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COPPER AND COPPER CONTAINING PESTICIDE AS COPPER OXYCHLORIDE TOXICITY AND ITS ADVERSE EFFECTS ON ANIMAL AND HUMAN HEALTH

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ABSTRACT

Copper is a trace element and has a vital role in homeostasis but it has cumulative effects and may be hazardous to the animal, human, and environment. Copper is a vital trace element to all living as it participates in various metabolic processes and is controlled via complicated homeostatic mechanisms, but excess copper has a deleterious effect in organisms as it stimulates free radical production in the living cell, lipid peroxidation, and disturbs the whole-body antioxidant capacity. Although its beneficial effect, it has a hazardous effect on health as it has genotoxic, developmental toxicity, and hepatic toxicity in experimental animals

REVIEW ARTICLE

INTRODUCTION

The sharp increase in populations usually coupled with an increase in the food demand which necessities the expansion of the agricultural lands (1). However, reduced water sources may represent an obstacle for such expansion which means the food availability may decrease that considered as a global alarm so to increase the food sources intensive innovation of agriculture methods must be designed as greenhouses to provide food all over the year even out of the cultivation season (2). At the same time, the human was engaged continuously to improve the life quality to achieve the basic needs through limiting the crop destroyer as weeds, insects, pests, etc (3).

So protected Agriculture (PA) considered as a sustainable source of food production especially in arid, hot environments, harsh climate and water-deficient regions as North Africa and the Middle East area (4) but the nature of the greenhouses as high humidity, high temperature and enclosed area all promote many plagues to come out that may justify the excessive usage of pesticides, however, such pesticides as organophosphates, pyrethroids and carbamates may induce significant toxic and occupational hazards depending on the

time required to spray a hectare, type of pesticide used and the toxic combinations **(5).**

The cultivation of greenhouse vegetables is dramatically increased throughout the world recently, but little information is available on the uptake and distribution of the pesticides used in the greenhouse's vegetables (6)**.**

Pesticides are chemicals that may have harmful effects on the environment and human beings, such chemicals known to remain in the water, air, soil, and food for a long time. Their catastrophic effect became noticed when it was widely used in considerable amounts all over the world also monitoring programs for the residues of pesticides are limited due to the lack of trained personnel, resources, rigorous legislation, and specialized equipment so education through guide books and international databases as WHO and EPA, successive monitoring and integrated pest management (IPM) programs must be taken in the consideration especially in food commodities in the developed countries (7)**.**

Several studies stated that the pesticides residues and behavior vary greatly between the greenhouses and conventional cultivation for example mainly fungicides and insecticides commonly used in greenhouses more than the open field system so more concentration of such pesticides residues were found in greenhouses vegetables as organochlorine pesticides that tend to be magnified through bioaccumulation in the animals or human body (8).

Pesticides can be classified according to the specific target into insecticides, herbicides, molluscicides, nematicides, rodenticides, fungicides, etc. and the application of such chemicals increased in the recent decades in agriculture, livestock, and household products even some of them are persistent in the environment so can be bio accumulated in the food chain with observable effect after many years of their application (9)**.**

The pesticides improper and extensive uses caused inevitable pollution to soil, air, water, and food with a detrimental hazard to non-target organisms and populations (10)**,** such agricultural pesticides used in large surfaces are washed off daily causing contamination of the environment with a significant amount of pesticides that reach the bodies through runoff water of the agricultural surfaces and greenhouses that pollute the drinking water and food supplies and pesticides type and amount determine the level of toxicity (11).

The current monitoring programs of pesticides usually concentrate on the possible leaching of such pesticides to the aquatic environment but mainly there is a lack of knowledge on possible consequences of water, soil contamination, and human risk especially in certain areas where there is excessive use of pesticides as in greenhouse crops when compared to the outdoor open or conventional cultivation (7)**.**

Vegetables and fruits that consumed semi-processed or raw are supposed to contain higher residue levels of pesticides when compared to the other food once reached to certain levels may cause ill health, genotoxic or teratogenic effect (6)**,** such teratogenic effect is multifactorial as genetic factors (mutations) or environmental factors (toxins, drugs, and radiation) or combination of the genetic and environmental factors (12)**.**

Fungicides, as a type of pesticide, made of different chemical formulations, are used extensively to control and eradicate the plant's fungal diseases and are applied on seeds directly or sprayed on field crops (3)**.**

Fungicides classified as fungi static that prevent fungi development or antisporulants that prevent reproductive spores formation and according to the chemical nature into organic and inorganic while according to the mode of action classified into systemic and unsystematic so fungicides act in different ways and the presence of high

quantity metallic ions in the fungal cells causes various disturbances and has a toxic effect **(13).**

Many extensively used fungicides are copper-based that have a significant historical value due to their fungicidal properties and applied annually worldwide with millions of tons so it can harm human health causing different cancer types, reproductive, hematological, neurological, and immune disorders (14)**.**

Copper is a vital trace element to all living as it participates in various metabolic processes and controlled via complicated homeostatic mechanisms but excess copper has a deleterious effect in organisms as it stimulates free radical production in the living cell, lipid peroxidation, and disturbs the whole body antioxidant capacity (15)**.** However, copper is relatively safe, there is a great concern due to its accumulation in the agricultural soils and environment with a significant toxic effect on population and animal in the form of copper-based fungicides as copper oxychloride

Copper Oxychloride

Although copper considered as an essential metal and involved in most of the physiological functions, excess copper may be toxic and interfere with enzyme activities (16) and environmental contamination by copper away from that occur naturally mainly due to industrial sources and agrochemicals containing copper that used in several applications as fungicides, paints, alloys and construction materials

Copper-based pesticides primarily used as fungicides to control fungal diseases of vegetables, fruits, and field crops as leaf spots, mildew, apple scab, and blights (15) but it may also affect non-target organisms even may be lethal upon several applications with the inability of tissues to synthesize copper-binding ligands and causing hepatotoxicity, gastrointestinal and blood-toxicity besides irritation of the skin and mucous membranes (17).

Copper Metabolism in Living Organisms

Copper is absorbed mainly through the GIT and about 20 to 60% of the dietary copper absorbed while the rest excreted via feces, the absorbed part then bounded mostly to copper-binding proteins in the liver that considered the major organ involved in the process of copper homeostasis and synthesis and storing of copper-containing protein ceruloplasmin (18)**.**

Copper is considered an essential micronutrient and many animals can cope with excess copper exposure by the liver but the overwhelming of such mechanism may occur with repeated exposure (15) resulting in hemolysis, anemia, diarrhea, hemorrhage, kidney failure, and death of animals (19)**.**

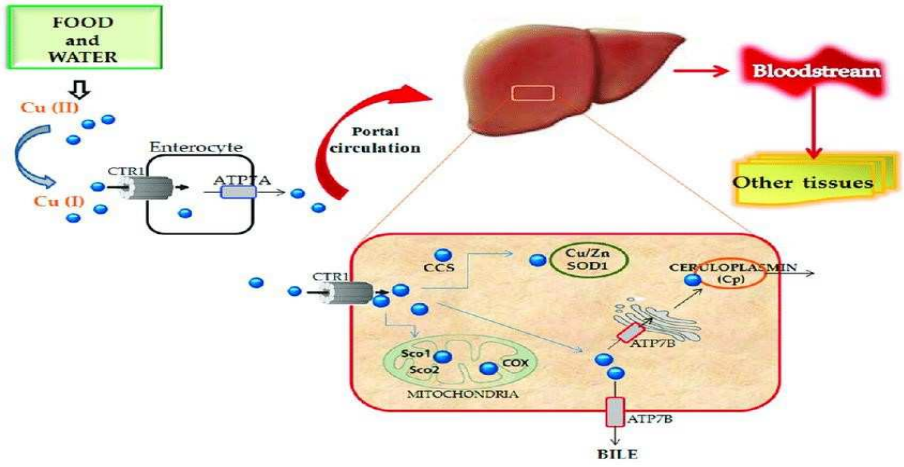

Fig (1): showing the normal metabolism of copper as essential micronutrient and copper-binding proteins in liver *identified by Antonucci et al.(20)*

MECHANISM OF ACTION OF COPPER OXYCHLORIDE

Copper oxychloride [3Cu (OH) $_2$ $CuCl_2$] is a broad-spectrum fungicide that protects fruits and vegetables against fungal diseases of fruits and vegetables as Coryneum beijerinckii Vesicatoria and Deuterophoma tracheiphila to be edible for consumption and improve their productivity (21)**.**

Copper oxychloride is applied during the vegetation period and once penetrates the fungal cell, copper ions cause cellular protoplasm coagulation, denatured the cellular membrane, depress the respiratory processes also act as anti-metabolites (13)**.**

Copper oxychloride toxicity in non-target organisms occurs through promoting the oxidative stress as it accumulates in various tissues as a heavy metal causing cellular damage due to release of ROS through Fenton reaction (22) that may also cause hepatocellular neoplasia, kidney dysfunction, and hemolytic anemia (23)**.**

Copper may also oxidize cellular macromolecule as proteins, lipids, and DNA forming DNA adducts with DNA fragmentation through Fenton reaction resulting in hydroxyl, ROS radicals production causing an increase in malondialdehyde especially in liver tissues, on the other hand, decrease in catalase activity, α-tocopherol and glutathione peroxidase besides mitochondrial swelling depressing the cellular respiration and normal cell growth and development (24)**.**

Copper toxicity and hepatocellular injuries were studied with an electron microscope in rats and revealed that lysosomes not responsible for the cytotoxic effect of copper as previously stated but nuclear irreversible damage due to copper accumulation that may be responsible for cell death of hepatocytes in the whole liver (25)**.**

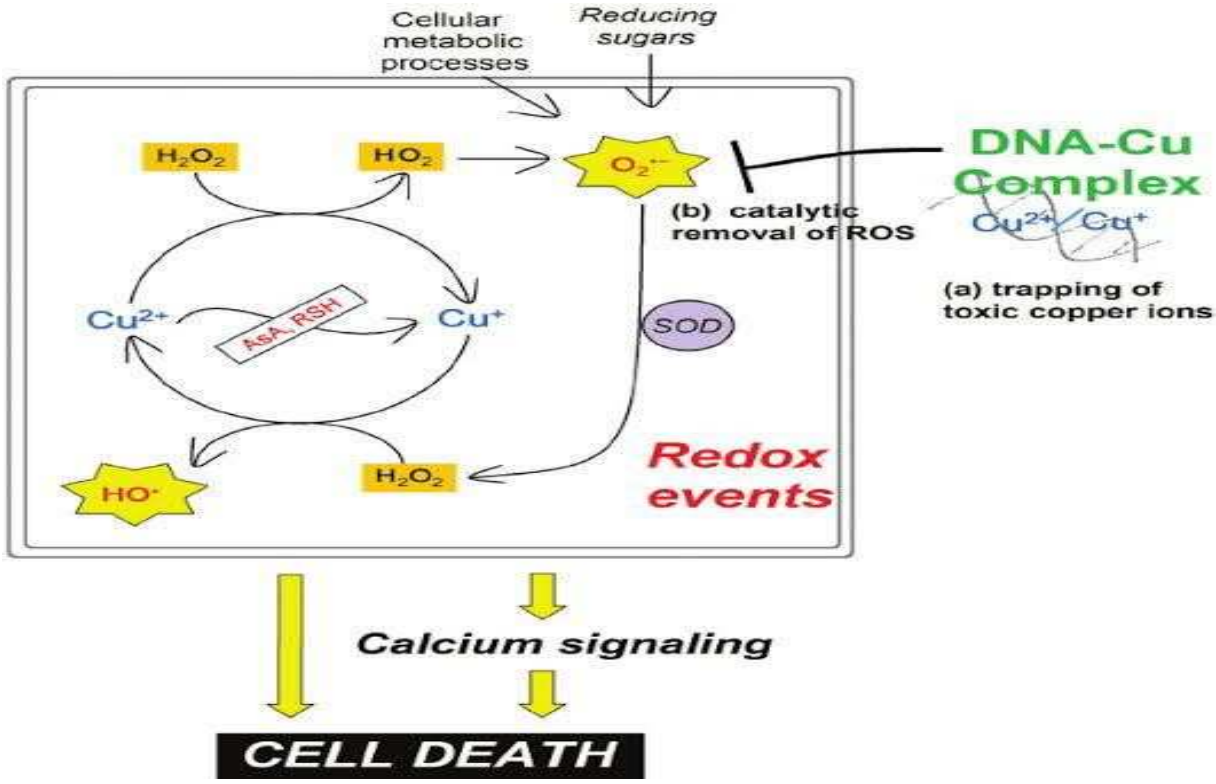

Fig (2): showing the mechanism of action copper oxychloride fungicide due to excess cellular copper toxic effect with the generation of free radicals, oxidative stress, and calcium signaling causing cellular death and DNA damage described by *Iwase et al.* **(26).**

General Toxicity of Copper Oxychloride

The liver considered as the target organ of copper toxicity and showed a significant increase in the lysosomes' numbers and diversity, mitochondrial swelling, rough endoplasmic reticulum fragmentation, and nuclear degeneration that is supposed to be the initiating event in hepatic toxicity (25)**.**

Acute exposure of rats as mammalian models to COC showed that COC may harm human and public health causing hepatotoxicity and lipid peroxidation with a significant increase in ALT and AST liver enzymes that considered as biomarkers of hepatic injury besides a marked increase in the liver MDA due to lipid peroxidation (27) also **Grădilă (28)** stated that copper accumulation in the aquatic system and soils my accounts for most of the hazard.

Copper oxychloride showed higher toxicity when compared to organic pesticide and that clarifies that the metal concentration might have a significant effect on the toxicity degree of pesticides and with higher metal content the toxicity became stronger (29)**.**

In a toxicity study of copper carried by **Chung et al. (30)** with different concentrations (5, 20, and 80 mg/kg orally daily) there was a significant dose-dependent decrease in food consumption besides decrease in the whole Hematological parameters as Hb, MCH, and red blood cell count, on the other hand, there was a severe increase in the white blood cell count and the oxidative stress parameters furthermore histopathologically stomach showed squamous cell hyperplasia due to sever copper irritation.

The copper level also increased significantly in copper oxychloride treated rats as (27) proposed with histopathological changes in the hepatic tissue as congestion with inflammatory cells and hepatic cell degeneration and necrosis due to inhibition of caspase 3. Also, copper oxychloride may increase the activity of some enzymes as AChE, GST, EROD, and CaE (31)**.** Furthermore, copper oxychloride showed severe oxidative stress damage with a drastic increase of malondialdehyde levels in *Helix aspersa* snail and a sharp decrease in reduced glutathione content besides severe fall in liver protein level

Upon all a significant cytotoxic effect on HepG2 and HaCaT cell lines after exposure to 20-450 µg/ml for 24 had been observed which confirmed the continuous exposure to this fungicide is harmful to human health (3)**.** Also, a subchronic toxicity study of copper oxychloride in common carp revealed that there was a significant increase in oxidative stress parameters as MDA with a decrease in antioxidant enzymes as GPx, and CAT levels, furthermore liver (the target organ in copper oxychloride toxicity) showed a severe pathological lesion as degenerations and swollen nuclei and mitochondria besides increase in levels of transaminases and ammonia, also found that copper oxychloride induced metabolic, hematological and behavioral impairment with an increase in glucose level and RBCs count and a significant decrease in leucocyte count due to immunosuppression (22).

A case series study as an intent of suicide carried by **Gunay et al. (32)**revealed that most of the signs related to GIT as nausea, vomiting, metallic taste, and abdominal pain in association with severe anemia and acute renal failure whereas in chronic exposure most effect observed in the liver due to copper accumulation with liver damage and hemolytic anemia also, chronic copper poisoning in human cause dizziness weakness, leg and back pain, abdominal pain, anemia, chronic kidney and liver damage .(33)

Toxic effect of copper in human observed at 11 mg/kg of copper with vomiting due to copper irritating effect on GIT and also may cause serious diseases as Wilson disease or Menkes syndrome (34)**.**

For the genotoxic effect, **Bayram et al. (35)** and **Pirtskhelani et al. (36)** hypothesized

that copper oxychloride confirmed to be genotoxic to cultured human lymphocytes with a significant increase in Micronuclei, chromosomal aberrations, and polymorphic bands and decrease in the mitotic and nuclear index and genetic stability so caution must be fallowed upon exposure to this fungicide.

Also, **Stanić (2008) (13)** showed that copper oxychloride at a concentration of 0.75% within72 hours after application on spermatid cellular lines of drosophila melanogaster has a potential genotoxic effect and induce recessive sex-linked lethal mutations on X chromosome of males with significant increase in germinative mutations than control, moreover, **Pirtskhelani et al. (36)** stated that copper oxychloride has a strong genotoxic effect in albino mice.

Additionally, the excess copper also may cause neuronal toxicity besides it was found that copper involved in binding to all molecules that form the neurofibrillary tangles and amyloid plaque as β-Amyloid that cause the Alzheimer's disease **(37).**

For the reproductive toxicity, copper oxychloride in male albino rats at dose level 50 mg/kg showed a significant decrease in the weight of treated rats' testes with a severe decline in sperm motility and density also glycogen, fructose, sialic acid decreased but cholesterol and protein levels increased significantly **(38).**

Furthermore, COC confirmed to inhibit spermatogenesis and affect the function of leydig cells with steroidogenic inhibition and cholesterol accumulation in such cells, such reproductive toxic effect may be due to Cu+ capability of interaction with other metals as selenium, iron and zinc and in turn affect their absorption through antagonism resulted in a deficiency of such element with subsequent cellular dysfunction with deleterious effect on the testes (19, 27)

The toxic effect of copper on the follicular waves also observed in female mouse ovaries (intraperitoneal injection of 70mg/ kg of $CuCl_2$in adult female mice) and the results revealed a significant increase in stage II follicles with degeneration and apoptosis of oocytes and oocyte fragmentation in treated groups than control **(39)** even in earthworm *Eisenia fetida* the cocoon production and hatchling were significantly reduced upon exposure for different concentrations of copper oxychloride **(40).**

Developmental Toxicity of Copper Oxychloride

Heavy metals adversely disrupt many metabolic processes in the developing embryos with functional and morphological abnormalities and even death **(41).**

The teratogenic effect of Copper investigated in vivo in both mouse and chick embryos and results showed that excess copper has a significant deleterious effect with developmental retardation, decrease in crown-rump length and limbs anomalies in mouse embryo besides a high rate of mortality, limb dysgenesis and tallness syndrome in a 5 days post-incubation chick embryo and the results confirmed microscopically with disorganization and necrosis in the neural tube and notochord further more mitochondrial vacuolization had been detected in the ultrastructural examination **(42,43).**

Also, in in vitro study in 9th days' mouse embryos cultured for 48 hours in rat serum then exposed to different concentrations of copper showed the failure of neural tube closure in the anterior region and exencephalia with significant embryonic developmental retardation and decrease in somites average numbers **(44).**

The fetal skeleton of mice embryo was also investigated from day 9 to day 19 of gestation with i.p. injection of $CuCl_2$ (0, 1 ml/10 g body weight) showed a significant teratogenic effect in some ossification centers, delayed ossification of bones and decrease in fetal weight **(45).**

The teratogenic effect of copper salts (sulphate and citrate) investigated in hamster

embryos through I / V injection in pregnant dams on 8 days of gestation and results showed an increased levels embryonic resorption and a high incidence of malformations as supraumbihical and thoracic hernias, facial cleft, microphthalmia and ectopia cordis furthermore in additional study confirmed the permeability of the placenta to radioactive copper during the organogenesis critical stages **(46).**

Chang and Tatum (47) found that intrauterine excess copper exposure in form of wires in rats had a significant effect on the normal implantation and blastocyst development

Copper citrate at dose level 2.7 mg/kg through I/P injection in pregnant hamster from day 8^{th} to day 13^{th} of gestation revealed different cardiac malformations in embryos as double outlet right ventricle, ventricular septal defect, and pulmonary trunk hypoplasia **(48).**

The fetal outcome of copper and zinc Interactions was studied in pregnant rats and revealed a significant malformation in embryos of dams fed in excess copper and zinc-deficient diets and that confirmed the antagonistic action between copper and zinc and all malformation resulted from excess copper resemble that of zinc deficiency **(49).**

On the other hand, excess dietary Copper at dose level 500 µg Cu/g diet fed to pregnant rats revealed no abnormal outcomes in embryos but fetal viability reduced with growth retardation that may only be due to a decrease in the food intake **(50).**

Copper developmental toxicity studied in different developmental stages of salmon fish causing delayed hatching, larval deformities as yolk sac abnormalities, craniofacial alterations, and deformed heads and spinal cords **(51).**

Furthermore, a developmental toxicity study of R6 fungicide (mixture of copper oxychloride and cymoxanil) at a concentration of 2.5 µg/ml on sea urchin showed that a significant increase of larval malformations with derangement of differentiation and embryogenesis **(52).**

Finally, we reported that copper oxychloride induced prenatal and postnatal toxicity in female albino rats (53,53,55)

On conclusions, copper and copper containing compounds may hazardous to animal and human health as it has genotoxic, developmental and hepatic toxicity.

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