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Cyanoacetanilides as efficient synthons in the heterocyclic synthesis of some inventive pyridones for potential use as insecticides against *Spodoptera littoralis* (Lepidoptera: Noctuidae) and *Aphis gossypii* (Hemiptera*:* Aphididae*)*

Reda, R. H. Abdullah; Nahla, H. Harraz; Shereen, A. Shoman and Abd El Salam, M.
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract

A novel series of anticipated insecticidal activity 2-pyridone derivatives incorporating a 3,4-(Methylenedioxy) aniline moiety were synthesized in a good yield through interaction of the readily available cyanoacetanilide, *N*-(benzo[*d*][1,3] dioxol-5-yl)-2-cyanoacetamide (1) with different arylidene ethyl cyanoacetate and arylidene malononitrile derivatives. The newly synthesized skeletones were elucidated by IR, MS and ^{1}H NMR spectral techniques. Toxicological and biochemical parameters of the examined compounds (**1-5**) against the cotton pests, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) as well as cotton aphid, *Aphis gossypii* (Glover) *(*Hemiptera*:* Aphididae*)* under laboratory conditions, were also inspected. As regards the indomitable LC_{50} values, cyanoacetanilides, **5**, **1**, **3**, **4** and **2**, showed proper toxic effects against the 3rd instar larvae of *S. littoralis* after 7 days post treatment with LC_{50} values of 970, 1075, 1088, 1574 and 1996 ppm, respectively, and toxicity index of 100%, 90%, 89%, 61% and 48%, respectively. Otherwise, cyanoacetanilides, **5**, **2**, **1**, **3** and **4**, showed a powerful toxicity against *A. gossypii* after 48 hours post treatment with LC_{50} values of 50, 58, 81, 103 and 343 ppm, respectively, and toxicity index of 100%, 86%, 61%, 48% and 14%, respectively. As well as, all tested compounds caused significant changes of estimated insect enzymes and total protein and lipids compared with control.

Introduction

There is an urgent need to increase the number of active ingredients of pesticides due to their scarcity around the world. In addition, the emergence of resistance in many types of pests towards many of the available active ingredients of pesticides. In order to obtain new active substances, there are several sources, including, extraction of some active ingredients from wild plants or the metabolic products of microorganisms, as well as synthesis new effective organic compounds.

Cyanoacetanilides are utilized as a perfect building block in heterocyclic synthesis of a wide-ranging scale of biologically active polyfunctionalized compounds (Fadda *et al.*, 2008) exhibiting both electrophilic and nucleophilic features (Fadda *et al.*, 2012). NH and methylene groups in cyanoactanilides possess

nucleophilic centers while electrophilic properties are associated with CO and CN functions (Fadda *et al.*, 2017). So, different reagents were reported for the regioselective attack on precursor 1 to yield flexible, vastly functionalized heterocyclic compounds of prospective insecticidal efficacy towards different pests such as the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) as well as cotton aphid, *Aphis gossypii* (Glover) (*Hemiptera*: *Aphididae*) under laboratory conditions. Likewise, assessment of the mode of action of the utmost effective tested insecticides by determination of biochemical enzymatic parameters, such as alkaline phosphatase (Alk-p), acid phosphatase, (Acid-p), lipase, amylase, alanine aminotranferase (ALT), asparate aminotransferase (AST), and acetylcholinesterase (AchE), total lipid and total protein as indicated by Soliman *et al.* (2020).

Toxicological and biochemical parameters of the examined cyanoacetanilides incorporating a 3,4-(Methylenedioxy) aniline moiety **(1-5)** against the cotton pest, *S. littoralis* as well as cotton aphid, *A. gossypii* under laboratory conditions, were also inspected.

Materials and methods

This study was conducted at Plant Protection Research Institute, Mansoura Branch, Agricultural Research Center, Egypt, in 2022 AD. Cyanoacetanilides incorporating a 3,4-(Methylenedioxy) aniline moiety **(1-5)** were synthesized and evaluated as insecticidal agents against *S. littoralis* and *A. gossypii* as well as the changes in insect enzymes activities were estimated of treated insects by synthesized compounds.

1. Synthesis process:

All spectroscopic carried out as stated by Soliman *et al*. (2020).

N-(benzo[d][1,3] dioxol-5-yl)-2-cyanoacetamide(1)

In three hours of refluxing dry benzene (30 ml), equimolar amounts of both 3,4-(Methylenedioxy) aniline (2g, 0.01 mol) and 1-cyano-acetyl-3,5-dimethylpyrazole (2.4g, 0.01mol) were added. A crystalline precipitate was acquired after cooling to r.t and was filtered and recrystallized from ethanol to give cyanoacetanilide **(1)**.

Reddish brown crystals; mp 195-200^{o} C; yield 90%. IR (KBr) *υ/cm*$^{-1}$: 3350 (NH), 2220 (CN), 1750 (CO). ^{1}H NMR (400 MHz, DMSO – d_6): δ ppm 3.35 (s, 2H, CH_2), 5.97 (s, CH_2 of dioxol ring), 6.84-7.25 (3H, Ar-H), 10.02 (s, 1H, NH). MS *m/z* (%): 204 (M^+, 8.63), 178 (40.17), 164 (43.16), 136 (32.40), 121 (100.00), 107 (39.83), 75 (45.56). Anal. for $C_{10}H_8N_2O_3$ (204.05). Calcd: C, 58.82; H, 3.95; N, 13.72%; Found: C, 59.02; H, 4.02; N, 13.85 %.

Preparation of pyridin-2-ones (2 and 4).

Method (A)

In 25 ml boiling ethanol, 2-(arylidene)-ethyl cyanoacetate [namely 2-(4-methoxybenzylidene)-ethyl cyanoactate and 2-(4-chlorobenzylidene)-ethyl cyanoactate] (0.0015 mol) were added to **1** (0.3 g, 0.0015 mol) in presence of catalytic piperidine (0.5 mL), after refluxing for 3 h. The reaction mixture was kept to cool. The resulted solid was filtered off and recrystallized from EtOH to get **2** and **4**.

Method (B)

In ethanol (25 mL), a mixture of **1** (0.3 g, 0.0015 mol) was refluxed for 3 h, with the appropriate aldehyde (namely *p*-methoxybenzaldehyde and *p*-chlorobenzaldehyde) (0.0015 mol), piperidine (3 drops), and ethyl cyanoacetate (0.0015 mol). After cooling, the formed ppt was purified by isolation and filtration, then

recrystallization from EtOH to get **2** and **4**.

1-(benzo[d][1,3]dioxol-5-yl)-6-hydroxy-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (2)

Yellowish brown crystals; mp 213-215 ºC; yield 84%. IR (KBr) *υ/cm*$^{-1}$: 3407 (OH), 2207 (CN), 2204 (CN), 1713 (CO). ^{1}H NMR (400 MHz, DMSO – d_6): δ ppm 3.81 (s, 3H, OCH_3), 5.97 (s, 2H, CH_2 of dioxol ring), 6.87-7.52 (m, 7H, Ar-H), 10.75 (s, 1H, OH). MS *m/z* (%): 387 (M^+, 8.63), 318 (40.17), 313 (43.16), 302 (32.40), 187 (100.00), 146 (39.83), 121 (45.56), 105 (44.78). Anal. for: $C_{21}H_{13}N_3O_5$ (387.09). Calcd: C, 65.12; H, 3.38; N, 10.85%. Found: C, 65.91; H, 3.35; N, 10.65%.

1-(benzo[d][1,3]dioxol-5-yl)-4-(4-chlorophenyl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile(4)

Dark yellow crystals; mp 220-222ºC; yield 76%. IR (KBr) *υ/cm*$^{-1}$: 3316 (OH), 2221 (CN), 2220 (CN), 1710 (CO). ^{1}H NMR (400 MHz, DMSO – d_6): δ ppm 5.97 (s, 2H, CH_2 of dioxol ring), 6.87-7.44 (m, 7H, Ar-H), 10.75 (s, 1H, OH). MS *m/z* (%): 391 (M^+, 8.63), 372.07 (13.11), 106.09 (12.79), 105.04 (100.00), 78.07 (3.59), 77.05 (50.91), 77.05 (50.81), 76.06 (3.67), 51.03 (4.49). Anal. for: $C_{20}H_{10}ClN_3O_4$ (391.04). Calcd: C, 61.32; H, 2.57; N, 10.73%. Found: C, 61.38; H, 2.64; N, 10.93%.

Preparation of pyridin-2-ones (3 and 5).

Method (A)

When cyanoacetanilide **1** (0.3 g, 0.0015 mol) was reluxed for 3hrs. in 25 ml EtOH and Pip. (0.5 ml) with the appropriate 2-(arylidene)-malononitrile [namely 2-(4-methoxybenzylidene)-malononitrile, and 2-(4-*N*, *N*-dimethylbenzylidene)-malononitrile] (0.0015 mol), and the reaction mixture was allowed to cool. The solid end product that was filtered off and recrystallized from ethanol to get **3** and **5**.

Method (B)

A mixture of **1** (0.3 g, 0.0015 mol), in 3h refluxing ethanol (25 mL), piperidine (0.5 mL), the appropriate aldehyde (namely *p*-methoxybenzaldehyde and 4-*N*, *N*-dimethylbenzaldehyde) (0.0015 mol) and malononitrile (0.0015 mol). The reaction content was allowed to cool. The output that designed was insulated by filtration, dried and purified by recrystallization from EtOH.

6-amino-1-(benzo[d][1,3]dioxol-5-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (3)

Off-white crystal; mp 225-227 ºC; yield 75%. IR (KBr) *υ/cm*$^{-1}$: 3200 (NH_2), 2217 (CN), 2204 (CN), 1715 (CO). ^{1}H NMR (400 MHz, DMSO – d_6): δ ppm 3.81 (s, 3H, OCH_3), 5.97 (s, 2H, CH_2 of dioxol ring), 6.49 (s, 2H, NH_2), 6.87-7.52 (m, 7H, Ar-H). MS *m/z* (%): 386 (M^+, 8.63), 372.07 (16.17), 105.04 (100.00), 77.05 (50.91), 55.03 (4.49). Anal. for $C_{21}H_{14}N_4O_4$ (386.10). Calcd: C, 65.28; H, 3.63; N, 14.50%. Found: C, 65.01; H, 3.55; N, 14.22%.

6-amino-1-(benzo[d][1,3]dioxol-5-yl)-4-(4-(dimethylamino)phenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (5)

yellow crystals; mp 230-232 ºC; yield 86%. IR (KBr) *υ/cm*$^{-1}$: 3343 (NH_2), 2213 (CN), 2208 (CN), 1715 (CO). ^{1}H NMR (400MHz, DMSO – d_6): δ ppm 3.02 (s, 6H, *N*Me_2), 6.49 (s, 2H, NH_2), 5.97 (s, 2H, CH_2 of dioxol ring), 6.87-7.16 (m, 7H, Ar-H). MS *m/z* (%): 399 (M^+, 8.63), 372.07 (23.17), 77.05 (44.31), Anal. for $C_{22}H_{17}N_5O_3$ (399.13). Calcd: C, 66.16; H, 4.29; N, 17.53%. Found: C, 66.25; H, 4.36; N, 17.59%.

2. Insect pests:

2.1. Cotton leafworm:

Spodoptera littoralis Boisd. (Lepidoptera: Noctuidae), a laboratory strain of cotton leafworm, was selected to check the efficacy of

cyanoacetanilides derivatives as pesticides. To rear cotton leafworm, eggs stage of the insect was obtained from Cotton Leafworm Department, Plant Protection Research Institute, Mansoura branch, Agriculture Research Center. Then they were incubated under controlled conditions at 27 ^{o}C, 60% humidity until hatching. After hatching, the larvae were reared on cleaned castor leaves as described by EL-Defrawi *et al.* (1964) for use them in experiments.

2.2. Cotton aphid:

Cotton aphid *Aphis gossypii* Glover (Homoptera; Aphididae) was used to study the insecticidal activity of synthesized cyanoacetanilides. The aphids were taken from the untreated cotton fields by pesticides and reared under controlled conditions for three generations in an insect incubator at 20 ^{o}C, 60% humidity, and 16L: 8D photoperiod in the Plant Protection Research Institute, Mansoura Branch, Agricultural Research Center. Wingless individuals from the third generation were used for each experiment (Dampc *et al*., 2020).

3. Bioassay of synthesized compound:

Five synthesized compounds were evaluated as pesticides against cotton leafworm and cotton aphids. 0.5 gram of each compound was dissolved in 1 ml of DMF. After complete dissolving, the volume was completed to 50 ml by adding distilled water for obtained to 10000 ppm as a stock solution. Serial dilutions were prepared from each compound as follows; the concentrations 500, 1000, 1500, 2000, and 2500 ppm were prepared for cotton leafworm treatment while the concentrations 100, 200, 400 and 1000 ppm were prepared for cotton aphids treatment.

3.1. Bioassay against cotton leafworm:

The third instar larvae of reared cotton leafworm were used to evaluate the efficiency of five synthesized compounds at six concentrations of each compound by leaf dipping method as mentioned by Tabashnik *et al.* (1991). The larvae were starved about two hours before treatment. Ten larvae were counted and transferred to plastic jar then discs of castor leaves were dipped in prepared concentrations of compounds for 30 seconds and leave to air dry. The treated castor leaves were presented to prepared larvae jars. Each treatment was repeated five times. Also, another castor leaf was dipped in water and DMF as control treatment and presented to prepare larvae jars. Dead larvae were counted everyday post treatment. Mortality percentages were calculated and corrected by Abbot's formula (Abbott, 1925). Toxicity index between the compounds was calculated as Sun's equation (1950).

3.2. Bioassay against cotton aphids:

Spray method was used for bioassay test against *A. gossypii*. Filter papers were put in Petri dishes (10 cm diameter). Then twenty individuals of adult stage were transferred to the Petri dishes. Three ml from each concentration of each compound were sprayed by atomizer in each Petri dish. The control treatment was conducted by spraying distilled water plus DMF. Each treatment was repeated five times. Dead insects were counted every day. Mortality percentages were calculated by Abbot's formula (Abbott, 1925). Toxicity index between compounds was calculated as Sun's equation (1950).

4. Effect of synthesized compounds on biochemical contents:

4.1. Effect of synthesized compounds on biochemical contents in cotton leafworm:

The fourth instar larvae of cotton leafworm were used for biochemical studies. Median lethal concentrations were prepared from each compound based on the results of

previous bioassay test as mentioned by **Finney (1971)**. The same method in bioassay test was used to treat larvae by LC_{50} only. Distilled water plus DMF was used in control treatment. After three days of treatment, one gram of live larvae was weighted in eppendorf tube and frozen then transferred to physiology laboratory for biochemical tests (Total Protein, Total Lipid, Amylase, Protease, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), Acid and Alkaline phosphatase and Acetyl Choline Esterase) in Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

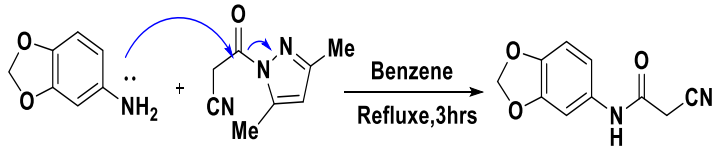
4.2. Effect of synthized compounds on biochemical contents in cotton aphid:

The median lethal concentrations (LC_{50}) from each compound were prepared based on mortality percentages of treated cotton aphids as mentioned by Finney (1971). Twenty individuals of untreated cotton aphids were sprayed with 3 ml from LC_{50} of each compound in petri dishes (10 cm diameter) as the same method in bioassay test. Distilled water plus DMF was used in the control treatment. After 24 hrs. post treatment, 0.5 gram of live insects were weighted in eppendorf tube and frozen until biochemical studies in physiology laboratory, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. Total Protein, Total Lipid, Amylase, Protease, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), Acid and Alkaline phosphatase were determined.

Results and discussion

1. Chemistry:

Schemes (1, 2) accentuated the synthetic approaches of the target compounds. Thus, the auspicious synthon, *N*-(benzo[*d*][1,3] dioxol-5-yl)-2-cyanoacetamide (1) was acquired upon cyano acetylation of 3,4-(Methylenedioxy)aniline by 1-cyano acetyl-3,5-dimethyl pyrazole in dry solvent benzene as reported by Abd El Salam *et al.* (2022).



1

Scheme1:Synthesis of Cyanoacetanilide 1

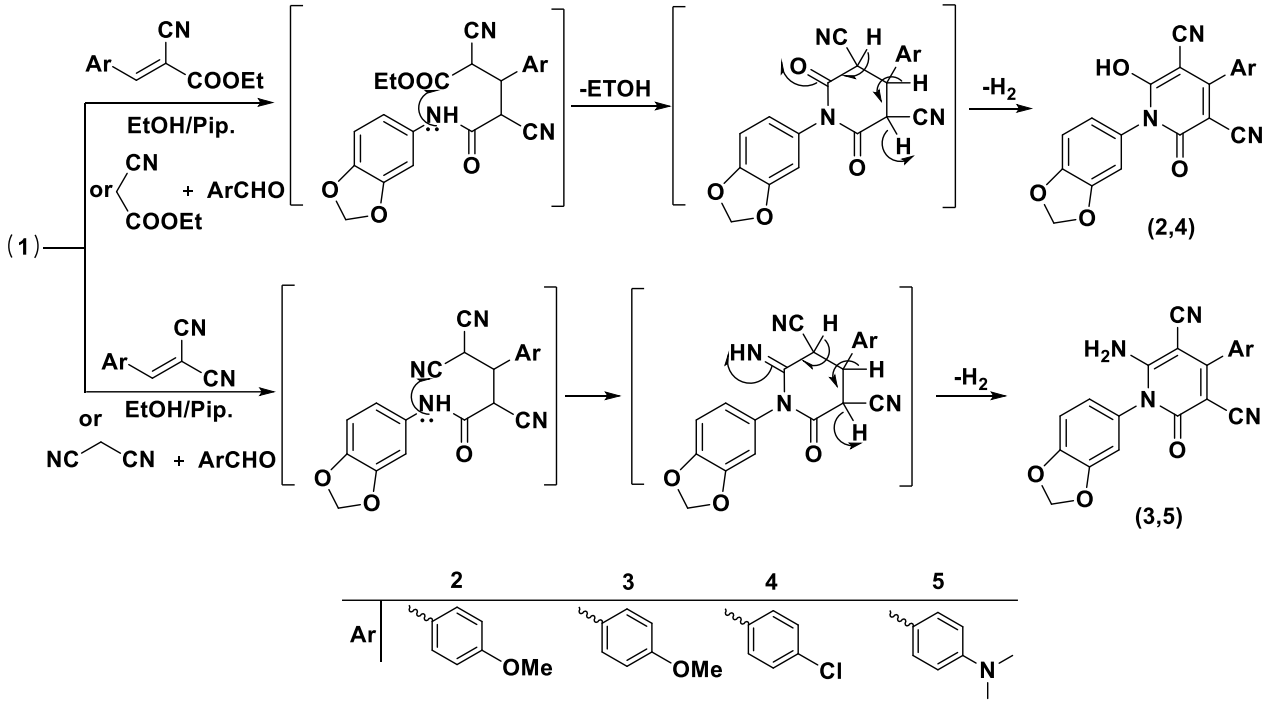
Data obtained from the spectra of 1 was in complete harmony with its proposed structure. Hence, absorption bands at 3350, 2220 and 1750 cm^{-1} in the IR spectrum of cyanoacetanilide **1**, refer to NH, CN and CO functions, respectively. Its MS showed a (M^+) at *m/z* 204 related to molecular formula $C_{10}H_8N_2O_3$. Also, ^1H NMR indicated a multiplet at δ_H 6.84-7.25 ppm due to three aromatic protons, three singlets at δ_H 3.35, 5.97 and 10.02 ppm ascribed to amidic methylene, methylene in dioxol ring and NH, respectively.

Subsequent, cyanoacetanilide derivative 1 has the synthetic potentiality and applicability to improve a simplistic and suitable path to some novel pyridine derivatives with an expected prevalent spectrum of bio-responses (Refat and Fadda, 2015).

One-pot reaction of the promising synthon 1 with ethyl cyanoacetate and different aromatic aldehydes, namely *p*-methoxybenzaldehyde and *p*-chlorobenzaldehyde (1: 1: 1 molar ratio), in a solution of catalytic boiling ethanol in piperidine generated the 2-pyridinones **2** and **4**. As well, the 2-pyridone derivatives **2** and **4** were also assimilated upon reaction of

cyanoacetanilide **1** with arylidene ethyl cyanoacetate in refluxed ethanol having catalytic piperidine.

Alternative path for the synthesis of 2-pyridone derivatives was the one-pot reaction of the cyanoacetanilide **1** with malononitrile and aromatic aldehydes, namely *p*-methoxybenzaldehyde and 4-*N*,*N*-dimethylbenzaldehyde (1 : 1 : 1 molar ratio) in refluxing EtOH/piperidine solution to provide **3** and **5**. Furthermore, when arylidene malononitrile was heated with the synthon 1 in ethanol in the company of piperidine, it gave 2-pyridone derivatives 3 and 5. The structures **2**, **3**, **4** and **5** were established according to spectral data.



Scheme 2: Synthesis of arylidene and 2-pyridone derivatives

Spectra of 2-pyridones **2**, **3**, **4** and **5** indicated no NH functions in the IR spectra and showed a molecular ion peak (M+), at *m/z* 387, 386, 391 and 399 attributable to the molecular formulas $C_{21}H_{13}N_3O_5$, $C_{21}H_{14}N_4O_4$, $C_{20}H_{10}ClN_3O_4$ and $C_{22}H_{17}N_5O_3$, respectively. ^{1}H NMR spectrum of **2** indicated a methoxyl singlet at δ_H 3.38 ppm and a multiplet at δ_H 6.87-7.52 ppm for seven aromatic protons in addition to two singlets at δ_H 5.97, 10.75 ppm due to methylene protons of the dioxol ring and OH proton. ^{1}H NMR spectrum of **5** revealed the presence of three singlet signals at δ_H 3.02, 5.97 and 6.49 ppm assignable to *N*Me$_2$, CH$_2$ and NH$_2$ protons, respectively, and a multiplet signal at δ_H 6.87–7.16 ppm assigned to aromatic protons.

2. Efficiency of synthesized compounds against *Spodoptera littoralis* and *Aphis gossypii*:

The presented data in Table (1) illustrate the efficiency of five synthesized compounds against *S. littoralis* at different concentrations. The results show that the mortality percentage increased with increasing concentrations and days post treatment. Also, the compounds (Com.); Com.1, Com.3 and Com.5 were more efficient compounds compared with Com.2 and Com.4. Where, the mortality percentage of treated *S. littoralis* by these compounds reached 81.5%, 78% and 86.31%, respectively. In addition,

the toxicity data in Table (2) and Figure (1) show the same results where the LC_{50} values of these compounds; Com.1, Com.3 and Com.5 were less than other compounds (1075, 1088 and 970 ppm, respectively). This refers to the compounds Com.1, Com.3 and Com.5 were more toxic against cotton leafworm *S. littoralis* compared with other compounds. The toxicity index and relative potency revealed that the more toxic compound was Com.5 followed by Com.1, Com.3, Com.4 and the less toxic compound was Com.2 as shown in Table (2). The results show that changing the starting compound (Com.1) increased the toxicity of Com.5 and slightly decreased the toxicity of Com.3, but caused a significant decrease in the toxicity of Coms.2 and 4.

Table (1): Mortality % of treated cotton leafworm 3rd instar larvae by synthesized compounds.

Tested compounds	Concentrations	Mortality % after days post treatment				General mean effect
		1day	3 days	5 days	7 days	
Com.1	500	0	3.33	13.3	26.67	10.82
	1000	0	13.33	30	43.33	21.66
	1500	0	26.67	50	58.33	33.75
	2000	3.33	33.33	56	73.33	41.49
	2500	13.33	53.33	70	81.5	**54.54**
Com.2	500	0	3.33	3.33	16.67	5.83
	1000	0	6.67	10	30	11.66
	1500	0	10	13.33	36.67	15.00
	2000	0	10	13.33	50	18.33
	2500	16.67	33.67	43	60.18	**38.38**
Com.3	500	0	3.33	6.67	33.33	10.83
	1000	0	3.33	16.67	43.33	15.83
	1500	3.33	10	23.33	50	21.66
	2000	6.67	23.67	53.33	70	38.41
	2500	43.67	67.33	71	78	**65.00**
Com.4	500	0	16.67	23.33	26.67	16.66
	1000	3.33	23.67	33.33	33.33	23.41
	1500	6.67	26.67	36.67	43.33	28.33
	2000	10	33.67	43.33	56.67	35.91
	2500	23.67	51.67	63.33	67.35	**51.50**
Com.5	500	3.33	20	30	33.33	21.66
	1000	6.67	26.67	36.67	43.33	28.33
	1500	6.67	33.67	43.33	63.33	36.75
	2000	10	43.33	63.33	70	46.66
	2500	33.67	67.35	71.33	86.31	**64.66**

Table (2): Toxicity of the synthesized compounds against 3rd instar larvae of cotton leafworm after 7 days post treatment.

Tested compounds	LC_{50} (ppm)	95% Confidence limit (ppm)		*Slope* ± *S.E.*	Toxicity Index (%)	Relative potency
		Lower	Upper			
Com.1	1075	931	1218	2.16 ± 0.25	90	1.85
Com.2	1996	1693	2514	1.71 ± 0.25	48	1
Com.3	1088	902	1273	1.67 ± 0.23	89	1.83
Com.4	1574	1326	1934	1.51 ± 0.24	61	1.26
Com.5	970	818	1113	1.99 ± 0.24	100	2.05

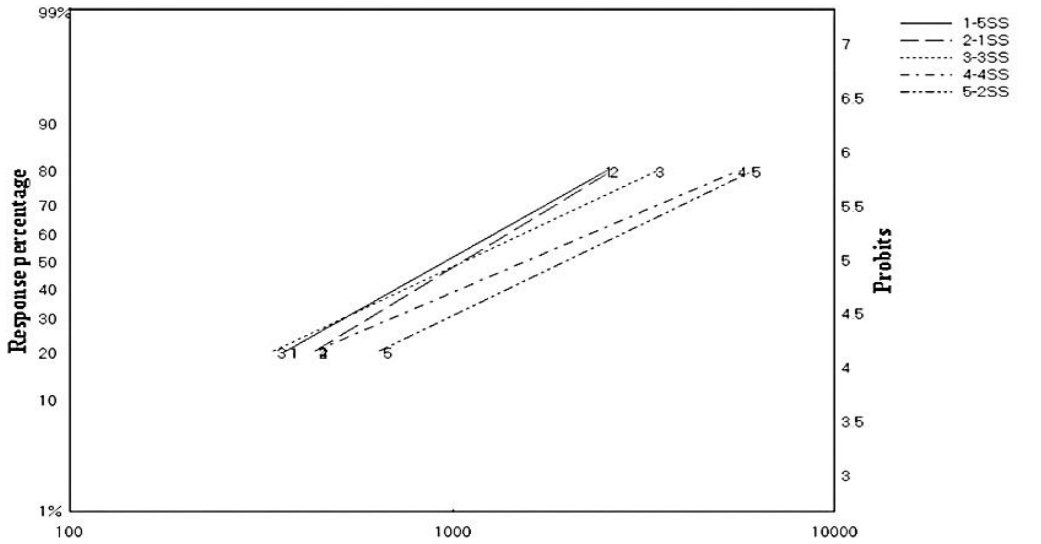


Figure (1): LD-P- Lines of synthesized compounds against the 3rd instar larvae of cotton leafworm.

On the other hand, mortality percentages of treated *A. gossypii* increased by the compounds; Com.1, Com.2 and Com.5 as shown in Table (3). Where, they reached 93%, 90% and 85% after 48 hrs. post treatment by Com.1, Com.2 and Com.5, respectively. Also, the LC_{50} values indicated that the more toxic compound was Com.5 followed by Com.2, Com.1 and Com.3 then the less toxic compound was Com.4. Where, LC_{50} values of these compounds were 50, 58, 81, 103 and 343 ppm, respectively as shown in Table (4) and Figure (2). In general, changing the starting compound (Com.1) increased the toxicity of Com.5 and Com.2, while decreasing the toxicity of Com.3 and greatly decreasing the toxicity of Com.4, as shown in Table (4).

Table (3): Mortality % of treated *Aphis gossypii* by the synthesized compounds.

Tested compounds	Concentrations	Mortality % after days post treatment			General mean effect
		24 hours	48 hours	72 hours	
Com.1	100	22	56	62	46.66
	200	42	68	73	61.00
	400	69	79	91	79.66
	1000	75	93	95	**87.66**
Com.2	100	15	58	65	46.00
	200	28	74	79	60.33
	400	33	84	91	69.33
	1000	57	90	98	**81.66**
Com.3	100	6	51	56	37.66
	200	14	63	67	48.00
	400	29	85	86	66.66
	1000	64	94	94	**84.00**
Com.4	100	7	19	24	16.66
	200	33	44	52	43.00
	400	51	52	63	55.33
	1000	63	73	81	**72.33**
Com.5	100	23	55	71	49.66
	200	28	77	83	62.66
	400	30	80	91	67.00
	1000	56	85	98	**79.66**

Table (4): Toxicity of the synthesized compounds against *Aphis gossypii* after 48 hours post treatment.

. Tested compounds	LC_{50} (ppm)	95% Confidence limit (ppm)		*Slope* ± *S.E.*	Toxicity Index (%)
		Lower	Upper		
Com.1	81	45	115	1.27 ± 0.21	61
Com.2	58	24	92	1.11 ± 0.21	86
Com.3	103	71	133	1.58 ± 0.21	48
Com.4	343	277	433	1.38 ± 0.18	14
Com.5	50	13	89	0.89 ± 0.19	100

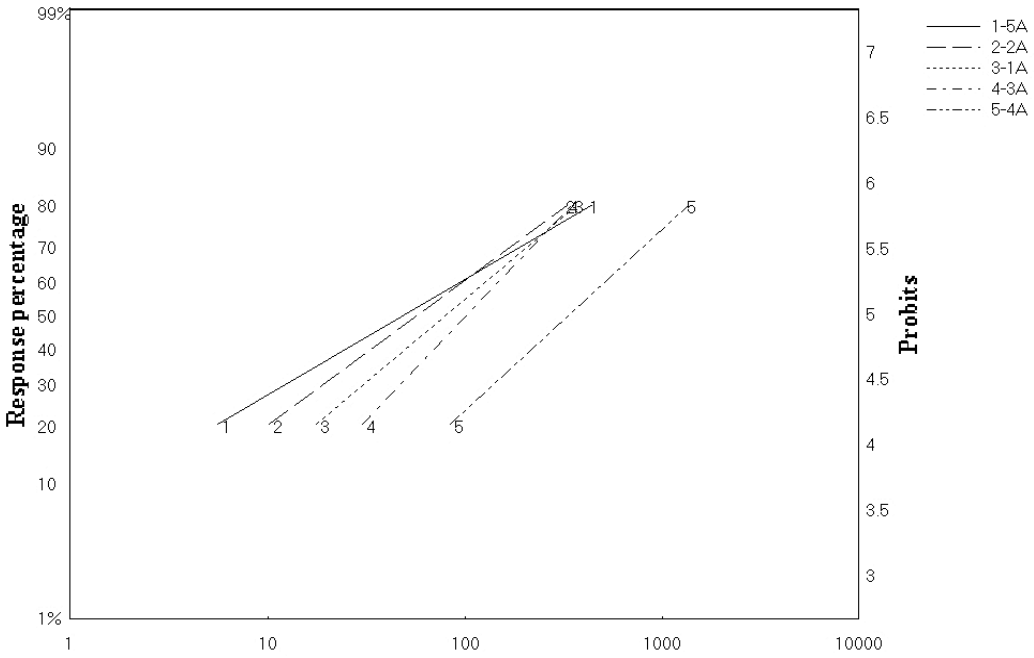


Figure (2): LD-P- Lines of synthesized compounds against adult stage of *Aphis gossypii*

The relationship between a molecule's chemical structure and its biological activity is known as the "Structure-activity relationship." Additionally, the structure-activity relationship makes it possible to pinpoint the chemical groups that are responsible for a compound's intended biological impact in an organism, which enables a compound's effect to be changed by altering its chemical structure. Thus, 2-pyridone derivative 5 was the most effective toxin and showed impressive biological responses, which led to the formation of possible indications for future development as candidates for insecticides (Bhongade, 2016).

This is most likely due to the presence of a pyridinonyl ring together with electron-withdrawing groups/atoms like cyano and amino functions in their skeletons, in addition to the other aromaticity properties that were necessary for enhancing insecticidal effect. All of these factors contributed to the development of active sites on molecules, which promote chemical react with various chemicals and the development of novel insecticides with greater activity.

3. Effect of the synthesized compounds on biochemical contents of tested insects:

In this study, authors focused on some enzymes activities of insects as digestive enzymes (Amylase and lipase), detoxifying enzymes (Acid phosphatase, alkaline phosphatase, ALT, AST and acetyl choline esterase

AchE) as well as, total soluble protein and total lipids in insect hemolymph. The highest three toxic compounds beside control treatment were investigated only. The compounds Com1, Com3 and Com5 were studied in case of *S. littoralis* but the compounds Com1, Com2 and Com5 were studied in case of *A. gossypii.*

As shown in Table (5) there was a significant decrease in total protein in larvae of *S. littoralis* which were treated by Com.1 and Com.3 but there was no significant effect with Com5. While, there was a significant decrease of total lipids in larvae that were treated by Com.1 and Com.5 but there was no significant effect with Com.3. On the other hand, there were significant changes in digestive enzymes activity with all treatments compared with the control treatment (Table 5). Where, amylase activity decreased in all larvae which were treated by Com1, Com.3 and Com.5. Also, lipase activity decreased in the case of Com.1 and Com3 treatments but increased in the case of Com5 treatments.

Table (5): Effect of the synthesized compounds on total protein, total lipids, lipase and amylase activity in the 4th instar larvae of *Spodoptera littoralis* .

Tested compounds	Total Protein (mg / gm body weight)	Total Lipids (mg / gm body weight)	Lipase activity U / ml	Amylase activity U / ml
Control	19.8 [a]	4.34 [a]	0.075 [b]	0.106 [a]
Com.1	5.80 [c]	2.45 [b]	0.011 [d]	0.028 [d]
Com.3	8.00 [b]	4.30 [a]	0.020 [c]	0.057 [c]
Com.5	19.8 [a]	2.03 [b]	0.117 [a]	0.073 [b]
LSD (5%)	**0.489**	**0.220**	**0.003**	**0.001**

Data in Table (6) show the changes in detoxification enzyme activities in treated larvae of *S. littoralis* compared with control treatment. There was significant increase of AST and ALT activities in larvae which were treated by Com3 and Com5 but there was a significant decrease in Com1 treatment. While, there was significant decrease of Acid and Alkaline phosphatase activities in all larvae that were treated by Com1, Com3 and Com5 compared with control treatment. Also, the same effect happened in the case of Acetyl choline esterase (AchE) activity in all treated larvae by the compounds. Where, there was high a significant decrease in the activity of AchE enzyme.

Table (6): Effect of the synthesized compounds on AST, ALT, acid phosphatase, alkaline phosphatase and acetyl choline esterase activity in the 4th instar larvae of *Spodoptera littoralis* .

Tested compounds	AST U / ml	ALT U / ml	Acid phosphatase U / ml	Alkaline phosphatase U / ml	Acetyl choline esterase (µg AchBr/min /gm body weight)
Control	0.202 [c]	0.036 [c]	0.132 [a]	0.091 [a]	776 [a]
1	0.063 [d]	0.027 [d]	0.010 [d]	0.068 [d]	343 [c]
3	0.207 [b]	0.041 [b]	0.021 [b]	0.071 [c]	566 [b]
5	0.702 [a]	0.105 [a]	0.019 [c]	0.078 [b]	272 [c]
LSD (5%)	0.001	0.001	0.001	0.001	160

On the other hand, the results in Table (7) illustrate that there was a significant decrease of total protein and lipids in the treated adult stage of *A. gossypii* compared with the control treatment. But there was a significant increase in lipase activity of the treated individuals by Com1 and Com5 while

there wasn't significant change in the treated individuals by Com2. Also, the results in Table (7)show that no significant changes of amylase activity in all treated individuals of *A. gossypii.*

Table (7): Effect of the synthesized compounds on total protein, total lipids, lipase and amylase activity in the adult stage of *Aphis gossypii.*

Tested compounds	Total Protein (mg / gm body weight)	Total Lipids (mg / gm body weight)	Lipase activity U / ml	Amylase activity U / ml
Control	6.60 [a]	2.28 [a]	0.016 [c]	0.002 [a]
1	3.90 [c]	1.32 [b]	0.028 [a]	0.0016 [a]
2	2.00 [d]	1.06 [d]	0.016 [c]	0.002 [a]
5	4.30 [b]	1.22 [c]	0.020 [b]	0.002 [a]
LSD (5%)	0.188	0.018	0.001	0.0016

The presented data in Table (8) show that all tested compounds led to a significant decrease of ALT activity in treated *A. gossypii* compared with control. Also, there was a significant decrease of AST activity in treated *A. gossypii* by Com.3 and Com.5 but the activity of AST enzyme increased in treated *A. gossypii* by Com1. On the other hand, Com.2 and Com5 caused significant decrease of acid and alkaline phosphatase activity in treated *A. gossypii* but the Com.1 led to significant increase of both enzymes in the same insect.

Table (8): Effect of the synthesized compounds on AST, ALT, Acid phosphatase and Alkaline phosphatase activity in the adult stage of *Aphis gossypii.*

Tested compounds	AST U / ml	ALT U / ml	Acid phosphatase (ACP) U / ml	Alkaline phosphatase (ALP) U / ml
Control	0.114 [b]	0.110 [a]	0.029 [b]	0.024 [b]
1	0.149 [a]	0.043 [c]	0.033 [a]	0.029 [a]
2	0.054 [c]	0.016 [d]	0.010 [d]	0.014 [d]
5	0.037 [d]	0.047 [b]	0.013 [c]	0.020 [c]
LSD (5%)	0.002	0.001	0.001	0.001

The primary building block of enzymes and hormones, proteins carry out a variety of biochemical processes in insects and are crucial for insect growth and metamorphosis (Avila *et al*., 2011). In our study, the tested compounds led to decrease total protein in the treated *S. littoralis and A. gossypii.* This decrease is related to either the insects' ability to cope with the stress or the effect of synthesized compounds, which completely suppress certain haemolymph enzymes. (Mirhaghparast *et al*., 2013).

Triglycerides are stored as anhydrous fatty acids, which are also used to produce energy through the process of -oxidation and are a component of the cuticle of insects. Triglycerides are produced from fatty acids, proteins, or carbohydrates (Arrese and Soulages, 2010). Also, in this study total lipids were decreased in the treated insect (*S. littoralis and A. gossypii*). This decrease may be caused by the use of lipids for energy synthesis and metabolic activity to battle the oxidative stress brought on by using synthesized compounds on insects (Canavoso *et al*., 2001).

Insects obtain energy from the digestion of food. Several enzymes are required to break down macromolecules into smaller ones that can be absorbed through the midgut epithelial cells. Amylase, protease, and lipase are examples of these enzymes. Any changes in their activity cause

injury to the insect (Zibaee and Bandani, 2010).

Additionally, a physiological challenge in the insect's body, such as the presence of poisonous substances, microbial infections, or tissue injury, is the cause of the shift in ALT and AST activity (Giboney, 2005). The decrease in total protein content that was observed may be connected to the decrease in ALT and AST activity that most tested compounds caused. These enzymes provide the amino acids with the building ingredients for the creation of proteins and may also help an organism undergo metamorphosis by synthesizing amino acids. One of the enzymes involved in the oxidative metabolism of proline, which was regarded as the primary source of energy in the early life of insects, is ALT. Additionally, aminotransferases enzymes are frequently utilized as markers for the metabolism of proteins and amino acids (Storey and Storey, 2012).

The hydrolytic enzymes, acid phosphatase and alkaline phosphatase, hydrolyze phosphomonoesters in alkaline or acidic environments, respectively. The animal intestinal epithelium is the primary location of ALP, which primarily serves to supply phosphate ions from mononucleotides and ribonucleoproteins for a number of metabolic processes. The midgut has the highest levels of ALP and ACP activity when compared to other tissues, and ALP is engaged in the transphosphorylation reaction (Sakharov *et al*., 1989).

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