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RESEARCH ARTICLE

Bioassay drugs and their Cu (II) ion-pairs, phenylazo compounds with Ni (II) and Cu (II) complexes on the flour beetle, *Triboliumconfusum* (Coleoptera : Curculionidae).

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Abstract

The flour beetles *Triboliumconfusum* were biologically affected after exposure to dose 10µg/insect of Ibuprofen, naproxen, lornoxican, Indomethacin, and phenylazo compounds. The survival adult of *T. confusum* was decreased by increasing chemical doses. Higher biorational effect of drugs was 73% of adult mortality after treatment with lornoxicam at dose 50µg; while the same compound had antagonistic effect when attached with Cu (II) which caused 38% of adult mortality. Contrary synergistic effect when Cu(II) ion was attached into Ibuprofen drug; where it produced 86% of adult mortality with Cu (II) and 41% without Cu(II). Also the bioactivity of phenylazo compounds, R1 (p-OCH_3) and R2 (p-NO_2) was increased by attachment to Ni (II) and Cu (II) ions; while it was decreased effect of compounds R3 (p-Br) and R4 (p-H) attachment to Ni (II) and Cu (II) ions. Bioactivity action of the compound R1 was 73 and 83 mortalities at dose of 50µg when attached to Ni (II) and Cu (II) ions respectively; while it was 68% without attachment to the same ions. The compound R3 was the most active one against *T. confusum*, where it recorded 100% of adult mortality at the dose of 30 µg. Therefore; it is concluded that the drugs and phenylazo compounds and their copper and nickel attachment are appropriate probe as bioactive compounds on Vertebrates and Invertebrates.

Introduction:-

The flour beetle, *Triboliumconfusum* (Coleoptera: Tenebrionidae) isamong the most common and destructive insectspecies in flour mills and related areas as given by Aitken [1], Buchelos[2-6], Trematerra [7] and Athanassiou*et al* [8] . Flour beetles are common in homes and grocery stores. They also infest mills and food processing facilities.

Drugs of ibuprofen, naproxen, lornoxicam and indomethacin are non-steroidal anti-inflammatory compounds studied by Johnson and Howard [9]. Many studies have been assessed about the mode of action of these drugs. Lornoxicam known as chlortenoxicam drug that decrease prostaglandin synthesis by inhibiting cyclooxygenase in animals given by Radhofer-Welt and Rabasseda [10]. Indomethacin probe was used for the physiological evaluation of the invertebrates by Jon and David [11]. Also ibuprofen and naproxen were very effective for the penetrating of the rat skin as studied by Irwin et al [12]. The biorational activity of the phenylazo compounds was investigated by many authors. Judit et al [13] reported that, using of synthetic peptide N-4[4-(dimethyamino) phenylazo] benzoyl, allowed precise measurement of the effects of several inhibitors on different biological samples. Contrary Anne et al [14] concluded that the collagenase from the larvae of *Hypodermalineatum* has no action on the synthetic peptide. On the other hand, 2-[5-chloro-2-(methoxyphenyl) azo]-1 H- imidazole (M6434) were lethal of rabbit by hemorrhage or coronary ligation as given by Ohnishi, et al [15].

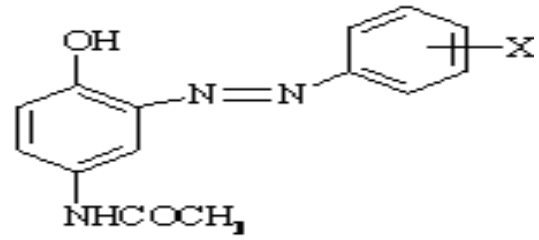
Materials and methods:-

Reagents:-

All the chemicals used in this work were of analytical grade. They included p-acetamidophenol, aniline, p-bromoaniline, p-methoxyaniline, and p-nitroaniline (Sigma) sodium nitrite and sodium hydroxide (Adwic). Organic solvents (spectroscopic pure from BDH) used included absolute ethyl alcohol, diethyl ether and dichloromethane.

Preparation and analysis of azocompounds:-

P-acetamido phenol-azo-derivatives (I-IV) were prepared by coupling p-acetamidophenol with aryl, p-bromo-, p-methoxy- and p-nitro- diazonium chloride, in an ice bath, in the presence of sodium hydroxide. The precipitates were left in refrigerator overnight, filtered and crystallized from acetic acid (yield 78-86 %). The elemental analyses of the prepared dyes referred to the general formulae of o-phenylazo- ($C_{14}H_{12}N_3O_2$) (I), p-bromo-o-phenylazo- ($C_{14}H_{13}BrN_3O_2$) (II), p-methoxy-o-phenaylazo- ($C_{15}H_{16}N_3O_3$) (III) and p-nitro-o-phenylazo-p-acetamidophenol ($C_{14}H_{13}N_4O_4$) (IV) compounds, respectively. Their proposed structural formulae are given in Fig 1.

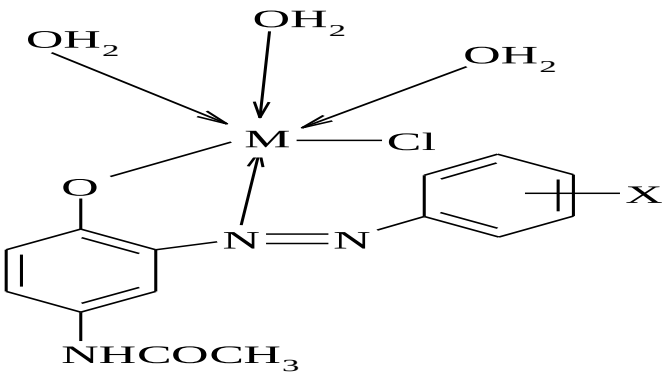


I, X = H
II, X = p-Br
III, X = p-OCH_3
IV, X = p-NO_2

Fig.1. Structure of azodyes (I-IV)

Synthesis and analysis of Copper (II) and Ni (II) complexes:-

The appropriate metal chloride (Cu(II) and Ni(II) ions) (10 mmol) in ethanol–water (1:1) (25 mL) mixture was added to the solution of the azo compound (0.40 g, 10 mmol) in the same solvent mixture (50 mL). The resulting solution was stirred under reflux for 0.5 h whereupon the complexes were precipitated. They were separated by filtration, washed with 1:1 ethanol/water and with diethyl ether. The structure information for these complexes is in agreement with the data reported in this paper based on the elemental analysis, IR, and electronic spectra measurements. Consequently, the structures proposed on octahedral geometry are shown in Fig. (2).



H_2L^1; X = OCH_3
H_2L^2; X = p-NO_2
H_2L^3; X = p-Br
H_2L^4; X = H

M = Ni(II) and Cu(II).

Fig.2. Proposed structural formulae of metal complexes

Synthesis and analysis of drugs-copper (II) ion-pairs:-

The solid ion-pairs of Ibuprofen and Lornoxicam drugs with copper (II) were prepared by addition of a solution of appropriate weight of metal salt of 0.125 g (0.5 mmol) copper sulphatepenta hydrate in 50 ml water to a 50 ml solution of 0.103 g (0.499 mmol) IBU and 0.186 g (0.5 mmol) LOR drugs, respectively. Appropriate weight of each drug dissolved in 2 ml of 0.05 M Na_2CO_3 and bidistelled water. The resulted solid ion-pairs appeared as colored precipitates. The precipitates leaved for 10 mins until completely settled. The obtained solid ion-pairs were separated, filtered and washed with suitable solvent using a Hearch funnel of suitable pores. Moreover, the obtained ion-pairs were dried in vacuum desiccator, separated and analyzed by elemental analyses and found to have general formulae of IBU-Cu (II) ($CuC_{13}H_{18}O_2.H_2O$) and that of LOR-Cu(II) ($CuC_{13}H_{10}ClN_3O_4S_2.3H_2O$).

Rearing of *T. confusum*

Adults of *Triboliumconfusum* were laboratory reared on wheat flour at 27.5 ± 1.5ºC and 70 % ± 5% (R.H.) according to the method of Frederic *et al*[16] with some modifications.

Bioassay and statistical analysis

Triboliumconfusum adult was topically treated with the doses of 10, 30 and 50µg of each compound according to the protocol described by Delobel*et al* [17] as follows: Thirty insects divided on three replicates (10 adult/replicate) were topically treated and biological effect was recorded after 24 hr. Thirty adults of control experiment were used in three replicates without treatment. Bioactivity was studied and the lethality was estimated according to Abbott[18]. Estimation of LD_{50} values was made by Finney [19] using probit analysis.

Results and Discussion

Effect of drugs on *Triboliumconfusum*:

The drugs of D1 (Ibuprofen), D2 (Naproxen), D3 (Lornoxicam), and D4 (Indomethacin) had biorational effect on the adult of *Triboliumconfusum.* From the Table (1) it is found that, lornoxicam compound was the most biologically effective on the *T. Confusum* which caused 21, 40, and 73% mortalities after adult was treated with the doses of 10, 30, and 50 µg of lornoxicam comparison to no effect of control. Least mortality (8, 20, and 41%) was obtained after adult beetles were treated with Ibuprofen compound at the same previous doses. Moderate similar bioactivity occurred by the effect of the naproxen and indomethacin drugs. In order of toxicity, LD_{50} were 55, 48, 35 and 46µg of the drugs D1, D2, D3, and D4 respectively (Table 1). On the other hand the addition of Cu (II) to the previous drugs of D1, D2, D3 and D4 with the condition of heating and room temperature its biological activity was decreased except the ibuprofen was increased (Table 2). Where the drug A1 compound represented the ibuprofen (D1+Cu (II) at room temperature), A2 is (D2 + Cu (II) at boiling point), A4 is(D2 + Cu (II) at room temp.), A5 is (

D4 + Cu (II) on heating), A6 is (D4 + Cu (II) at room temp.) and A7 (D3 + Cu (II) room temp.). Data recorded in Table (2) revealed that the highest mortality effect was 86 against *T. confusum* occurred after treatment adult with the compound A1 (ibuprofen + Cu (II)) of dose 50µg/insect. Meanwhile the compounds A2, A4, A5 and A7 were produced 36, 38, 18 and 40 of percent mortalities of the adult at the same previous dose. Moreover the compound A6 had no effect on the adult of *T. confusum*at the two doses of 10 and 30 µg (Table 2). Similar results were obtained by Marcelo and Moacyr [20]; they demonstrated that treatment of *Aedesalpopictus* cells (mosquito) with indomethacin results in reduction of normal growth rate. After injection of indomethacin into the hemocoels of tobacco hornworms,*Manducasexta* insect, it rapidly moved from the hemolymph circulation into all major tissues and was slowly metabolized within tissue (Jon and David [11]). Johnson and Howard [9] revealed that indomethacin and eicosatetraynoic acid was inhibited growth due to toxic the cells of insects, *Maducasexta, Choristoneurafumiferana, and Plodiainterpunctella* at the high dose of 20µM. N-Alkatonic N, N-dimethyl amides were found to act as penetration enhancers for the transport of ibuprofen and naproxen from suspensions in 50% aqueous propylene glycol vehicles across rat skin. Greatest enhancement was observed by Irwin et al. [12] with naproxen than ibuprofen but both drugs effectively for penetrated the skin. EC_{50} is 10-100 mg due to effect of the non-steroidal anti-inflammatory drug (NSAID) ibuprofen on *Daphnia magna*; but no chronic ibuprofen toxicity data on Arthropod populations are reported by Lars-Henrik et al. [21]. On the other hand nonsteroidal anti-inflammatory ibuprofen increases sulfates renal clearance and decreases the fractional reabsorption of sulfate by kidney of rat as detected by Kazuko et al. [22]. Also Radhofer-Welte and Rabasseda at [10] concluded that the nonsteroidal anti-inflammatory lornoxicam drug decreased animal prostaglandin synthesis by inhibition of cyclooxygenase. Moreover Lornoxicam is a novel non –steroidal, its toxicity was observed in 1.0 and 2.0mg/Kg/day dose in monkeys. Histopathologically, Lornoxicam was gastrointestinal erosions, ulcerations and inflammation in males and females monkeys at 0.5 / 0.6 mg / kg / day dose as detected by Atzpodienet. al [23].

Assessment biological effect of the different phenylazo compounds on *Triboliumconfusum*:-

Data represented in Table (3) concluded the compound R3 (p-Br) was the most biologically active compound where it produced 38, and 100% mortalities of the *T. confusum* at dose of 10 and 30µg respectively. The same previous two doses of the chemical compound R1(P-OCH_3) caused 15 and 40 mortalities of adult *T. confusum*. And the chemical compounds R2 (p-NO_3) caused 12 and 30 mortality and finally the chemical compound R_4 (P-H) caused 9 and 20 of *T. confusum* mortality respectively. On the other hand the most effective dose was 30 in case of chemical compound R_3 while the dose of 50µg was the highest bioactive in case of compounds R_1, R_2 and R4 respectively. In order of toxicity, LD_{50} of the phenylazo compounds was 36, 48, 15 and 64of the compounds, R1, R2, R3 and R4 respectively.

Effect of Phenylazo-complex on *T. confusum*:-

Different derivatives of the compounds R1, R2, R3 and R_4 were obtained by different attachments of Ni and Cu ions. The derivatives became [R1 -p-methoxy-o-phenylazo-acetamidophenol] 485(R1-Ni) and 486(R1-Cu), [R2 –p-nitro-o-phenylazo-p-acetamidophenol] 487(R2-Ni), 488(R2-Cu), [R3 –p- bromo-o- phenylazo-p-acetamidophenol] 497(R3-Ni), 489(R3-Cu) and [R4 –o-phenylazo-p-acetamidophenol] 499(R4-Ni) , 500(R4-Cu). Their Biorational activities are represented in Table (4), where the chemical compounds 486 was the most bioactive compound caused 18, 50 and 83% of the adultmortality at dose of 10, 30 and 50µg respectively. The highest dose of 50µg produced 73, 57, 37, 54, 56, 16 and 74 of adult mortalitiesby the effect of compounds 485,487,488,498, 497, 499 and 500 respectively. The bioactivity of these compounds may due to the endotoxin and inhibition of insect metabolism. Similar results were obtained by the effect of similar compounds on insect and animals. The compound 2-[(5-chloro-2-methoxyphenyl)azo]-1H-imidazole (M6434) produced lethal shock of survival rates in hemorrhagic and endotoxin-shock rats at the dose of 3 or 10 µg/kg/min. Also it was decreased the blood pressure and the urine output of shocked rabbits (Ohnish et al. [15]). Survival rates of cardiogenic shock rats improved, and the content of ATP and creatine phosphate in myocardium of these animals were restored by the treatment with 1 or 3 µg/kg/min of M6434. On the other hand Taiwei Chu *et al* at [24] reported that, the bio-distribution of 11-(1-imidazolyl)-undecanoic acid (IUA) and 11-(2-(4-bromo-phenylazo)-1-imidazolyl)-undecanoic acid (BPIUA) in mice demonstrated poor heart blood ratios. In addition these complexes were used for the metabolic myocardial imaging in these animals. Similar results were obtained by US patent [25]; they conducted that the invention relates to novel 6486191- Nitrophenyl-sulphonyl-imidazoles (6486191) used for the controlling of vegetable and animal pests. These compounds had fungicidal properties. Moreover Anne Lecroise et al. at [14] reported that the synthetic 4-phenylazobenzyloxycarbonyl-L-leucyl-glycyl-L-prolyl-D-arginine was inhibited the metabolism of stiochiometrically collagenase enzyme of *Hypodermalineatum*insect. Meanwhile the synthetic peptide of N-(4 [4' (dimethylamino) phenylazo] benzoyl-Evyaves-5-[(2 amino ethyl) amino] naphthalene-1-sulfonic acid was bioactive

substrate inhibitor for serralysin- type enzyme of *Phtorhabdus luminescent* insect larvae as given by JuditMarokhazi et al.[13].

Table (1): Effect of some drugs on*Triboliumconfusum*

%bioactivity of drugs compounds on *T. confusum*				
Dose µg/Insect	**D1** **(Ibuprofen)**	**D2** **(Naproxen)**	**D3** **(Lornoxicam)**	**D4** **(Indomethacin)**
10	**8**	**15**	**21**	**17**
30	**20**	**30**	**40**	**30**
50	**41**	**58**	**73**	**56**
LD_{50}	**55**	**48**	**35**	**46**
Control	**00**	**00**	**00**	**00**

Table (2): Effect of copper (II)-drugs ion-pairs on*Triboliumconfusum*

% bioactivity of Drugs with Cu (II) **compounds on *T. confusum***						
Dose µg/insect	**A1** **(D1+**Cu (II)**)** **Room temperature**	**A2** **(D2+**Cu(II)**)** **Boiling point**	**A4** **(D2+**Cu (II)**)** **Room temperature**	**A5** **(D4 +** Cu (II)**)** **On heating**	**A6** **(D4 +** Cu (II)**)** **Room temperature**	**A7** **(D3 +** Cu (II)**)** **Room temperature**
10	**21**	**8**	**7**	**5**	**00**	**3**
30	**50**	**20**	**20**	**10**	**00**	**20**
50	**86**	**36**	**38**	**18**	**8**	**41**
Control	**00**	**00**	**00**	**00**	**00**	**00**

Table (3): Effect of the phenylazo compounds on*Triboliumconfusum*

% bioactivity of Phenylazo compounds on *T. confusum*				
Dose µg/insect	**R1X** **= p-OCH_3**	**R2X** **= p-NO_2**	**R3X** **= p-Br**	**R4X** **= p-H**
10	**15**	**12**	**38**	**9**
30	**40**	**30**	**100**	**20**
50	**68**	**55**	**00**	**36**
LD_{50}	**36**	**48**	**15**	**64**
Control	**00**	**00**	**00**	**00**

Table (4): Effect of phenylazo- complexes with Ni (II) or Cu (II) on*Triboliumconfusum*

% bioactivity of Phenylazo-complexes with Cu (II) and Ni (II) on *T. confusum*								
Dose µg/insect	**485** **R1-Ni**	**486** **R1-Cu**	**487** **R2-Ni**	**488** **R2-Cu**	**497** **R3-Ni**	**498** **R3-Cu**	**499** **R4-Ni**	**500** **R4-Cu**
10	**14**	**18**	**13**	**8**	**14**	**13**	**5**	**15**
30	**40**	**50**	**30**	**20**	**30**	**30**	**10**	**40**
50	**73**	**83**	**57**	**37**	**56**	**54**	**16**	**74**
Control	**00**	**00**	**00**	**00**	**00**	**00**	**00**	**00**

Conclusion:-

Combination of some drugs and their copper (II) ion-pairs with phenylazo compounds attached with copper and nickel introduced a powerful method for appropriate probe as bioactive compounds on Arthropods as well as flour beetles, *Triboliumconfusum*. The method was successfully applied for the new trend of the bioactive compounds.

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