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**Faculty of
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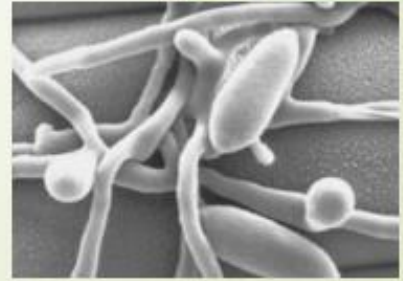
Occupational Poisoning of Pesticides to Spray Workers

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Plant Pathogens & Pest Management Methods



Pesticides

- ❑ Pesticides applied extensively to control pathogenic pests of various crops
- ❑ The intensity of pesticide use has increased considerably by a 15–20-fold worldwide in the last decade
- ❑ It help farmers reducing the lose of their crops
- ❑ It is a key factor in promoting food security
- ❑ Yearly, agricultural producers use pesticides that worth 40 billion USD
- ❑ About 998 pesticides are registered in Egypt to control different pests
- ❑ However, the use of pesticides on crops had always been a serious concern
- ❑ Adverse effects of pesticides on liver, cardiac, kidney function, haematological and biochemical characteristics - all body parameters

Study Rational & Objectives

- ❑ In developing countries, farmers are exposed to pesticides due to:
 - ❑ The incorrect application techniques,
 - ❑ Poor or inappropriate spraying equipment,
 - ❑ Inadequate storage practices,
 - ❑ Lack of personal protective equipment, and
 - ❑ Reuse of old pesticide containers for food and water storage
- ❑ Additionally, the application of pesticides on crops was done by non-certified applicators (farmers) using hand held, back held, or motors-drawn sprayers, which put farmers under a huge exposure stress



Study Rational & Objectives

- ❑ In 2012, approximately 14,000 tons of pesticides (4,808, 2,809, and 6,374 tons of insecticides, fungicides, and herbicides, respectively) were used in Egypt (FAOSTAT 2015)
- ❑ Therefore, the objectives of current study were to:
 - ❑ determine the pesticide residues in blood samples of volunteers,
 - ❑ assess the adverse effects of pesticide exposure on human health in occupational and residential settings, and
 - ❑ use the discriminant analysis to interpret and predict variable(s) that might be used for fast detection of toxicity



Study Design

Three groups of volunteers were recruited:

Treatment

**Occupationally
-exposed**

**Farmers apply
pesticides
regularly (once
every week at
least)**

Positive Control

**Indirectly-
exposed**

**Farmers live in
rural area but
do not apply the
pesticides**

Negative Control

Not-exposed

City people

Subjects were further categorized based on smoking



Methodology



**Blood Samples
Collection**



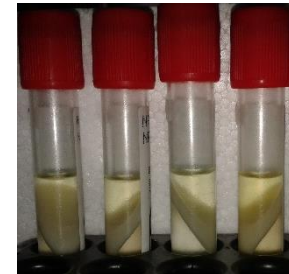
**Serum Separation for
Hormone Estimation**



**Blood Samples to be analyzed for
pesticide residue and complete
blood picture**



**Determination of Pesticide
Residues by GC-MS**



**Extraction of Pesticide Residues
by QuEChERS method**

Methodology



Analysis of Blood Picture was done in a certified clinical laboratory

S/L	Parameter	Result	Normal Range / Reference Value	Unit	Group
1	Haemoglobin	15.3	<5 yrs: 11.0 g/dL, 5 to <12 yrs: 11.5 g/dL, 12 yrs to <15 yrs : g/dL		
2	ESR (Westergreen)		Adult(M:0-10mm,F:0-15mm), Child(0-10 mm) in 1st hr	mm/1st Hour	
3	Total WBC Count	5.21	4 - 11 X 10 ³ /uL	X 10 ³ /uL	Total Count
4	Neutrophils	3.44	2.16 - 6.04 X 10 ³ /uL	X 10 ³ /uL	Total Count
5	Lymphocytes	0.02	0.60 - 3.06 X 10 ³ /uL	X 10 ³ /uL	Total Count
6	Monocytes	0.00	0.00 - 0.60 X 10 ³ /uL	X 10 ³ /uL	Total Count
7	Eosinophils	1.75	0.00 - 0.40 X 10 ³ /uL	X 10 ³ /uL	Total Count
8	Basophils	0.00	0.00 - 0.08 X 10 ³ /uL	X 10 ³ /uL	Total Count
9	Neutrophils	66	Child (25 - 66 %), Adult (40 - 70 %)	%	Differential Count
10	Lymphocytes	0.00	Child (25 - 62 %), Adult (20 - 40 %)	%	Differential Count
11	Monocytes	0.00	Child (02 - 07 %), Adult (02 - 08 %)	%	Differential Count
12	Eosinophils	33.6	Child (00 - 03 %), Adult (00 - 04 %)	%	Differential Count
13	Basophils	0.00	0 - 01%	%	Differential Count
14	Total RBC Count	5.35	3.5 - 5.5 X 10 ⁶ /uL	X 10 ⁶ /uL	RBC Panel
15	HCT	43.4	37.00 - 47.00 %	%	RBC Panel
16	MCV	81.1	76.00 - 96.00 fL	fL	RBC Panel
17	MCH	28.6	27 - 32 pg	pg	RBC Panel
18	MCHC	35.3	32.00 - 36.00 g/dL	g/dL	RBC Panel
19	RDW-SD	42.2	42.5 ± 3.5 fL	fL	RBC Panel
20	RDW-CV	14.4	12.8 ± 1.2 %	%	RBC Panel
21	Total Platelet Count	303	150 - 450 X 10 ³ /uL	X 10 ³ /uL	PLT Panel
22	MPV	10.5	9- 13 fL	fL	PLT Panel
23	P-LCR	27.7	13 - 43 %	%	PLT Panel



Methodology



Determination of Thyroid and Reproductive Hormones in Serum Samples

- ❑ Testosterone hormone was determined using the International Immuno Diagnostics Kits (Granoff and Abraham 1979 and Tietz 1995)
- ❑ Triiodothyronine (T3) hormone determination was carried out using the International Immuno Diagnostics (Burke and Eastman 1974)
- ❑ T4 hormone was carried out using the International Immuno Diagnostics (Skelley et al. 1973)

Results



Pesticides Residue Analysis Using GC-MS

Common Name	Uses		LOQ (ppb)	Common Name	Uses		LOQ (ppb)
Bifenazate	A	ND	50	Clodinafop	H	ND	25
Dicofol	A	ND	100	Ethofumesate	H	D	50
Hexythiazox	A	ND	25	Flucarbazon	H	ND	50
Chlorpyrifos-methyl	A, I	ND	10	Oxadiazon	H	ND	100
Dimethoate	A, I	ND	50	Pendimethalin	H	ND	50
Diazinon	A, I	ND	10	Aldrin	I	ND	10
Dichlorvos	A, I	ND	5	Azinphos-methyl	I	ND	20
Ethion	A, I	ND	5	a-BHC	I	ND	15
Endosulfan	A, I	ND	15	Bioallethrin	I	ND	50
Malathion	A, I	ND	10	Cadusafos	I	ND	50
Pirimiphos-methyl	A, I	ND	10	Chlorfluazuron	I	ND	50
Phenthoate	A, I	ND	10	Cyfluthrin	I	ND	15
Profenofos	A, I	ND	15	delta-BHC	I	ND	10
Propetamphos	A, I	ND	15	Chlorpyrifos	I	ND	10
Quinalophos	A, I	ND	15	gamma-cyhalothrin	I	ND	5
Triazophos	A, I, N	ND	10	gamma-BHC	I	ND	25
Bromuconazole	F	ND	30	lambda-Cyhalothrin	I	ND	10
Chlorothalonil	F	ND	25	gamma-Chlordane	I	ND	25
Cyflufenamid	F	ND	50	Cypermethrin	I	ND	25
Dicloran	F	ND	20	Deltamethrin	I	ND	15
Difenoconazole	F	ND	20	Dieldrin	I	ND	5
Diniconazole	F	ND	10	op-DDT	I	ND	5
Epoxiconazole	F	ND	20	pp-DDD	I	ND	10
Fenarimol	F	ND	10	pp-DDT	I	ND	10
Fluazinam	F	ND	20	Endrin	I	ND	50
Myclobutanil	F	ND	15	Esfenvalerate	I	ND	10
Propiconazole	F	ND	15	Fenamiphos	I	ND	15
Proquinazid	F	ND	20	Heptachlor	I	ND	10
Penconazole	F	ND	30	Heptachlor-epoxide	I	ND	10
Tetraconazole	F	ND	30	Methoxychlor	I	ND	25
Triticonazole	F	ND	30	Permethrin	I	ND	10
Triforine	F	ND	30	Prothiofos	I	ND	10
Triflumizole	F	ND	50	Tetramethrin	I	ND	20
Acetochlor	H	ND	30	Thiocyclam	I	ND	25
Atrazine	H	ND	10	Thiamethoxam	N, I	ND	50



Effects on White Blood Cell & Platelets Counts and Differentials

Criteria	City Inhabitants (-Control)		Rural Inhabitants (+Control)		Applicators	
	Non-Smoking	Smoking	Non-Smoking	Smoking	Non-Smoking	Smoking
WBC# (X*10 ³ µL)	12.80 ^a	7.32 ^c	9.68 ^b	6.82 ^c	7.31 ^c	6.60 ^c
LYM (%)	29.30 ^{ab}	29.41 ^{ab}	33.58 ^a	32.38 ^{ab}	31.89 ^{ab}	27.58 ^b
MXD# (X*10 ³ µL)	0.55 ^{ab}	0.76 ^{ab}	0.88 ^a	0.60 ^{ab}	0.42 ^b	0.40 ^b
PLT (X*10 ³ µL)	281.00 ^a	276.00 ^a	261.25 ^a	285.17 ^a	157.10 ^b	156.00 ^b
PDW (fL)	14.10 ^a	13.53 ^a	13.63 ^a	14.68 ^a	9.77 ^b	9.42 ^b
MPV (fL)	10.80 ^a	10.73 ^a	10.53 ^a	10.52 ^a	8.31 ^b	8.38 ^b
P-LCR (%)	32.25 ^a	29.32 ^a	29.82 ^a	27.38 ^{ab}	18.17 ^c	19.08 ^{bc}

Within each row, numbers followed by the same superscript letter(s) were not significantly different based on Dunnett's *post hoc* specific comparison ($P < 0.05$)

Adverse Effects on Red Blood Cell Counts and Differentials

Criteria	City Inhabitants (-ve Control)		Rural Inhabitants (+ve Control)		Applicators	
	Non-Smoking	Smoking	Non-Smoking	Smoking	Non-Smoking	Smoking
RBC ($X \cdot 10^6 \mu\text{L}$)	5.00 ^{ab}	4.78 ^{ab}	4.14 ^b	5.19 ^a	5.18 ^a	5.08 ^a
HGB (gdL^{-1})	14.05 ^{abc}	13.82 ^{bc}	12.32 ^c	15.10 ^{ab}	14.98 ^{ab}	15.86 ^a
HCT (%)	39.05 ^{bc}	41.67 ^{ab}	35.13 ^c	43.60 ^{ab}	43.69 ^{ab}	45.18 ^a
MCH (pg)	27.30 ^b	27.37 ^b	29.93 ^a	30.42 ^a	30.38 ^a	30.66 ^a

Within each row, numbers followed by the same superscript letter(s) were not significantly different based on Dunnett's *post hoc* specific comparison ($P < 0.05$)

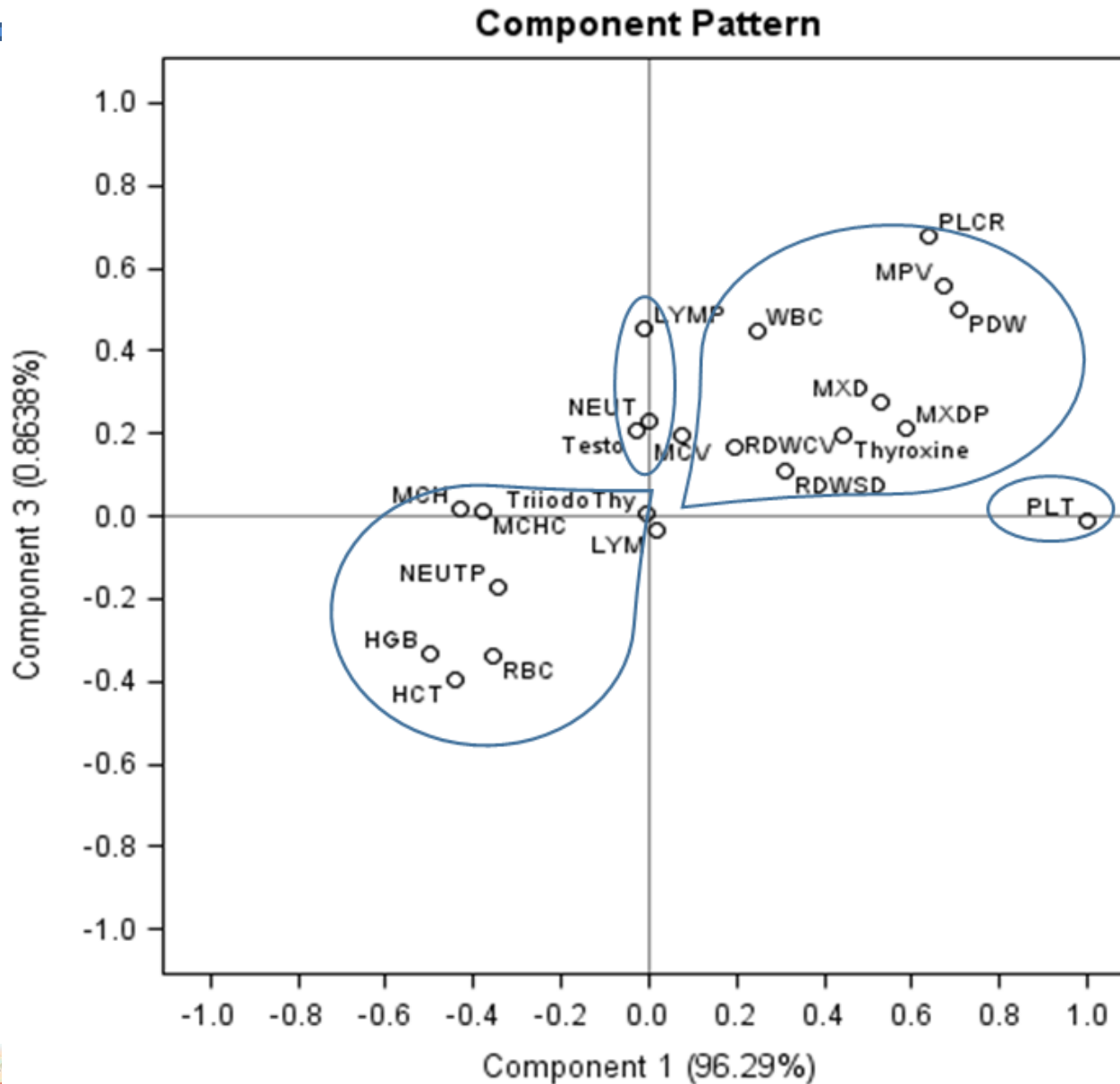
Adverse Effects on Hormones

Concentration (ng/ml)	City Inhabitants (-ve Control)				Rural Inhabitants (+ve Control)				Applicators			
	Non-Smoking		Smoking		Non-Smoking		Smoking		Non-Smoking		Smoking	
Testosterone	5.57	a	3.29	ab	2.83	bc	1.54	c	2.46	bc	3.27	ab
Triiodothyronine (T3)	1.52	a	1.09	a	1.49	a	1.14	a	1.34	a	1.41	a
L-Thyroxine (T4)	10.81	a	6.49	b	6.50	b	5.49	bc	3.40	c	5.94	b

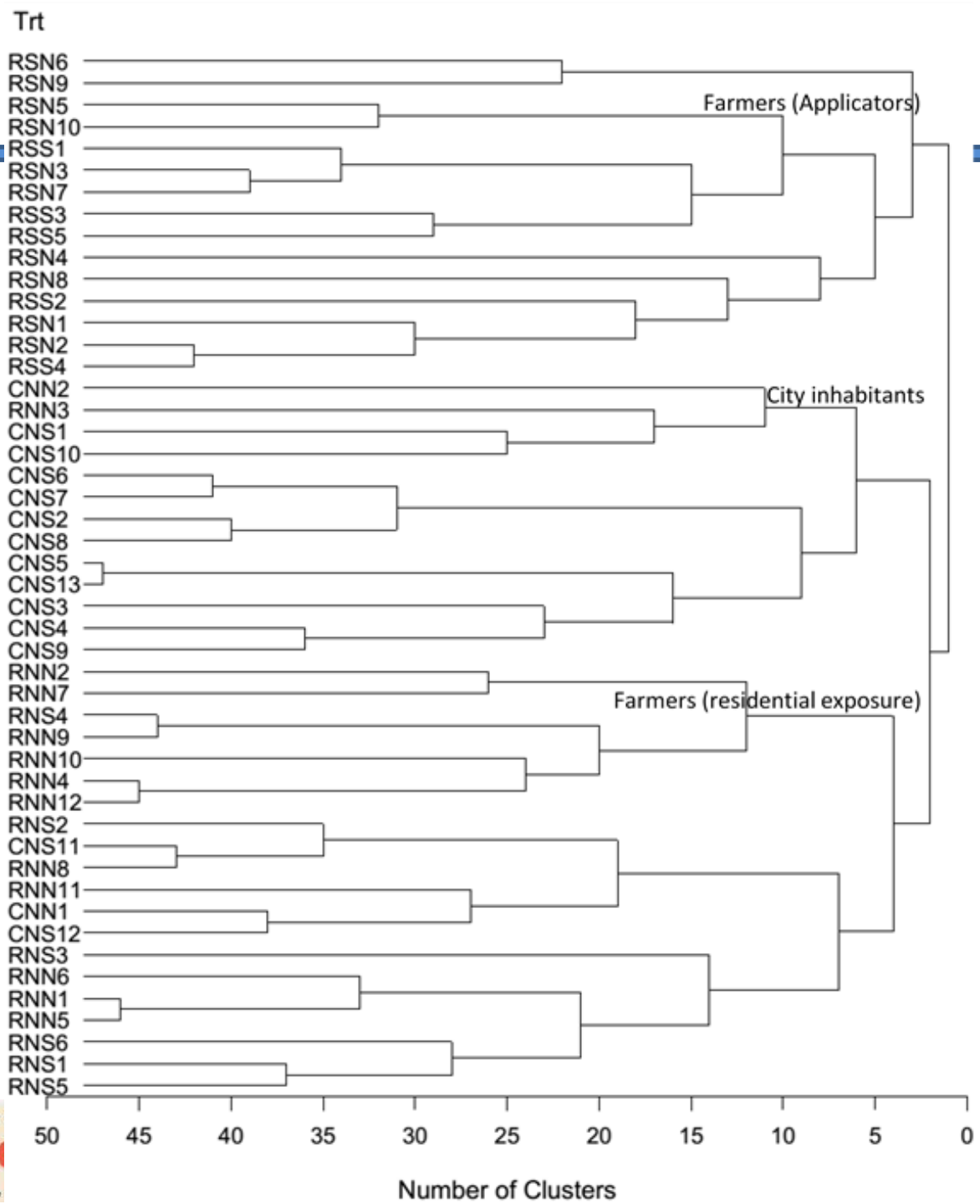
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Component Pattern Analysis Plot of The PCA



Tree dendrogram of the clustering patterns and their proportion of explained variance



Conclusions

- ❑ Lack of the use of personal protective equipment and follow a restrict personal hygiene maximized the pesticide occupational exposure
- ❑ Pesticides residues were not detected in the serum samples
- ❑ No significant differences between RBC, platelet count and HGB values in blood samples of the sprayer group compared to control groups
- ❑ WBC count was significantly decreased in blood samples of pesticide sprayers
- ❑ Pesticide exposure has altered the content of the thyroid and reproductive hormones
- ❑ Smoking revealed a unique effect on the groups of volunteers and not just pesticide applicators
- ❑ Farmers who smoke and were exposed to pesticides were under greater risk than non-smokers



Conclusions

- ❑ **Multivariate analysis showed an association between smoking and pesticide occupational exposure**
- ❑ **The hierarchical cluster analysis efficiently classified groups of treatments; applicator (smokers or not), farmers (residential exposure; smokers or not), and city residents (control)**
- ❑ **The principal component analysis showed that the hematological and hormonal parameters might be good predictor variables in subsequent analyses**
- ❑ **Current study highlights the critical need for official regulations and enforced interventions to reduce overexposure of spray workers throughout Egypt**



Personal Protective Equipment





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Thank You!
Comments?



4th International Conference and Exhibition on
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