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Toxicity and biochemical activity of chlorobenzothiazole and nitrobenzothiazole derivatives as IGR$_S$ against *Spodoptera littoralis* (Boisd.)

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Abstract
Benzothiazoles have a broad spectrum of agricultural biological activities, which are important as heterocyclic structures in agrochemical applications. The benzothiazole-dihydropyridine and benzothiazole-dihydropyramidine derivatives are new active ingredients compounds that were synthesized in a solvent-free by employing a Brønsted acidic ionic liquid (BAIL gel) as an effective heterogeneous catalyst. Five compounds were prepared in a range of concentrations (50, 150, 250, 500 and 1000 ppm). Toxicological assay were studied. Data indicated that, diethyl 1-(6-chlorobenzo [d]thiazol-2-yl)-2, 6-diethoxy-4-(4-hydroxyphenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (4) was approved to be the most toxic compound with LC_{50} value of 34.02 ppm against 4th instar larvae. compound (1) showed less toxicity with LC_{50} value of 191.10 ppm. Also, in case of 1-(6 nitrobenzo [d] thiazol-2-yl)-3-phenyl-2 thioxodihydro pyrimidine-4, 6 (1H, 5H)-dione (5) showed close toxicity value to that of compound 4, its LC_{50} value was 35.29 ppm. By using the leaf dip method, the antifeedant activity of the newly synthesized compounds was evaluated against *S. littoralis* larvae. According to enzymes Activities data, all tested compound raised the level of acetyl cholinesterase in homogenates of larvae in their fourth instar. It was observed that, compound (4) demonstrated a significant difference when compared to the control. In α-esterases data, Compounds (4) and (3) significantly increased the values to79.9 and 62.2%. The results showed that, the LC_{50} of compounds (4) resulted in a highly significant decrease in total protein (-38.2 %). Compounds 1, 2, and 3's LC_{50} resulted in notable drops of 12.6, -15.8, and 2.5.4%. Also, compound (5) showed significantly increased on chitinase activity of 21.2 %. Compounds (5) caused significant decrease (-29.87%) on activity of phenoloxidase in comparision with control.

Keywords: Benzothiazole, the insecticida activity, the antifeedant activity, biochemical activities

Introduction

Insects are the primary source of crop yield loss in global agriculture [35, 8]. Every year, pests [15, 28] cause enormous financial losses to the world's agricultural sector. Using agrochemicals is still one of the best ways to manage pest resistance and resistant weeds, as well as plant diseases, insects, and damage to grass nowadays. More importantly, the best course of action for quick pest control is to use highly effective chemical pesticides when pests (like armyworms) break out in large areas [38]. But continued use of conventional agrochemicals will not only contaminate the environment but also make pathogens more resistant [29], making it harder to control weeds, insects, and plant diseases [43, 9, 36].

A species of moth belonging to the Noctuidae family, *Spodoptera littoralis* (Boisd., 1833) is widely distributed throughout Africa, Mediterranean Europe, and the Middle East [17]. Many nations are known to suffer significant financial losses as a result of the cotton leaf worm [33]. The extremely hazardous *S. littoralis* feeds on valuable plants, such as cotton, potatoes, corn, and vegetables [30, 40].

The bioisostere of benzoxazole is benzothiazole. Benzothiazole have atoms of sulfur and nitrogen; provide special and adaptable scaffolds for designing the toxicity of experiments. Benzothiazole was regarded as a weak base with a variety of biological effects. According to Racané *et al.* (2012) [34], Patel and Shaikh (2010) [31], Patel and Khan (2011) [32], Munirajasekhar *et al.* (2011) [23], Catalano *et al.* (2013) [10], Arora *et al.* (2010) [1], Chaudhary *et al.* (2010) [11], benzothiazole exhibit a broad biological activities. Twenty years ago, the majority of benzothiazole and benzoxazole research was conducted in the medical field [37, 24, 7]. In contrast, the field of agrochemicals received little research attention. Benzothiazoles are important in the hunt for novel pesticides because they have seven modifiable sites in addition to their bicyclic structure. Recently, there has been a greater focus on benzothiazoles and their derivatives, with more research being done on the topic. The present status of research on the mechanism of action and the identification of novel targets of benzothiazole derivative compounds is one of the main obstacles to the development of new environmentally friendly pesticides.

The study of benzothiazoles has advanced significantly in recent years, particularly in the creation of insecticides and herbicides. Because benzothiazoles are so widely used, more benzothiazole containing products might be released in the future. We talked about the active compounds' structural–activity relationship. The goal of this work is to offer motivation and suggestions for the identification of novel agrochemicals derived from benzothiazole derivatives. In order to use in the field of insect control *Spodoptera littoralis* (Boisd.), we were inspired to synthesize a set of modified benzothiazole to evaluate their efficacy as insecticides, one of the most promising compounds, after conducting the required studies in the future [44].

The present study is to through light on synthesis, toxicological and biochemical activities of five chlorobenzothiazole-dihydropyridine and nitrobenzothiazole-dihydropyrimidine derivatives as insect growth regulators (IGR$_S$) agents against 4th instar larvae of *Spodoptera littoralis* (Boisd.).

Materials and methods

1. Chemicals

Sigma-Aldrich provided the chemicals and solvents needed for this investigation**.** Tests were conducted on *S.littoralis* 4th instar larvae to determine the insecticidal activity of the target synthesized compounds.

2. Synthesis of Benzothiazole-Dihydropyridine and Benzothiazole Dihydro pyramidine derivatives

The productions of benzothiazole-dihydropyridine and benzothiazole- dihydropyramidine derivatives are carried out in a solvent-free environment by employing a Brønsted Acidic Ionic Liquid Gel as an effective heterogeneous catalyst. The reusable Brønsted acid ionic liquid gel (BAIL gel) that we present here is made from 1-methyl-3-(4-sulfobutyl)-1H-imidazolium hydrogen sulfate that has been treated with tetraethyl orthosilicate (TEOS). The surface of TEOS was grafted with the Brønsted acidic ionic liquid, which enhanced the material's catalytic activity and made catalyst recovery from the reaction mixture easier. The BAIL gel is a novel catalyst for the synthesis of benzoxazoles, benzimidazoles, and benzothiazoles. This reaction has a broad substrate range. The process exhibits appealing qualities like simple work-up, high yields, and recyclable catalyst [41].

3. Breeding Larva Insects and Analytical Statistics.

During the 2023–2024 season, *S. littoralis* insects were collected from the fields of the agricultural research center farm at the Mansoura branch. The prepared compounds insecticide's efficacy against the *S. littoralis* insect was then evaluated Using a statistical (LDP line) equation, the mortality data of larval insects were computed through probit analysis.

4. The insecticidal and the antifeedant activity

Using standard leaf dip bioassay techniques, the insecticidal bioactivity of all prepared derivatives of benzothiazole-dihydropyridine and benzothiazole- dihydropyramidine derivativesis were screened as shown in figure (1). Target compound results for lab testing were documented, and the concentrations needed to kill 50% (LC_{50}) of *S. littoralis* larvae were calculated. This article used 0.2% Tween-80 as a surfactant with five synthetic compounds of benzothiazole-dihydropyridine and benzothiazole-dihydropyramidine derivatives. Castor leaf discs with a diameter of 10 cm were dipped in the concentration under test for 10 seconds, dried, and then fed to the fourth larvae. The larvae were then put in glass jars and each treatment was carried out three times with 10 larvae per treatment. The control disks were then moved to the untreated ones after being submerged in water and Tween-80. After 48 hours of feeding on castor, the larvae were moved to the untreated one**.** For every synthetic compound, the mortality was determined after 72 hours at 25 ± 2 °C and 60 ± 5% relative humidity. Abbott's formula was utilized to compute the mortality [2]. By using probity analysis, the mortality relapse line in the measurements was quantifiably broken down [14]. Sun's equations were used to determine the harmfulness index [39]. Moreover, the antifeedant activity of these compounds was tested using *Spodoptera littoralis* larvae in their fourth instar using the leaf dip method [18, 25]. After being prepared, the roughly 25 cm^2 leaf discs were dipped in a variety of test compounds for thirty seconds. After the excess compound solution was removed by air drying the leaf discs, they were made available for feeding. For a full day, the insects were permitted to feed. Using a leaf area meter, the amount of uneaten leaf area was measured after 24 hours. The amount of leaf area consumed is determined by subtracting the amount of uneaten leaf area from the amount of leaf area provided. The maximum likelihood programmer MLP 3.01 was used to calculate the feeding inhibition, which was then used to calculate the effective concentration (LC_{50}).

5. Biochemical study and determination of enzymes activities

1. Determination of acetylcholinesterase (AChE).
2. Determination of glutathion s-transferase (GST) and α-esterases (α-EST) 3- Determination of chitinase.
3. Determination of phenoloxidase.
4. Determination of total protein.
5. Determination of Aspartate (AST) and alanine (ALT) amino transferases.
6. Determination of α-esterases.

Discussion

1.Synthesis

1.1. Synthesis of Dimethyl 1-(6-ethoxybenzo [d]thiazol-2-yl)-4-(4-methoxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (1).

2-amino-5-ethoxy benzenethiol was added to dimethyl 1-formyl-4-(4 methoxy phenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate in an equimolecular amount and stirring with 10mg BAIL gel (catalyst) and refluxed for 7 h at 130˚C to give product (1) in a 89−93% yield. Chemical formula of the product (1) was $C_{27}H_{28}N_2O_6S$, Molecular Weight=508.59 and Elemental analysis (C, 63.76;H, 5.55;N, 5.51;O, 18.87;S, 6.30).

1.2. Synthesis of Diethyl 1-(6-chlorobenzo [d]thiazol-2-yl)-4-(4-methoxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (2)

An equimolecular amount of 2-amino-5-chlorobenzenethiol was added to diethyl 1-formyl-4-(4-methoxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate dropwise and stirring with 10mg BAIL gel (catalyst) and refluxed for 7 h at 130˚C to give product (2) in a 91−95% yield as shown in scheme 2. Chemical formula of the product (2) was $C_{27}H_{27}ClN_2O_5S$, Molecular Weight=527.03and Elemental analysis (C, 61.53; H, 5.16; Cl, 6.73; N, 5.32; O, 15.18; S, 6.08).

1.3 Synthesis of Dimethyl 1-(6-chlorobenzo [d]thiazol-2-yl)-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (3)

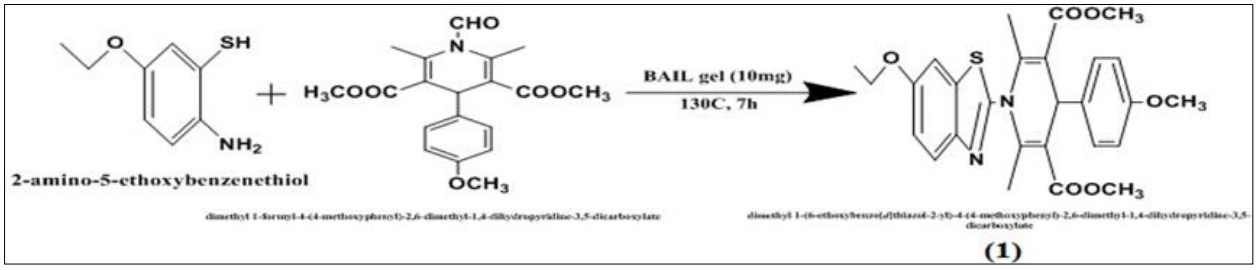
2-amino-5-chlorobenzenethiol was added to dimethyl 1-formyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate dropwise and stirring with 10mg BAIL gel (catalyst) and refluxed for 7 h at 130˚C to give product (3) in a 88−91% yield as shown in scheme 2. Chemical formula of the product (3) was $C_{24}H_{21}ClN_2O_5S$, Molecular Weight=484.95 and Elemental analysis (C, 59.44; H, 4.36; Cl, 7.31; N, 5.78; O, 16.50; S, 6.61).

1.4. Synthesis of Diethyl 1-(6-chlorobenzo [d] thiazol-2-yl)-2, 6-diethoxy-4- (4-hydroxyphenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (4)

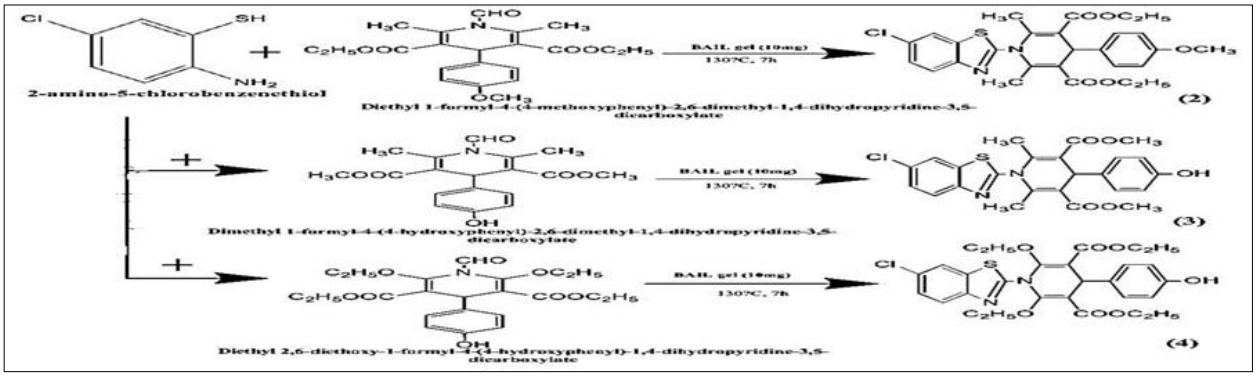
An equimolecular amount of 2-amino-5-chlorobenzenethiol was added to diethyl 2, 6-diethoxy-1-formyl-4-(4-hydroxyphenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate dropwise and stirring with 10mg BAIL gel (catalyst) and refluxed for 7 h at 130˚C to give product (4) in a 92−96% yield as shown in scheme 2. Chemical formula of the product (4) was $C_{28}H_{29}ClN_2O_7S$, Molecular Weight=573.06 and Elemental analysis (C, 58.69; H, 5.10; Cl, 6.19; N, 4.89; O, 19.54; S, 5.59).

1.5 Synthesis of 1-(6-nitrobenzo [d]thiazol-2-yl)-3-phenyl-2 thioxodihydro pyrimi dine -4, 6(1H, 5H)-dione (5)

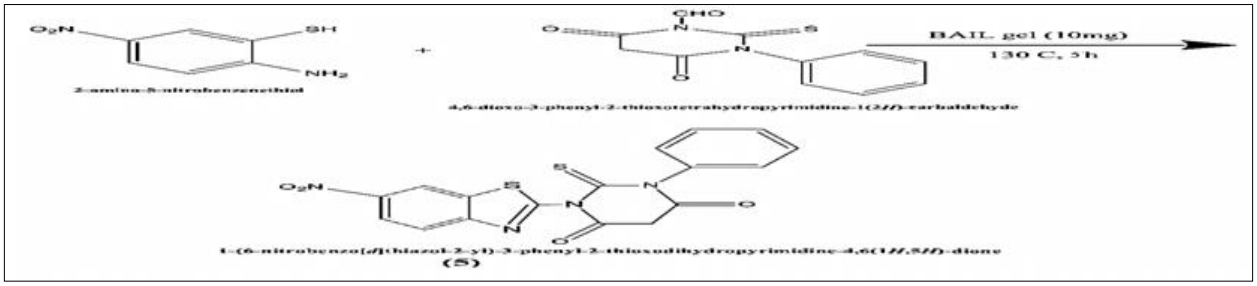
2-amino-5-nitrobenzenethiol was added to 4, 6-dioxo-3-phenyl-2-thioxotetrahydropyrimidine-1(2H)-carbaldhyde dropwise and stirring with 10mg BAIL gel (catalyst) and refluxed for 5 h at 130˚C to give product (3) in a 85−89% yield as shown in scheme 3. Chemical formula of the product (4) was $C_{17}H_{10}N_4O_4S$, Molecular Weight=398.41 and Elemental analysis (C, 51.25; H, 2.53; N, 14.06; O, 16.06; S, 16.09).



Scheme 1: Synthesis of Ethoxy Benzothiazoles Derivative (1)



Scheme 2: Synthesis of Chloro Benzothiazoles Derivatives



Scheme 3: Synthesis of 1-(6-nitrobenzo [d]thiazol-2-yl)-3-phenyl-2 thioxodihydropyrimidine-4, 6(1H, 5H)-dione

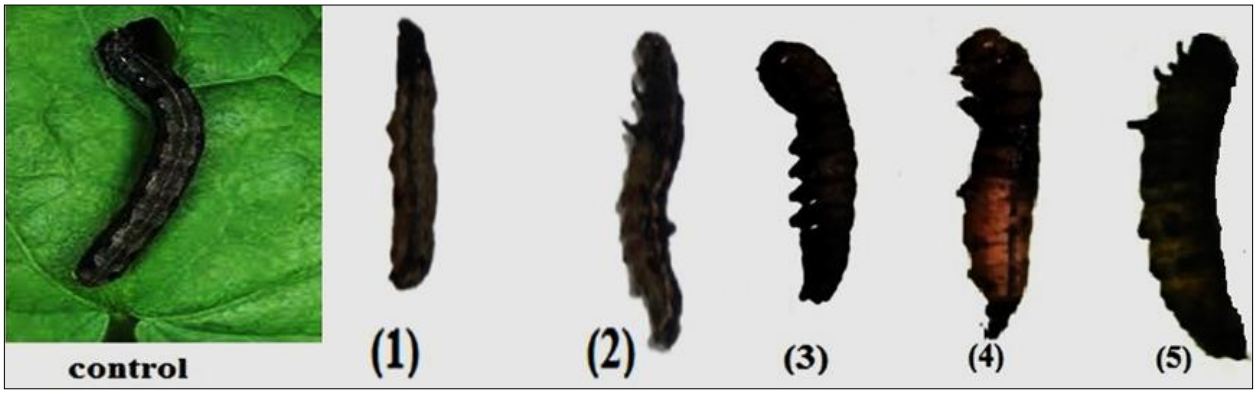


Fig 1: Insecticidal activity of Tested Compounds against 4th instar larvae of cotton leafworm, *Spodoptera littoralis.*

2. Toxicity tests

Each compound was prepared in a range of concentrations (50, 150, 250, 500 and 1000 ppm) by dissolving it in DMSO and then adding water to complete the volume. Four caster-bean leaves were dipped in each attention for thirty seconds, then left to dry. For 48 hours, the larvae in their fourth instar had been enclosed in muslin. Additionally, analyze included a non-treated control group in which the leaves were submerged in water. Each of the four replicates, or roughly ten larvae, was screened for every concentration. Daily assessments were conducted for numerous treatments, and mortality rates were recorded at the end of each day and hour of treatment. The mortality percentage average was calculated using Abbott's formula (1925). The adjusted death component of the compound was statistically calculated in accordance with Finney (1971), from which the equivalent attentiveness probit outlines and dedication regarding fifty and ninety percent mortalities were estimated; slope values with the screened substances were also estimated. Additionally, the efficacy of your screening process for unique substances was evaluated by comparing the most prevalent screened substances to the strongest compound using the following formula: toxicity list = LC_{50} of the strongest compound / LC_{50} of the screened compound × 100 (Sun, 1950).

Toxicological assay chloro ethoxy benzothiazol dihydropyridine and its derivatives and nitro benzothiazol dihydropyrimidine were studied. Data in Table (1) indicate that, diethyl 1-(6-chlorobenzo [d]thiazol-2-yl)-2, 6-diethoxy-4-(4-hydroxyphenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (4) was approved to be the most toxic compound with LC_{50} value of 34.02 ppm against 4th instar larvae and this may be due to the presence of four (OC_2H_5) and pyridine groups. It is clear that the toxicity increased in case of electron withdrawing groups of aromatic amines compared with electron donating groups [3]. In case of aromatic amines, compounds 3 showed high toxicity values near compound 4 with LC_{50} value of 37.09 ppm respectively, followed by 2 with LC_{50} value of 54.46 ppm. compound (1) showed less toxicity with LC_{50} value of 191.10. But in case of 1-(6 nitrobenzo [d] thiazol-2-yl)-3-phenyl-2 thioxodihydro pyrimidine-4, 6 (1H, 5H)-dione (5) showed close toxicity value to that of compound 4, its LC_{50} value was 35.29 ppm. This may be due to the presence of pyrimidine and dione groups.

3. Antifeedant Activity

Using the leaf dip method, the antifeedant activity of the newly synthesized compounds was evaluated against *Spodoptera lituralis* larvae. The findings unequivocally show that the compounds exhibit varying degrees of antifeedant activity against the insect larvae. Compounds 2 and 3 displayed moderate activity, while compounds 4 and 5 displayed higher activity. These substances may interact with the active site of the enzyme that causes nervous breakdown in insects, potentially causing a spasm condition in insects (Table 2).

Table 1: Insecticidal activity of Chlorobenzothiazole-Dihydropyridine derivatives and Nitrobenzothiazole-Dihydropyrimidine against 4th instar larvae of cotton leafworm, *Spodoptera littoralis.*

Tested compounds	LC_{50} Its limits at 95%	LC_{90} Its limits at 95%	Slope	Toxicity index %
1	191.10 149.72 238.10	546.03 407.46 802.16	2.991 ±0.491	17.80
2	54.46 19.09 96.14	145.17 123.50 352.43	2.482 ±0.761	62.47
3	37.09 16.20 85.24	559.03 337.33 3322.05	1.201 ±0.342	91.72
4	34.02 9.12 62.31	254.19 182.95 559.21	1.812 ±0.540	100.00
5	35.29 20.83 83.05	625.17 298.91 1990.73	1.141 ±0.262	96.40

Table 2: Antifeedant Activity of Chlorobenzothiazole Dihydropyridine derivatives and Nitrobenzothiazole-Dihydropyrimidine against 4th instar larvae of cotton leafworm, *Spodoptera littoralis.*

Compounds	Fiducial Limits	LC_{50} at 24 hrs.	Slope
1	0.84 - 2.14	1.62	1.06 ± 0.15
2	0.65 - 1.56	0.83	1.47 ± 0.16
3	0.39 - 0.81	0.48	1.03 ± 0.14
4	0.25 - 0.49	0.31	1.17 ± 0.14
5	0.26 - 0.55	0.35	1.01 ± 0.13

4. Biochemical study and determination of enzymes activities

4.1 Effect on acetylcholinesterase, glutathione s-transferase (GST) and α-esterases (α-EST) activity

According to Table (3)'s data, every tested compound raised the level of acetylcholinesterase in homogenates of larvae in their fourth instar. It was observed that compound (1) caused an insignificant difference, whereas compound (4) demonstrated a significant difference when compared to the control. The acetylcholine esterase enzyme's activity effects on the target site and be sensitive to chlorobenzothiazole-dihydropyridine as well as nitrobenzothiazole - dihydropyrimidine derivatives. Otherwise, Compounds (4 and 5) demonstrated an insignificant decrease in glutathione s-transferase (GST) levels, while compound (1) significantly decreased GST levels (-51.2%) when compared to the control. The acquired data supported the findings of Abou-Taleb et al. (2015) [4] regarding the reduction in GST activity in *S. littoralis* caused by lufenuron.

Table (3) presents the effects of all tested compounds on α-esterases (α-EST). The data showed that compounds (4 and 3) significantly increased the values of 79.9 and 62.2%, respectively, when compared to the control. Through fast binding, the insect esterases can lead to resistance to some active ingredients (Karunaratne et al., 1995) [19]. El-Sheikh (2012) [13] demonstrated that resistance to some insecticides may be caused by resistance mechanisms such as increased detoxification. The increased detoxification caused by elevated activity of glutathione S-transferases, oxides, and/or esterases is thought to be the cause of cotton leafworm resistance. Since glutathione contains cysteine, glycerin, and glutamic acid, it catalyzes the separation (R) of the foreign compound RX by complexing in multiple reactions and transferring it to the thiol group of glutathione. The substance HX is created when the hydrogen atom of the cysteine thiol group is moved to the residue X. As is well known, glutathione is a tripeptide that forms complexes with other compounds through conjugation. According to Walker (1975), while glutamic acid is hydrolytically eliminated, the

foreign compounds' cysteine derivatives are left behind. However, in insects, glutathione conjugates are either excreted unaltered or agree to undergo cysteine substitution in place of acetyl cysteine derivatives; for this reason, GST can conjugate with compounds via the (-SH) group. The compounds that have an OH group conjugated to them inhibit the enzyme [26].

4.2 Effect of tested compounds on total protein, aspartate amino transferase (AST) and alanine amino transferases (ALT) activities

The larvae's total protein content is displayed in Table (4). The results showed that, in comparison to the control, the LC_{50} of compounds (4 and 5) resulted in a highly significant decrease in total protein -38.2 and -37.5%, respectively. Compounds 1, 2, and 3's LC_{50} resulted in notable drops of 12.6, -15.8, and 2.5.4%. According to Awadalla et al. (2017) [5], treating the fourth larval instar of *S. littoralis* with the insecticide teflubenzuron, which is part of the IGRs group, resulted in a decrease in the total protein content. These findings also corroborate those of Megahed et al. (2013) [27], who discovered that spinosad significantly reduced total protein and caused a reduction in protein content, possibly as a result of inhibiting the synthesis of DNA and RNA. The aspartate amino transferases (AST) in homogenates of larvae in their fourth instar are displayed in Table (4). According to the data, compounds (5 and 4) had LC_{50} values that considerably raised the enzyme activity (60.8% and 59.3%, respectively). For alanine amino transferases (ALT), the data indicate that the LC_{50} of compounds (5, 4, 3, and 2) resulted in a high significant increase (172.73, 158.35, 124.05, and 107.1%) compared with control. According to Li and Liu (2007) [20], the detoxification of these enzymes in insects which is thought of as a defense against foreign substances that preserves their regular physiological functions may be the cause of the increase in activity of these enzymes against insecticides. The AST enzyme activity was greatly increased by all tested compounds, which may have been caused by the enzyme detoxifying in response to these compounds. The two subunits of AST are identical, and the large domain of AST binds the PLP cofactor to Lys's E-amino group through an aldmine linkage. In these compounds, the carboxylic group can react with pyridine, and in their structures, the (-OH) group can conjugate with oxygen. These findings concur with those of Megahed et al. (2013) [27], who discovered that administering spinosad to *S. littoralis* larvae in their fourth instar increased the activity of (AST), which in turn caused changes in GOT activities. Lufenuron treatment for 48 and 72 hours resulted in an increase in (AST) activity in *S. littoralis* larvae [4].

4.3 Effect of tested compounds on chitinase and phenoloxidase activities

The activity of chitinase in homogenates of fourth-instar larvae is displayed in Table (5), compounds (5 and 4) significantly increased its activity (21.2 and 15.5%), respectively, at the LC_{50}. Compounds 1, 2, and 3 each experienced a non significant increase its activity (2.6, 4.9, and 8.01%), respectively .

The results obtained did not align with the findings of Gelbic et al. (2011) [16], who demonstrated that the insecticides lufenuron and tebufenozide inhibited chitin synthesis at *S. littoralis*. Lufenuron is more active than tebufenozide, according to the data. The results obtained align with the findings of Mery M.S. Shenouda et al. (2019) [26] who proved that the activity of chitinase in homogenates of the 2nd instar larvae since LC_{25} of spinosad and lufenuron led to significant increase in its activity. Data in Table (5) show the activity of phenoloxidase. LC_{50} of compounds (5 and 4) caused significant decrease (-29.87 and -22.74%), respectively in comparision with control. The LC_{50} of compounds (1) caused nonsignificant decrease in the activity of phenoloxidase (-5.03%). This results agree with that obtained by Mery M.S. Shenouda et al. (2019) [26] who evaluated the activity of phenoloxidase of spinosad and lufenuron caused significant decrease (-7.02%) and (-33.27%), respectively. The five compounds decreased the activity of phenoloxidase enzyme. The (PO) enzyme is oxidative agent and can reduce oxygen in the five compounds producing (-OH) group. This factor leads to decrease the activity of phenoloxidase enzyme.

Table 3: Effect of the tested compounds on the activities of some enzymes acetylcholinesterase, Glutathione S-transferase and α-esterases in tested 4th instar larvae of cotton leafworm at LC_{50}.

Treatments	AChE (µmol / per min / mg total Protein)		GST (nm / min / mg total protein)		α-EST (ug α-naphthol/min/ml/mg total protein)	
	Mean	Change %	Mean	Change %	Mean	Change%
Control	0.009	---	0.379	---	113.42	---
1	0.0098	8.9	0.185	-51.2	119.92	5.7
2	0.011	22.2	0.241	-36.4	146.18	28.9
3	0.013	44.4	0.286	-24.5	184.02	62.2
4	0.017	88.9	0.355	-6.3	204.13	79.9
5	0.016	77.8	0.312	-17.7	171.19	50.9

Table 4: Effect of the tested compounds on the activities of total protein, aspartate amino transferase (AST) and alanine amino transferases (ALT) in tested 4th instar larvae of cotton leafworm at LC_{50}.

Treatments	Total protein (mg/ml)		AST (units / ml)		ALT (u x 10^3 / ml)	
	Mean	Change %	Mean	Change%	Mean	Change %
Control	8.013	---	5.409	---	1210	---
1	7.031	-12.6	5.933	9.6	1862	53.9
2	6.744	-15.8	6.113	13.01	2506	107.1
3	5.980	-25.4	7.001	29.4	2711	124.05
4	4.950	-38.2	8.615	59.3	3126	158.35
5	5.010	-37.5	8.700	60.8	3300	172.73

Table 5: Effect of the tested compounds on the activities of Chitinase and Phenoloxidase activity in tested 4th instar larvae of cotton leafworm at LC_{50}.

Treatments	Chitinase (ng /min/mg total protein)		Phenoloxidase (O.D. units/min/mg total protein)	
	Change %	Mean	Change %	Mean
Control	899	---	15.70	---
1	922	2.6	14.91	-5.03
2	943	4.9	14.24	-9.30
3	971	8.01	13.98	-10.96
4	1038	15.5	12.13	-22.74
5	1090	21.2	11.01	-29.87

Conclusions

Traditional insecticides were indispensable in the fight against pests, but their continued use pollutes the environment and endangers public health in addition to hastening the rise in pest resistance [12, 6, 21]. In pesticide research, the discovery of new active ingrediants have always been a hot topic [42]. The insecticidal activity of benzothiazole was not take much attention as it will be in the future. The compounds' antifeedant activity may be enhanced by the combination of pyridine and benzothiazole, according to the Maximum Likelihood Programmer (MLP) computation. Compound, diethyl1-(6-chlorobenzo [d]thiazol-2-yl)-2, 6-diethoxy-4-(4-hydroxy phenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (4) exhibited a significantly higher insecticidal activity against 4th instar larvae. It is clear that the toxicity increased in case of electron withdrawing groups of aromatic amines compared with electron donating groups. Compounds 3 showed high toxicity values near compound 4 followed by compound 2. Compound (1) showed less toxicity. While in case of 1-(6 nitrobenzo [d] thiazol-2-yl)-3-phenyl-2 thioxodihydro pyrimidine-4, 6 (1H, 5H)-dione (5) showed close toxicity value to that of compound (4). This may be due to the presence of pyrimidine and dione groups. The compounds exhibit varying degrees of antifeedant activity against the insect larvae. Compounds 2 and 3 displayed moderate activity, while compounds 4 and 5 displayed higher activity. These substances may interact with the active site of the enzyme that causes nervous breakdown in insects, potentially causing a spasm condition in insects. According to biochemical studies, all the tested compounds raised the level of acetylcholinesterase of larvae in their fourth instar. Compound (4) demonstrated a significant difference when compared to the control. The acetylcholine esterase enzyme's activity causes the target site to be sensitive to derivatives of chlorobenzothiazole-dihydropyridine as well as nitrobenzothiazole -dihydropyrimidine. Compounds (4 and 5) demonstrated an insignificant decrease in GST levels, while compound (1) significantly decreased GST levels when compared to the control. Also the results showed that, compounds (4 and 5) resulted in a highly significant decrease in total protein, possibly as a result of inhibiting the synthesis of DNA and RNA. In the AST, compounds (5 and 4) had raised to enzyme activity. For ALT, the data indicate that compounds (5, 4, 3, and 2) resulted in a high significant increase compared with control. The AST enzyme activity was greatly increased caused by the enzyme detoxifying in response to these compounds. The activity of chitinase in homogenates of fourth-instar larvae is displayed, compounds (5 and 4) significantly increased its activity. But, compounds (5 and 4) caused significant decrease on activity of phenoloxidase. The five compounds decreased the activity of phenoloxidase enzyme. The phenoloxidase enzyme is oxidative agent and can reduce oxygen in the five compounds producing (-OH) group.

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