot recall what happened during the sleep. (Complete amnesia of what he did). (Nielsen, et al., 2009). It also constitutes one of the actions during barbiturates' abstinence syndrome.

d. Sleep Talking: This abnormal behaviour may take the form of few words or few sentences. It usually occurs during the non REM sleep. The patient then continues sleeping. The subject cannot recall what happened (Mahowald, et al., 2009; Rechtschaffen, et al., 1962).

e. Bruxism: Bruxism is the term given to the condition of grinding the teeth and contractions of Jaws' muscles during sleep. It mainly occurs during the REM sleep stage. It may occur in the slow-wave sleep (Reding, et al., 1964). It may be related to dreaming.

f. Enuresis: Enuresis is the term given to the involuntary passage of urine or bed-wetting during sleep. It is usually observed in some children below the age of 8 years. It occurs mainly in the non-REM sleep stage (Mahowald, et al., 2009). It can be treated by drugs that contract the urinary bladder destrusor muscles e.g. sympathomimetics.

g. Leg Kicking: This is a parasomnia usually observed in both REM & non-REM sleep. It consists of involuntary movement of leg or hand skeletal muscles. The kick may hurt a near-by subject.

h. Sleep Apnea: This sleep disturbance usually occurs in subjects over 50 years of age who are sufferring from hypertension and obesity. In such subjects the respiratory airways are obstructed during sleep. This results following relaxation of the muscles of the tongue and the soft palate at the base of the throat resulting in a decrease in the airflow. As a result O_2 supply decreases. This awakens the subject to take a breath which is heard as a grasping strong breath producing what is called a snort. The decrease in O_2 supply if severe may lead to sleep death. The process is believed to involve afferent vagal nerve reflexes with their induced broncho constriction (Mahowald, et al., 2009). The disturbance can occur during REM sleep.

12. SLEEP DISORDERS

The major sleep disorders are narcolepsy, sleepiness and insomnia. The following lines define these disorders and the probable mechanisms underlying them:

a. Narcolepsy: This disorder is simply defined as excessive daytime sleeping accompanied by EEG changes similar to those observed in REM sleep at the onset of sleeping. Its major symptoms beside the excessive sleepiness together with cataplexy which is sudden skeletal muscle relaxation, hypnagogic hallucinations and dreams (visual hallucination) experienced at the onset of the

sleep together with partial or total reversible skeletal muscles paralysis at the onset of sleep (Bassetti, et al., 1996). It is classified as an auto-immune disease that probably involves two specific HLA (Human Leukocyte Antigen) alleles known as DQB1–0602 and DQA1–0102. It is an inherited disease (Rogers, et al., 1997). The chromosomal defect is in chcromosome 6 that controls the HLA antigen immune complex (Wurtman, 2006). The biochemical defect lies in the partial or full degeneration of the lateral hypothalamic neurones that release the wakefulness polypeptide neurotransmitter hypocretin / orexin, resulting in decrease in the release of the peptide (Samson et al., 2005) and probably a decrease in its receptors as shown in mice and dogs (Baumann, et al., 2005). The most danger of this disorder is when it occurs when the patients fall asleep during driving (Nish, et al., 1997).

b. Sleepiness: Another sleep disorder with a magnitude lesser than that of narcolepsy is sleepiness. This is defined as periodic or frequent attempts to fall asleep during the day. Persons with tendency for sleepiness show prior symptoms such as yawning, eye rubbing, dropping of eyelids, nodding and fatigue. They cannot concentrate in their work. Various factors predispose to this disease that include deprivation of sleep, sleep restriction to less than 4 hours per day, fragmentation of normal sleep via loud sounds or very shinning lights, intake of sedatives during the day e.g. H_1 histamine receptor blockers that pass the blood-brain barrier or via intake of drugs that have sedation as one of their side effects e.g. propranolol. It is normally treated by correcting the causes or simply by having adequate sleep (Chase, et al., 1983; Mahowald, et al., 2009). The timing of this disorder is not specific. It can occur while reading, working, driving a car or even during sexual activity!! The most prone subjects are shift labours who don't sleep well before they start their work.

c. Insomina:

Insomnia is encountered by more than 20% of the population. It is most common in subjects confronted with life stress or suffering from chronic diseases, asthma, depression, peptic ulcers, arthritis, anger and fear especially in the elderly and females. It is simply defined as difficulty in falling or staying sleep. It may also be observed as frequent awakening during sleep or short sleep times (Chase, et al., 1983; Mahowald, et al., 2009).

13. DREAMING DURING SLEEP

Although various reports consistently linked the experience of human dreaming during sleep with the REM sleep yet recent reports presented evidence that dreams can occur in both phases of sleep and even at the onset of sleep (Admin, 2003; Rosenlicht, et al., 1994). Thus, dreaming is shown to occur at the onset of sleep (the first minutes) in babies, children, napping adults and in patients with narcolepsy. In this connection it should be recalled that in infants and babies, all of their sleep is of the REM sleep (Siegel, 1999) and that in narcoleptic patients the EEG at the onset of sleep is of the REM type. Dreams can also occur in the non REM sleep (Admin, 2003; Rosenlicht, et al., 1994) and the REM sleep phases (Admin, 2003; Rosenlicht, et al., 1994).

Generally in humans all age groups do dream during sleep. Even those who are congenitally blind do experience auditory dreams. Although it was previously believed that the time in dreaming is compressed i.e. dreaming takes seconds but describing the dream takes several minutes, yet recent evidence points to the fact that time in dreams is not compressed and that time taken to narrate a dream correlated very well with the time taken to experience the whole dream.

Regarding dreams recalling in the

morning–after, many people insist that they never dream as they cannot remember any dream. It should be noted that dream recalling is directly related to the time of awakening following the experienced dream in the REM sleep. Indeed Kleitman observed that if a dreamer is awaken more than 10 minutes following the end of his dream, the probability of forgetting the dream is more than 90%!! (Kleitman, 1963). To recall dreams one should have a highly active hippocampus to store the dream rapidly in the short memory to be transferred later to the permanent memory in the cortex.

One question related to dreams is do dreams serve any useful function for the human being? To answer this question we have to note that some dreams are mere reflections to what one sees in his awakening hours, some dreams are a gift from God to discharge tensions and harmful impulses whereas other peoples are highly privileged by dreaming for future events that actually come true. Such type of dreams usually alert the fortunate dreamer to be careful about certain aspects of his acts in the comming few hours, days or even months. By such types of dreams one can arrange and prepare himself for the appropriate actions to take if he is confronted by his real dream in action. Furthermore it can also be pointed that dreaming may also act to enrich children or even adults skills in solving certain problems in life and probably enhancing the capabilities of digesting or assimilating new knowledge. Thus, no one of us wants to be deprived of dreams in his sleep. Indeed thirty five years ago Ernest Hartman wrote "dreaming is essential for normal personality functions and stable behaviours (Hartman, 1973). Dreaming indeed is a fruitful outcome of indulging in sleep. For this purpose many people thought for foods, drugs or chemical or herbs to increase the probability of experiencing enjoyable dreams. Some of the knowledge gained in this regard included consumption of cheese, tryptophan-rich foods and vitamin B_6 supplements, nutmeg or even glutamine and choline-rich herbs. Some peoples revealed that these foods fulfilled the hopes.

Finally, it is hoped that this concise review together with the previous widely scattered reviews (Maquet, et al., 1997; Admin, 2003; Mc Ginty, et al., 1990) may help the reader to gain the recent understanding of the pharmacological and neurophysiological developments in the field of sleep in the animal kingdom.

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النوم: تطورات حديثة في الإنسان والحيوان ، أنواعه ، الموصلات العصبية والاضطرابات.

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المُلخّص

تختص هذه الدراسة المراجعة العلمية بالتطورات الحديثة الدوائية والفيزيولوجية العصبية في النوم واضطراباته ويتم تفعيل كل البواعث الكيميائية ذات العلاقة بحالة اليقضة والمتمثلة في البواعث من أمثال ببتيد الغدة النخامية المعروف باسم هايبوكربتين / أوركسين والبواعث الآخر مثل استيل كولين ونور أددرينالين والهستامين وتتطرق المراجعة إلى دورتي النوم المعروفتين باسم نوم الأمواج البطيئة ونوع حركة العين السريعة وبواعثهما الكيميائية حيث نجد أن من بواعث الدورة الأولى كل من غابا والادينوزين والسروتونين والاناندمايد والملوتانين ومن بواعث الدورة الثانية نور أدرينلين وأستيل كولين وببتيد هايبوكريتيين / أوركسين ، وتتطرق المراجعة أيضاً إلى اختلافات دورتي النوم في الحيوانات المختلفة ونوم نصف الدماغ في حيوانات البحار والطيور بعين واحدة مغلقة وتناقش المراجعة أيضاً النتائج السلبية عند الامتناع عن النوم والتي تشمل تثبيط النمو الجسدي والعقلي وكذلك كل ما يحدث من اضطرابات أثناء النوم مثل السرنمة والأحلام المرعبة والكلام وصكيك الأسنان والتبول وفقدان التنفس وإضافة لهذا تتطرق المراجعة إلى الأرق والتناوم وعدم النوم كلية وكذلك تناقش المراجعة أوقات حدوث الأحلام أثناء دورتي النوم.

كلمات مفتاحية : النوم - البواعث الكيميائية - غابا - نورأدرينالين - أستيل كولين - هايبوكريتين - أوركسين الأحلام.

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Floral Biology and Visitors Behaviour of Caralluma Acutangula (Decne.) N.E.Br. in Jazan Region, Southwestern Saudi Arabia

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Abstract

Caralluma acutangula (Decne.) N.E.Br. is one of the most succulent species of Asclepiadoideae(Apocynaceae) in the Tihama hill slopes of Jazan, Southwestern Saudi Arabia. The flowers of the plant attract many visitors, some of which are pollinators which are represented by Diptera, especially from families Muscidae, Sarcophagidae, and Calliphoridae. To attract the pollinators flowers use mimicry and deception for brood and food site (Sapromyophily) thus the female flies are the most frequent visitors. This mimicry with structure of flowers affects the behaviour of flies and leads to the attachment of pollinia to the proboscis and transfers them to receptors (guide rails). Other visitors are butterflies, represented only by Plain Tiger (*Danaus chrysippus* – Nymphalidae) whose larvae feed on flowers. Some predators of Thomisidae spiders hide between flowers to catch Diptera prey. This study sheds some light on a complex system between flowers and their visitors in arid habitats.

Keywords: *Caralluma acutangula*,Flowers,Diptera,Mimicry, Pollination,*Danaus chrysippus*, Thomisidae.Jazan,Saudi Arabia.

1. INTRODUCTION

The Asclepiadoideae (subfamily Apocynaceae) constitute a group showing complex floral structures and pollination in angiosperms (Kunze,1991; Albers and Meve,2002). Caralluma is a stem succulent genus of Asclepiadoideae, comprises about 60 species distributed in arid and semiarid regions of Africa and Asia (Bruyns,2000b; Müller and Albers,2002; Gilbert,2003). In Saudi Arabia, this genus is represented by 6 species, restricted to the southwestern part of the country (Müller and Albers,2002; Masrahi,2011).

Reproduction success in higher plants depends on the activity of pollinators in pollination. The flowers of many angiosperms have evolved many intricate mechanisms to attract pollinators including highly scented floral parts, mimicry of brood and food sites, colour patterns, and structural morphologies (Faegri and Van der Pijl,1979;Sakai,2002). On the other hand, some flowers represent sites to attract many kind of visitors vary in the behavior, some of them are pollinators,while others prey on the pollinators or even feed on the flowers itself (Dukas,2004).

No studies have been published on pollination and flower visitors of Caralluma, except some scattered observations (see Meve and Liede,1994), compared with other genera like Asclepias, Calotropis, and Ceropegia (Kunze,1991 and references within; Masinde, 2004).

This study documents floral biology and visitors behaviour in Caralluma acutangula,which represents one of the most succulent species of Asclepiadoideae in Southwestern Saudi Arabia.

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2. MATERIALS and METHODS

Study species and sites

Caralluma acutangula is a perennial erect stem succulent, 0.4-1m tall. Flowers are crowded in terminal dark globose heads on stems. Flowers bloom after summer rain,from July- October. Field observations were conducted in rocky habitats (Tihama hill and slopes), 3 km east of Abu-Arish city,Jazan district,southwestern Saudi Arabia, between 100-400 m a.s.l. Plant distribution is patchy in these areas, where the plant grows in microhabitats between rocks and beneath shrubs. Average annual temperature is 32.3°C , whereas annual rainfall is between 186-328 mm . The study was carried out in the flowering stage of summer rainfall (July-October 2010).

Flower morphology

Flower morphology was described based on concepts of Asclepiadoideae (Liede and Kunze,1993; Meve and Liede,1994). Flowers were collected from the field and immediately transferred to the laboratory to be examined under stereomicroscope.

Flower visitors and their behaviour

Sampling (in total 104 flowers) was undertaken in the period (07:00 a.m. – 16:00 p.m.). At the same times, the behavior and movement of the visitors were observed closely. The frequency of visitors were recorded as (very frequent, frequent, infrequent) (Taroda and Gibbs,1982). Specimens collected were killed in chloroform tubes (insects bearing pollinia) and examined immediately under stereomicroscope or preserved in 70% ethanol for subsequent examination. Samples were identified by Amoudi(1997) in the case of flies ; Larsen(1984) in case of butterflies; Taher and Faragalla (1990); Hawkeswood (2003) in case of spiders (at family level).

Statistical Analysis

Data of visitors are represented as mean ± SE (visitors to N of flowers = 104). Statistical significance was evaluated using Student's t-test.

3. RESULTS

Flower morphology

Flowers of Caralluma acutangula are arranged in dark large globose heads,pseudumbles inflorescence (fig.1,A). Flowers emit fetid scents,resembling rotten meat or decaying organic matter. The single flower consists of five petals(corolla lobes). The surface of the corolla lobe is wrinkled. The corona (the centre of the flower) includes white coloured stigma head surrounded by five staminal coronas. Five pollination units, the pollinia,are located on the margins of the stigma head, just above guide rails. Guide rails represent narrow slits (to received pollinia)(fig.1,E). These guide rails produce droplets of nectar as seen under the microscope. The droplets drop and accumulate in the nectar cavity below the guide rail (fig.1,C).

Pollination and visitors behavior

C. acutangula flowers were visited mainly by insects in orders Diptera and Lepidoptera as well as Arachnoidae (Thomosidae-crab spiders).

Diptera were the most frequent visitors to flowers (table 1.) followed by Plain Tiger butterfly (as observed by larval stage feeding on flowers), and finally crab spiders (Thomisidae).

Diptera visitors belonged to three families: Muscidae, Sarcophagidae, and Calliphoridae. Female were more frequent than males ($P<0.001$) except Chrysomya marginalis which male and female were equall in percentage of visiting (fig. 2.).

The behavior of all fly species was almost similar whereby the fly lands on the flower attracted to the corona at the center of the flower. In order to reach the nectar cavities inside,the fly then extends its proboscis and probe the surface of these narrow chambers which are wide enough only for

the proboscis (fig. 1,B). This activity leads to attachment of the pollinia to the proboscis (fig.1,D). In all cases,pollinia were found attached only to the proboscis. Upon repeating this process with the nectar cavity of another flower, the pollinia enter the slit of guide rail and hence the flower ensures pollination (fig1, F). No pollinia were ever detected adhering to the bodies of other groups of visitors.

Other visitors to the flowers of C. acutangula were the Plain Tiger butterfly (Danaus chrysippus-Nymphalidae) and crab spiders (Thomisidae)(fig.3). Larvae of D. chrysippus were frequently found on flowers and feed on corolla lobes (fig.3,A). The duration of feeding behavior is not included in this study. On reaching maturity,the larvae left the flowers to search for suitable pupation sites (usually part of the stem just below the flowers-fig.3,B,C).

Thomisidae spiders were infrequently found on the flowers. They hide between flowers in inflorescence, waiting for Diptera prey (fig.3,D,E).

4. DISCUSSION

Faegri and Van der Pijl (1979) have distinguished two types of pollination syndrome for fly-pollinated flowers: myophily and sapromyophily. General features of myophily are simple structures ,light colours, imperceptible odour,easy access to nectar and exposed sexual organs (anthers and stigma). Sapromyophily is characterized by dark or brown-purple colour (or blotched dark spots), pollination units frequently have great depth, and odour resembling that of decaying organic matter. Attraction in sapromyophilous flowers is by deceit, with flies confusing the flowers for decaying organic matter as a suitable brood and food site (Faegri and Van der Pijl,1979; Dafni,1984; Jürgens et al.,2006). Typical examples of sapromyophily are found in the genera Stapelia (Apocynaceae-Asclepiadoideae), Arum (Araceae), Rafflesia (Rafflesiaceae), and some Orchids (Kevan and Baker,1983; Beaman et al.,1988; Albre et al.,2003).

On the basis of the above concepts,the flowers of Caralluma acutangula belong to sapromyophily syndrome since they have dark colour, pollination units not exposed, and odour resembling that of decaying organic matter.

In entomophillous flowers,structures vary and are adapted to fit the structure and behavior of insect pollinators. By analogy, flower-visiting insects have many modifications to get flower attractants (nectar and pollen) especially in the mouthparts (Krenn et al.,2005). Flowers of C. acutangula don't have easy access to nectar and pollinia, so they must have many adaptations in shape,colour and odour to attract pollinators. Pollinators of C. acutangula are flies from families Muscidae,Calliphoridae, and Sarcophagidae. These flies have a short proboscis. Most flower-visiting Diptera with a short proboscis take nectar from flowers with open and easily accessible nectarines (Brackenbury,1995; Krenn et al.,2005). On the other hand,flies of Muscidae,Calliphoridae, and Sarcophagidae,especially species visiting C. acutangula flowers, common in slaughter-

Table.1. Frequencies of visitors for flowers of ***C. acutangula***

Visitors	Frequency
Flies	Very frequent
Plain tiger butterfly	Frequent
Crab spiders	Infrequent

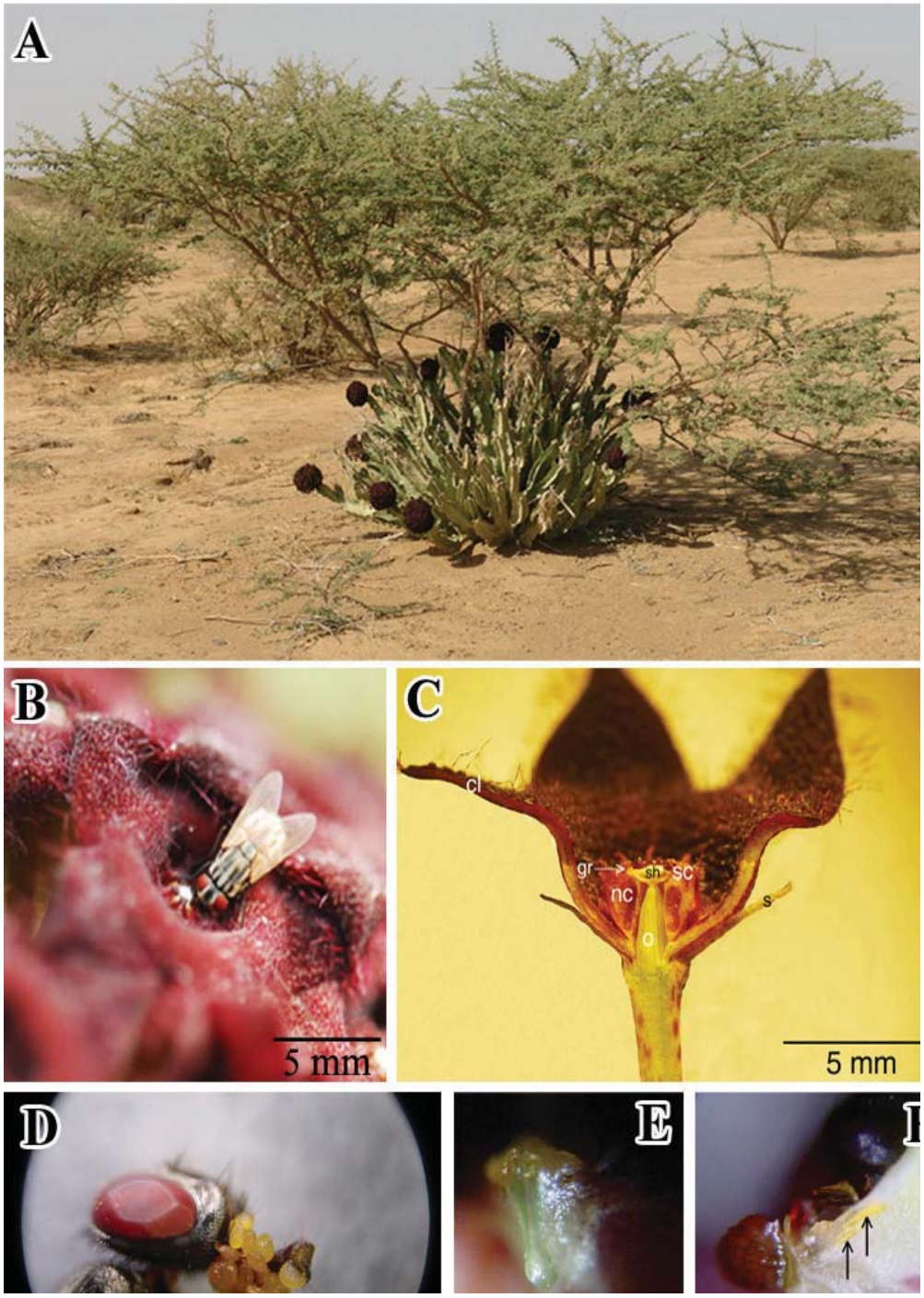

Fig.1. A. *Caralluma acutangula* in its natural habitat. **B.** *Musca domestica* on a flower. **C.** L.S. in the flower. **D.** Pollinia attached to *Musca domestica* proboscis. **E.** guide rail. **F.** Guide rail after entrance of pollinium (L.S) (arrows indicate germination tubes). (abbreviations in **C.** cl. Corolla lobe, gr. guide rail, sh. stigma head, nc. nectar cavity, sc. staminal corona, o. ovary, s. sepal).

houses and garbage dumps, being share in attracted to meat in which the eggs are deposited (Buttiker et al.,1979; Gadallah and Bosly, 2006). Flowers of C. acutangula with odour resembling rotten meat (or carcasses), and appearance with dark colour and wrinkled surfaces, mimic brood and food sites of these flies.

After the attraction and landing on the flowers, the fly may try to search for a suitable location to lay eggs(most of flies visiting are females). In the behaviour of laying eggs in females, choosing a site correlates to two stimulators: general stimulator to site (odour) and special stimulator to specific location (particular concentrations of cations in excretion decaying matter sensed by receptors on ovipositor of the flies) (Rice,1977; Byrd and Castner, 2001; Amendt et al.,2004). Therefore, fly females cannot lay eggs on dry decaying matter (Byrd and Castner,2001; Amendt et al.,2004). In our field observations,we did not observed eggs on the flowers, which may confirm the above mentioned. Flowers of C. acutangula seem to mimic general stimulator to brood site (odour), whereas the dry appearance of corolla lobes surfaces does not motivate the special stimulator to lay eggs.

Nectar in most succulents of Asclepiadoideae is found in the guide rail and flows to the bottom of the nectar cavity(Meve and Liede,1994; Bruyns,2000a). Nectar cavities of C. acutangula are narrow,and nectar is not easily accessible. In this case, the presence of nectar may be detected by taste sensory organs on the fly's labellae, maxillary palps and tarsi. Touching of these organs, especially tarsi, with flowing nectar stimulates the proboscis to extend and probe (Chapman,1971; Daly et al.,1978; Vogel,1983). On searching for nectar in the nectar cavity, the fly must extend its proboscis in this narrow chamber and probe cavity surfaces. This behavior leads to attachment of pollinium to the proboscis. On repeating this process with another cavity, the chance is suitable to entrance of pollinium into the guide rail, and thus the flower ensures pollination. The dependence on flies for pollination seems to be an adaptive strategy, because these insects are widespread, prefer high temperatures, and are present at all times of the year (Büttiker et al.,1979; Faegri et al.,1979).

Another visitor to the flowers of C. acutangula is the Plain Tiger butterfly (Danaus chrysippus-Nymphalidae). It is well known that butterflies of Danaus spp. feed exclusively on poisonous plants of Apocynaceae (especially Asclepiadoideae) which contain cardiac glycosides (cardenolides) (Larsen,1984; Zalucki and Brower,1992; Mebs et al.,2005). The larvae sequester these poisons and pass them to the adults in concentrated form. Small mammals and birds react with vomiting when they attempt to eat them (Mebs et al.,2005). The female of D. chrysippus lays eggs on the flowers. After hatching, larvae feed on corolla lobes. It is not known if the flowers of C. acutangula contain cardenolides or not. Some samples of D. chrysippus feeding on Apocynaceae plants do not contain cardenolides (Mebs et al.,2005) indicating a lack of them in eaten plant parts. On the other hand,some chemicals (like flavonol glycosides) in Apocynaceae plants act as oviposition stimulants for Danaus spp.,which means that recognition of host plants by these butterflies depends on the unique chemistry of these plants (Haribal and Renwick,1998). Existence of the larvae on the flowers of C. acutangula, not on any plant species in the same habitat, confirm the above mentioned.

Crab spiders (Thomisidae) are infrequently found on flowers (as compared with other visitors). This observation may be misleading, because these spiders hide in flowers (below petals), especially light colour species (Hawkeswood,2003). They sit

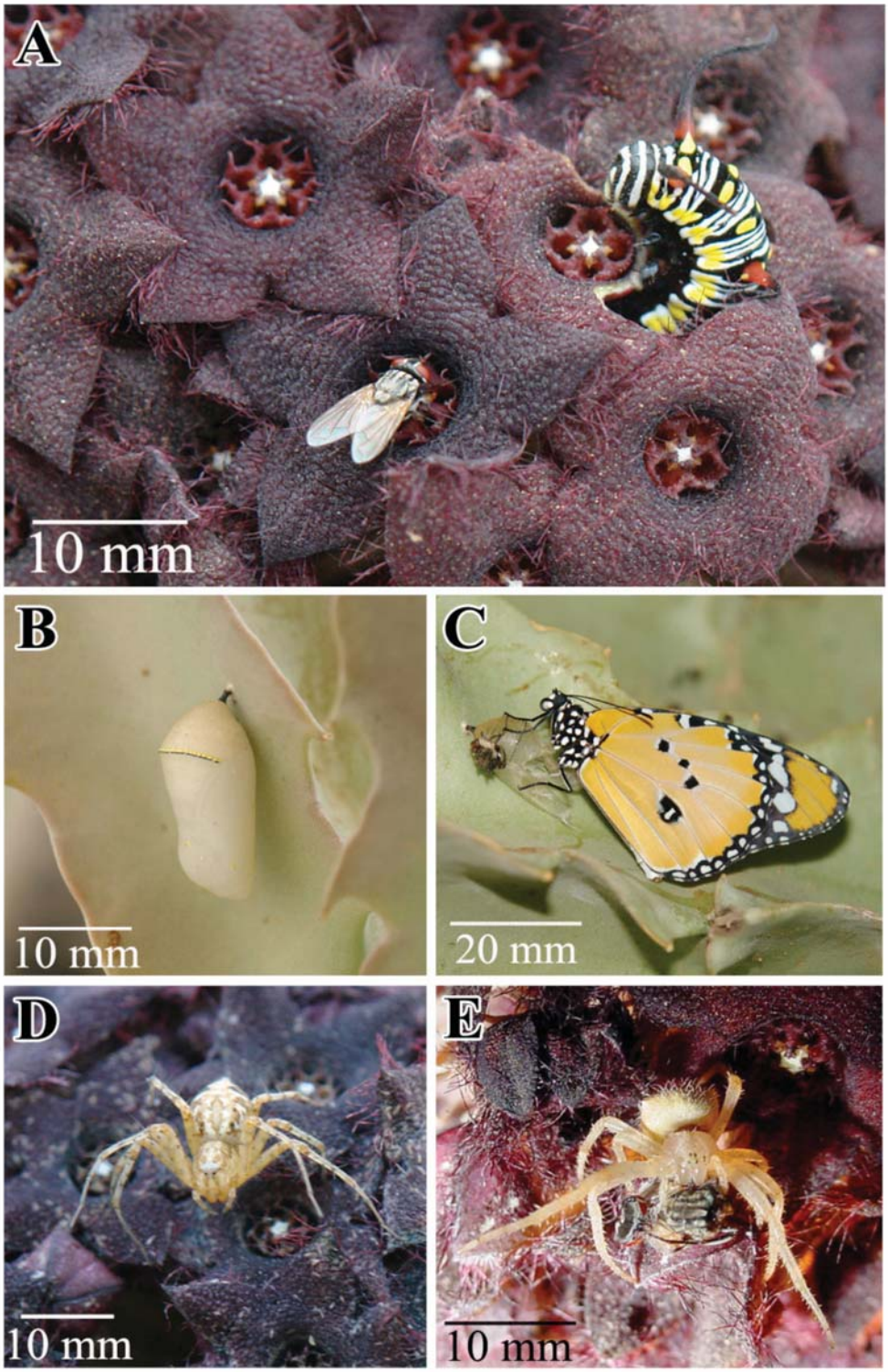

Fig. 3. A. Larva of *Danaus chrysippus* feeds on a flower of ***C. acutangula*** **. B.** Pupa on a stem of ***C. acutangula.*** **C.** The adult butterfly is shown minutes after it emerged from the pupa. **D,E.** Thomisidae spiders on the flowers (in E spider has caught and is feeding on *Musca domestica*).

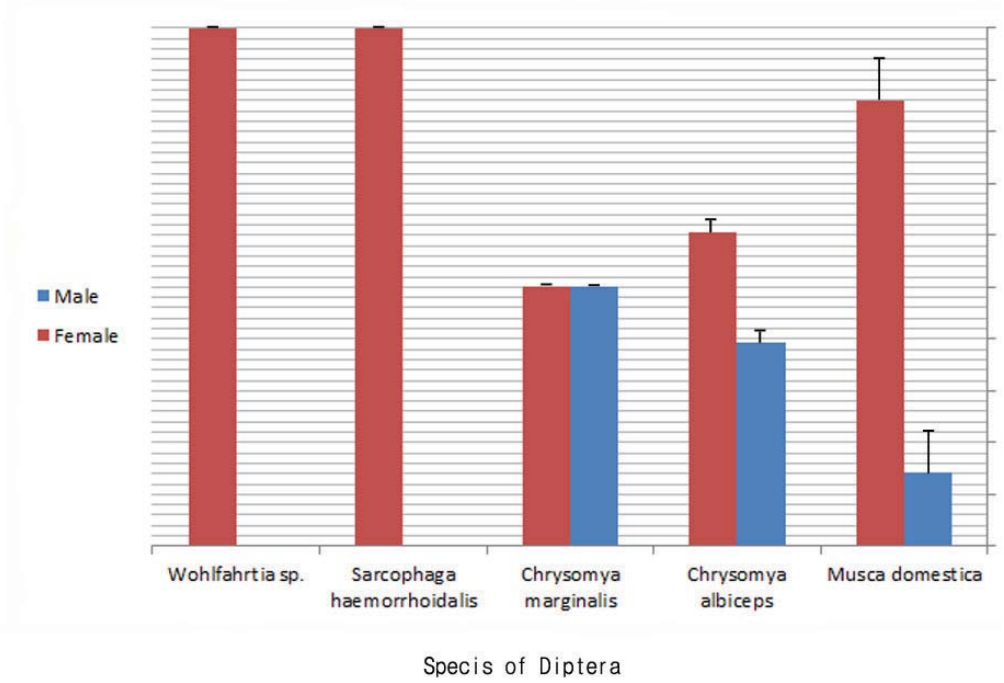

Fig.2. Percentage of sex in Diptera visiting to flowers of *C. acutangula*

motionless to wait for landing insects, and then seize them by strong front legs,bite and inject a rapidly acting poison (Foelix,1996). Crab spiders represent most frequent ambush predators that sit on or near the flowers (Dukas,2004). Crab spiders respond to the floral signals in the same manner as pollinators, including olfactory signals (Aldrich and Barros,1995; Heiling et al.,2004).

Flowers of C. acutangula represent three types of plant-visitors interactions :

1-Seduction and deception of Diptera for pollination,

2-Predation of flowers (larvae of D. chrysippus),

3- Act as an attractive microhabitat to insect predators (Thomisidae spiders).

These three types of interactions seem to share in the same manner of visitors attractants: olfactory and chemical signals, and we emphasize that further work is required.

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بيولوجيا الأزهار وسلوك الزائرات لنبات الغلفي في منطقة جازان،جنوب غرب المملكة العربية السعودية

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المُلخّص

يعتبر نبات الغلفي أحد أكثر عصاريات تحت الفصيلة العشارية تواجداً في منحدرات سهول تهامة جازان،جنوب غرب المملكة العربية السعودية. تجذب أزهار النبات العديد من أنواع الذباب، خصوصاً ضمن فصائل الذباب المنزلي،ذباب اللحم، والذباب المعدني. لجذب الملقّحات تسلك الأزهار سلوك الخداع والمحاكاة لأماكن وضع البيض والغذاء؛ ولذا فإن إناث الذباب هي أكثر الزائرات تردداً. تؤثر المحاكاة التي تبديها الأزهار على سلوك الذباب مؤدية إلى التصاق الكتل اللقاحية بخرطوم الحشرة ونقلها إلى المستقبل الميسمي(الشق الموجّه). من الزائرات الأخرى للأزهار الفراشات، وتتمثل بنوع وحيد هو فراشة نمر السهول؛ حيث تتغذى يرقاتها على الأزهار. بعض أنواع العناكب(من فصيلة عناكب السرطان) تنجذب أيضاً للأزهار؛ حيث تختبئ بين الأزهار لاقتناص الفرائس من الذباب الزائر. تلقي هذه الدراسة بعض الضوء على العلاقة المعقدة بين الأزهار وزائراتها في البيئات الجافة.

كلمات مفتاحية : نبات الغلفي – الأزهار – الذباب المنزلي – ذباب اللحم – الذباب المعدني – جازان – المملكة العربية السعودية.

فهرس المحتويات

الموضوع صفحة

مجلة جامعة جازان
فرع العلوم التطبيقية

دورية علمية محكمة

المجلد ١ العدد ١ محرم ١٤٣٣هـ (ديسمبر ٢٠١١م)

المراسلات

توجه جميع المراسلات إلى:

رئيس هيئة التحرير

مجلة جامعة جازان

٤٤٢١- حي الروابي

وحدة رقم ٨

جازان ٨٢٨٢٢-٦٥٦١

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رقم الإيداع : ١٤٣٣/٢٢٨

مطابع مدينة الملك عبدالعزيز للعلوم والتقنية
الرقم: ٣٣٠٥١٠

ردمد : ١٦٥٨-٦٠٥٠

قواعد النشر في المجلة

مجلة جامعة جازان دورية محكمة تنشرها الجامعة، وهي تهدف إلى إتاحة الفرصة للباحثين لنشر إنتاجهم العلمي وتقوم المجلة بنشر المواد الآتية:

١- البحث: ويندرج تحت تخصص الباحث ويجب أن يحتوي على إضافة للمعرفة في مجاله.

٢- المقالة الاستعراضية التي تتضمن عرضاً نقدياً لبحوث سبق إجراؤها في مجال معين أو أجريت في خلال فترة زمنية محددة.

٣- البحث المختصر.

٤- نقد الكتب.

٥- الخطابات الموجهة إلى المحرر، والملاحظات والردود، والنتائج الأولية.

تقوم هيئة التحرير، بالنظر في نشر المواد المعرفية ذات الصلة بذلك الفرع، وتقدم البحوث الأصلية، التي لم يسبق نشرها، وفي حال قبول البحث للنشر، لا يجوز نشره في أي منفذ نشر آخر ورقياً أو إليكترونياً، دون إذن كتابي من رئيس هيئة التحرير.

تعليمات النشر في المجلة

١- تقديم المواد: يقدم أصل البحث مخرجاً في صورته النهائية متضمناً الإشارة إلى أماكن الجداول والأشكال داخل المتن ومطبوعاً على هيئة صفحات مرقمة ترقيماً متسلسلاً، مع ضرورة إرفاق قرص ممغنط مطبوع عليه البحث على برنامج Ms Word باستخدام النظام المتوافق مع IBM، وسيعتذر عن قبول أي بحث لا يلتزم مؤلفه بهذه التعليمات.

٢- الملخصات: يرفق ملخصان بالعربية والإنجليزية للبحوث والمقالات الاستعراضية والبحوث المختصرة. على ألا يزيد عدد كلمات كل منهما على ٢٠٠ كلمة، وعلى عمود واحد بعرض كتابة ١٣ سم.

٣- لا بد من احتواء كل بحث على كلمات مفتاحية (Key Words) توضع أسفل الملخصين العربي والإنجليزي على ألا تزيد عن عشر كلمات.

٤- الجداول والمواد التوضيحية: يجب أن تكون الجداول والرسومات واللوحات مناسبة لمساحة الصف في صفحة المجلة (١٦ × ٢٤سم بالحواشي، ويتم إعداد الأشكال الخطية على برامج الحاسب الآلي، ولا تقبل إلا أصول الأشكال. كما يجب أن تكون الخطوط واضحة ومحددة ومنتظمة من حيث كثافة الحبر وتناسب سمكها مع حجم الرسم، ويراعى أن تكون الصور الفوتوغرافية (الضوئية) الملونة وغير الملونة مطبوعة على ورق لَمّاع، أو محملة على برنامج (Adobe Photoshop). مع كتابة عنوان لكل جدول، وتعليق لكل شكل وصورة، والإشارة إلى مصدر المادة إن كانت مقتبسة.

٥- الاختصارات: يجب استخدام الاختصارات المقننة دولياً مثل: سم، مم، م، كم، سم٢، مل، مجم، كجم...إلخ.

٦- المراجع: يشار إلى المراجع داخل المتن بنظام الاسم والتاريخ، وتوضع المراجع جميعها في قائمة المراجع بنهاية المادة مرقمة ومتبعة نظام ترتيب البيانات الببليوجرافية التالي:

أ) يشار إلى الدوريات في المتن بنظام الاسم والتاريخ بين قوسين على مستوى السطر. أما في قائمة المراجع فيبدأ المرجع بذكر الاسم الأخير للمؤلف، ثم الاسم الأول، ثم الأسماء الأخرى أو اختصاراتها بالخط الأسود، ثم سنة النشر بين قوسين، فعنوان البحث كاملاً بين علامتي تنصيص " "، فاسم الدورية، فرقم المجلد، ثم رقم العدد: ثم أرقام الصفحات تفصل بشرطة.

مثال:

هادي، أحمد بن جابر. (٢٠١١م)، "استخدام تقنية النانو لتعريف الشفرات الوراثية" مجلة جامعة جازان، ١، ١: ٢٠٠-٢٢٠.

ب) يشار إلى الكتب في المتن داخل قوسين بالاسم والتاريخ.

أما في قائمة المراجع، فيكتب الاسم الأخير للمؤلف، ثم الاسم الأول، ثم الأسماء الأخرى أو اختصاراتها، ثم سنة النشر بين قوسين، فعنوان الكتاب بين علامتي تنصيص، ثم بيان الطبعة، فالناشر، فمدينة النشر: ثم صفحات الكتاب إن وجدت.

مثال:

عبدالهادي، محمد علي، (١٤٣٣هـ)، "مقدمة في التقنية الحيوية"، جامعة جازان، جازان.

ويجب عدم استخدام الاختصارات المرجعية مثل: المرجع نفسه. المرجع السابق...إلخ.

٧ – أ. الحواشي: تستخدم لتزويد القارئ بمعلومات توضيحية، ويشار إليها في المتن بأرقام مرتفعة عن السطر. وترقيم التعليقات متسلسلة داخل المتن. وفي حال الضرورة؛ يمكن الإشارة إلى مرجع داخل الحاشية عن طريق استخدام كتابة الاسم والتاريخ بين قوسين وبنفس طريقة استخدامها في المتن، وتوضع الحواشي أسفل الصفحة التي تخصها والتي ذكرت بها و تفصل بخط عن المتن وبخط أصغر.

ب. يستخدم في تخريج الأحاديث والآثار الطريقة المنهجية المعتمدة في هذا الفن وهي كالآتي/ اسم المؤلف – اسم الكتاب – رقم الجزء والصفحة والحديث)

٨- المواد المنشورة في المجلة لا تعبر؛ بالضرورة، عن رأي جامعة جازان.

٩- المستلات: يعطى المؤلف (٢٠) عشرين نسخة مجانية من بحثه.

١٠- المراسلات: توجه جميع المراسلات إلى:

رئيس هيئة التحرير
مجلة جامعة جازان
٤٤٢١- حي الروابي
وحدة رقم ٨
جازان ٨٢٨٢٢-٦٥٦١
المملكة العربية السعودية

١١- تصدر المجلة مرتين في العام.

المملكة العربية السعودية
وزارة التعليم العالي
جامعة جازان

مجلة جامعة جازان

فرع العلوم التطبيقية

دورية علمية محكمة

المجلد ١ العدد ١ محرم ١٤٣٣هـ (ديسمبر ٢٠١١م)

ردمد : ١٦٥٨-٦٠٥٠