





Ashraf M. Ahmed

King Saud University, 2015





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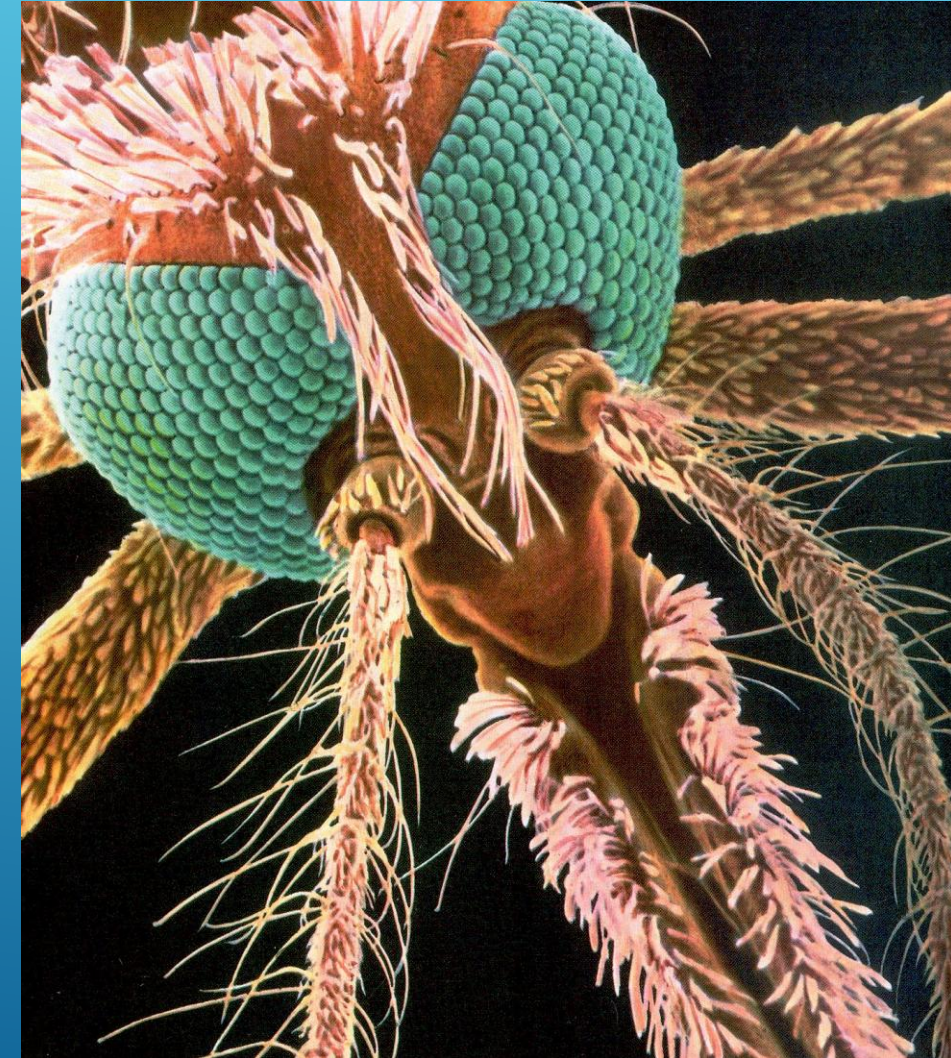
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



الخطة الوطنية للعلوم والتقنية
The National Plan for Sciences & Technology

***Bacillus thuringiensis* strains native to Saudi Arabia with enhanced larvicidal toxicity against the Rift Valley Fever mosquito vector, *Aedes caspius*.**

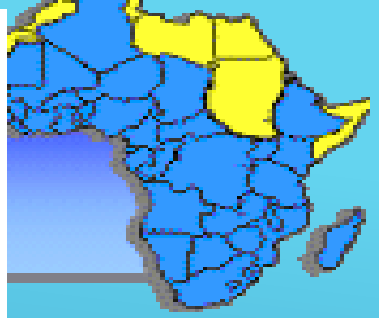
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Yazeed A. Al-Sheikh, Fahd A. Al-Mekhlafi



Filaria

- Transmitted by **Culex** mosquitoes
- *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*
- Nearly **1.4 billion** people in 73 countries worldwide are threatened by filariasis.
- Over **120 million** people are currently infected.





13

47



5

6



Dengue Fever

- Viral disease transmitted by *Aedes aegypti*





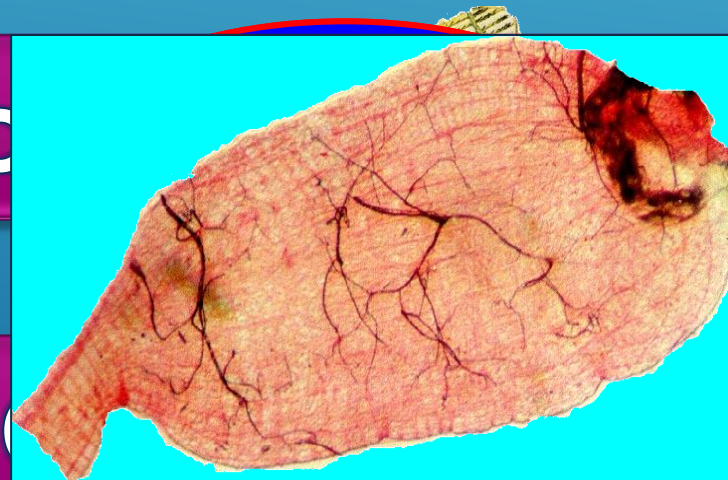
Immuno-Control Strategy of Mosquito-borne Diseases



Malaria Control

A) Immuno-control

B) Malaria prevention





Paskew
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A. O. and
432-439.

n (Refractory)

A) Immuno-control (**Vector Refractoriness**)

Dimop
Kafato



ller, H-M. and
: 11508-11513

ceptible)



Immuno-control



Shahabuddin, M.; Fields, I.; Bulet, P.; Hoffman, J. A.
and M. *Parasitology*. 89:
103-112



Ramiro
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B)- Mosquito Transgenesis



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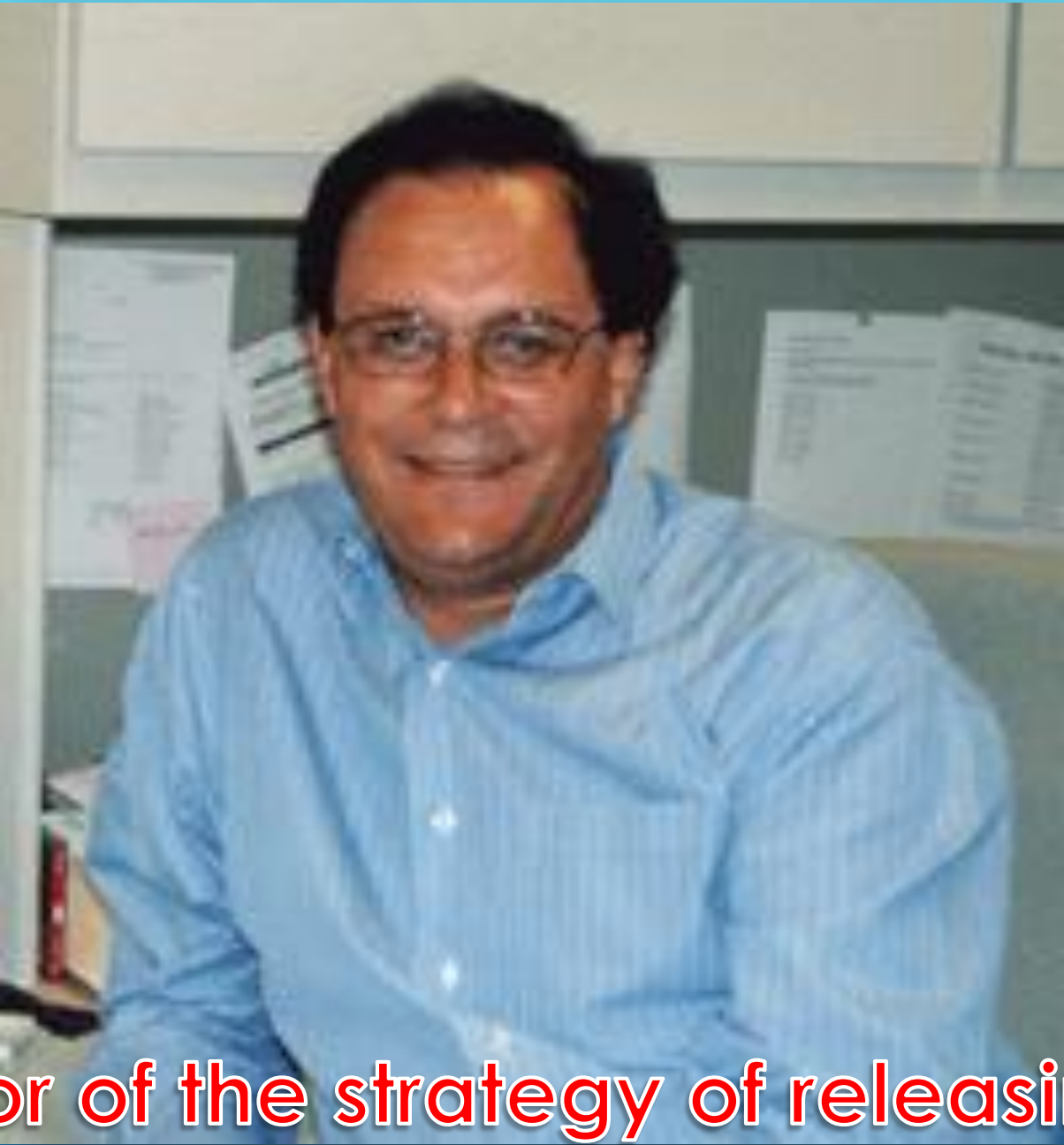
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from An.

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iene, N.;

, USA. 97:

refractory)

This is in the favor of the strategy of releasing transgenic mosquitoes into the field.



Refractoriness: disadvantage



Yan G, et al. (1997). *Evolution*, 51:441-450.

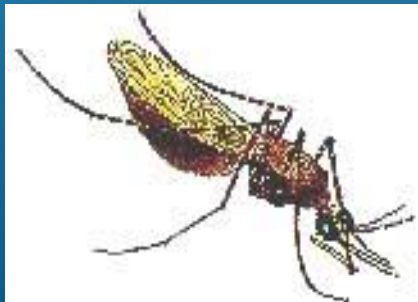
Ae. aegypti
gallinaria
and la

refractoriness to *P.*
density, smaller body sizes
susceptible ones.

Hurd H, et al.
An. gambiae
nigeriensis
ones af



2.
refractoriness to *P. y.*
spring than susceptible



Somboon, et al. (1999). *Medical and Veterinary Entomology*. 13: 355-361.

An. dirus selection for refractoriness and
susceptibility to *P. y. nigeriensis*.



More Risks



How these would be introduced into the wild (**Field Release**)?



How GMM would be **competent** with the naturally existed wild-mosquitoes



Transgenic mosquito will require **careful assessment** before the approach could become reality.



C) Approaching the nature: Entomopathogenic bacteria



Targeted mosquitocidal bacteria

Bacillus thuringiensis (Bt):

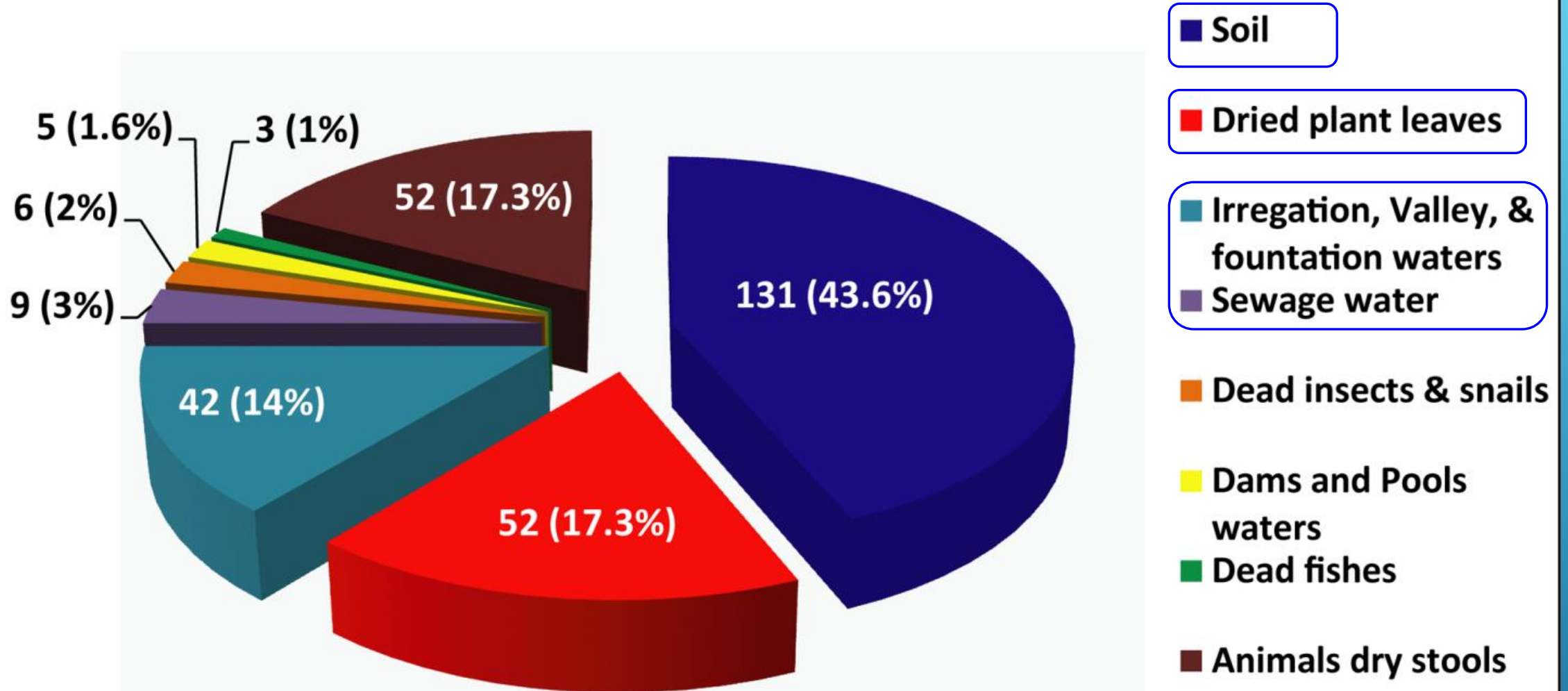
- Gram-positive spore forming bacterium belongs to Genus *Bacillum*.
- Known with its mosquito larvicidal activity (Goldberg and Margalit, 1977).
- Isolated from the Saudi environment (El-Kersh *et al.*, 2012).





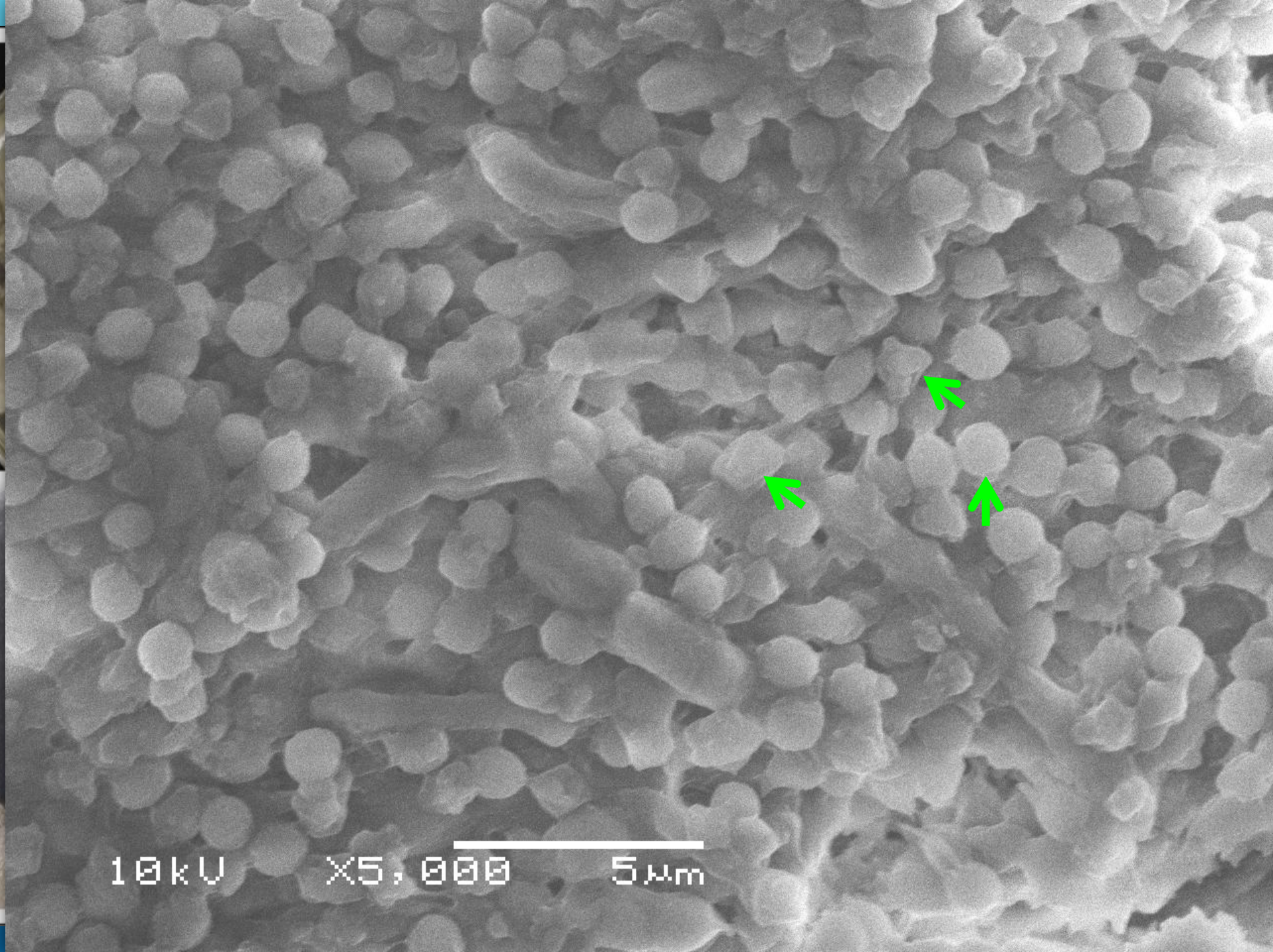
Geographical distribution of processed samples

Types and numbers of collected samples



A

B



10kV

X5,000

5 μm



BIOASSAY TEST

Bt-code	City of collection	Hrs P-T	LC ₅₀ (µg/ml) (lower - upper)*	LC ₉₅ (µg/ml) (lower to upper)	X 10 ⁵ CFU/µg	Slope ± SE
Bt-63	Makkah	24	0.91 (0.7 – 1.07) ^a	5.51 (4.1 – 8.2)	0.7 ± 0.2	2.1 ± 0.053
		48	0.5 (0.41 – 0.58)	1.12 (0.95 – 1.4)		4.72 ± 0.006
Bt-55	Madinah	24	1.7 (1.5 – 1.98) ^b	7.9 (6.11 – 10.68)	1.95 ± 0.3	2.5 ± 0.059
		48	1.06 (0.91 – 1.2)	2.29 (2.5 – 3.8)		3.65 ± 0.21
Bt-53	Madinah	24	1.9 (1.7 – 2.2) ^b	8.08 (5.8 – 11.7)	1.3 ± 0.4	2.6 ± 0.0011
		48	0.72 (0.6 – 0.8)	2.5 (2.08 – 3.6)		2.9 ± 0.0014
Bt-60	Madinah	24	1.9 (1.7 – 2.2) ^b	5.01 (4.2 – 6.15)	0.13 ± 0.1	4.05 ± 0.002
		48	0.94 (0.8 – 1.05)	2.9 (2.5 – 3.8)		3.3 ± 0.09
Bt-68	Madinah	24	2.3 (2.06 – 2.7) ^c	10.7 (7.8 – 15.7)	1.05 ± 0.3	2.5 ± 0.055
		48	1.1 (0.97 – 1.3)	5.4 (4.2 – 7.5)		2.4 ± 0.056
Bt-42	Jezan	24	2.5 (2.2 – 2.9) ^c	15.4 (10.9 – 22.8)	0.93 ± 0.3	2.1 ± 0.048
		48	1.2 (1.04 – 1.35)	3.07 (2.5 – 3.8)		4.02 ± 0.26
Bt-44	Jezan	24	3.03 (2.7 – 3.4) ^d	9.4 (7.7 – 11.6)	0.63 ± 0.4	3.35 ± 0.075
		48	2.08 (1.8 – 2.3)	6.12 (5.1 – 7.5)		3.5 ± 0.094
Bt-29	Madinah	24	4.2 (3.5 – 5.0) ^e	32.6 (22.8 – 47.8)	0.52 ± 0.1	1.8 ± 0.03
		48	2.2 (1.8 – 2.7)	14.05 (10.6 – 19.2)		2.05 ± 0.037
Bt-12	Yanboa	24	4.6 (4.2 – 5.1) ^e	9.4 (7.9 – 11.08)	0.53 ± 0.4	5.4 ± 0.005
		48	2.4 (2.04 – 2.8)	10.4 (8.01 – 13.9)		2.6 ± 0.06
Bt-26	Madinah	24	4.8 (4.1 – 5.6) ^e	33.9 (21.8 – 54.5)	4.1 ± 0.7	1.94 ± 0.044
		48	1.9 (1.5 – 2.4)	14.6 (10.2 – 22.01)		1.9 ± 0.051
Bt-15	Bti-H14	24	4.88 (4.2 – 5.4) ^e	21.93 (17.3 – 28.03)	4.5 ± 0.3	2.52 ± 0.044
		48	2.62 (2.2 – 3.1)	10.01 (7.8 – 13.2)		2.82 ± 0.092

Toxicity of *Bt* isolates against 3rd larval stage of *Cx. pepiens* at 24h post-infection. *Bt* subsp. *israelensis* strain (H14) is the positive control.

KEY; LC₅₀ = Lethal concentration (concentration to kills 50% of test organisms); LC₉₅ = Lethal concentration (concentration to kills 95% of test organisms); CFU = colony forming unit; * significant different compared to the reference positive control *Bti*-H14 (based on the non-overlapping confidence limits).



Bt-code	City of collection	LC ₅₀ (µg/ml) (lower to upper)	LC ₉₅ (µg/ml) (lower to upper)	Slope ± SE	× 10 ⁵ CFU/µg
Bt-H14	H14(Reference)	13.33 (11.2-15.9)	63.7 (40.4-102.3)	2.4 ± 0.071	4.5±0.3
Bt-05	Kharj	13.4 (11.07-16.5)	78.5 (46.0-137.0)	2.15 ± 0.0612	1.0±0.1
Bt-07	Asseer	15.0 (12.2-18.8)	90.4 (50.5-166.0)	2.12 ± 0.065	1.0±0.2
Bt-10	Qassim	4.96 (4.41-5.58)*	18.59 (14.6-23.8)	2.9 ± 0.062	1.2±0.3
Bt-11	Younboa	12.3 (10.2-14.8)*	72.6 (43.5-124.25)	2.1 ± 0.057	0.65±0.3
Bt-12	Younboa	9.5 (8.3-10.9)*	41.6 (29.4-59.6)	2.6 ± 0.0613	0.53±0.4
Bt-16	Asser	13.0 (10.6-16.0)	84.2 (47.8-152.6)	2.02 ± 0.0563	0.92±0.3
Bt-17	Hafr-elbaten	22.0 (18.2-26.7)	128.5 (76.4-219.7)	2.14 ± 0.059	8.1±0.4
Bt-26	Madinah	12.9 (9.9-16.9)	149.7 (64.7-367.2)	1.54 ± 0.0456	4.1±0.7
Bt-27	Madinah	7.1 (6.1-8.2)*	40.1 (27.3-60.2)	2.2 ± 0.048	5.9±0.4
Bt-28	Makkah	8.6 (7.7-9.6)*	27.5 (22.4-33.9)	3.3 ± 0.073	6.2±0.3
Bt-29	Madinah	14.4 (12.4-16.73)	49.4 (34.1-72.3)	3.1 ± 0.12	0.52±0.1
Bt-42	Jezaan	5.9 (5.2-6.8)*	24.3 (18.9-31.3)	2.7 ± 0.067	0.93±0.3
Bt-44	Jezaan	16.1 (13.7-18.9)	91.9 (59.3-144.2)	2.2 ± 0.052	0.63±0.4
Bt-53	Madinah	13.8 (11.7-16.4)	101.7 (61.3-171.9)	1.9 ± 0.0452	1.3±0.4
Bt-55	Madinah	4.11 (3.6-4.6)*	14.4 (11.6-18.1)	3.02 ± 0.071	1.95±0.3
Bt-56	Madinah	11.7 (10.2-13.5)*	43.8 (31.2-62.2)	2.9 ± 0.083	0.15±0.3
Bt-57	Madinah	7.4 (6.5-8.25)*	26.12 (20.9-32.9)	2.98 ± 0.068	1.27±0.3
Bt-58	Madinah	5.73 (5.06-6.5)*	24.62 (18.6-32.9)	2.6 ± 0.05	1.24±0.2
Bt-59	Madinah	10.3 (8.5-12.6)*	84.9 (46.3-161.6)	1.8 ± 0.047	1.28±0.2
Bt-60	Madinah	14.7 (12.5-17.5)	58.2 (38.1-89.9)	2.75 ± 0.096	0.13±0.1
Bt-63	Makkah	3.9 (3.4-4.4)*	15.7 (12.3-20.3)	2.7 ± 0.063	0.7±0.2
Bt-67	Madinah	6.9 (5.9-8.2)*	50.6 (31.5-83.4)	1.9 ± 0.045	0.6±0.1
Bt-68	Madinah	5.16 (4.5-5.8)*	21.6 (16.6-28.5)	2.64 ± 0.0564	1.05±0.3

BIOASSAY TEST

Toxicity of Bt isolates against 3rd larval stage of *An. gambiae* at 24h post-infection. *Bt* subsp. *israelensis* strain (H14) is the positive control.

KEY; LC₅₀= Lethal concentration (concentration to kills 50% of test organisms); **LC₉₅** = Lethal concentration (concentration to kills 95% of test organisms); **CFU**= colony forming unit; * significant different compared to the reference positive control *Bti*-H14 (based on the non-overlapping confidence limits).



BIOASSAY TEST

Toxicity of *Bt* isolates against 3rd larval stage of *Ae. aegypti* at 24h post-infection. *Bt* subsp. *israelensis* strain (H14) is the positive control.

KEY; LC₅₀= Lethal concentration (concentration to kills 50% of test organisms); **LC₉₅** = Lethal concentration (concentration to kills 95% of test organisms); **CFU**= colony forming unit; * significant different compared to the reference positive control *Bti*-H14 (based on the non-overlapping confidence limits).



Bt-code	City of collection	Hrs P-T	LC ₅₀ (µg/ml) (lower to upper)	LC ₉₅ (µg/ml) (lower to upper)	X 10 ⁵ CFU/µg	Slope ± SE
Bt-15	B.t.i. (H14) (Reference)	24	13.02(11.9-14.2)	28.74(24.4-33.8)	4.5 ± 0.3	4.78± 0.2
		48	4.1(3.5-4.7)	15.1(11.9-19.2)		2.9±0.064
Bt-05	Kharj	24	1.3(1.2-1.5)	4.7(3.8-6.05)	1.0 ± 0.1	3.02±0.0673
		48	0.54(0.43-0.64)	1.9(1.5-2.5)		3.06±0.16
Bt-07	Asseer	24	9.9(9.2-10.7)	19.37(16.77-22.41)	1.0 ± 0.2	5.64±0.26
		48	5.71(5.09-6.4)	15.25(12.5-18.7)		3.85±0.2
Bt-10	Qassim	24	3.67(3.03-4.42)	30.05(20.88-44.65)	1.2 ± 0.3	1.8±0.034
		48	1.4(1.07-1.75)	8.8(6.37-12.91)		2.05±0.059
Bt-11	Yanboa	24	3.98(3.4-4.6)	20.3(15.6-26.8)	0.65 ± 0.3	2.32±0.038
		48	2.01(1.6-2.4)	11.9(8.6-17.4)		2.12±0.052
Bt-12	Yanboa	24	4.79(4.13-5.54)	14.05(11.32-17.53)	0.53 ± 0.4	3.52±0.20
		48	1.43(1.15-1.73)	6.58(5.04-9.03)		2.52±0.47
Bt-16	Asser	24	3.88(3.46-4.35)	14.5(11.3-18.7)	0.92 ± 0.3	2.9±0.06
		48	1.44(1.3-1.6)	3.41(2.86-4.16)		4.4±0.32
Bt-17	Hafr-Elbaten	24	1.32(1.2-1.5)	5.8(4.5-8.01)	8.1 ± 0.4	2.5±0.058
		48	0.58(0.45-0.71)	2.98(2.35-4.23)		2.3±0.082
Bt-27	Madinah	24	4.58(3.96-5.29)	21.19(16.45-27.7)	5.9 ± 0.4	2.47±0.043
		48	1.25(0.95-1.58)	7.5(5.5-10.8)		2.11±0.065
Bt-28	Makkah	24	5.5(4.7-6.4)	23.3(17.5-31.4)	6.2 ± 0.3	2.62±0.064
		48	2.2(1.6-2.8)	15.9(11.1-23.9)		1.9±0.054
Bt-44	Jezan	24	2.24(1.96-2.56)	10.58(7.77-15.26)	0.63 ± 0.4	2.44±0.052
		48	1.44(0.98-1.31)	5.04(3.97-6.84)		2.55±0.0613
Bt-53	Madinah	24	2.23(1.9-2.6)	11.78(8.0-19.5)	1.3 ± 0.4	2.3±0.056
		48	1.06(0.94-1.2)	3.93(3.2-5.2)		2.9±0.062
Bt-60	Madinah	24	1.17(1.03-1.32)	4.81(3.81-6.56)	0.13 ± 0.1	2.7±0.057
		48	0.41(0.28-0.52)	2.76(2.09-4.27)		2.0±0.071
Bt-63	Makkah	24	1.28(1.12-1.46)	5.93(4.5-8.4)	0.7 ± 0.2	2.4±0.053
		48	0.7(0.6-0.8)	2.63(2.14-3.5)		2.8±0.08
Bt-68	Madinah	24	1.7(1.5-1.9)	6.7(5.2-9.2)	1.05 ± 0.3	2.7±0.0603
		48	1.07(0.95-1.2)	3.7(3.0-4.7)		3.06±0.069

Conclusion and recommendation

- We isolated 68 native *Bt* isolates, 23 of them are mosquitocidal, and only 8 of them showed higher mosquitocidal activity compared to *Bti*.
- These native mosquitocidal *Bt* could be utilized in the battle against mosquito vector in Saudi Arabia and worldwide.
- Molecular characterization of these potentially active isolates and their toxic parasporal protein crystals are currently being investigated.
- We strongly recommend to continuous use this eco-friendly biocontrol agent in the battle against mosquito vectors.



Collaborators



Prof. A. Alkhalifa

Dr. A. Mashaly

Dr. F. Almekhlafy



Collaborators



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Collaborators



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