Biochemical Impacts of Rynaxypyr (Coragen) and Spinetoram (Radiant) on *Spodoptera littoralis* (Boisd.)

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Abstract: In laboratory study the impact of rynaxypyr (coragen) and spinetoram (radiant) on the activity of acetylcholine E and non-specific esterases (α and β esterase), carbohydrate hydrolyzing enzymes (amylase, trehalase and invertase), chitinase and lactate dehydrogenase (LDH) and on major biochemical components was studied when *Spodoptera littoralis*, 5th instar larvae of the laboratory strain was treated by LC_{50} of both insecticides. The effect of LC_{50} of both insecticides on major biochemical components of 5th larval instar after 24 hours showed that, the amount of total carbohydrates, total protein, total lipids, carbohydrate hydrolyzing enzymes (invertase, trehalase and amylase) were significantly decreased, where spinetoram resulted in slight increase in α–esterase and rynaxypyr resulted in moderate increase in activity AchE. In contrast both insecticides induce pronounced increase in activity of chitinase and lactate dehydrogenase (LDH).

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1. Introduction:

Calcium is a universal interacellular messenger and its release from interacellular stores is modulated by Ca^{2+}- channels such as ryanodine receptor (RyR). As a result the RYR mediates many cellular and physiological activities such as neurotransmitter release, hormone secretion, gene expression and muscle contraction.

The RyR has been known as a potential insecticide relevant compounds addressing this target been developed (Nauen, 2006). This resulted from the discovery of a new chemical class of insecicide based on insecticidal diamide scaffolds that bind to insect RyR at a site distinct from ryanodine and strongly interfere with the receptor's role in calcium homeostasis (Cordova *et al.,* 2006; Ebbinghaus, Kintscher *et al.,* 2006). However, Diamide insecticides were recently introduced to the market, and are represented by two commercial compounds : flubendiamide and chlorantraniliprole (Hirooka *et al.,* 2007) and are particularly useful against those species which have developed resistance to other chemical classes of insecticides (Nauen *et al.,* 2007).

Both newly developed diamide compounds, flubendiamide and chlorantraniliprole show specificity for insect RyR's and not affect isoforms of their mammalian counterparts, which show significant differences in their amino acid sequence, thus explaining the excellent toxicological profile of both compounds (Sattelle *et al.,* 2008).

The other tested compound i.e., Spinetoram was included to represent a new generation of spinosyn group. It causes excitation of the insect nervous system by altering the function of nicotine and GABA-gated ion channels. (Crouse and Sparks, 1998), with low environmental human risk (Thempson and Sparks, 2002).

Lepidopteran larvae treated with diamide insecticides show unique symptoms of poisoning including feeding cessation, complete contraction paralysis and ultimately death (Tohnishi *et al.,* 2005). The cotton leafworm *Spodoptera littoralis* (Boisd.) represents a major lepidopteran pest of cotton, field crops and vegetables in Egypt as well in many parts of the world and extremely destructive if once the infestation thresholds exceeded. However, based on its significance values on agricultural production, it felt necessary to study the response of its digestive enzymes to two of the newly developed insecticides.

Generally, insect digestive enzymes could be used as a parameter for determining feeding and growth deterrent activity of certain compounds, e.g. phenyltin on cotton leafworm, *Spodoptera littoralis* (Boisd) larvae (Ishaaya *et al.,* 1974).

Enzymes hydrolyzing carbohydrates such as trehalase, which is activated during moulting to generate production of glucose for chitin build-up (Candy and Kilby, 1962), and two important digestive enzymes, amylase and invertase were investigated in this study. In insect amylase and invertase are the enzymes most frequent in the salivary glands; trehalase is present in the haemolymph and fat body (Wigglesworth, 1972).

In addition the activity of non-specific estrases, α-esterases and β–esterases were also determined. However, the effect of rynaxypyr (coragen) on these enzymes and the possibilities of

relationship between enzyme inhibition and larval growth deterrence was discussed.

2. Material and Methods

2.1. Insects:

The cotton leafworm *Spodoptera littoralis* (Boisd.) larvae used in the present study were obtained from a colony initiated in cotton leafworm Department, Plant Protection Institute, Agric., Res. Center, Giza, from eggs masses reared for more than ten years on insecticide-free castor bean leaves under laboratory conditions of 27 ± 2 °C and 65 ± 5% R.H. according to El-Defrawi *et al.* (1964) rearing technique.

2. Chemicals:

Chlorantraniliprole (rynaxypyr) formulated as Coragen 20% Sc., Supplied by S.A.E. DuPont. Spinetoram formulated as Radiant 12% Sc., Supplied by Dow Agrosciences. The tested insecticides were diluted with water to make stock solutions and appropriate water - diluted concentrations were prepared freshly before treatments.

3. Treatment procedure:

Preliminary bioassay was carried out by dipping castor bean leaves in the appropriate concentration solutions for 5 Sec., the treated leaves were allowed to dry at room temperature, then the leaves were offered to the larvae in 3 replicates (10 fifth instar larvae/each for only 24 hrs and then the mortality percent was recorded and corrected by Abbott formula (Abbott, 1925) based on death in control. Probit analysis for mortality data at 24 h (Finney, 1971) was adopted to obtain the LC_{50} value.

4. Preparation of larval enzymes solution:

The samples of larvae used in enzyme assays were obtained from those subjected and fed on the sub lethal concentration (LC_{50}) of the experimental insecticides (Coragen 20% SC) and Spinetoram (Radiant 12% SC).

The larval enzyme solution was prepared according to the method described by Ishaaya *et al.* (1971). The enzyme solutions were obtained by homogenizing 10-15 fifth-instar larvae, representing ca. 2 g larval weight, in 20 ml distilled water, using a chilled glass Teflon grinder. The homogenate was centrifuged at 8000 r.p.m. for 15 min at 5°C, the deposits were discarded and the supernatants were kept in deep freezer till use.

5. Determination of enzymes activities:

a- Digestive enzymes

The determinations of invertase, amylase and trehalase activities were based on the digestion of sucrose, starch and trehalose, respectively, by spectrophotometric methods (Ishaaya and Swirski, 1970, 1976). Briefly, invertase, amylase and trehalase were assayed using 3, 5 dinitrosalicylic acid reagent for determining the free aldehyde groups of glucose formed after sucrose, starch or trehalose digestion, respectively. This reaction is based on the reduction of dinitrosalicylic acid by the aldehydic groups of glucose units in basic medium. The reduced dinitrosalicylic acid is measured spectrophorometrically at an absorbency of 550 nm.

b- Non-specific estrases

α-estrases and β-estrases, as non-specific estrases, were determined colorimetrically according to the method described by van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrate, respectively. Naphthol produced as a result of hydrolysis of substrate can be identified by the addition of diazoblue sodium lauryl sulphate solution, producing a strong blue color in the case of α-naphthol or a strong red color in the case of β-naphthol at which colors are measured spectrophorometrically at an absorbency of 600 and 555 nm for α-naphthol and of β-nophthol, respectively.

c- Acetylcholinesterase

Ach E is measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (Ach Br) as substrate.

d- Lactate dehydrogenase

LDH was determine according to the formulation recommended by the German Society for clinical chemistry (DGKC, 1972).

e- Total carbohydrates

Total carbohydrates were estimated in acid extract of treated samples by the phenol-sulphuric acid reaction of Dubois *et al.* (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

f- Total protein

Total proteins were determined by the method of Bradford (1976).

g- Total lipids

Total lipids were estimated by the method of Knight *et al.* (1972).

3. Results and Discussion

3.1. Total carbohydrate, protein and lipids

Total carbohydrates and total protein are major biochemical components necessary for an organism development, growth and performance of its vital activities, thus the mean values of homogenate contents of carbohydrate and protein were estimated in the 5th instars treated with LC_{50} of either rynaxypyr or and spinetoram after 24 hours.

a- Total protein

Data in Table (1) showed that the mean total protein reached 16.37 and 20.17 mg/g. b.wt for rynaxypyr and spinetoram versus 26.30 mg/g.b.wt in untreated control at 24 hrs after treatment with LC_{50}. Both treatments recorded significant reduction of -37.75 and -23.3% for rynaxypyr and spinetoram, respectively.

Elbarky *et al.* (2008) recorded significant decrease in protein content by -69.87% after treating *Spodoptera littoralis* larvae by LC_{50} of spinosad compared to the control group, they indicated that the reduction in protein content may be due to inhibition of DNA and RNA Synthesis. However, the decrease of the total protein in treated 5th larval instar may reflect the decrease in the enzymatic activities of various enzymes.

These results are in agreement with those demonstrated by Abd El-Aziz *et al.* (2007). Also, agree with results of El-Shershaby *et al.* (2008) indicating that treatment of *Spodoptera littoralis* larvae by the bacterial formulation Dipel-2x resulted in negative changes in the total protein content and that this may be due to bacterial toxins which led to inhibition of protein synthesis by forming a protein complex. Similarly the total protein level exhibited significant decrease in haemolymph of 3rd instar larvae of *Cephalopina titillator* treated with LC_{50} of either pyriproxyfen or chlorfluazuron (El-Bassiony *et al.,* 2005).

b- Total carbohydrates

The results obtained for total carbohydrates at 24 hrs after treatment with LC_{50} of rynaxypyr and spinetoram are shown in Table (1). It was obvious that mean total carbohydrate was significantly and highly reduced after treatment by LC_{50} of rynaxypyr by -73.93% while the reduction was -54.97% for spinetoram relative to control.

The same results were found by Bennet and Shotwell (1972) who reported a rapid reduction in the haemolymph carbohydrates was observed following injection of bacteria into some insect species, the Japanese beetle larvae *Popillia japonica.*

Also, similar trend was recorded in the Indian meal moth larvae, *Plodia interpunctella* (El-Kattan, 1995) and the lesser cotton leafworm larvae *S. exigua* (Younes *et al.*, 2002).

Likewise, Elbarky *et al.* (2008) recorded significant decrease in carbohydrates, after treatment by radiant (Spinetoram) at Lc_{50}.

c- Total lipids

Data in Table (1) revealed that the mean total lipids was 4.24 and 5.63 mg/g.b.wt after 24 hrs treatment with LC_{50} with rynaxypyr and spinetoram, respectively compared with 7.98 mg/g. b.wt for untreated larvae. However, both treatments recorded significant reduction reached -46.86% and -29.44% for rynaxypyr and spinetoram, respectively.

Abo Elghar *et al.* (1995) indicated that both the acetone and ethanol extracts from *Melia azedarach* highly reduced haemolymph lipids by -66.2% and -55.6% reduction, respectively below that of control. Also, Abdel-Aal (2006) found that chlorfluazuron caused significant decrease of total protein, lipids and carbohydrates in *Spodoptera littoralis* larva.

Non specific esterases

General esterases are a large and diverse group of hydrolases that hydrolyze numerous substances including esters and certain non-ester compounds. Numerous studies have demonstrated that esterases play an important role in conferring or contributing to insecticide detoxifications in insect and arthropod species (Motoyama and Dauterman, 1974; Mouches *et al.,* 1986; Saleh *et al.,* 1986).

Esterases are hydrolyzing enzymes,which split ester compounds with the addition of water to yield alcohol and acids (Shaurub *et al.,* 1999).

Results in Table (2) demonstrate the effect of both rynaxypyr and spinetoram on alpha-esterase and beta-esterase activity in total homogenate of *Spodoptera littoralis* 5th instar larvae laboratory strain. It was obvious that treatment with LC_{50} of both insecticides resulted in mean activity of α-estrases reacted 497.33 and 755.0 µg α-naphthol/min/g. b.wt for rynaxypyr and spinetoram, respectively compared with 728.33 µg α-naphthol/min/g.b.wt for control group. However, considering the change in activity it was obvious that rynaxypyr exhibited remarkably significant reduction of -31.71 relative to control.

As for the effect of the two insecticides on beta-esterase activity, the obtained data were 443.66 and 1011.0 versus 1138.33 µg β raphthol/ min/g. b.wt for rynaxypyr, spinetoram and untreated check, respectively. It was obvious that both treatments resulted in remarkable reduction in beta -esterase activity which was significantly high -61.81% in rynaxypyr treatment and low -11.18% in spinetoram treatment.

El-Kawas *et al.* (2009) found that diflubenzuron reduced activity of non specific esterases (α and β-esterases) in immature stages of *Tetranyehus urticae* relative to control, whereas chlorfluazuron caused slight decrease in the activity of α and β-esterase.

Salem *et al.* (1995) and Mead (2006) found reduction in the activity of α and β-esterases in the larvae of *S. littoralis* as affected by IGRs, buprofezin, diafenthiuron and triflumuron. However, Elevating the activity of α and β-esterases was observed also by Al-Elimi and Eid (1998) when tested two IGRs against susceptible strain of *S. littoralis*.

The general decrease in the activity of the studied enzymes (alpha-esterase, beta- esterase and AchE) in the present work may indicate that general esterases are not involved in the detoxification process of rynaxypyr and spinetoram. These findings were in agreement with Fahmy and Dahi (2009) and Wang *et al.* (2009) who found that esterases and GST might be

unimportant in conferring in the *S. exigua* field population.

In this respect, Zhang *et al.* (2003) reported that there was no obvious relationship between the sensitivity of the beet armyworm to spinosad and the activities of endogenous enzymes of protective system.

Acetylcholinesterase (Ach E)

Ach E has a key role in neurotransmitter by hydrolyzing the neurotransmitter achetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides.

Data in Table (2) demonstrate Ach E activity in total homogenate of *Spodoptera littoralis* 5th larvae untreated and those treated with LC_{50} of rynaxypyr and spinetoram. It was obvious that the Ach E activity recorded mean activity of 152.85 and 76.01 versus 121.56 µg AchBr/min/g.b.wt for rynaxypyr, spinetoram and control, respectively. It was of interest to note that rynaxypyr exhibited significant increase in Ach E activity reached + 25.74% while spinetoram recorded a remarkable reduction of -37.47%.

On the contrary Fahmy and Dahi (2009) recorded a significant increase of + 4.94% and + 18.65% in spinetoram-treated larvae of Kalyobia and in Behiara, respectively.

Abd El-Mageed and Elgohary (2006) indicated that the change of response to spinosad could be associated with the decrease in Ach E activity, Likewise the tested compounds caused a disturbance in the activities of other tested enzymes either with increase (Ali-E, β-E and amylase activities) or with decrease.

Carbobydrate hydrolyzing enzymes

a- Trehalase enzyme

In most insects, carbohydrates reserves are present as glycogen and trehalose which can be ready converted into glucose for the support of all life processes. Metamorphic changes in insect are usually accompanied by substantial depletion of their carbohydrate reserves. During this period, glycogen and treholase supply glucose which provides an energy source and a substrate for the synthesis of pupal and adult tissues, especially the cuticle.

Table (3) indicate that, the activity of trehalase enzyme in the supernatant of the homogenated larvae was generally decreased as affected by all treatments than control. The mean trehalase activities recorded after 24 hrs for rynaxypyr and spinetoram were 281.66 and 437.0 µg glucose/min/g.b.wt respectively compared with 637 µg glucose/min/g.b.wt for control. It was obvious that both insecticides at LC_{50} reduced trehalase activity remarkably than control. The reduction reached -55.78 and -31.39% than control for rynaxypyr and spinetoram, respectively.

b- Invertase enzyme

Regarding to invertase enzyme, there was remarkable decreases in the activity in the supernatant of homogenated larvae resulted from both treatments. The activity reached mean of 1133.66 and 1236.66 µg glucose/min/g.b.wt for rynaxypyr and spinetoram, respectively, compared with 1519.0 µg glucose/min/g.b.wt for control. Based on the change relative to control, it was obvious that rynaxypyr at LC_{50} resulted in moderate reduction of -25.36% whereas spinetoram exhibited relatively less reduction of -18.56% relative to control.

C- Amylase enzyme

The obtained results show that the activity of amylase in the supernatant of *Spodoptera littoralis* larval homogenate was affected in both treatments and was lower than that obtained with untreated larvae. The activity recorded 97.66 and 118.66 µg glucose/min/g.b.wt. for rynaxypyr and spinetoram, respectively versus 165.66 µg glucose/min/g.b.wt. for control. However, both treatments exhibited considerable reduction relative to control, reached -41.04 and -28.37% for rynaxypyr and spinetoram, respectively. In previous study.

Abo El-Ghar *et al.* (1995) found that feeding *Spodoptera littoralis* larvae 5 ppm abamectin caused remarkable decrease in invertase, amylase and trehalase activities. In addition the same treatment caused a reduction in the activities of both α- esterase and β-esterase.

Also, El-Naggar (1999) found that exposure of *Earias insulana* to gamma irradiation lowered the activity of the haemolymph digestive enzymes, amylase, invertase and trehalase. Recently when Reyad and Ibrahim (2008) studied the effect of infection by four nematode species on certain enzymes activities, the data indicated that infection with *Steinernema abbassi* and *S. glaseri* dereased significantly the activity of invertase and trehalase in adult of *Euborellia annulips.*

Generally, the trend of both treatments (rynaxypyr and spinetoram) caused significant decrease in the activities of trehalase, invertase and amylase as compared to control group. However, the general disturbance in carbohydrates metabolism as expressed by reduction of trehalase, invertase and amylase activities could be result from a chain effect originating primarily from inhibition of chitin synthesis (Salem *et al.,* 1995). The disturbance of trehalase activity might hamper the supply of glucose needed for chitin build up (Candy and Kilby, 1962). These results are in agreement partly with Eid (2002) who found that Consult (hexafluromuron) and runner (methoxyfenozide) decreased the invertase activity after 5 days of treatment, whereas consult, Attabron

and Cascade (IGRs) exhibited reduction in trehalase and invertase activities.

Chitinase enzyme activity

From the results in Table (4) it can be seen that all tested insecticides caused an increase in chitinase activity reached + 24.30 and + 11.73% more than in untreated control.

In agreement Abd El-Mageed and Shalaby (2011) found that chitinase activity was increased by range of + 17.14% to + 53.34% more than control after treatment of cotton leafworm larvae by mixtures of chlorpyrifos, cypermethrin, lufenuron, flufenoxuron, alpha-cypermethrin, thiamethoxam and lambda-cyhalothrin.

Similar results were reported by Lee *et al.* (1994) was found an increase in the chitinase activity of larvae of *Hyphantria cunea* treated with the (chitin synthesis inhibitors) diflubenzuron and chlorofluazuron. Moreover, the chitinase activity was markedly increased when 4th instar larvae of *Spodoptera littoralis* was treated with diflubenzuran (Farag, 2001). Also, Tolba (2006) found an increase in chitinase activity in pupae of *Agrotis ipsilon* treated with flufenoxuron. Likewise, Abdel-Aal (2006) found that treatment of *Spodoptera littoralis* larvae with either chlorfluazuron or two biocides caused significant increase in chitinase activity compared to control.

In addition, the increase in chitinase activity of *Spodoptera littoralis* pupae treated with lufenuron could be attributed to the secondary effect of chitin synthesis inhibitor, or may be a secondary effect for the reduced activity of β-ecdysone metabolizing enzymes, followed by β-ecdysone accumulation which result in hyperchitinase activity. (Yu and Terriere, 1977).

Lactate dehydrogenase (LDH) activity

LDH is an important glycolytic enzyme and has been used as an indicative criterion of exposure to chemical stress (Wu and Lam, 1977 and Diamantino *et al.*, 2001).

Data in Table (4) indicate that LDH activity increased slightly by + 3.3% after treatment by LC_{50} of rynaxypyr compared with + 11.46% more than control after treatment by Lc_{50} of spinetoram.

On contrary, Abd El-Aziz (2007) found that azadirachtin treatment significantly suppressed the amylase and lactate dehydrogenase (LDH) activity. Consistent results were also obtained by Senthil Nathan (2006) who reported that LDH activity of *Cnaphalocrocis midinalis* showed maximum reduction after treatment with 2% *Melia azedrach* extract.

Similarly, Nathan *et al.* (2005) showed that treatment of *Spodoptera littoralis* with azadirachtin highly decreased this enzyme in the mid gut.

Table (1): Total carbohydrate, protein and lipids contents of homogenate of *Spodoptera littoralis* 5th instar larvae lab-strain after 24 h (treatment with LC_{50} of rynaxypyr and spinetoram).

Treatments	Mean total content (mg/g.b.wt) (% change to control)		
	Carbohydrate	Protein	Lipids
Rynaxypyr	3.85 [c] (-73.93)	16.37 [c] (-37.75)	4.24 [c] (-46.86)
Spinetoram	6.65 [b] (-54.97)	20.17 [b] (-23.30)	5.63 [b] (-29.44)
Control	14.77 [a]	26.30 [a]	7.98 [a]

Table (2): Non specific esterases activity and Ach E in homogenate of *Spodoptera littoralis* 5th instar larvae lab-strain after 24 hrs of treatment with LC_{50} of rynaxypyr and spinetoram.

Treatments	Enzymes activity (% Change to control)		
	α-esterase 1	β-esterase 2	Ach E 3
Rynaxypyr	497.33 [b] (- 31.71)	443.66 (- 61.81)	152.85 [a] (+ 25.74)
Spinetoram	755.0 [a] (+ 3.53)	1011.0 (- 11.18)	76.01 [c] (- 37.47)
Control	728.33 [a]	1138.33	121.56 [b]

1. Expressed as (µg α-naphthol /min/g.b.wt.)
2. Expressed as (µg β-naphthol /min/g.b.wt.)
3. Expressed as (µg Ach Br /min/g.b.wt.)

Table (3): Mean carbohydrate digestive enzymes activity in homogenate of *Spodoptera littoralis* 5th instar larvae lab-strain after 24 hrs of treatment with LC_{50} of rynaxypyr and spinetoram.

Treatments	Enzyme activity (µg glucose/min/g.b.wt)					
	Invertase	% Change to control	Trehalase	% Change to control	Amylase	% Change to control
Rynaxypyr	1133.66 c	-25.36	281.66 c	-55.78	97.66 c	-41.04
Spinetoram	123.66 b	-18.58	437.00 b	-31.39	118.66 b	-28.37
Control	1519.00 a		637.00 a		165.66 a	

Table (4): Chitinase and Lactate dehydrogenase (LDH) activity in homogenate of *Spodoptera littoralis* 5th instar larvae lab-strain after 24 hrs of treatment with LC_{50} of rynaxypyr and spinetoram.

Treatments	Chitinase		Lactate dehydrogenase	
	Activity (µg NAGA/min/g.b.wt)	% Change to control	Activity (µg $Ux10^3$/g.b.wt)	% Change to control
Rynaxypyr	148.33 a	+ 24.36	2063.66 b	+ 3.30
Spinetoram	133.33 b	+ 11.73	2226.66 a	+ 11.46
Control	119.33 b		1997.66 c	

Generally, it should be pointed out that applications of both insecticides specially rynaxypyr (Coragen 20%) led to significant reduction in carbohydrates content, associated with general disturbances in carbohydrates metabolism, as expressed by significant inhibition of digestive hydrolyzing enzymes activities, which could be result from a chain effect originating primarily from inhibition of chitin synthesis.

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