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Study the Effect of Some Chemicals Used Locally in Agricultural Processes in Iraq on the DNAof some non-targeted Organisms

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Abstract
Agricultural chemicals are widely used in the world, although harmful to the human and other living organisms. Our research is designed to study damaging effect of these chemicals on different DNA sources on different organisms in Iraq including human. This study showed that (snails and slug) killer material called (Metaldehyde) was strongly binding and cleavage with two DNA sources from human and plant. However, herbicide materials revealed obvious DNA binding with plant DNA. In the other hand fertilizer material showed cleavage effect with plant DNA only, while the other samples seem normal as control.

Keywords: Binding, DNA, agricultural chemicals

1.Introduction
Agricultural chemicals as a term refer to the wide variety of chemical products used in agriculture worldwide, such as pesticides (including insecticides, herbicides and fungicides), as well as synthetic fertilizers, hormones and antibiotics[1].Because of lack of rain , the state of desertification , frequent plant diseases , agricultural pests and lack of arable land in Iraq, the agricultural chemicals has become widely used here to minimize the damage to crops produced, some of them are locally made or imported from unknown sources,regarding to desertification and dust storms that blowing seasonally in the middle east and hot seasons which lead to drought, these chemicals may enter the body of organisms and go to river water during crops irrigation threatened other organisms and entering food chain.Worldwide comet assays has been used to investigate the level of DNA damage in farmworkers [2, 3], also such test is used for monitoring the DNA-damaging effects of environmental pollutants in potato plants [4]. Bacterial cells (plate count), and disc diffusion method were used to determine fungicide effect [5]. Different cells model is used to evaluate pesticides toxicity [6], other recent study showed biochemical parameters and growth in soil *Escherichia coli* isolate indicate the cytotoxicity of pesticides [7]. This study was designed to use the DNA extraction from different sources to describe the damaging effect of agricultural chemicals at DNA damage.

2.Experimental
2.1 *Agricultural chemicals:*
Different agricultural chemicals collected randomly from the markets, orchards and gardens from the city of Baghdad,these chemicals have active ingredients that take effect in mode of action and work effectively when usedwith agricultural activity (Table 1).

2.2 *Specimens for DNA isolation*:
Plant (Pepper: *Capsicum annuum*), and human blood.

Table 1: Commercial agricultural chemicals active ingredients, and mode of action.

No.	Material	Active ingredients/Contents	use	Mode of Action
1	Deltamethrin	Deltamethrin 25 gm (W/v)	Insecticide	Sodium channel modulators
2	Metaldehyde	Metaldehyde 1.5 % W/W	Snails and Slug Killer	Neurotoxicant
3	Organic Fertilizer	Organic	Fertilizer	Fertilizer
4	Quizalofop-p-efuryl	Quizalofop-p-efuryl Haloxyfop-R Methyl Ester108 g/L (pyridinyl-oxyphenoxy compound)	herbicide	Lipid synthesis inhibitors
5	Glyphosate	Glyphosate 48% SL (N-phosphono-methyl glycine)	herbicide	amino acid synthesis inhibitor
6	Glyphosate 2	Glyphosate 48% W/V	herbicide	amino acid synthesis inhibitor
7	EDDHA	EDDHA , EDTTI	Fertilizer	
8	Chlorpyrifos + Cypermethrin	Chlorpyrifos + Cypermethrin 20%+2%W/V	Insecticide	Nerve action (protein)Acetyl-choline esterase inhibitors
9	Chlorpyriphos	Chlorpyriphos	Insecticide	Insecticide
10	NAA	Pure NAA (Alpha Naphtalene Acetic Acid) 0,5 g	Growth hormone	Hormone

2.2.1*Total DNA for human Blood*:
Promega Genomic DNA Purification Kit (A1120), used for extraction total DNA from human blood according to the kit user manual [8].
2.2.2 *Plant DNA*:
Plant DNA extracted according to Ogunkanmi *et al* method (2008) [9].

2.3*DNA purity*:
Estimation DNA purity by (Nanodrop) Spectrophotometer (ACT gene, USA). The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for DNA was measured to check its purity. The ratio A_{260}/ A_{280} was ranged from 1.65 to 1.84.

2.4 *DNA and agricultural chemicals mix preparation*:
Using concentration of 1material: 2DNA incubation at 37^0C for 2 hours. The agricultural chemicals prepared according to the manufacturing labeling.

3. **Results and discussion**

The results showed that the plant DNA was broken when treated with Chlorpyrifos + Cypermethrin and the gel result showed ghost smear alongside of the sample and band is in the molecular weight the same as in control. Also, the result of EDDHA treatment showed that the DNA breakage is clear and the smear is along in the gel as an indicator for this breakage increasing the DNA cleavage efficacy compared to the control. Inthetreatment of organicfertilizer, smearis obvious and the band looks weak as an indicator for DNA breakage. Full breakage of DNAtreated with NAA,band is not clear but there is obvious smear of DNA breakage. DNA breakage is clear compared withcontrol when treated with Quizalofop-p-efuryl, the band is very weak and the smear is along the gel. There was obvious effect on the plant DNA when treated with Deltamethrin. The result showed that most of DNA fragments in the bottom of the gel and most of the DNA fragments size are close to 200 bp.The Glyphosate / DNA complex in the gel indicates that this complex showed smear along the gel compared with

control and there are DNA small fragments in the bottom of the gel. The treatment of DNA with Chlorpyriphos showed that there is a smear along the sample movement in the gel and DNA breakage looks as small fragments like a spot showedin the bottom of the gel.Glyphosate2 sample, as in Glyphosate, the same DNA /material complex the band is clear as in control sample but there is a smear along the gel and the DNA breakage to small fragments which look like a spot in the bottom of the gel. Full break of Plant DNA with different molecular weight fragments looks like a smear in the lane when DNA treated with Metaldehyde (Figure 1).

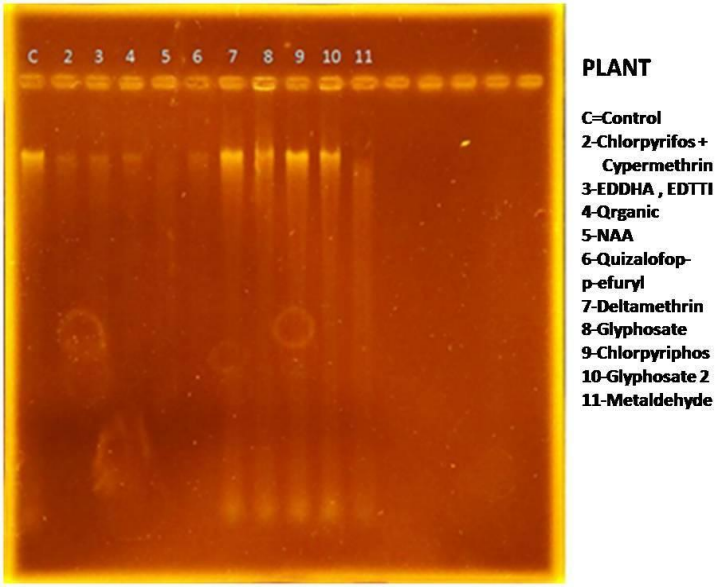

Figure1: Plant DNA vs. different chemicals

Human DNA / agricultural chemicals reaction did not show any breakage for Chlorpyrifos + Cypermethrin, EDDHA, Organic Fertilizer, Quizalofop-p-efuryl, and Deltamethrin. Little break for material /DNA complex as in NAA, Glyphosate, Chlorpyriphos, Glyphosate2 andMetaldehyde (Figure 2).

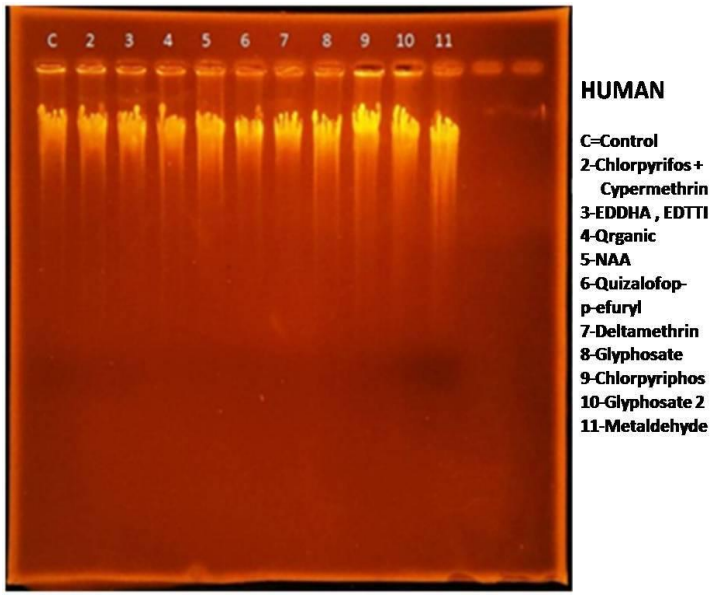

Figure2: Human DNA vs. different chemicals

The insecticides materials such as Chlorpyrifos + Cypermethrin and Chlorpyriphos which act as poisoning and oxidative stress materials [10], although some papers mentioned about its effect on spermatozoan DNA damage

[11], or DNA damage in liver or brain of other mammals like rats[12, 13]. Recently about DNA damage in lymphocytes of mice [14]. That does not comply with the report of U.S. EPA [15]. Deltamethrin also insecticide activity which significantly induced DNA damage examination of Deltamethrin-treated rats [16],and even if it sprayed, it damaging DNA on children [17], or to other organisms that live in water where the material finally goes to like Zebra fish where it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis [18].

Fertilizer material such as EDDHA, the active materials are the chelating agents EDDHA (ethylenediamine di-o-hydroxyphenylacetic acid), and no information about EDTTI that linked with metals like copper or iron. Fertilizer material EDDHA /plant DNA complex, showedobvious breakage and the smear is along in the gel as an indicator for this breakage compared with the control. These results agree with [19] and [20].

Herbicides materials such as Quizalofop-p-efuryl, most papers like [21] give the results of using Quizalofop-p-ethyl. This study showed damaging effect of Quizalofop-p-efuryl when treated with plant DNA, seems that there is no effect on DNA but when we look at the bottom of the gel, we can see obvious spot as an indicator for DNA breakage to very small fragment, and the material/DNA complex in this case may need more incubation time to complete clearage operation. Some researchers published that the strong effect of the active material on DNA damage may be due totime- and dose-dependent that increase (21–300%) in DNA fragmentation [22].

The active material of Glyphosate2 and Glyphosateis Glyphosate (N-phosphono-methyl glycine). The large use of Glyphosate is partly explained by its application to genetically modified plant varieties [23] that have a gene which confers resistance to the herbicide molecule, thus the metabolic pathway is not interrupted and the plants develop normally [24], as mentioned in the results , the Glyphosate showed DNA damage scores significantly higher than control [25].

Snails and slug killer materials such as metaldehyde, which interacts with the slug mucocytes and stimulates the mucous production [26]. On the other hand, metaldehyde is poisoned to the non-target organisms such as: hedgehogs [27], cows [28], dogs [29, 30], birds [31] and humans [32]. Poisoning has occurred by consumption of slug pellets of Metaldehyde [27]. Our study showed Metaldehyde highly DNA binding with all DNA sources.

Conclusions

A lot of studies about the impact of chemicals used in agriculture focus on human health and not on biodiversity,most of the chemicals used in agriculture, damaging the genetic content of the cells of living organisms, the chemicals used in agriculture may be sprayed with dust and can reach the respiratory organs of animals and may enter cells and lead to this adverse effect.This simple method can detect damage of DNA by the chemicals used in agriculture.While a lot of chemicals that publicationsmentioned as safe, shows that their low concentrations affect the genetic content of cells. We expected that the results are almost identical for all living beings according to DNA composition, but the emergence of different results need additional studies which focus on the effect of different components and the nitrogenous bases on the molecular level.

References

1. Agricultural Chemical Usage Swine and Swine Facilities (ACUSSF). U.S. Department of Agriculture, National Agricultural Statistics Service. (2006). http://usda.mannlib.cornell.edu/usda/current/AgChemUseSwine/AgChemUseSwine-12-20-2006.txt
2. McCauley L.A., Lasarev M., Muniz J., Stewart V.N., Kisby G., *J. Agromedicine*. 13 (2008) 237.
3. Khayat C.B., Costa E.O., Gonçalves M.W., da Cruz D.M.C., da Cruz A.S., de Araújo-Melo C.O., Bastos R.P., da Cruz A.D., de Melo e Silve D., *Environ. Sci. Pollut. Res. Int*. 20 (2013) 7334.
4. Gichner T., Patkov Z., Szakov J., Znidar I., Mukherjee A., *Envir. Exper. Botany*. 62 (2008) 113.
5. Mubeen F., Shiekh M.A., IqbalT., Khan Q.M., Malik K.A., Hafeez F.Y. Pak., *J. Bot*. 38 (2006) 1261.
6. Hreljac A.I., Zajc I., Lah T., Filipic M., *Environ. Mol. Mutagen*. 49 (2008) 360.

7. Shetti A.A., Kulkarni A.G., Kaliwa R.B., Shivasharana C.T., Kaliwa B.B., *Int. J. Pharm. Bio. Sci*. 3 (2012) 1155.
8. LondonoJ., Santos A. M., Peña P., CalvoE., Espinosa L. R., Reveille J. D., Vargas-Alarcon G., Jaramillo C. A., Valle-Oñate R., AvilaM., Romero C., and Medina J. F., *BMJ Open*, 5 (2015) e009092.
9. Ogunkanmi A.L., Oboh B., Onifaole B., Ogunjobi A.A., Taiwo I.A., Ogundipe O.T., *Eurasia J. Bio. Sci.*, 2 (2008) 115.
10. Idris S.B., Ambali S.F., Ayo J.O., *African J. Biotechnol*. 11 (2012) 16461.
11. Wang X., Sharma R.K., Sikka S.C., Thomas J., Falcone T., Agarawa A., *Fertil. Steril*. 80 (2003) 531.
12. Mehta A., Verma R.S., Srivastava N., *Environ. Mol. Mutagen*., 49 (2008) 426.
13. Muthuviveganandavel V., Muthuraman P., Muthu S., Srikumar K., Wooder M.F., *J. Appl. Pharm. Sci*. 1 (2011) 121.
14. Rahman M.F., Mahboob M.K., Danadevi B., Banu S., Grover P., *Mut. Res*. 516 (2002) 139.
15. EPA. Chlorpyrifos (Pc Code 059101), *Toxicology Data Review*. (2000).
16. Abdul-Hamid M., Salah M., *J. Basic Appl. Zool*., 66 (2013) 155.
17. Ortiz-Pérez M.D., Torres-Dosal A.,Batres L.E., López-Guzmán O.D., Grimaldo M., Carranza C., Pérez-Maldonado I.N., Martínez F., Pérez-Urizar J., Díaz-Barriga F., *Envir. Health Perspectives*, 113 (2005) 782.
18. Sharma D.K., Ansari B.A., *Res. J. Chem. Sci*., 1 (2011) 125.
19. Ahmed H, Khalil M.K.,Abd El-Rahman A.M., Hamed N.A.M.,*J. Applied Sci. Res*. 8 (2012) 1271.
20. Sevinc M.S., Pag W.J., *J. General Microbiol*., 138 (1992) 587 .
21. Mustafa Y., Arikan E.S., *Caryologia*, 61 (2008) 45.
22. Hossain M.M., Richardson J.R., *Toxi. Sci*., 122 (2011) 512.
23. Williams G.M., Kroes R., Munro I.C., *Regul. Toxicol. Pharmacol*., 31 (2000) 117.
24. Coutinho C.F.B., Tanimoto S.T., Galli A., Garbellini G.S., Takayama M., Amaral R.B., Luiz Mazo H., Avaca L.A., Machado S.A.S., *Rev. Ecotoxicol Meioambiente*, 15 (2005) 65.
25. Moreno N.C., Sofia S.H., Martinez C.B.R., *Envir. Toxi. Pharm*., 37 (2014) 448.
26. Triebskorn R., *Malacologia*, 31 (1989) 141.
27. KeymerI.F., Gibson E.A., Reynolds D.J., *Veter. Record*. 128 (1991) 245.
28. Valentine B.A., Rumbeiha W.K., Hensley T.S., Halse R.R., *J. Veter. Diag. Inves*., 19 (2007) 212.
29. Campbell A., *Veter. Record*. 163 (11) 343.
30. Andreasen J.R., *J. Veter. Diag. Invest*. 5 (1993) 500.
31. Shih C.C., Chang S.S., Chan Y.L., Chen J.C., Chang M.W., Tung M.S., Deng J.F., Yang C.C., *Veter. Human. Toxi*. 46 (2004) 140.
32. Bleakley C., Ferrie E., Collum N., Burke L., *Emerg Med J*., 25 (2008) 381.