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The impact of organophosphorus and carbamate compounds on cholinesterase among Gandato agricultural scheme farmers

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Abstract
This analytical study was carried out to detect the impact of organophosphorus and carbamate compounds on cholinesterase among Gandato agricultural scheme farmers.this agricultural scheme is one of two major schemes in Shandi town,178km northern Khartoum. an interview was done with the farmers to detect the type of insecticides that used by the farmers.
sample size was detected using the formula:

$$\frac{n= no}{(1+no/N)}$$ where:

n:$Sample\ size.$
no:$initial\ sample\ size.$
N:$population\ size.$
A questionnaire also was designed and filled by farmers (both users and nonusers of Ops and carbamates compounds) then the blood test for two groups was done to detect the effect of such compounds on acetyl chorine esterase (AChE) activity, and it was found that (24.4%) of them has a depletion in their AChE activity.while only (2.2 %) of the control group were affected.
In spite of that, (70.8%) of the exposed group were not trained about spraying insecticides, 74.5% of them did not received any caution measures or guidance from the sellers.
The study showed that there is a clear relation between (AChE) depletion and preventive measures used by exposed group after using insecticides (P.V< 0.05).

Consent: The objectives of this study was clearly explained to the general manager of the farmers' union in Shendi agricultural schemes(Gandato and Kabosheyah) who explained the scientific goals of the study to all farmers and acquired their consent and voluntary participation in blood examination.

Keywords: Acetyle Choline esterase, organophosphorus, carbamate

1. Introduction
Insecticides are substances which are used to kill insects; the word pesticide is a general term that includes insecticides, fungicides, rodenticides, herbicides, disinfectant, repellents, and other chemicals used for the control of pests. Insecticides are classified into 3 groups: contact poisons, stomach poisons and fumigants. Contact poisons are those which kill insects primarily by contact e.g. pyrethrum DDT, HCH, dieldren. Stomach poisons are those which when ingested cause the death of the insects e.g. paris green, sodium floride. Fumigants are those which give off vapours which have a lethal effect on the insects e.g. sulfur dioxide. This classification is by no means a rigid one, because a contact poisons can also be a stomach poisons.[1]
Pesticides can be classified according to chemical groups; this is useful for determining their effects on man and animal. Major chemical categories of pesticides include Carbamates, organochlorines, organomercury compounds, organophosphates, pyrethroids and biological agents.[2]

1.1.1. Organophosphates
These insecticides contain carbon, hydrogen, oxygen and phosphorous and some contain other elements such as chlorine, bromine and sculpture. They kill insects by inhibiting the enzyme cholinesterase and blocking transmission of nerve impulses. This group has been

increasingly used because of the increase of organochlorine resistance in medically important insects These are biodegradable insecticides which do not accumulate and persist in the environment and consequently have much less effect on non-target organisms. It kills a large variety of insects not all of which are pests.[3]

The most Organophosphates insecticides which used are, malathion, dichlorvos, (DD VP), naled (Dibvom), trichlorphon (Dipterex), fenthion (Baytex), temiphos (Abate), fenitiothion (Sumithion), diazinon and chlorpyrifos (Dursban).[4]

Organophosphates (Ops) is currently used generic term that includes all insecticides containing phosphorus, other names used but no longer in vogue, are organic phosphates, phosphorus insecticides, nerve gas relatives, and phosphoric acidesters. All organophosphates are derived from one of the phosphorus acids, aand as a class are generally the most toxic of all pesticides to vertebrates. Because of the similarity of OP chemical structures to the (nerve gases) their mode of action are also similar. Their insecticidal qualities were observed in Germany during world war II.

The Ops have two destructives features: they are generally much more toxic to vertebrates than other classes of insecticides, and most are chemically unstable or nonpersistent. It is this latter characteristic that brought them into agricultural use as substitutes for the persistent Organochlorines.[5]

1.1.2. Mode of action

The Ops work by tying up or inhibiting certain important enzymes of the nervous system, namely cholinesterase (ChE). The enzyme is said to by phosphorylated when it becomes attached to the phosphorous moiety of insecticide, a binding that is irreversible. This inhibition results in accumulation of acetylcholine (Ach) at the neuron/neuron and neuron/muscle (neuromuscular) junctions, causing rapid twitching of voluentary muscles and finally paralysis.[6]

1.1.3. Carbamates.

This group contains carbon, hydrogen, oxygen and nitrogen but not chlorine or phosphorus. Many carbamates acts as nerve poison and lower the cholinesterase levels, they are usually slow acting , but a few such as carbonyl (sevin) and propoxur (Arprocarb) produce a quick knock-down of insects similar to that produced by pyrethrum compounds.[7]

Carparyl is widely used in public health, veterinary and agricultural programs to kill a variety of insect's pests. It can be formulated as sprays to kill adult of mosquitoes and because of it's low mammalian toxicity, dust formulations have been used on animals to kill fleas and lice, it can also be incorporated into lotions for head lice control.[4]

The carbamate insecticides are derivatives of phosphoric acid. And like the Ops, their mode of action is that of inhibiting the vital enzyme cholinesterase (ChE). The first successful carbamate insecticide carbaryl (sevin) was introduced in 1956. more of it has been used world wide than all the remaining carbamates combined. Two distinct qualities have made it the most popular carbamate: it is very low mammalian oral and dermal toxicity and an exceptionally broad spectrum of insect control. Othr carbamates are methomyl (lannate), carbofuran (furdan), aldicarb (temik), examyl (vydate) and carbosulfan.[7]

1.1.4. Mode of action

Carbamates inhibit cholinesterase (ChE) as Ops do, and they behave in almost identical manner in biological systems, but with two main differences. First, some Carbamates are potent inhibitors of aliesterase, and their selectivity is sometimes more pronounced against the ChE of different species. Second, ChE inhibition by carabamates is reversible, when ChE is inhibited by carbamates, it is said to be carbomylated, as when an Ops results in the enzyme being phosphorylated . in insects the effect of Ops and carbamates are primarily those of poisoning of the central nervous system, since the insects neuromuscular junction is not cholinergic, as in mammals. The only cholinergic synapses known in insects are in the central nervous system. (the chemical neuromuscular junction transmitter in insects is thought to be glutamic acid, but that has not been proved).[8]

1.2. Cholinesterase

In biochemistry, a cholinesterase or choline esterase is an esterase that lyses choline-based esters, several of which serve as neurotransmitters. Thus, it is either of two enzymes that catalyze the hydrolysis of these cholinergic neurotransmitters, such as breaking acetylcholine into choline and acetic acid. These reactions are necessary to allow a cholinergic neuron to return to its resting state after activation. For example, in muscle contraction, acetylcholine at a neuromuscular junction triggers a contraction; but for the muscle to relax afterward, rather than remaining locked in a tense state, the acetylcholine must be broken down by a choline esterase. The main type for that purpose is acetylcholinesterase (also called choline esterase or erythrocyte cholinesterase).[9]

1.3. Cholinesterase inhibitors

A cholinesterase inhibitor (or "anticholinesterase") suppresses the action of the enzyme. Because of its essential function, chemicals that interfere with the action of cholinesterase are potent neurotoxins, causing excessive salivation and eye-watering in low doses, followed by muscle spasms and ultimately death**.**

Among the most common acetylcholinesterase inhibitors are phosphorus-based compounds, which are designed to bind to the active site of the enzyme. The structural requirements are a phosphorus atom bearing two lipophilic groups, a leaving group (such as a halide or thiocyanate), and a terminal oxygen.[10]

2. Study Methods and Materials

This study has two phases for data collection: firstly, a questionnaire was designed and filled by farmers (both users and nonusers of Ops and carbamates compounds) then the blood test for two groups was done to detect the effect of such compounds on acetyl chorine esterase activity.

2.1. Sample selection technique

Gandato agriculture scheme consists of three sectors denoted as ABC and contain 918 farmers in total

Sector	No of farmers	Cumulative
A	311	311
B	299	610
C	308	918

the sample size was detected using the formula:

$$n = \frac{no}{(1+no/N)}$$

n:*Sample size.*
no:*initial sample size.*
N:*population size.*

$no = Z2pq/d2 = \frac{(1.96)20.5\times0.5}{(0.05)2} = 384 = 384$

$n = no/(1+no/N) = 384(1+384/918) = 270$
$nA = A/N\times n = 311/918\times270 = 91$
$nB = B/N\times n = 299/918\times270 = 88$
$nC = C/N\times n = 308/918\times270 = 91$

So, the initial sample size desired was 270 farmers among them only 90 were found using Ops and carbamates and those were subjected to blood test for cholinesterase activity, on the other hand another 90 farmers who were not using any type of insecticides were also subjected to the same test as a control group.

2.2. Blood test method summary

An initial check in the quality of the reagent was performed, and then blood samples were mixed with PH indicator (bromothymol blue) solution after which *acetylecholine* per-chlorate (substrate) solution is added and the solution obtained inculcated under specified conditions, the cholinesterase enzymes present in the blood liberate acetic acid from *acetylecholine* there by changing the PH and consequently the indicator color. The resulting color compared usually with those of a series of glass corresponding cholinesterase activity, express in steps of 12.5 from 0 to 100 read of from the scale.

2.3. Interpretation of results

Cholinesterase level below 75 on the titno-meter scale indicate exposure to *organophosphorus* or carbonate compounds
and should be investigated, followed by corrective action, when the cholinesterase level drop to 50 or below the test should be repeated the next day before starting work, if the level is still 50 or below, the person should be suspended from work with *organophosphorus* or *carbonate* compounds.

3. Results

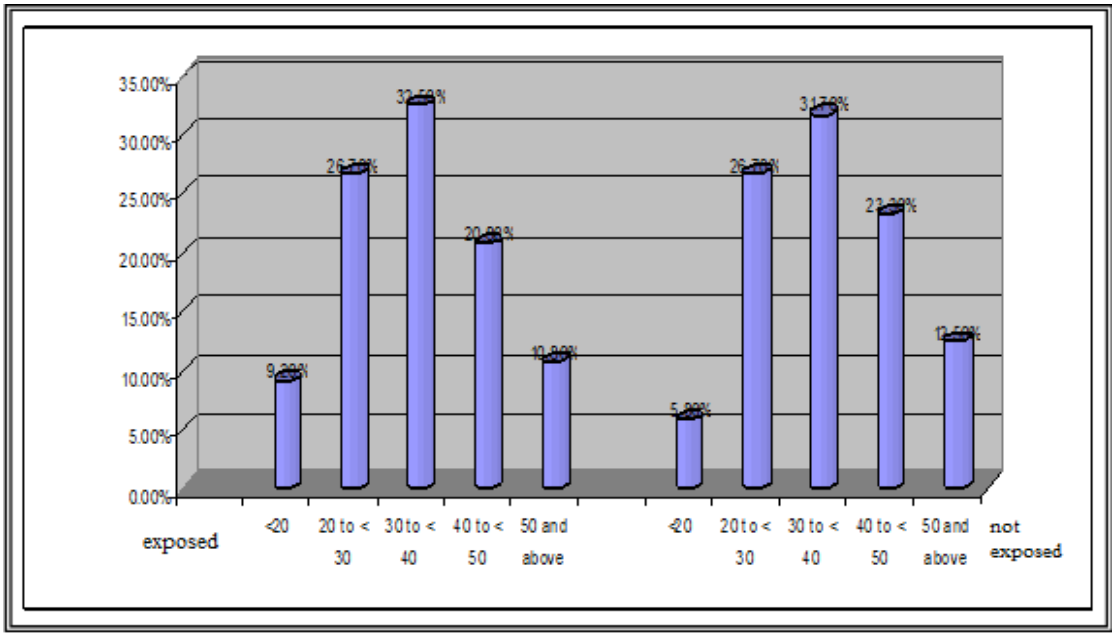

Fig 1: Age of the farmers/years
N= 90

As shown, the age group (30 - < 40 years) represents the high percentage among exposed & control group (32.5% & 31.7%) respectively and few farmers among both groups (9.2% of exposed & 5.8% of control group) were under 20 years of age

Table 1: type of insecticides used by farmers

Type of insecticides used	Frequency	Percentage (%)
Ops &carbamates compounds	90	33.5
Other type of insecticides	115	42.5
Didn't use any type of insecticides	65	24
Total	270	100

Table 2: Caution measures received from sellers:
N=90

	Frequency	Percent
Yes	23	25.5
No	67	74.5
Total	**90**	**100**

The table shows that 74.5% of the exposed group did not receive any caution measures or guidance from the sellers.

Table 3: Using protective wears by farmers under study:
N= 90

Condition	Frequency	Percent
Face shield	18	20.2

Gloves & face shield	20	22.2
P.Shoes & Face shield	1	1.1
Gloves, P.shoes & Face shield	1	1.1
None	50	55.4
Total	**90**	**100**

As stated above more than the half of the exposed group(55.4%) did not use any protective wears when spraying insecticides but; 22.2% of them used gloves and face shields, and 20.2% of them used face shields only during spraying.

Table 4: Preventive measures used by exposed group after using (Ops) and Carbamates compounds:
N= 90

	Frequency	**Percent**
Wash hands with water	11	12.3
Wash hands with soap & water	30	33.3
Wash legs with water	4	4.5
Wash legs with soap & water	17	18.8
Wash the whole body with water	7	7.8
Wash whole body with soap & water	21	23.3
Total	**90**	**100**

The majority of the study cases (31.7%) wash their legs with water only as a preventive measure after spraying insecticides; others (23.3%) wash their whole body with soap and water.

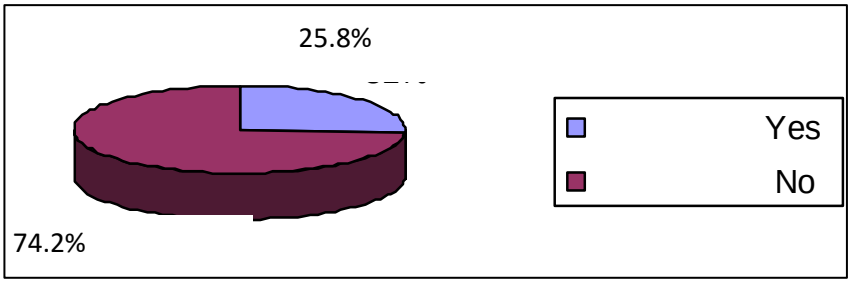

Fig 2: Training of the exposed group about mixing and preparing insecticides:
N= 90

The majority of farmers (74.2%) were not trained about mixing and preparing insecticides.

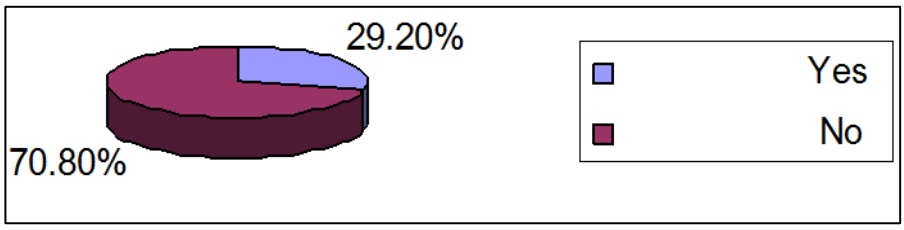

Fig 3: Training of the exposed group about spraying insecticides:
N= 90

70.8% of the cases were not trained about spraying insecticides.

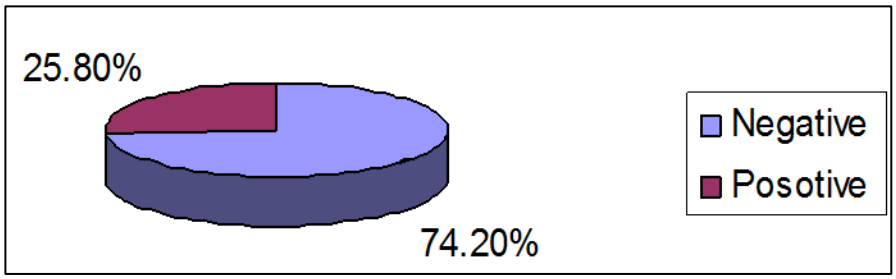

Fig 4: Acetyle Choline estrase test for exposed farmers.
N= 90

The figure shows that 25.8% of the exposed farmers gave a positive test for acetyle choline esterase.

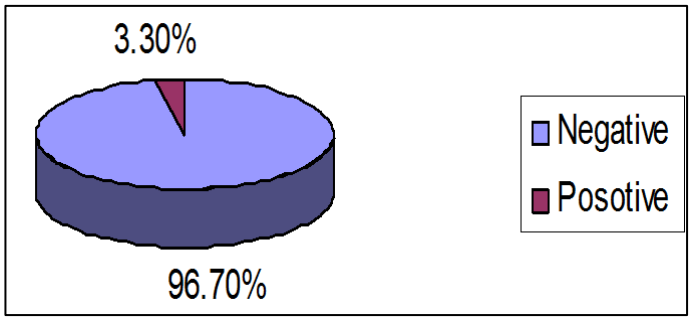

Fig 5: Acetyle Choline esterase test for control group.

The above figure indicates that only 3.3% of control group have a positive test of acetyle choline esterase.

Table 5: Acetyle Choline esterase activity among the study group.
N= 180

	Acetyle Choline esterase activity (%)			Total
Condition	75	87.5	100	
Exposed	9	13	68	90
Not exposed	-	2	88	90

The table shows that the enzyme activity declined to 75% among 9 of the exposed farmers, and to 87.5% among 13 of them, rather than only 2 of the not exposed farmers, while the rest of the exposed farmers and controls their enzyme activity was normal (100%)

Table 6: The association between age and acetyle choline esterase test result:
N= 180

Condition	Age of the farmers	Acetyle choline esterase test result		Total
		Positive	Negative	
Exposed	< 20	5	21	26
	20 - < 30	14	19	33
	30 - < 40	3	16	19
	40 - < 50	2	10	12
	50 and above	24	66	90
Not exposed	< 20	1	23	24
	20 - < 30	1	33	34
	30 - < 40	1	16	17
	40 - < 50	1	14	15
	50 and above	4	86	90

	Exposed	Not expose
P =	0.20	0.94

The table shows that most of the +ve cases are found among the age group 20 - < 30 years "14 cases" and distributed equally between all age groups of the control group (not exposed) so there is no association between age group and acetyle choline esterase test result of the controls (p value >0.01), while the +ve tests among cases may be due to their exposure rather than their age. The difference is not significant among both exposed and control group.

Table 7: The relation between (AChE) test result and preventive measures after using (Ops) and Carbamates.

	Preventive measures after using (Ops) and carbamates						Total
	Wash hands with water only	Wash hands with soap and water	Wash legs with water only	Wash legs with soap & water	Wash the whole body with water only	Wash whole body with soap & water	
Acetyle Choline +ve	9	7	1	0	2	1	20
Estrase test - ve	2	23	3	17	5	20	70
Total	11	30	4	17	7	21	90

P.value = 0.000

The table shows that there is a clear relation between acetyle chE esterase test and preventive measures after using insecticides (P.V< 0.05).

4. Discussion

The study showed that 75% of the exposed farmers had not received any caution measures from sellers and this may result in poor awareness among them towards the safe measures of insecticide handling. This result is different from what was mentioned by Rozendal "Special precautions must be taken during transport, storage, and handling". Also it is in disagreement with the ISO guide " alert the user to potential hazards and provide information on preventive safety and health measures.

55% of the exposed farmers did not use any protective wears during insecticides applications(table3).This behavior make them vulnerable to potential hazard that may arise as an outcome of using such compounds.

As shown in "Fig iv and v" the acetyle choline esterase change was high among the exposed group and the difference of the enzyme change between exposed and controls was highly significant (P= 0.000) which may be associated to the contact with the insecticides. This result is in agreement with what was stated by (Charles D.) "carbamates inhibit choline esterase as Ops do"

Table (5) illustrate Acetyle Choline esterase activity among the study group,it was found that 22 of the exposed group their enzyme was declined up to 75% .

Although there was no significant difference in the acetyle choline suppression among the different age group (P= 0.20) it was apparent that the suppression of the enzyme was high among the young cases table (5). This may refer to the large number of the exposed group within this age (33%)

As table (7) shows the association between acetyle choline esterase and preventive measures after using insecticide was highly significant (P= 0.000), therefore the suppression of acetyle chE was high among those who did not wash their whole body with soap and.

5. Conclusion

All types of insecticides were used by the exposed group but only (33.5%) of them were used Ops and Carbamates while the majority of them(42.5%)used other types (organochorines, and pyrethroids) on the other hand some of the farmers(24%) were not used any type of insecticide.

The majority of the exposed groups (74.2%) were not trained about mixing and preparing insecticides.in the same time more than the half of them (55.4%) did not used any protective wears when spraying insecticides.

the age group 20 - < 30 years was more affected by using Ops and Carbamates compounds, however there was no statistical significance between age and AChE depletion.($p>0.05$)

6. Acknowledgements

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