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Joint action of certain insecticides by sub lethal dose effect on the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae

Seham M. Ismail

Insect Ppulation Toxicology Department, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Egypt

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Abstract :

Toxicity of the four compounds belong to different groups of insecticides: indoxicarb, imidacloprid, pyridalyl and lufenuron against the 2nd and 4th larval instars of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) . The joint toxic action of lufenuron with tested compounds against 2nd instar larvae *S. littoralis* was studied. The activities of polyphenol oxidase (PPO) and chitinase were also studied. Indoxicarb against 2nd and 4th larval instars (LC_{50} = 0.087 and 0.31 ppm, respectively) was more toxic than lufenuron (LC_{50} = 0.23 and 0.62 ppm, respectively), followed by imidacloprid (LC_{50} = 0.93 and 1.23 ppm, respectively) and pyridalyl (LC_{50} = 1.28 and 3.13 ppm, respectively) after 96 hours of treatment. Lufenuron/ indoxicarb mixtures resulted in potentiation effect more than the lufenuron/imidacloprid mixtures and lufenuron/pyridalyl mixtures respectively, the co-toxicity factor (CTFs) gave potentiation effect with three mixtures tested, the CTF ranged from +62.6 to +20. The *in vivo* interaction of LC_{25} values of each tested compounds, with polyphenol oxidase (PPO) and chitinase caused significant increase in the activities in all treatment. Therefore, mixtures of lufenuron with these tested insecticides can be used for cotton leafworm control. The results generally indicate that indoxicarb, imidacloprid and pyridalyl are a successful insecticides at sub lethal dose, which may be used to prevent or delay appearance of resistance to conventional pesticides and save the environment.

In respect with the joint toxic action, the combination of lufenuron with tested compounds results in a synergistic effect; this will shed some lights in the possible joint toxic action and the sequence of alternative spraying programs. So, indoxicarb, imidacloprid and pyridalyl are a promising compounds in integrated pest control programmes.

Introduction

The extensive use of insecticides to control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae, the major lepidopterous cotton leafworm **(**CLW) infesting more than 150 crop in Egypt, the rate of development to multiple insecticide resistance toward the majority of compounds (Johnson and Gnanadhas 2016). Therefore, the determination of the most effective safe methods and practices for decreasing the level of infestation with CLW on economically important crops, by used a great need to develop noval alternative control agents with new mode of action where they disrupt the development of target pest, or functional combinations of pest control techniques is emphatically a product of this decade to reduce resistance to conventional insecticides (Yongqiang *et al*., 2016). Attention was therefore paid to control insect using different non-traditional insecticides, e.g., insect growth regulators (lufenuron) (El-Helaly and El–Bendary 2015), and **s**elective insecticides with modes of action differed from older classes of insecticides, these insecticides are: indoxicarb, imidacloprid, pyridalyl and lufenuron. If this trend continues, new compounds will be required to replace these insecticides (Haijing *et al*., 2017).

Insecticides mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests attack simultaneously. Mixtures are available as pre-mixes from pesticides companies or they are tank-mixed by farmers. Ideally, the insecticides with different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pests**.** When two compounds are mixed, they can be potentiating, additive or antagonistic in an insect species. These effects can be varied on different insect species or strains depending upon their physiology and the mechanisms of resistance developed. Nowadays, the scientists of pest control and environmental protection oriented their activities to limit the environmental pollution. The efficiency of broad-spectrum neurotoxic insecticides and their mixture with insect growth regulators (IGRs) against the CLW affected several investigators (Bushra *et al*., 2017).

The present work aimed to investigate the effect of pre-treatment of sub lethal dose of lufenuron on the efficacy of indoxicarb, imidacloprid and pyridalyl against 2nd and 4th instars larvae of *S. littoralis.* polyphenol oxidase and chitinase activities as biochemical parameter were studied.

Materials and Methods

1. Test insects:

Larvae of *S.littoralis* were obtained from Central Lab. of Pesticides, Agricultural Research Center, Cairo, Egypt and reared on castor oil leaves under laboratory conditions (27 ± 2 ^{o}C and RH 65 % ± 5) for several years, according to Eldefrawi *et al*. (1964).

2. Test insecticides:

Commercial formulations of indoxicarb (Steward, 15% EC); imidacloprid (Sinodor, 70% W.G) were supplied by Du Pont Co.; pyridalyl (Pleo, 50% EC) was supplied by Sumitomo Chem., Co., and lufenuron (Match, 5% EC) was supplied by Syngenta Co.

3. Toxicity tests:

3.1. Toxicity of insecticides against *Spodoptera littoralis* larvae:

Toxicity of the four-mentioned insecticides against the 2nd and 4th larval instars of *S. littoralis* was evaluated. To assess the insecticidal activity of the tested compound, series of aqueous concentrations for each compounds were prepared using the commercial formulations. The leaf dipping technique was adopted according to Eldefrawi *et al*. (1964) where fresh castor oil leaves were cut into discs (2 cm^2) each disc was dipped for 30 seconds in one of the prepared concentrations. The treated leaves had dried under laboratory conditions before being offer to *S. littoralis* larvae. Ten larvae in three replicated, were used for each concentration. Larvae were fed on leaves immersed in only water as a control. Newly moulted 2nd and 4th larval instars were fed on the treated leaves in

a glass jar covered with muslin for 24 hrs for the tested compound. The treated leaves were replaced by another untreated ones. Mortality percentages were recorded after 24, 48, 72 and 96 hrs of treatment, percent mortality was corrected according to Abbott equation (Abbott, 1925). The LC_{25}, LC_{50} and slope values of the tested compounds where calculated using Finney's equation (1971), through software computer program.

3.2. Joint toxic action of lufenuron with tested insecticides against *Spodoptera littoralis* larvae:

Joint toxic action of lufenuron with tested insecticides (indoxicarb, imidacloprid and pyridalyl) against the 2nd larvae of *S. littoralis* was investigated. LC_{25} of lufenuron was mixed with the LC_{25} of the other insecticides after 96 hrs of treatment. The percent mortality of each mixture was recorded after 24 hrs. The combined effect of the different mixtures was expressed as the co-toxicity factor (CTF) which was estimated according to the equation given by Mansour *et al*. (1966).

4. Biochemical studies:

4.1. Polyphenol oxidase activity assay:

Surviving larvae treated with LC_{25} value of each tested insecticide, after 24 hrs of treatment. The larvae were homogenized in 10 ml of 0.1 M potassium phosphate buffer (pH 7.0) using polytron Kinemetica on ice. The homogenate was filtered through two layers of cheesecloth and centrifuged at 10,000 rpm for 10 min at 4 ^{o}C using Beckman J2-21 hotor centrifuge. The supernatant was used as the crude enzyme extract. The activity of PPO (EC 1.10.3.2) was determined according to Zhi-qing *et al*. (2008) by mixing of 1.5 ml of 0.2 mol/L pyrocatechol, 1.4 mL enzyme extract, respectively. The mixture was incubated at 25 ^{o}C for 25 min and the absorbance was measured λ420 nm by spectrophotometer (Unico 1200- Spectrophotometer, USA). The specific activity of PPO was calculated and expressed as OD_{420}. 30 min^{-1} .mg $protein^{-1}$.

4.2. Chitinase activity assay:

Chitinase (EC 3.2.1.14) is specific hydrolyze enzyme which hydrolyse chitin (chitobiose polymer) to N-acetyl-D-glucosamine (reduced sugar monomer). The specific activity was determined in the surviving larvae treated with LC_{25} value of each tested insecticide, after 24 hrs of treatment. The larvae were homogenized in 0.1 M phosphate buffer (pH 7.0) with a tissue Tearor on ice. The homogenates were then centrifuged at 5000 rpm for 20 min at 0 ^{o}C. The supernatants were used as enzyme source for chitinase activity assay. Enzyme activity was measured according to Monreal and Reese (1969**)** method. One ml of colloidal chitin, as a substrate, in 5 M citrate phosphate buffer (pH 6.6) was mixed with 1 ml of enzyme extract. Colloidal chitin was prepared by Shimahara and Takiguchi (1988). A suspension containing 1% (w/v) of moist colloidal chitin is prepared in appropriate buffer and pH. The vials were placed sufficient to keep the chitin in suspension. Subsecquently the vials were placed into a boiling water bath for 5 min then were cooled to room temperature by placing the vials in a cold water bath. The reaction mixtures were centrifuged at 5000 rpm for 10 min at 0 ^{o}C. The supernatants was retained. Enzyme activity was assayed by measuring the amount of reducing sugar that produced by enzyme reaction (Miller, 1959). Reducing sugar was determined by mixing of 1 ml of the supernatant with 2 ml phosphate buffer (pH 6.8) and 1.5 ml of 3,5-dinitrosalicylic acid (96 mM, 438 mg of 3,5-dinitrosalicylic acid in 20 mL of deionized water and heat in a boiling water bath to dissolve). The tubes were boiling for 5 min. After cooling the tubes, the optical density (OD) was measured at λ450 nm using a Unico 1200- Spectrophotometer, USA. The specific activity of chitinase was calculated as OD_{450}. mg $^{-1}$ protein. $1h^{-1}$. Blank sample was determined in the manner described above without enzyme solution.

4.3. Determination of total protein:

The Lowry *et al*. (1951) method was used to determine protein content in the supernatant comparing to the standard curve of Bovine Serum Albumin (BSA).

5. Statistical analysis:

All the quantitative estimation of toxicity and biochemical parameters were based on three replicated and the values are expressed as mean ± standard error. The data were statistically analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) using SPSS 12.0 software (Statistical Package for Social Sciences, USA). Mean values were compared using Duncan's multiple rang test (1955).

Results and Discussion

1. Toxicity of tested insecticides against *Spodoptera littoralis* larvae:

Toxicity of the indoxicarb, imidacloprid, pyridalyl and lufenuron against the larval instars were recorded. Tables (1 and 2) shown that the toxicity of indoxicarb was the most effective at LC_{50} level after 96 hours of treatment whereas pyridalyl was the least active with LC_{50} level 0.087; 1.28 ppm for 2nd instar larvae, and 0.31; 3.13 ppm for 4th instar larvae respectively. The lufenuron and imidacloprid gave LC_{50} 0.23; 0.93 ppm for 2nd instar larvae, and 0.62; 1.23 ppm for 4th instar larvae respectively. From these data, it was clear that the toxicity of the tested compounds against two larval instars of *S. littoralis* were increased with the increasing of the exposure time and decreased with the advancement of larval instar**,** also, Von Keyserlink (1988) stated that sublethal concentration of pesticides can provide useful information concerning the basic physiological and behavioral responses of the target insect pest and this could be of high important value when new compounds are evaluated for potential application in pest management program. These results are in agreement with that obtained by many investigators, Cox, 2001; Sakamoto *et al.*, 2005; Lisa, 2015; Silva *et al.*, 2016 and Mushtaq and Sanobar, 2017. They reported that emamectin benzoate is the most potent compound followed by indoxacarb, imidacloprid, pyridalyl and chlorantraniliprole of *S. littoralis*.

Table (1): Toxicity of some insecticides against 2nd larval instar of *Spodoptera littoralis* at different exposure times.

Insecticides	Time (hrs)	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits		Slope values ± SE
				Lower	Upper	
Indoxicarb	24	4.42	19.35	2.91	7.68	1.05 ± 0.22
	48	0.55	2.22	0.25	0.86	1.11 ± 0.18
	72	0.13	0.562	0.027	0.28	1.06 ± 0.20
	96	0.010	0.087	0.001	0.07	0.71 ± 0.21
Imidacloprid	24	7.99	77.55	4.03	56.7	0.68 ± 0.22
	48	1.88	14.42	0.92	3.18	0.76 ± 0.19
	72	0.4704	2.365	0.17	0.80	0.96 ± 0.18
	96	0.19	0.93	0.04	0.39	0.97 ± 0.19
Pyridalyl	24	4.73	49.18	2.51	16.54	0.66 ± 0.20
	48	3.05	19.03	1.81	5.40	0.85 ± 0.20
	72	0.98	8.17	0.32	1.68	0.73 ± 0.18
	96	0.32	1.28	0.12	0.55	1.13 ± 0.19
Lufenuron	24	10.2	88.25	4.98	93.5	0.72 ± 0.23
	48	5.20	56.31	2.72	21.6	0.65 ± 0.20
	72	1.61	11.32	0.77	2.62	0.79 ± 0.19
	96	0.07	0.23	0.010	0.18	1.31 ± 0.27

Table (2): Toxicity of some insecticides against 4th larval instar of *Spodoptera littoralis* at different exposure times.

Insecticides	Time (hrs)	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits		Slope values ± SE
				Lower	Upper	
Indoxicarb	24	3.50	26.54	1.99	7.26	0.77 ± 0.20
	48	1.44	10.83	1.44	0.63	0.77 ± 0.19
	72	0.31	1.21	0.12	0.53	1.14 ± 0.19
	96	0.076	0.31	0.007	0.20	1.09 ± 0.26
Imidacloprid	24	9.66	84.78	4.78	81.9	0.72 ± 0.22
	48	2.32	21.21	1.13	4.33	0.70 ± 0.19
	72	1.42	6.42	0.81	2.09	1.03 ± 0.19
	96	0.33	1.23	0.14	0.55	1.19 ± 0.19
Pyridalyl	24	5.37	55.57	2.84	22.1	0.66 ± 0.21
	48	3.53	26.11	2.03	7.28	0.78 ± 0.20
	72	1.65	12.96	0.75	2.76	0.75 ± 0.19
	96	0.77	3.13	0.39	1.16	1.11 ± 0.18
Lufenuron	24	8.26	98.5	8.55	95.86	0.63 ± 0.21
	48	7.80	75.7	3.96	52.96	0.68 ± 0.22
	72	0.99	9.93	0.28	1.78	0.68 ± 0.18
	96	0.13	0.62	0.023	0.29	0.99 ± 0.19

2. Joint toxic action of lufenuron with tested insecticides against *Spodoptera littoralis* 2nd larvae:

The joint toxic action of lufenuron with the tested insecticides at LC_{25} against *S. littoralis* 2nd larvae is shown in Table (3). It is clear that, all mixtures of lufenuron (at LC_{25}) with the tested insecticides (at LC_{25}) resulted in potentiation effect with co-toxicity factors (CTFs) ranged between +62.6 to +20. The highest potentiation effect was observed, when lufenuron mixed with indoxicarb CTF value was +62.6 while CTFs were +40.4 and +20 when lufenuron was mixed with the imidacloprid and pyridalyl respectively. So, it could be concluded that all tested combinations positive effect, these effect depending upon their different modes of action for these insecticides are mixed on the assumption that they would complement the action of each other for killing the target pest. Also, these mixtures are potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance, in this case, there may be potential for reducing the application rate of one or both components of the mixture, so, favorable to mix lufenuron with tested insecticides. These results were compatible with the results obtained by El-Helaly and El–Bendary 2015 and Bushra *et al*., 2017. They reported that insect growth regulator mixtures with the insecticides resulted in additive effect, these positive effect may due to the insecticides from different chemical groups with different mod of action to act influenced on *S. littoralis*.

Table (3): Joint action for three mixed tested insecticides against 2nd instar *Spodoptera littoralis*.

Combination	Concentration levels[1]	Observed (%)Mortality	CTFs	Type of interaction
Lufenuron + Indoxicarb	$LC_{25} + LC_{25}$	81.3	62.6	Potentiatio
Lufenuron + Imidacloprid	$LC_{25} + LC_{25}$	70.2	40.4	Potentiatio
Lufenuron + Pyridalyl	$LC_{25} + LC_{25}$	60	20	Potentiatio

[1]Concentration level of each insecticide in the paired combination was calculated from its corresponding LC-p lines at 96 hrs of exposure.

3. Influence of tested insecticides on the polyphenol oxidase and chitinase activity in the *Spodoptera littoralis* larvae:

The *in vivo* effects of tested insecticides when applied to the 2nd instar larvae of *S. littoralis* on the PPO and chitinase activities after 4 days of treatment the results are shown in Tables (4 and 5). In general, the PPO activity and chitinase with all treatments significantly decreased compared to the control. The inhibition was recorded with LC_{25} of lufenuron followed by indoxicarb; imidacloprid and pyridalyl with inhibition percentages of 58.97, 43.83, 30.87 and 23.43% of PPO activity respectively. On the other hand, the inhibition percentages of 64.13, 38.63, 27.67 and 21.33% of chitinase activity respectively. Ishaaya and Casida (1974) reported that house fly larvae showed an increase of both the cuticle chitinase and phenoloxidse activities up to about 180 and 155% respectively, when treated with one ppm of the compound TH-6040. Moreover, insect excluded exotic substances by means of melanin. As the key enzyme to compose melanin, PPO was important for insect immunoreaction. Usually, PPO was located in insect blood lymph in the form of hydroxybenzene oxidase, which was activated by specific cascade reaction of serine protease and hydrolysis (Jing *et al*., 1998). Luo *et al*. (2005) reported that, the inhibition of PPO and chitinase by insecticides were concentration dependent. The present results are confirmed by the results of investigators, McKinley, 2002; Merzendorfer and Zimoch, 2003; Liu *et al*., 2014; Xing-Liang *et al*., 2015 and Shi, 2017, where they reported that PPO was important for insect immunoreaction and chitinase plays an essential role during ecdysis chitin, therefore, the PPO and chitinase had become one of the targets of pesticides studies,

Table (4): *In vivo* effect of tested insecticides at their LC_{25} values on polyphenol oxidase activity in larvae of *Spodoptera littoralis* after 4 days of exposure.

Insecticides	LC_{25} (mg./L^{-1})	Specific activity (OD_{420}. min^{-1} .mg $protein^{-1}$)± SE	Inhibition %± SE
Control	--------	17.33±0.612 a	----------
Indoxicarb	0.010	9.17±0.606 b	43.83±0.353
Imidacloprid	0.19	11.67±0.617 cd	30.87±0.809
Pyridalyl	0.32	12.93±0.636 c	23.43±0.740
Lufenuron	0.07	6.53±0.491 b	58.97±0.851

Means in the same column followed by the same letter are not significantly at P = 0.05.

Table (5): *In vivo* effect of tested insecticides at their LC_{25} values on chitinase activity in larvae of *Spodoptera littoralis* after 4 days of exposure.

Insecticides	LC_{25} (mg./L^{-1})	Specific activity (OD_{450}. mg $protein^{-1}$. $1h^{-1}$)± SE	Inhibition %± SE
Control	--------	14.83±0.524 d	----------
Indoxicarb	0.010	7.43±0.996 ab	38.63±0.769
Imidacloprid	0.19	10.43±0.348 ab	27.67±1.39
Pyridalyl	0.32	11.07±0.491 cd	21.33±0.674
Lufenuron	0.07	7.0±0.173 a	64.13±0.120

Means in the same column followed by the same letter are not significantly at P = 0.05.

It is concluded that the results presented, indoxicarb, imidacloprid, pyridalyl and lufenuron are potentially potent insecticides for controlling *S. littoralis*. Their high activity all mixtures of lufenuron with the tested insecticides, so, it is preferred to use these mixtures for controlling *S. littoralis*, which can lead to increase the efficacy of insecticides. The use of these insecticides with low doses may introduce good control results. Such treatments will reduce the used doses of this groups to not effect on non-target organisms and to save the environment, on the other hand, the

alternation between these insecticides can reduce resistance and avoid increasing selection pressure of *S. littoralis* populations to these insecticides.

References

Abbott, W. S. (1925): A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Bushra, S.; Masood, E.; Sarfraz, A. S. and Muhammad, A. (2017): Assessing combined toxicity of conventional and newer insecticides on the cotton mealybug *Phenacoccus solenopsis*. Ecotoxicologym, 26: 9-15.

Cox, C. (2001) : Imidacloprid. J. of Pestic. Reform., 21: 15-21.

Duncan, D. B. (1955) : Multiple rang and multiple F test. Biometerics, 11: 1-42.

Eldefrawi, M. E.; Toppozada, A.; Mansour, N., and Zeid, M. (1964) : Toxicological studies on the Egyption cotton leafworm, *Prodenia litura*. I. Susceptibility to different larval instars of *Prodenia* to insecticides. J. Econ. Entomol., 57: 591-593.

El-Helaly, A. A., and El–Bendary, H. M. (2015) : Field study to evaluate the joint action of certain insecticides, IGR's and baculoviruses against *Spodoptera littoralis* (Bosid.). J. Entomol. Zoology Studies, 3: 289-293.

Finney, D. J. (1971) : Probit analysis, 3rd edn, Cambridge Univ. Press, Cambridge, England. pp: 318.

Haijing, C.; Wenjing, L.; Yuansen, C.; Hui, C.; Yang, Z.; Wenping, X. and Limong, T. (2017): 5′- *Epi* - SPA-6952A, a new insecticidal 24-membered macrolide produced by *treptomyces diastatochromogenes* SSPRC-11339. Natural Product Research, 16: 1-6.

Ishaaya, I., A.; and Casida, J. E. (1974) : Dietary TH6040 alters composition and enzyme activity of housefly larval cuticle. Pestic. Biochem. Physiol., 4: 484-490.

Jing, H. B.; Wang, Y., and Kanost, M. R. (1998) : Pro-phenol oxidase activating proteinase from an insect, *Manduca sexta*, a bacteria-infducible protin similar to *Drosophila easter*. In Proceedings of the National Academy of Sciences of the USA, 95: 12220-12225.

Johnson, S., and Gnanadhas, P. (2016) : Pesticide toxicity to parasitoids: exposure,toxicity and risk assessment methodologies. Pesticide Toxicity to Non-Target Organisms, 99-152.

Lisa, J. B. (2015) : Baseline susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to indoxacarb, emamectin benzoate, and chlorantraniliprole in Australia. J. Economi. Entomology, 108: 294-300. **Liu, X. L.; Gulijimila, M. J. T.; Wang, Z. Y. and Wu, H. Q. (2014) :** Effects of several pesticides on the activities of *in vitro* enzymes in *Clostera anastomosis*. J. Northeast For. Univ., 42: 128–133.

Luo, W. C.; Gao, X. X.; Yu, T. U. and Wang, S. D. (2005) : Inhibitory effect of quercetin on the activity of phenoloxidase in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Acta Entomologica Sinica, 48: 36-41.

Lowry, O. H.; Rosebrough, N. J.; Farry, A. L. and Randall, R. J. (1951) : Protein measurements with folin phenol reagent. J. Bio. Chem., 193: 265-271.

Mansour, N. A.; Eldefrawi, M. E.; Toppozada, A. and Zeid, M. (1966) : Toxicological studies in the Egyption cotton leafworm, *Prodenia litura*. VI. Potentiation and antagonism of

organophosphorus and carbamate insecticides. J. Econ. Entomol., 59: 307-311.

McKinley, N.; Kijima, S.; Cook, G. and Sherrod, D. (2002) : Avaunt (Indoxacarb): a new mode of action insecticide for control of several key orchard pests. Proc. 76th Ann.

Merzendorfer, H. and Zimoch, L. (2003) : Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinase. J. Experimental Biol., 206: 4393-4412.

Miller, G. L. (1959) : Use of dinitrosalicyclic acid reagent for the measurement of reducing sugar. Analytical Chem., 31: 426-428.

Monreal, J. and Reese, E. (1969) : The chitinase of *Serratia marcescens*. Canadian J. Microbiol., 15: 689-696.

Mushtaq, A. and Sanobar, G. (2017) : Susceptibility of armyworm *Spodoptera litura* (Lepidoptera: Noctuidae) to novel insecticides in Pakistan. The Canadian Entomologist, 149: 649-661.

Sakamoto, N.; Ueda, N.; Umeda, K.; Matsuot, S.; Hagat, T.; Fujisawa, T. and Tomigahara, Y. (2005) : Research and development of a novel insecticide "pyridalyl" "Sumitomo Kagaku" Chem. Abst., 1: 133-144.

Shi, X.; Tang, F.; Zhou, X. and Bu, X. (2017) : *In vitro* inhibition of polyphenol oxidase activity by insecticides and allelochemicals in *Clostera anastomosis* (Lepidoptera: Notodontidae) larvae and poplar trees. J. Entomol Sci., 52: 239-247.

Shimahara, K., and Takiguchi, Y. (1988) : Preparation of crustacean chitin. Methods in Enzymol., 161: 417-423.

Silva, T. B. M. ; Silva, W. M. ; Campos, M. R. ; Silva, J. E. ; Ribeiro, L. M. S. and Siqueira, H. A. A. (2016) : Susceptibility levels of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to minor classes of insecticides in Brazil. Crop Protection, 79: 80-86.

Von Keyserlink, H. C. (1988) : The significance of behavior studies in insecticides Research. Pestic. Sci., 16: 558-559.

Xing-Liang, W.; Wen, S.; Jian-Heng, Z.; Yi-Hua, Y.; Ke, D. and Yi-Dong, W. (2015) : Two novel sodium channel mutations associated with resistance to indoxacarb and metaflumizone in the diamondback moth, *Plutella xylostella*. Insect Sci., 19: 537-545.

Yongqiang, L.; Xiangying, L.; Chao, Z.; Feng, L. and Wei, M. (2016) : Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. Sci. Reports., 6: 1-10.

Zhi-qing, M. A.; Xiu-ling, H.; Jun-tao, F.; Guang-ze, L. and Xing, Z., (2008) : Effects of Terpinen-4-ol on four metabolic enzymes and polyphenol oxidase (PPO) in *Mythimna separta* Walker. Agric. Sci. China., 7: 726-730