International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 28-06-2021, Accepted: 14-07-2021, Published: 29-07-2021
Volume 6, Issue 4, 2021, Page No. 100-107

Insecticidal, acaricidal and biochemical evaluation of some synthetic azobenzene-hydrazone derivatives with nano cationic adjuvant

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Abstract
The present study undertakes to evaluate the effect of used cationic new nano surfactant ((1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate(II) complex(S)) upon addition to synthetic Azobenzene-hydrazone Derivatives ((*E*)-2-oxo-2-((4-((Z)-phenyldiazenyl)phenyl)amino)-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetohydrazonoyl cyanide (1) and ((*E*)-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((Z)-phenyldiazenyl)phenyl)amino) acetohydrazonoyl cyanide (2). The physico-chemical properties for adjuvants and formulations were both determined separately. The effect of these synthetic complexes formulations has been measured against 3rd instar larvae of cotton leafworm, *Spodoptera littoralis (Boisd.)* and against two spotted spider mite, *Tetranychus urticae Koch,* LC_{50}s and LC_{90}s values were measured and tabulated under semi field conditions. The results indicated that, the surface tension and pH values of the spray solution decreased in the two formulations. Results showed that coated 1and 2 with applied cationic nano surfactant gave highest increase in the insecticidal activity than 1 and 2 alone solutions. These results indicated that cationic adjuvant (S) increased the effectiveness of formulations. Therefore, cationic adjuvant (S) may be used to reduce the number of applications per season and the application rates of chemicals works as insecticides. The toxicity index as well as enzymes activity must be considered when investigating the total effects of new tested chemical formulations. Therefore, the aim of the present work is to clarify toxicity and the role played by the sub lethal dose LC_{50} of two new synthetic chemical formulations on the 3rd instar larvae of *S. littoralis* and two spotted spider mite *Tetranychus urtica* Koch, in order to better understand of the action of these compounds on lepidopterous larvae and spider mite through studying their effect on biochemical parameters. These parameters are attained by estimating activity of some enzyme affecting in cuticle formation.

Keywords: adjuvant, physico-chemical properties, insecticidal efficiency, cotton leaf worm, two spotted spider mite

Introduction
The active ingredients in synthetic pesticides are often under fire for their toxic effects. This study takes a look at the nano additives, called (hallo cuprate complex) with new synthetic active ingredients formulations. Adjuvants may increase the toxicity or penetration of the active ingredients, and they are frighteningly unregulated [1].
The producer of newly environmentally friendly pesticides have made good progress. These formulations is based on oil/water emulsions and aqueous suspension concentrates instead of conventional pesticide. These formulations are purposed not only to change toxic and non-degradable ingredients, but also to raise the efficacy of products and balance of all components in the formulation [2]. The essential roles of formulation additives and adjuvants are to raise the biological activity and to give a product which is safe for use, minimize the residues of pesticides on food crops [3, 4]. Adjuvants are achieve more environmentally acceptable pest control, effective, best targeted and helping shift use from preventative, high dose applications to low dose [5]. The use of other modified ingredients and formulations with a Nano sized encapsulated to control release,improve efficacy and lowering costs associated with produced new active ingredients [6]. Nuruzzaman etal. (2016) [7] reviewed fundamental and critical information for researchers and engineers in the field of nanotechnology and especially the use of nano encapsulation techniques to deliver pesticides [7].
Nanotechnology implementations in the agro-industry area (pesticides, fungicides, and nano-sensors) and food contact materials were developed [8]. Nanotechnology includes working with characterization, industrialization and manipulation of nanometer sized materials. these nanomaterial have a change in the physicochemical properties compared to the same material [9]. In the future, Applications of these technology can help overcome future demands in agriculture, crop yield, quality, deceasing pollution caused by chemicals and protecting crops against environmental stresses [10]. However, its application without care can lead to many problems to plants, animals, environmental and humankind [11].
There are around 9000 species of insects and mites that are responsible for the damage to crops worldwide [12]. The cotton leaf worm, *Spodoptera littoralis* (Boised) (Lepidoptera: Noctuidae) is swarming polyphagous, foliage feeding insect that is distributed throughout the world. This insect is one of the major cotton pests which cause considerable damage to various ravages not only for cotton plants but also for other field crops and vegetables [13]. It is a destructive prolific and highly polyphagous insect in Egypt. Also, the two spotted spider mite, *Tetranychus urticae koch*, has been controversial in its taxonomic placement. About 60 synonyms included under this species have compounded the controversy. Spider mites feed by penetrating the plant tissue with their piercing and sucking mouth parts and are found primarily on the underside of the leaf. For that reason, the empolied of pesticides is necessary in order to maximise the agricultural activities and to protect the crops [14, 15].

The main objective of this research was to specify the effect of additive((1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate(II) complex(S)) cationic surfactant in increasing the effectiveness of synthetic Azobenzene-hydrazone Derivatives, ((*E*)-2-oxo-2-((4-((Z)-phenyldiazenyl)phenyl)amino*)-N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetohydrazonoyl cyanide) (1) and ((*E*)-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((Z)-phenyldiazenyl) phenyl)amino) acetohydrazonoyl cyanide (2)) against cotton leaf worm, *Spodoptera littoralis* (Boisd.) and two spotted spider mite, *Tetranychus urticae* Koch under laboratory and semi-field conditions.

Materials and Methods

1. ***Adjuvant:*** Cationic new nano surfactant (1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate(II) salt complex (S) *was applied.*
2. ***Tested* chemical formulations**: Azobenzene-hydrazone Derivatives: ((*E*)-2-oxo-2-((4-((Z)-phenyldiazenyl)phenyl)amino)-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl) phenyl) acet hydrazonoyl cyanide (1) and ((*E*)-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((Z) phenyl diazenyl) phenyl)amino) acetohydrazonoyl cyanide (2).
3. ***Insect:***

Cotton leaf worm, Spodoptera littoralis* (Boisd.)*: Insect larvae used were obtained from laboratory strains of S. littoralis reared on castor oil leaves for several generations under controlled conditions of 25± 2˚C and 65 ± 5 % R.H. Four replicates were prepared for each concentrate [16].

Two spotted spider mite*, *tetranychus urticae* koch*: In the Laboratory of Plant Protection Research Institute, ARC, Dakahlia, Egypt in season (2021). The cotton seeds were planted in Plastic bags (25X 40X15 cm) contained soil with peatmus. The seeds were planted at 1-2 cm deep and followed with irrigation and fertilizers as required. Mites, *Tetranychus urticae* koch) were collected from infected cotton plants from different places in Egypt. The mites were identified in the Acarology Lab by following the detailed descriptions mentioned [17]. Both of them were maintained on cotton leaves upside down on moisten cotton pads in Petri-dishes (12 cm in diameter) and kept under controlled conditions at 25±2°C, 80±5% R.H. and 16:8 h (L:D) in the Acarology Laboratory. The cotton pads were moistened daily and all the ends of the leaves were covered with wet cotton to avoid disc dryness and to prevent mite escape. Mites were transferred on fresh cotton leaves every 3 days.

4. Experimental work

4.1 Synthesis of the starting compound (E)-2-cyano-N-(4-(phenyldiazenyl)phenyl) acetamide (Active Ingredient): A solution of p-amino azobenzene (0.01 mol) was added to 1-cyano-acetyl-3,5- dimethylpyrazole (0.01 mol) in dry benzene (30 ml). The reaction mixture was heated under reflux for 3 hrs. The precipitate (solid portion) was separated after cooling and recrystallizedfrom dry EtoH to get *(E)-2-cyano-N-(4-(phenyldiazenyl)phenyl) acetamide* (Golden yellow crystals) [18]. The product (1) was characterized by IR, ^{1}H NMR spectrum and elemental analysis assigned to the molecular formula $C_{15}H_{12}N_4O$, fig.(1).

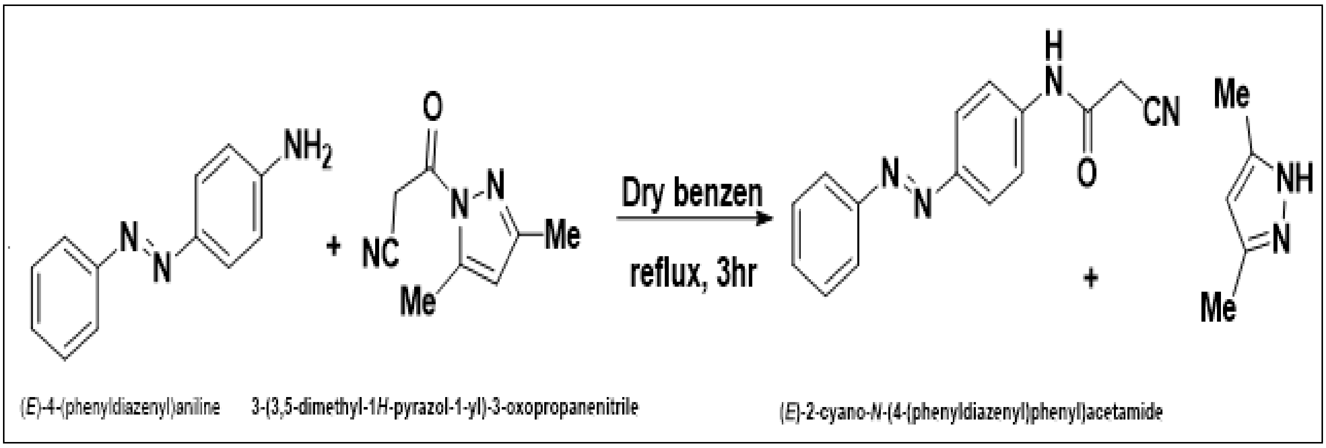

Fig 1: *Synthesis of starting compound ((E)-2-cyano-N-(4-(phenyldiazenyl)phenyl) acetamide)*

4.2 Synthesis of cationic nano surfactant (1-(2-chlorophenyl)diazane hexadecyltrimethyl azane, bromo trichloro cuprate(II) salt complex(additive): Equal molar reaction in solid state of cupper chloride (II) with 2-chloro phenyl hydrazine hydrochloride (0.05mole) in presence of (0.05mole) of 1-hexadecyl trimethyl ammonium bromide by hard grinding in the mortar for an hour until all solids mixed well. The mixture was kept tube inside glass jar containing $Cacl_2$ to prevent moisture for about month until color change from buff powder to light brown block, fig.(2).

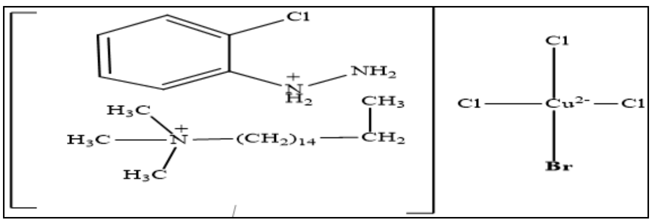

Fig 2: Chemical structure of cationic nano surfactant (additive).

5. Investigation the Physico-Chemical Properties for New Synthetic Chemical Formulations

5.1 Chemical characterization of the synthetic new chemical formulations: We approved the chemical structures of the synthetic new chemical formulations by IR spectrum, elemental analysis, ^{1}H NMR spectrum and and melting point to assign the molecular formulas of synthetic compounds and of Synthesis of cationic new nano surfactant was measured.

5.2 Physical studies of *the synthetic new chemical formulations*

PH measurement: The pH value of a mixture of a sample with water or of an undiluted aqueous formulation is determined by means of a pH meter and an electrode system, it was measured by using a pH Meter (Model: Jeway 3510) was initially standardized using buffered solution.

Surface tension measurement: The surface tension of surfactant was measured using Force tensiomate (sigma 700, USA) by du Nouy method, a platinum-iridium ring. Osipow, (1964) [19]; Tadros, (1995) [20] and Laurier *et al.*, (2003) [18] evaluated the critical micelle concentration (CMC) dyne/cm for surfactant [19, 20, 18]. The surface tension of surfactant was measured when 2.5 grams of surfactant were added into 250 ml of distilled water and then different concentrations from (0.1to 1%) were prepared and measured by tensiometer. The value of the CMC is the point maximum surface activity. The surface tension of the solution does not decrease further with an increase in surfactant concentration.

***Evaluation of effectiveness of* new synthetic chemical formulations *against 3rdinstars larvae of the cotton leaf worm, Spodoptera littoralis (Boisd.) and Two spotted spider mite*, *Tetranychus urticae* Koch:** Semi field experiments were conducted according to Ministry of Agriculture protocol, 1993, in cotton plants cultivated in Central Agricultural pesticides laboratory (CAPL) Dokki, Giza, Egypt. A single nozzle hand sprayer was used for spraying. The effectiveness of the two formulations (2 and 1) was evaluated with and without adding cationic nano surfactant (S) against 3rd instars larvae of the cotton leaf worm and Two spotted spider mite. Cotton leaves were collected from each treatment immediately after spraying and after 24 h post-treatment against 3rd instars larvae of the cotton leaf worm and after only 6h for Two spotted spider mite. The leaves were transferred to the laboratory for measuring the insecticidal toxicity.

Statistical Analysis

Mortality data were corrected according to Abbott's formula, 1925 [21]. The improvement insecticidal efficiency for synthetic azobenzene hydrazone derivatives (1 and 2) treatments were analyzed, the level of significance was expressed as $p < 0.05$. Larvae were fed on castor leaves and kept in 1 liter glass jars covered with muslin which was fixed tightly by a rubber band. Leaf dipping bioassay method was used to determine the median lethal concentration (LC_{50}) value of the tested formulated compounds were prepared. Castor leaves were dipped for ten seconds in each concentration then left to dry. The treated leaves were offered to the 3rd instar larvae for 12hr, then replaced by untreated ones daily. Mortality percentage were recorded after 24hr, the obtained data subjected to LDP line analysis and the toxicity index then estimated. The biochemical changes were studied.

Preparation of Samples for Biochemical Studies

The samples were prepared by use of the 4th instar larvae of *S. littoralis* after 96 hr of all treatments at LC_{50} level and control. The unfeeded larvae were homogenized in distilled water covered with crushed ice for three minutes. The samples were centrifuged at 500 r.p.m. for 10 minutes at 5˚C to remove supernatants and haemocytes. Then the samples were analyzed for the biochemical studies [22].

Enzymes measurements

The physiological response to active ingredient (as insecticide) with or without additive against the 4th instar larvae of S. littoralis associated with the changes in the values of insect enzymes and that can be measured by:

- Determination of AST (GOT) & ALT (GPT) activities.
- Determination of acid &alkaline phosphatase activity.
- Determination of total proteins, carbohydrates and lipids.

Results and Discussion

1. **Synthesis of *tested* chemical formulations: *coupling reaction of (E)-2-cyano-N-(4-(Phenyldiazenyl)Phenyl)acetamide with different primary aromatic amine diazonium salts (Azo compounds derivatives):*** Diazonium chloride ((prepared by dissolving sodium nitrite (0.001 mol)) in 3 ml cold water] was added to a cold (0-5 ºC) solution of compound (E)-2-cyano-N-(4-(phenyldiazenyl) phenyl) acetamide (0.001 mol in 20 ml pyridine) adding to a cold solution aromatic amine as, p- amino sulfamethazine and p-amino sulfathiazole (0.001 mol)under continuous stirring conditions for half an hour. The mixture was kept all night in the refrigerator and thereafter diluted by water. The precipitated (formed solid) was filtered, washing by water and dried. the purified preciptate was recrystallized from EtOH and DMF (2:1), fig.(3). (*E*)-2-oxo-2-((4-((Z)-phenyldiazenyl)phenyl)amino)-*N*-(4-(*N*-(thiazol-2-yl)sulfamoyl)phenyl)acetohydra zonoyl cyanide)1 is an orange crystals with yield 87%. While, (*E*)-*N*-(4-(*N*-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((Z)-phenyldiazenyl) phenyl) amino) acetohydrazonoyl cyanide 2is a Red crystals with yield 85% [23,24].

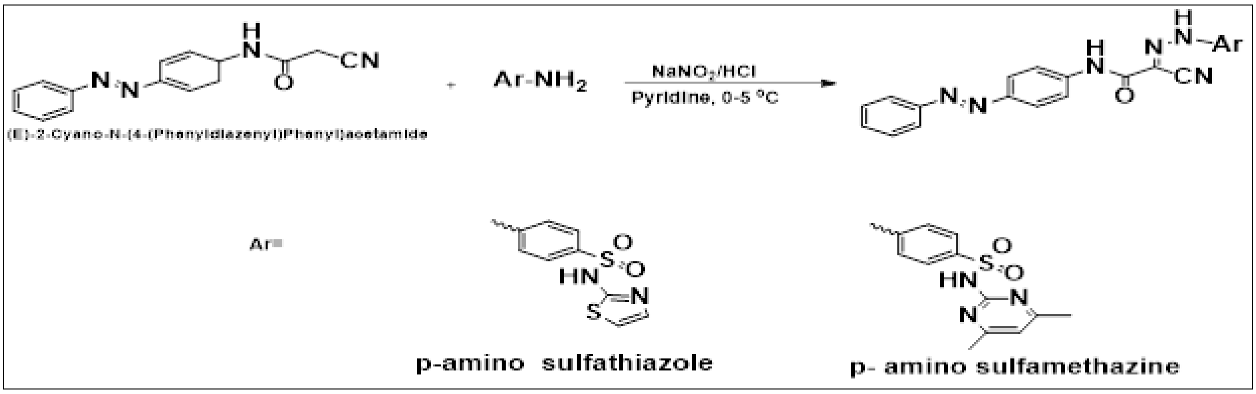

Fig 3: Coupling reaction of (*E*)-2-cyano-*N*-(4-(Phenyldiazenyl)Phenyl) acetamide with different primary aromatic amine diazonium salts.

2. Description of the new chemical formulations

Chemical characterization of the synthetic new chemical formulations:

Table (1) shows the chemical characterization of the synthetic Azobenzene-hydrazone Derivatives and cationic nano surfactant (additive) which were approved by IR spectrum, elemental analysis, ^{1}H NMR spectrum and melting point to assign the molecular formulas of synthetic compounds and additive.

Table 1: chemical analysis measurements of the synthetic new chemical formulations.

Chemical Analysis	Tested compound			
	(E)-2-Cyano-N-(4-(Phenyldiazenyl) Phenyl)acetamide	**1**	**2**	***Additive(S):***
IR (ν_{max}/cm^{-1})	3280 (NH), 2256 (CN), 1669 (CO)	3486, 3448, 3440 (3NH), 2215 (CN), 1670 (CO)	3448, 3333, 3228 (NH), 2218 (CN), 1687 (CO)	3421, (C-NH$_2$), 3018, 2916, 2850 (C-H) alkane, 1631, 1469 (C=C) aromatic, 788,719 meta aromatic, 600-800 C-Cl
^{1}H NMR (DMSO-*d*$_6$):	δ_H 3.97 (s, 2H, CH$_2$), 7.56-7.93 (m, 9H, Ar-H), 10.66 (s, ^{1}H, NH).	δ_H ppm 6.83 (br.s, 1H, olefinic H), 7.25 (br.s, 1H, olefinic H), 7.59-8.01 (m, 13H, aromatic H), 10.27 (s, 1H, NH amidic), 12.20 (s, 1H, NH hydrazo), 12.69 (s, 1H, NH SO$_2$)	δ_H ppm 2.26 (s, 6H, 2CH$_3$), 6.76 (s, 1H, olefinic H), 7.57-8.01 (m, 15H, aromatic H), 10.28 (s, 1H, NH amidic), 12.17 (s, 1H, NH hydrazo). 12.69 (s, 1H, NH SO$_2$)	
Elemental analysis	Calcd: C 68.16; H 4.56; N 21.18 %. Found: C 68.17; H 4.58; N 21.20%.	Calcd.: C, 54.12; H, 3.79; N, 21.04% Found: C, 54.15; H, 3.80; N, 21.06%.	Calcd.: C, 56.47; H, 3.16; N, 24.69% Found: C, 56.49; H, 3.18; N, 24.70%.	Calcd.: C,44.29; H,7.37; Br,11.80;Cl,20.91; N,6.20; Cu,9.37% Found: C,44.29; H,7.43; Br,11.79;Cl,20.92; N,6.20; Cu,9.37%
Melting point	189-190℃	260-265 °C	255-260 °C	179-188℃
Chemical Formula, (Molecular weight)	$C_{15}H_{12}N_4O$, (264.29).	$C_{24}H_{20}N_8O_3S_2$, (530.58).	$C_{27}H_{23}N_9O_3S$, (553.60)	$C_{25}H_{50}BrCl_4CuN_3$, (677.95)
Colour	Golden yellow crystals	Orange crystals	Red crystals	light brown block

1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide),2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide).
*Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate (II) salt.

3. Physical studies of *the synthetic new chemical* formulation

PH measurement

Data in table (2) shows that the pH values of cationic complexe and synthetic two chemical formulations. The cationic surfactant (has a less pH value (more acidic).The acidity of additive cationic surfactant increase the acidity of synthetic two chemical formulations. Also, data in table (2) indicates that 1 +S has pH value less than 2 +S, 1 and 2 respectively.

Surface tension measurement

Surface tension appeared originally from the attraction between the molecules at the surface. Generally by increasing the surfactant concentration, the surface tension of the resulted solution decreased. The surface tensions of the prepared metal cationic surfactant was decreased remarkably. So, The surface tensions of the synthetic two chemical formulations (1 +S, 2 +S) were decreased than its individual solutions without adding surfactant additive.

Table 2: Physical properties of the synthetic new chemical formulations in bi distilled water at 25 °C.

Compound Name	*pH*	*Surface Tension γ_{CMC} (mN/m)*
Additive S	2.91	37.80
1	*5.43*	*50.97*
2	*5.97*	*55.60*
1 +S	*3.99*	*42.06*
2 +S	*4.54*	*47.93*

γ_{CMC} the surface tension of the critical micelle concentration.
1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide), 2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-

yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide).
*Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate (II) salt.

4. Toxicological studies

The median lethal concentrations with their confidence limits on 3rd instar larvae of *S. littoralis* of tested new synthetic chemical formulations 1 ((E)-2-oxo-2-((4-((Z) phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide)) and 2 ((E)-N-(4-(N-(4,6-dimethylpyrimidin-2 yl)sulfamoyl) phenyl)-2-oxo-2-((4-((Z) phenyldiazenyl)phenyl)amino) acetohydrazonoyl cyanide)).And after adding cationic nano surfactant (1-(2-chlorophenyl)-1λ4-diazane hexadecyltrimethyl-λ4-azane, bromo trichloro *cuprate (II) (S)* after 24 hrs of exposure is represented in Tables (3,4). The experiments were carried out in the laboratory-field of Plant Protection Research Institute, ARC, Dakahlia, Egypt. The method of indirect exposure was used throughout the present investigation. Five concentrations of each chemical new formulation of 1 (25, 50,100, 150 and 300 ppm), for 2 (37.5, 75,150, 300 and 500 ppm), 1 +S (12.5, 25, 50,100 and 200 ppm) and 2 +S (18.5, 37.5, 75,150 and 250 ppm) were used; leaves of cotton plants were divided into five replicates. Each 10 of 3rd instar larvae of cotton leaf worm, *S. littoralis* (Boisd.) were transferred to the treated Plastic bags contained leaves of cotton plants, which then covered with muslin cloth. After 24 hours the alive larvae were counted. Also, Each 10 of two spotted spider mite *Tetranychus urticae* Koch, were transferred to leaves of cotton plants, which then covered with muslin cloth. Five concentrations of each chemical new formulation of 1 (18.5, 37.5,75, 150 and 300 ppm), for 2 (37.5, 75,150, 300 and 400 ppm), 1+S (5, 10, 37.5, 75and 150 ppm) and 2 +S (5, 10, 37.5, 75and 150 ppm) were used; leaves of cotton plants were divided into five replicates. After only 6 hours the alive spider mites were counted. The mortality were calculated and plotted by LDP line program. The tested compounds were compared for their efficiency on spotted spider mite and 3rd instar larvae of cotton leaf worm according to their LC_{50} and LC_{90} of the toxicity lines.

Compound 1 with cationic nano surfactant (1-(2-chlorophenyl)-1λ4-diazane hexadecyltrimethyl-λ4-azane, bromo trichloro *cuprate (II) (S)* was the highest toxic action followed by compound 2 with cationic nano surfactant (S) while, 1 and 2 individual solutions were the least toxic action, the LC_{50} values were (31.20, 35.97, 46.54 and 76.24 ppm) for 3rd instar larvae, respectively and (18.70, 33.93,58.45 and 102.68 ppm) for two spotted spider mite, respectively. The wet ability and spreading of new synthetic chemical formulations on the treated surface increase by reducing of the surface tension of the spray solution, and thus increases insecticidal activityc[25]. Also, the decrease in pH value indicates an increase in positive charge of spray solution leading to increase of the attraction between spray solution and treated plants leaves surface, which have a negative charge, thus increase the insecticidal efficiency [26, 27]. Additives can be used to enhance the biological performance of new synthetic chemical formulations as well to improve the physical characteristics of its formulations. The cationic surfactant improved the spreading and the speed of penetration of chemical formulations into the cuticle layer of surface leaf. Also, The synthetic azobenzene hydrazone derivatives moiety with the cationic nano surfactants were most potent toxic derivatives(1,2+s) and showed good biological and insecticidal activities [28] against target pests (cotton leaf worm S. littoralis and two spotted spider mite Tetranychus urticae). The presence of sulfonamide group in addition to cyano group in their structures. Furthermore, the presence of electron withdrawing groups/atoms is major for increasing the insecticidal activity [29].The end outcome is a successful pest control with less environmental damage.

Table 3: Susceptibility of 3rd instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested new synthetic chemical formulations after 24 hrs. from treatment.

Treatment	*LC50(ppm)*	*LC90(ppm)*	*Toxicity index* (Ti)	*Slope*
1	46.54	362.13	67.05	1.294
1 + *Additive(S)*	31.20	238.17	100	1.254
2	76.24	454.87	40.97	1.893
2 + *Additive(S)*	35.97	328.12	86.75	2.185

Table 4: Susceptibility of two spotted spider mite, *Tetranychus urticae* Koch to tested new synthetic chemical formulations after 6 hrs. from treatment.

Treatment	*LC50(ppm)*	*LC90(ppm)*	*Toxicity index* (Ti)	*Slope*
1	58.45	268.40	31.98	1.936
1 + *Additive(S)*	18.70	130.33	100	1.520
2	102.68	461.39	18.21	1.964
2 + *Additive(S)*	33.93	179.76	55.11	1.770

1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide),2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide).
*Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate (II) salt.

5. Biochemical Studies

1. Determination of AST (GOT) & ALT (GPT) activities

Data in table (5) indicate that 1 with cationic nano surfactant (1-(2-chlorophenyl) diazane hexadecyltrimethyl azane, bromo trichloro *cuprate (II) (S)* produced a significantly higher increase in AST (GOT) activity than the control it was 26.37%, followed by p- amino sulfa methazine with cationic nano surfactant (S) 24.64%, while the lowest increase in AST (GOT) activity was induced by 1 and 2 (11.33, 9.30 %). But a decrease in ALT (GPT) activity was observed, according to data in table 2, compound 2 exhibited the most observed decrease -28.24 % than in the control, followed by compound 1 (-4.58%) and increase in ALT(GPT) activity was observed, according to data in table 2, compound 2 and 1 exhibited the most observed increase (39.69, 48.60 %) than in the control [30-32].

Table 5: AST (GOT) and ALT (GPT) activity in hemolymph of the 4th instar larvae, *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each insecticide.

Tested compound	AST(GOT)		ALT(GPT)	
	Mean enzyme activity (U/L/g.b. wt)	Change %	Mean enzyme activity (U/L/g.b. wt)	Change %
1	2191 b	11.33	375 b	-4.58
1 + *Additive(S)*	2487 a	26.37	549 a	39.69
2	2151 b	9.30	282 c	-28.24
2 + *Additive(S)*	2453 a	24.64	584 a	48.60
Control	1968 c		393 b	

1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide), 2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide).
*Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, romo trichloro cuprate (II) salt.

The activity of AST (GOT) improved and activity of ALT (GPT) declined in haemolymph. These results are agreement with the reported data of (Zohry, (2006) [33]; Sokar, (1995) [34]; Mohamed and Azab (2002) [35]; El-Kordy *et al.*, (1995)) [36] against *S. littoralis* after treatment with several IGRs or insecticides, e.g. flufenoxuron, hexaflumuron, hexaflumuron alone or its binary mixture with chlorpyriphos, flufenoxuron, pyriproxyfen or teflubenzuron and pyriproxyfen, flufenoxuron or chlorfluazuron [33-36]. The tested new chemical formulations (p-amino sulfathiazole and p- amino sulfamethazine) inhibited activity of ALT (GPT) in haemolymph of *S. littoralis* larvae due to their effects on the synthesis or functional levels of this enzyme. The opposite was true when they applied insect growth regulators (IGRs), as Atabron [chlorfluazuron], Match [difenzoquat] and Mimic [tebufenozide], against 2nd and 4th instar larvae of *Spodoptera littoralis* [31]. In case of the 4th instar, the tested compounds 1 and 2 with 1-(2-chlorophenyl) diazane hexadecyltrimethyl azane, bromo trichloro cuprate (II) (S) increased the activity of the two enzymes after 48h of treatment.

5.2 Determination of Acid and Alkaline Phosphatase Activity

The obtained data in table (6) show that tested synthetic compound (1, 1 with cationic nano surfactant (S)) caused reduction in (ALK-P) activity -25.99, -23.24 % followed tested synthetic compound 2 with cationic nano surfactant (S), 2 22.93, -5.50 % compared to control. At the same respect, tested synthetic compound (1, 1 +S) caused significant decrease in acid phosphatase (AC-P) activity ranging between -45.31% and -44.90 % followed tested synthetic compound 2 with cationic nano surfactant (S) and 2 -32.24 and-18.50% compared to the control. These results are in agreement with data was measured by El-Barky *et al.*, (2008) on *S. littoralis* [32]. The resistance level was a positive correlated with alkaline phosphatase and it was negative correlated with Acid phosphatase activity.

Table 6: Alkaline phosphatase and acid phosphatase activity in haemolymph of the 4th instar larvae, *Spodoptera littoralis (Boisd.)* after treatment with LC_{50} of each insecticide.

Tested compound	Alkaline phosphatase		Acid phosphatase	
	Mean enzyme activity (U/L/g.b. wt)	Change %	Mean enzyme activity (U/L/g.b. wt)	Change %
1	242^{d}	-25.99	40.2^{e}	-45.31
1 + *Additive(S)*	251^{c}	-23.24	40.5^{d}	-44.90
2	309^{b}	-5.50	59.9^{b}	-18.50
2 + *Additive(S)*	252^{c}	-22.93	49.8^{c}	-32.24
Control	327^{a}	---	73.5^{a}	---
LSD 0.05	1.82	---	0.18	---

1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide), 2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide). *Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate (II) salt.

5.3 Determination of total proteins and lipids

Proteins are the most important components of biochemical of insect that bind the foreign compounds. Data in table (7) show that all tested new chemical formulations have an observed significantly decrease in total proteins -53.85, -46.15 and -30.77 for tested synthetic compounds (1 and 2 with cationic nano surfactant (S)), tested synthetic compounds (1and 2) alone solutions respectively, compared with control. This result agreed with hexaflumuron and teflubenzuron which were applied by Assar *et al.*, (2010) [37] on 4th instars of *S. littoralis* [37].

Also, Data in table (7) show that all tested new chemical formulations have an observed significantly decrease in total lipids -41.15, -31.89, -30.13 and -20.52 for 1 with cationic nano surfactant (S), tested synthetic compound (1, 2 with cationic nano surfactant (S)) and compound 2 respectively, compared with control. This result agreed with hexaflumuron and teflubenzuron as IGR s which were applied by Assar *et al.*, (2012) [38] against 4th instar larvae of *S. littoralis* [38]. All tested new chemical formulations reduce lipid content in *S. littoralis* treated due to the separation of lipids or fatty acids.

Table 7: Determination of total proteins and lipids in haemolymph of the 4th instar larvae of *Spodoptera littoralis (Boisd.)* after treatment with LC_{50} of each tested insecticide.

Tested compound	Total proteins		Total lipids	
	(mg/g.b. wt)	Change %	(mg/g.b. wt)	Change %
1	0.7[c]	-46.15	5.81[d]	-31.89
1 + *Additive(S)*	0.6[c]	-53.85	5.02[e]	-41.15
2	0.9[b]	-30.77	6.78[b]	-20.52
2+ *Additive(S)*	0.6[c]	-53.85	5.96[c]	-30.13
Control	1.3[a]		8.5[a]	
LSD 0.05	0.18		0.02	

1:(*E*)-2-oxo-2-((4-((*Z*)-phenyldiazenyl)phenyl)amino)-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl) acetohydrazonoyl cyanide), 2:(*E*)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl)sulfamoyl)phenyl)-2-oxo-2-((4-((*Z*)phenyldiazenyl)phenyl) amino) acetohydrazonoyl cyanide). *Additive(S):*1-(2-chlorophenyl) diazane hexadecyl trimethyl azane, bromo trichloro cuprate (II) salt.

Conclusion

There is a relationship between chemical structure and activity. The synthetic azobenzene hydrazone derivatives moiety with the cationic nano surfactants were most potent toxic derivatives (1,2+s) and showed good biological and insecticidal activities against target pests (cotton leaf worm S. littoralis and two spotted spider mite Tetranychus urticae). The presence of sulfonamide group in addition to cyano group in their structures. Furthermore, the presence of electron withdrawing groups/atoms is major for increasing the insecticidal activity. So, the heterocyclic compounds (1, 2) hydrazon derivatives and cyano group work as important port for the designing and development of new active ingredients to control pests. Nano cationic surfactant Additive can be also used to enhance the biological performance of these active ingredients and improve the physical characteristics of its formulations. In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of insect's body. On the basis of overall findings, it can be concluded all tested new chemical formulations are toxic to some developmental stages of *S. littoralis*, as well as caused many biochemical effects at its sub lethal level. Unlike insects, digestion in some mites occurs (at least partially) intercellularly at epithelial cells of midget were, cells cytoplasm act as haemolymph in insects.

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