



Novel Synthetic 2-Cyano-N-(3-(N-(Thiazol-2-yl) Sulfamoyl) Phenyl) Acetamide Derivatives against Spinning Silk Threads in *Spodoptera frugiperda*

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Novel Synthetic 2-Cyano-N-(3-(N-(Thiazol-2-yl) Sulfamoyl) Phenyl) Acetamide Derivatives against Spinning Silk Threads in *Spodoptera frugiperda*

Abstract:

There is a need to control *Spodoptera frugiperda* with new pesticides that have unusual modes of action. *Spodoptera frugiperda* initial larval instars can spin silk threads which would be the target of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide. The toxicity of synthetic derivatives (Ar1, Ar2, and Ar3) was tested against 1st and 2nd larval instars. Besides, the silk spinning ratio was examined after treatments with LC50s of certain derivatives, and different shapes of produced silk were detected in comparison with the control by SEM. In addition, malformation percentages of initial instars and latent effects were recorded. Biochemical experiments were done upon treatments with LC50s of derivatives. Results showed that Ar1 was the most effective derivative against *S. frugiperda* 1st and 2nd larval instars with LC50 values of 781.65 and 904.39 μLL^{-1} , respectively. Spinning ratios were 6.67, 13.33, 26.67 and 93.33 % for Ar1, Ar2, Ar3, and control, respectively, and differences in silk strands were detected morphologically upon potent interaction with the induced treatments. Results were explained upon more biochemical analysis which showed that Ar1 recorded the highest values of total protein and total lipids, while it was the lowest in the case of enzymes such as protease, amylase, and Na+K+ATPase. It could be concluded that synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives would be used as promising insecticides and anti-spinning silk threads compounds effectively against

Spodoptera frugiperda larvae with registered high mortality percentages.

KEYWORDS: Acetamide; Biochemical Analysis; Malformation; Silk Spinning; *Spodoptera frugiperda*

المستخلص:

هناك حاجة للسيطرة على *Spodoptera frugiperda* بمبيدات حشرية جديدة لها طرق عمل غير عادية، حيث يمكن للأطوار اليرقية الأولى لـ *Spodoptera frugiperda* أن تغزل خيوط الحرير التي ستكون هدفاً لأسيتاميد (3-Cyano-N-(2-Thiazolyl) Sulfamoyl) Phenyl الاصطناعي. تم اختبار سمية المشتقات الاصطناعية (Ar1, Ar2, Ar3) ضد أعمار اليرقات الأولى والثانية. علاوة على ذلك، تم فحص نسبة غزل الحرير بعد المعالجة بـ LC50s لهذه المشتقات، وتم الكشف عن أشكال الخيوط المختلفة للحرير الناتجة مقارنة بالكنترول بواسطة SEM. بالإضافة إلى ذلك، تم تسجيل نسب التشوه في الأعمار الأولية والتأثيرات الكامنة، وكذلك إجراء التجارب البيوكيميائية للمعاملات باستخدام LC50s من المشتقات. أظهرت النتائج أن Ar1 كان المشتق الأكثر فعالية ضد العمر اليرقي الأول والثاني لـ *S. frugiperda* بـ قيم LC50 تبلغ 781.65 و 904.39 ميكرو لتر⁻¹، على التوالي. وكانت نسب الغزل 6.67، 13.33، 26.67 و 93.33% لـ Ar1، Ar2، Ar3، والكنترول، على التوالي. وأيضاً تم الكشف عن الاختلافات المورفولوجية في خيوط الحرير عند التفاعل القوي مع المشتقات المختلفة. تم تفسير النتائج بعد المزيد من التحاليل البيوكيميائية التي أظهرت أن Ar1 سجل أعلى القيم للبروتين الكلي والدهون الكلية، بينما كان الأدنى في حالة الإنزيمات مثل البروتياز، الأميليز، Na+K+ATPase. يمكن الاستنتاج أن مشتقات أسيتاميد (3-Cyano-N-(2-Thiazolyl) Sulfamoyl) Phenyl الاصطناعية يمكن استخدامها كمبيدات حشرية واعدة ومركبات مضادة لغزل خيوط الحرير ضد يرقات *Spodoptera frugiperda*.

INTRODUCTION

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is a widespread polyphagous invasive pest that damages maize (*Zea mays* L.), a summer and winter crop farmed globally (Campos et al. 2014). It is primarily damaging to sweet corn and vegetable crops, but it also affects other non-host crops (Lewter et al., 2006), hence increasing its host range (Shylesha et al., 2018). FAW larvae primarily feed on plant whorls, reducing plant vitality and resulting in inadequate establishment, growth, development, and crop loss (Plem et al., 2016). Unlike other lepidopteran pests, FAW does not hibernate as a winter adaption, but as an advantage for survival dictated by weather patterns and host plant availability. Every year, they relocate from one location to another. (Sparks 1979).

Further, silk is known to be produced by many arthropods belonging to different phylogenetic groups, from spiders or pseudoscorpions to insects, including Diptera, Lepidoptera, Hymenoptera, and Trichoptera (Ruf et al., 2001). There are many types of silk with very diverse properties and chemical composition. Silk has many roles, from netting, protection, and thermoregulation to interpersonal communication. Silk threads laid by an individual can also be used as tracks for other species to follow, as shown by caterpillars (Fitzgerald 1983). These monitoring and silk-setting behaviors can trigger and expand the recruitment process by creating positive feedback mechanisms.

There were main causes of silk gland regression which either destructed it as alcohol or prevented silk spinning as cold shock. The development of silk glands is influenced by hormonal treatments applied to larvae. Juvenile hormone (JH) inhibits silk gland action, prevents degeneration, and can indirectly enhance silk output. Ecdysteroids at low concentrations promote silk gland growth and function, but excessive levels induce regression and degeneration (Sehnal and Akai, 1990). Further, JH was able to generate malformed pupae and adults and be as chemosterilant after treatment with juvenile hormone analogues as sulphonamide against *phthorimaea opercula* (Awasthi and Mahajan, 2008). Subsequently, when the silk

gland becomes afflicted, the synthesis of silk fibroin energy in the silk gland is disrupted, compromising the silk's quality. When the strength and length of the silk are reduced, the insect's hanging silk migration may be curtailed. Silk formation is linked to the growth of silk glands and the synthesis of silk protein (Zhao et al., 2022).

In particular, sulfonamide derivatives are sulfur-containing pesticide chemical families that have recently been in the spotlight for their potential to modulate the properties of novel crop protection compounds (i.e., fungicide, herbicide, insecticide, etc.), as modern agricultural chemistry seeks to support farmers with innovative agrochemicals. Sulphonamide compounds exhibit a wide range of biological activities, including antibacterial activity, carbonic (anhydrase) inhibitory activity, and insulin release induction. Besides, sulfonamide is one of the most versatile moieties for its pharmacological activities and actions as antiviral, antifungal, anticancer, and anti-inflammatory (Apaydin and Török, 2019). It is well known that sulfonamide reduces the biosynthesis (or inhibitory action) of dihydrophosphonamide (a sulfonamide moiety), which prevents the growth and reproduction (or inhibitory activity) of the dihydropterates synthase (DPS) enzyme. Further, sulphonamide moiety is essential for presented derivatives.

So the main target of this paper is the evaluation of the innovated synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives against *Spodoptera frugiperda* and its spinning behavior under laboratory conditions.

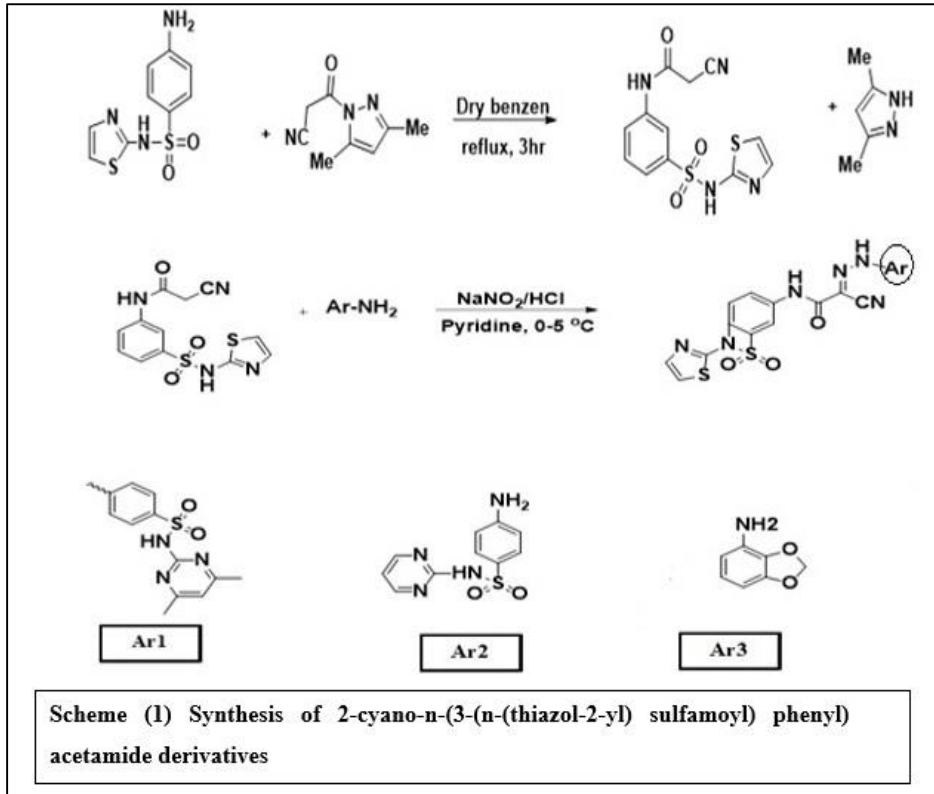
MATERIALS AND METHODS

-Experimental of derivatives

Coupling reaction of compound 1 with aromatic amine diazonium salts Compounds (General procedures)

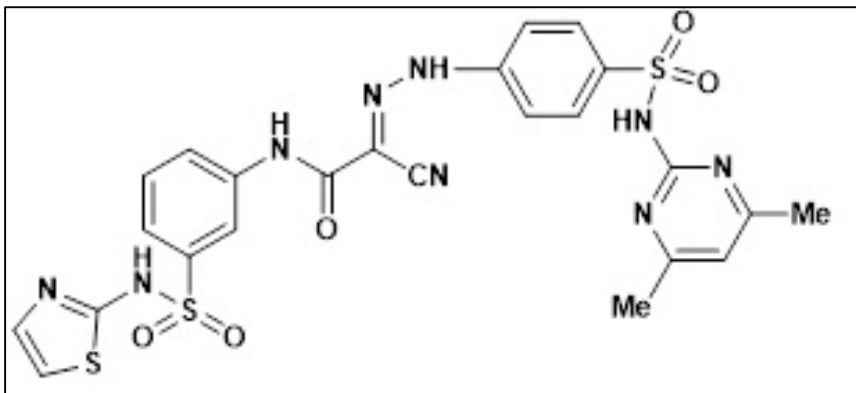
A cold solution of the appropriate diazonium chloride (0.002mol; was prepared by adding cold sodium nitrate solution (0.5 g, 0.002 mol) to a cold suspension of appropriate aromatic amine (0.002mol). in conc. HCl (1.5ml) with stirring was added, with continuous stirring, to a cold solution (0.4g, 0.002mol) at 0-5°C in pyridine (20ml). The mixture was

left to stand for two hours. Then diluted with water, filtration, and recrystallized from ethanol. The products Ar1, Ar2, and Ar3 were obtained as shown in scheme (1).



-(Ar) Substitutions

Ar1: (E)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl) sulfamoyl)phenyl)-2-oxo-2-((3-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)acetohydrizonoyl cyanide



Red Crystals; mp230-232C⁰; yeild 90%.

IR(KBr) ν /cm⁻¹:3440,3331,3128(3NH),1661(CO).

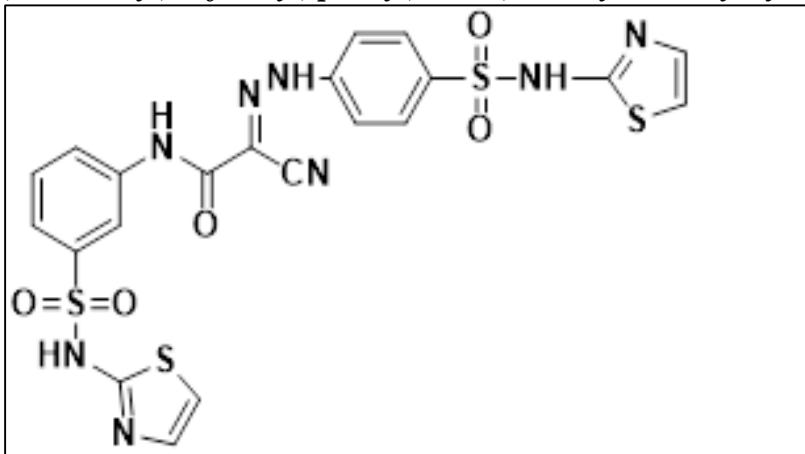
¹HNMR(400MHZ,DMSO-d6): δ_{H} ppm 2.25(t,3H, CH3),2,25(t,3H,CH3),9.88(s,1H,NHCO),7,75-8.00(m ,8H,Ar-rings),8.93(s,1H,=N-NH),11.36(s,1H,NHSO₂),12.64(s,1H,NHSO₂)

Analysis for C₂₄H₂₁N₉O₅S₃

Calcd:C,47.13;H,3.46;N,20.61;O,13.08;S,15.72%.

Found C,47.03;H,3.26;N,20.01;O,13.02;S,15.02%.

Ar2:(E)-2-oxo-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-2-((3-(N-(thiazol-2-yl)sulfamoyl) phenyl)amino)acetonitrile



Pale yellow crystals; mp=268-270 C⁰; yield 87%.

IR(KBr) ν/cm^{-1} : 3449, 3440, 3433, 3406(4NH), 2217(CN), 1661(CO).

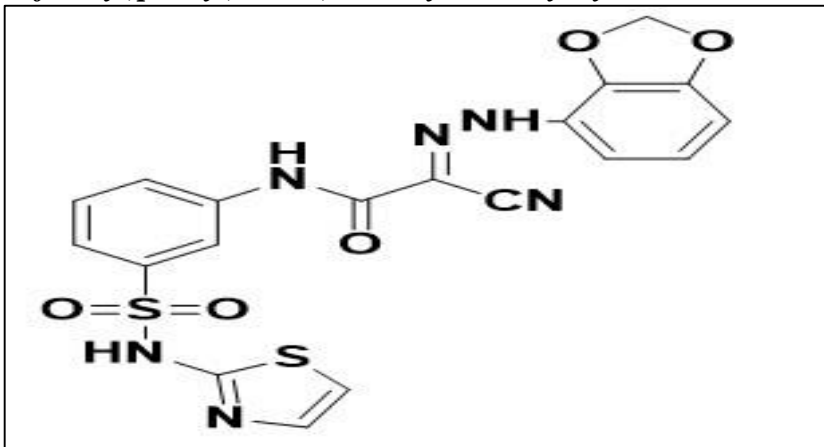
¹H NMR (400 MHz, DMSO-d₆): δ_{H} ppm 6.81(d, 1H, of thiazole ring), 7.21(d, 1H, of thiazole) and other thiazole ring in st. have same spectra. 7.67-8.00(m, 8H, Ar), 9.88 (s, 1H, NHCO), 11.61 (s, 1H, =N-NH), 12.30(s, 1H, NHSO₂), 12.64(s, 1H, NHSO₂).

Analysis for C₂₁H₁₆N₈O₅S₄

Calcd: C, 42.85; H, 2.74; N, 19.04; O, 13.59; S, 21.79%.

Found: C, 42.45; H, 2.34; N, 19.00; O, 13.39; S, 21.09%.

Ar3: (E)-N-(benzo[d][1,3]dioxol-4-yl)-2-oxo-2-((3-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino) acetohydrizonoyl cyanide



White crystals; mp 220-222 C⁰ ; yield 92%.

IR(KBr) ν/cm^{-1} : 3400, 3100, 3000(3H), 1600(CO), 2258(CN).

Analysis for C₁₉H₁₄N₆O₅S₅

Calcd: C, 48.51; H, 3.09; N, 17.86; O, 17.09; S, 13.63%.

Found: C, 48.01; H, 3.00; N, 17.46; O, 17.00; S, 13.33%.

-Docking procedure

Molecular modeling calculations and docking were done using “Molecular Operating Environment (MOE)”. Ligands were drawn on ChemDraw and imported in MOE.

-Spodoptera frugiperda

- *Spodoptera frugiperda* Rearing

S. frugiperda newly emerged larvae were incubated at regulated circumstances (27°C, 70% RH, and 12 hrs of incidence light). They were placed in transparent plastic pots (25 cm), covered with filter paper, and fed on castor oil leaves according to EL-Defrawi *et al.* (1964) with slight modifications to be suitable to *S. frugiperda*, until the insects reached the pupal stage. The pupae were then put in plastic pots (16× 10 cm) coated with wet filter paper and incubated for around 12 days. When the adults emerged, 30 couples were divided in bond paper-covered PVC cages (20 ×25 cm). Each cage got a plastic plate (25× 25 cm) coated in filter paper to act as an ovipositional substrate, and the cages were sealed with PVC plastic film. The couples were given a 10% (v/v) honey solution in the form of a hydrophilic cotton swab in 10 mL plastic tubes. Until hatching, the eggs were collected daily and moved to transparent plastic pots (16 ×10 cm) lined with filter paper, and produced caterpillars were fed in the method indicated before.

-Bioassay of synthetic derivatives against *Spodoptera frugiperda*

The leaf-dip technique was used to examine the efficacy of three synthetic chemicals against *S. frugiperda* larvae in both the first and second larval instars. To assess mortality percentages after 24 hours of exposure to various concentrations of fundamental components, in comparison to the control, which was dipping leaves in water only. Each treatment contained three identical replicates with 30 larvae. Likewise, the toxicity index was determined by using the sun's equation (1950), and relative potency values were measured according to Zidan and Abdel-Megeed (1988).

-Estimation of spinning behavior

The effect of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives against spinning behavior in *Spodoptera frugiperda* was determined. 1st and 2nd larval instars were exposed to LC50s of the three compounds and compared with control for 72 hours after treatments. Each treatment was triple-replicated and every replicate had 30 exposed larvae. The spinning rate was calculated with the following formula:

$$S (\%) = N/M * 100$$

S: Spinning rate,

N: Number of *S.frugiperda* larvae spinning,

M: Total number of living *S.frugiperda* larvae.

Silk strands were isolated from each treatment and photographed by SEM, in the National Research Center in Egypt. The estimation of the diameter of strands and morphological differences were detected.

-Biochemical experiments

-Biochemical test of certain contents

1st and 2nd larval instars were exposed to LC50s of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives. Live 1st and 2nd larval instars were collected after three days of treatments, weighted, frozen, and prepared for each test. Total protein, total lipids, protease, and amylase were examined and compared with the control.

-Activity assay of Na⁺,K⁺-ATPase:

Spodoptera frugiperda was used to analyze Na⁺,K⁺-ATPase. Larvae in their second instar were homogenized in a buffer with 40 mM Tris-HCl, 0.32 M sucrose, and 1 mM EDTA (pH 7.4). Cheesecloth was used to filter the homogenate through two layers. The differential centrifugation of the homogenate at 8000 Xg for ten minutes was used to prepare mitochondrial ATPase by Koch *et al.* (1969). After that, the supernatant was centrifuged for 30 minutes at 20000 Xg. The pellets that were made were then suspended in the same buffer and kept at -20 °C until they could be used. The method described by Koch *et al.* (1969) was used to measure ATPase activity, with a slight modification made by Morshedy (1980). The method that was described by Taussky and Shorr (1953) for determining inorganic phosphate (Pi) was used. After adding 1 mM Ouabain, the Mg²⁺-ATPase activity was measured, and the Na⁺, K⁺-ATPase activity was calculated as the difference between the total ATPase and Mg²⁺-ATPase activities. The protein content of prepared *S. frugiperda* homogenates was measured spectrophotometrically according to Lowery *et al.* (1951), using Bovine Serum Albumin (BSA) as the standard protein at less than 750 nm.

-Statistical Analysis

The analysis of variances between the examined compounds against *Spodoptera frugiperda*, in comparison with control, was conducted using SPSS (V.16). The statistical tests were applied to determine the difference significance level at probabilities of 5% and 1%.

RESULTS

-Toxicity estimation of tested compounds

The main target of this paper is to show the effect of synthetic derivatives as anti-spinning webs of *S.frugiperda*. So revealed toxicity assessment of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives in Table (1), showed that Ar1 was the most toxic with the least LC50s against 1st and 2nd larval instars. Then followed by LC50s of Ar2 and Ar3. Both toxicity index and relative potency were upon Ar1 followed by Ar2 and Ar3 by direct spray method bioassay.

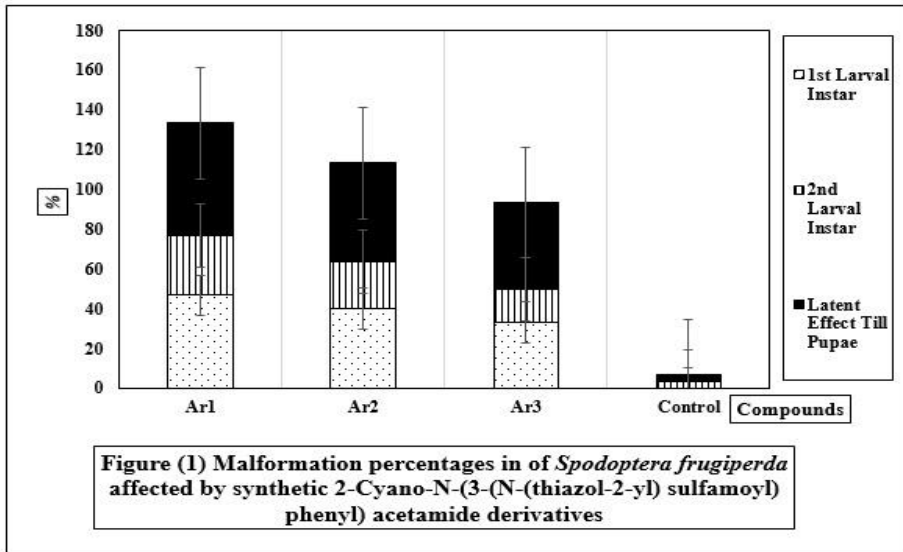
Table (1): Toxicity of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives against first and second larval instars of *Spodoptera frugiperda* under laboratory conditions

Larval Instars	Tested Compound	LC50 μLL^{-1}	CL 95%		Slope	Toxicity Index %	Relative Potency
			Lower	Upper			
1st	Ar1	781.65	534.74	994.42	2.01±0.89	100	1.27
	Ar2	844.37	612.39	1312.22	1.95±0.37	92.57	1.17
	Ar3	990.39	746.67	1490.07	1.91±0.24	78.29	1
2nd	Ar1	904.39	720.19	1340.32	1.94±0.11	100	1.28
	Ar2	1112.76	883.53	1512.77	1.81±0.58	81.27	1.04
	Ar3	1159.33	904.75	1606.40	1.90±0.33	78.01	1

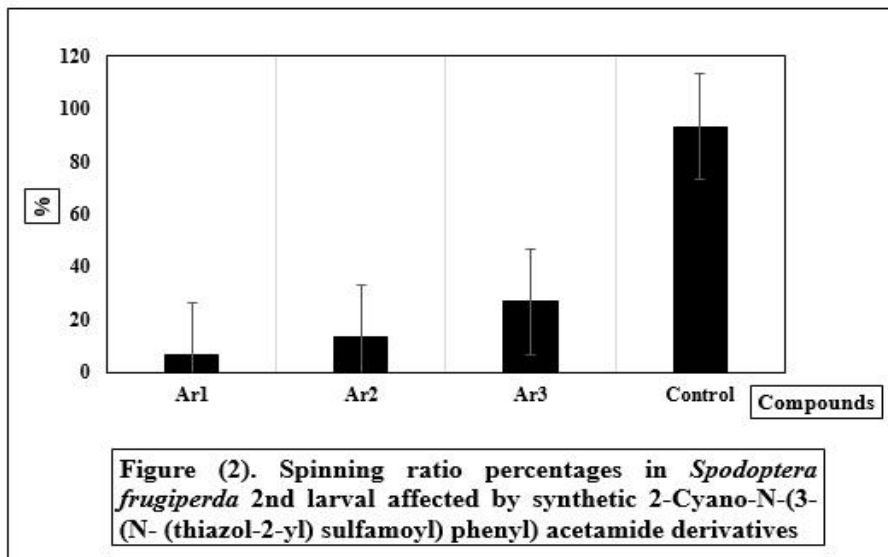
-Effect on silk spinning

The response to LC50s was shown in Figure (1) as malformed larvae after treatments of synthetic compounds against 1st, and 2nd larval instars and latent effect till adult release. and others of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide. Ar1 caused the highest malformation percentage 46.67% against 1st larval instar and 30% against 2nd larval instar. Subsequently, the latent effect of treatments affected malformed pupae percentages was detected

highly in Ar1(56.67%), and Ar2 (50%) followed by Ar3(43.33%)in comparison with the control (3.33%). Significant differences were shown among treatments and they were all for Ar1 which was shown by Kendall's tau_b(.004**).

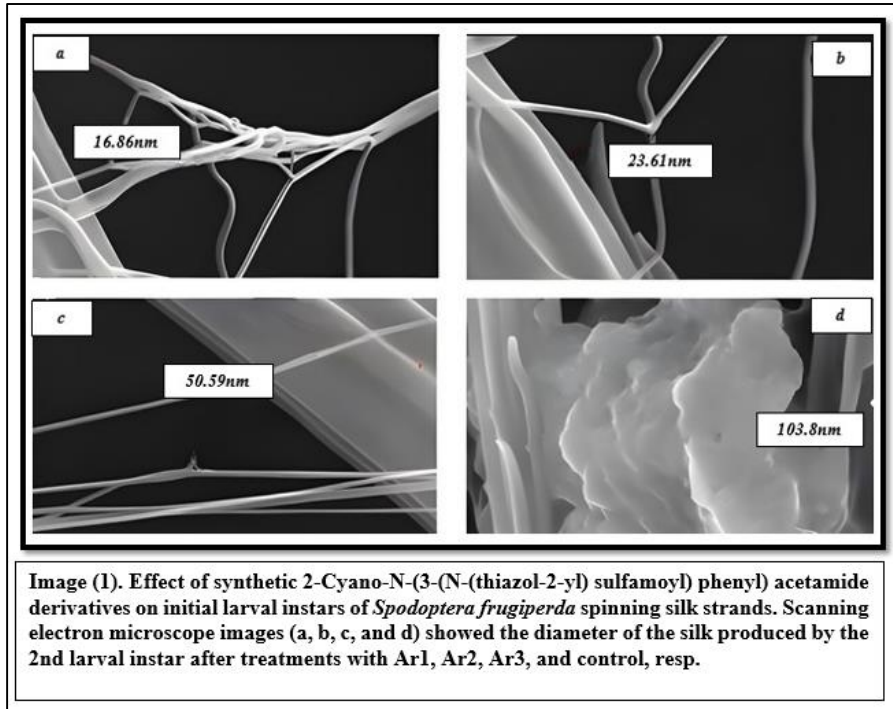


The spinning rate of 2nd larval instar *S. frugiperda* exposed to LC50s of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives was generally reduced than control, Figure 2. Reduction rates were 6.67,13.33, and 26.67 in the case of Ar1, Ar2and Ar3, resp., compared with control (93.33%). While all derivatives caused zero spinning rate in the case of 1st larval instar *S. frugiperda* exposed to LC50s. Kendall's Wa Coefficient of concordance=.661** and Chi-Square =7.223** were calculated to confirm significance for the differences among treatments, Ar1; Ar2; and Ar3 compared with the control.



SEM showed that the diameter of silk fibers produced by all larvae at the 2nd instar *S.frugiperda* treated with synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives was decreased than control as shown at Image 1. Ar1 caused the production of silk fibers with a diameter of 16.86nm (Image 1a), then Ar2 (Image 1b), and Ar3 (Image 1c), with 23.61 and 50.59nm, resp. Besides, all fibers produced in all treatments were silk fibers that are immensely thin and weak. While control of *S.frugiperda* produced silk fibers with bigger diameters (103.8 nm), and different shapes than treatments.

Nevertheless, all larvae at the 1st instar *S.frugiperda* treated with synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives were not produced silk.

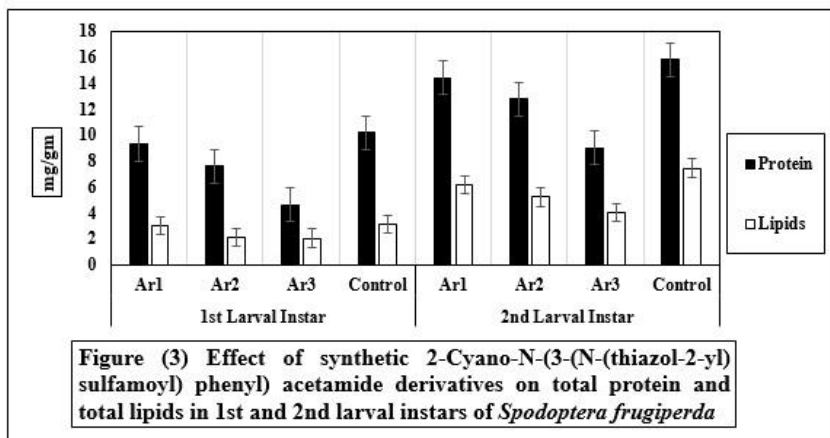


-Biochemical Studies

-Effect on protein and lipids

Apropos of the effect of synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives on protein and lipids in initial larval instars were detected in (fig.3). The closest compound to control was Ar1 followed by Ar2 and Ar3. Significant differences were estimated upon LSD5%. Besides, one-sample statistics showed that tested compounds and protein std. error means were 1.77944 and 1.10501, rep. Significant difference mainly among treatments was detected at 5% Sig. (2-tailed) with $t=2.5101^*$ and for protein $t=3.144^*$. It is recorded among pesticides, 0.50 and 0.41 in case of total protein determination for tested compounds against 1st and 2nd larval instars, resp. Also, it is recorded among pesticides, 0.17 and 0.11 in case of total lipids determination for tested compounds against 1st and 2nd larval instars, resp. Moreover, one-sample statistics showed that tested

compounds and lipids std. error means were 1.50123 and 1.02411, rep. Significant difference mainly among treatments was detected at 1% Sig. (2-tailed) with $t=2.0101^{**}$ and for lipids $t=2.144^{**}$.



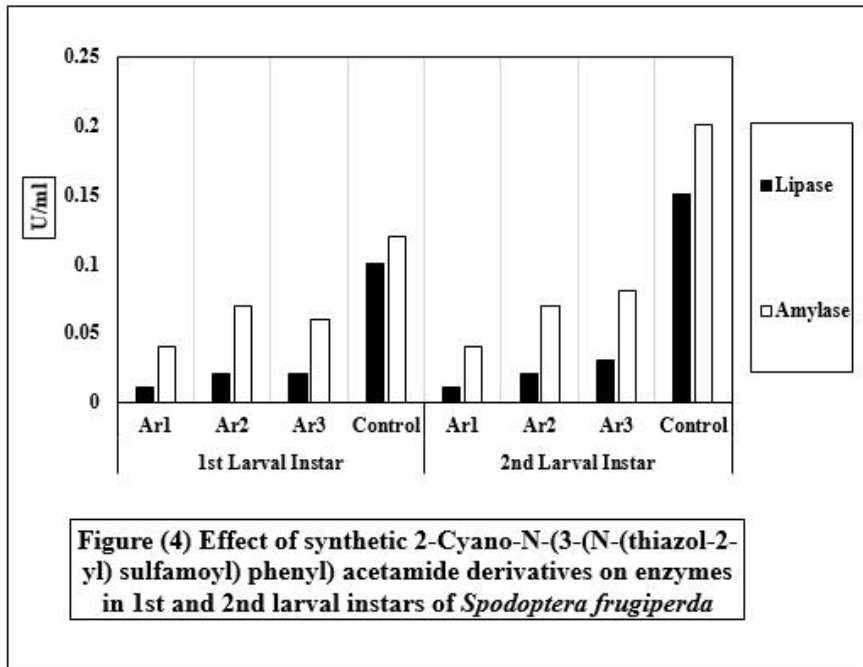
-Effect on lipase and amylase

Figure (4) refers to the changes in the rate of lipase in initial larval instars of *Spodoptera frugiperda* homogenates. The data generally revealed that all treatments caused significantly decreased differences in enzyme activity compared with the control. Ar1 recorded the lowest level of lipase in the first (0.01) and second (0.02) larval instars. While Ar2 caused a decrease to the control with 80 and 73.33%, and Ar3 reduced the enzyme activity with 60 and 66.67%, resp., for the 1st and 2nd larval instars. Significant differences upon LSD5% among pesticides were 0.003 and 0.005 in the case of lipase determination for tested compounds against 1st and 2nd larval instars, resp. significant responses differences were confirmed by the Jonckheere-Terpstra (Std. Deviation of J-T Statistic) = 2.301* and Kruskal Wallis (Chi-Square=1.22*) at 5%.

Accordant amylase activity, there was a reduction occurred in all treatments of *S. frugiperda* with synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl)sulfamoyl) phenyl) acetamide derivatives (fig. 4).

Ar1 recorded the lowest level of amylase (0.04) in the first and second larval instars. While Ar2 caused a decrease than control with 41.67 and 65%, and Ar3 reduced the enzyme activity with 50 and 60%, resp., for

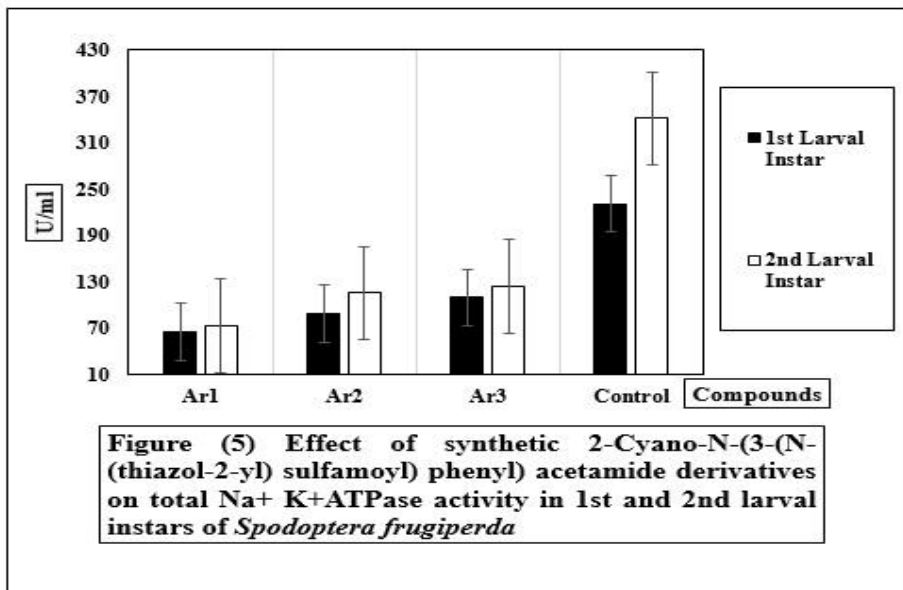
the 1st and 2nd larval instars. Significant differences upon LSD5% among pesticides were 0.007 and 0.004 in the case of lipase determination for tested compounds against 1st and 2nd larval instars, resp To confirm significant responses differences, the Jonckheere-Terpstra (Std. Deviation of J-T Statistic) = 2.15* and Kruskal Wallis (Chi-Square=1.59*).



-Effect on the total activity of Na⁺K⁺-ATPase

The total activity of Na⁺K⁺-ATPase in 1st and 2nd larval instars of *Spodoptera frugiperda* treated with 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives were determined and compared with the control as appeared in Figure (5). Data showed that the total activity of Na⁺ K⁺-ATPase was generally lower in treatments than in control. Compound Ar1 induced a remarkable reduction in the total activity of enzyme activity, of -71.85% and -78.63% for the 1st and 2nd larval instars, respectively, lower than the control, with a significant difference between each other. Therefore, Ar2 recorded a decrease

from the control with -61.81% and -66.18%, followed by Ar3 with -52.63% and -63.71%, resp., for the same mentioned arrangement. Significant differences upon LSD5% among pesticides were 0.13 and 0.42 in the case of ATPase determination for tested compounds against 1st and 2nd larval instars, resp.



DISCUSSION:

A wide array of factors can influence silk protein production. Damage to the silk gland may impair the synthesis of silk fibroin, sericin, and other compounds. Acetamiprid induces oxidative stress and inflammation, which are toxic to the silk gland epithelium and have an influence on silk cocoon formation (Lu et al.,2021). Exposure to organophosphorus pesticides (OP), particularly phoxim, can harm the silk gland and alter silk protein synthesis, significantly slowing cocooning in *Bombyx mori*. (Cheng et al.,2018). In this research, synthetic compounds affected the silk strands' shapes and biochemical composition of exposed

larvae which means that these compounds caused damage to silk glands in *S. frugiperda*.

Thus, pesticides are also able to affect silk production in *S. frugiperda*. Azadirachtin has excellent efficiency, low toxicity, zero residue, and no pesticide resistance (Kumar et al.,2010). Therefore, azadirachtin exerts antifeeding, contact killing, and stomach poisoning effects, slowing growth and development and disrupting ovarian development in a range of pests (Schmutterer,1990). Nevertheless, azadirachtin impacts the spinning of *S. frugiperda*, and after 24 hours, histological section examination revealed no evident pathological alterations in the silk gland of the larvae. After 48 and 72 hours, the silk glands were injured, protein aggregates in the silk glands were diminished, and glandular membranes were extensively perforated (Zhao et al., 2022). Moreover, Zhao et al. (2022) found that the surface of 3rd instar *S.frugiperda* the control silk was smooth and free of cracks by scanning electron microscopy, but after the insect silk is synthesized in the bilateral silk glands, it is processed and compressed by the silk press and spigot to a solid state. In this process, it is possible for the double-sided silk gathered in the spinner not to be well fused, resulting in depressions in the middle of the silk. After exposure to traces of 0.3 mg/kg azadirachtin for 48 h, there was obvious unevenness on the surface of the silk. The sericin in the anterior silk gland did not perfectly encapsulate the silk broin. Compared with the control group, the sol phenomenon was more prone to occur, and swelling and rupture occurred more easily. Analysis of the spinning of the 3rd instar *S. frugiperda* showed that there was no significant difference in spitting ability after 24 h of feeding on azadirachtin. The spinning rate

of *S. frugiperda* fed 0.3 mg/kg azadirachtin increased at 48 h, while no difference was observed at 3 mg/kg, however, the spinning rate was significantly reduced after 72 h at both doses (Zhao et al.,2022). As azadirachtin impacted the silk gland of *S. frugiperda*, the lumen of the silk gland in *S. frugiperda* exhibited vacuolation. KEGG analysis revealed the identification of 31 distinct metabolites, with 12 being upregulated and 19 being downregulated. These metabolites were enriched in 15 metabolic pathways, suggesting a close association between the silk gland of *S. frugiperda* and the formation of fatty acids and energy metabolism during the silk formation process. (Zhao et al.,2022). We found in the current research that a reduction in ATPase translated to a decrease in quantity and quality of produced silk from the treated 2nd larval instar, while it disappeared totally in the first instar.

Even though, spider mite webbing was mediating an anti-predator behavior (Lemos et al.,2010), so use of pesticides as ant-webbing pesticides could play an important role in controlling mites and insects and at the same time open the destination to predators and parasitoids.

At the same time, the juvenile hormone is released by the endocrine system and combines with carrier proteins known as Juvenile Hormone Binding Proteins (JHBPs), which have a strong affinity for JH molecules (Goodman and Granger,2005). When they bind to JH, JHBPs undergo a significant change in shape. Juvenile hormone analogs (JHAs) are thought to imitate the effects of JH such as Sulfonamides (Sharma and Awasthi,2021), and 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives. Because of their chemical structures, they were

able to cause malformation clearly till the pupal stage, and silk production in the initial larval stages also.

Therefore, the silk gland morphology of *S.frugiperda* showed that no silk gland was developed like *Bombyx mori* (Zhao et al., 2022), so *S. frugiperda* cannot spin silk like it. The posterior silk gland in *S.frugiperda* is miniature and thin, with no fall or twist. Subsequently, fibroin is the central silk protein of the fiber which is hydrophobic and attached to the silk gland in the back, secreted in the lumen of the silk gland in the back, then transferred to the middle silk gland and stored in the lumen until it spins. Sericin is wrapped around the silk fiber and produced in the central silk gland. Moreover, the small silk gland produced thin silk fibers which were thinner in the case of treatments with tested synthetic compounds than the control as gained through our results.

The direct relation between the production of silk strands and juvenile hormone (JH) that protects the metamorphosing juvenile (Sehna and Sutherland,2008), could be exploited to control spinning insects with specific compounds affected silk spinning and JH as shown in our paper results.

Concerning the relationship between the chemical structure and biological activity, all compounds have an occupation of the allosteric binding region, and ATP binding domain with different efficiency for all derivatives, which would be able to deal effectively with the target enzymes. Moreover, the diazene and/or sulfonamide linkers produce H-bonds with amino residues as H-bond acceptors and donors through N--N and NH, respectively. The hydrophobic pocket generated by non-polar amino acids was also occupied by the

terminal (un)substituted hydrophobic tails. Furthermore, the distal hydrophobic moieties were connected to sulfonamide linkers in order to lengthen the structure and allow these distal moieties to occupy additional hydrophobic grooves which contributed to control *S.frugiperda* through interaction with bioactive targets such as protein, lipids, enzymes and others. Within alongside, the aromaticity features required for increasing insecticidal efficacy. All of these characteristics aided in the development of active sites on molecules, which encourage chemical reactions with diverse chemicals and the development of new insecticides with increased activity.

Notably, carbonic anhydrases (CAs) are metalloenzymes with a zinc ion active site. CAs at physiological pH catalyzed CO₂ hydration to bicarbonate at physiological pH (Supuran et al.,2003). This chemical interconversion is critical because bicarbonate is the substrate for multiple carboxylation processes in a variety of important metabolic pathways, including gluconeogenesis, amino acid biosynthesis, lipogenesis, and pyrimidine synthesis. Aside from these biosynthetic events, several CAs are engaged in a variety of physiological processes linked to respiration and CO₂/bicarbonate transfer. β -CAs are target proteins of 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives which are considered as specific inhibitors for β -CAs and α -CAs. They affect processes such as fatty acid synthesis, respiration, neuronal signaling, nucleotide synthesis, gluconeogenesis, and others which explain the affected total protein and total lipid besides enzymes such as protease, lipase, and Na⁺, K⁺-ATPase. The mentioned reasons extended to explain such an effect on ATPase which

is inversely proportional to glucose, triglyceride, and protein concentrations. This tendency was especially pronounced for proteins. This is most likely due to a very potent inhibition of membrane pumps (e.g. Na⁺K⁺-ATPase) which prevents active transport and results in the deposition of some metabolites. Moreover, synthesized derivatives were able to cause microtubule disruption which caused the destruction of silk production glands that translated into malformed silk strands and matrix metalloproteinase but the extrusive mechanism is inhibition of β -CAs as explained.

CONCLUSION

Purposefully, synthetic 2-Cyano-N-(3-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide derivatives could be used effectively to control *S.frugiperda* even directly through high mortality percentages or indirectly by affecting its ability to silk spinning. So such derivatives are classified as pesticides and anti-spinning silk in initial larval instars of *S.frugiperda* by interacting with specific enzymes with unusual modes of action at the same time.

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