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# RESISTANCE CATEGORIES OF KALUBIA AND MENUFIA DIAMONDBACK MOTH STRAINS TO SOME INSECTICIDES

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## ABSTRACT

*Diamond back moth Plutella xylostella* (Linn.), is the most destructive and regular pest of cabbage and cauliflower universally. Protection of crucifer crops from damage often requires application of insecticide to plant foliage, sometimes as frequently as twice per week. However, resistance to insecticides is widespread, and includes most classes of insecticides. Therefore, the studies were carried out to monitor their resistance factor form two region Kalubia and Menufia against seventeen insecticides having different mode of actions to develop strategies for its management. Results show that Menufia and Kalubia were susceptible to IGRs and pyrethroids except for pymetrozine in kalubia. Spinosad in Menufia was 108.54 folds and considered the higher levels of resistance attained, followed by prothiophos, abamectin and imidacloprid, 17.69, 26.18, 11.43, folds respectively. But in kalubia the higher folds of resistance was thiocyclam 42.24 followed by methomyl 20.96 folds.

## INTRODUCTION

*Diamond back moth Plutella xylostella* (Linn.), a butterfly of the family Plutellidae, injurious to cruciferous plants. Where cabbages are a major food crop and insecticides are inefficient for control. The pest has developed resistance to almost all the recommended insecticides belonging to major groups of insecticides and several new molecule within a few years of its introduction (Tang *et al.,* 1988, Liu *et al.* 1982, Hama 1987 and Shelton *et al.* 1993a).The pest has also developed cross resistance and multiple-resistance to different chemical pesticides (Shelton *et al.* 2000) including *Bacillus thuringiensis* Berliner (BT) formulations. The highest levels of resistance were generally associated with areas of intensive brassica cultivation (Tabashnik *et al.* 1987 and Cheng 1988). Many searches cleaved to identify and mitigate the resistance in this pest with high diversity of spots, recently different ISSR markers have been tested as a tool for population discrimination and genetic variations among 19 *P. xylostella* populations around the world and in Egypt the result interpreted that this pest did not reflect geographical distances between them (Roux *et al.,* 2006). The Identifying of the most prospective compounds for insecticide resistance management and determining which class of insecticides would provide control in a field situation where the presence of resistance was suspected as well as fortunately maintain pyrethroid susceptibility of this pest to pesticides were the most powerful tool. Therefore the baseline susceptibility institution, dose-mortality response and the use of a discriminating dose assay (DC) for commercial formulations product insecticides belonging to different chemical groups were bioassayed which tells what proportion of the population is resistant (Roush and Miller 1986), in the way the properties of the Ldp line (in slope and relative position)

should give a measure of the three major components of the resistance allele in that population.

## MATERIALS AND METHODS

**Insects and rearing:**

A laboratory strain of *P. xylostella* was developed by rearing the collected larvae from kalubia cabbage fields in the laboratory for 20 generations without insecticide exposure on cabbage plants at room temperature (25-27$^{o}$C with 75% RH) and at a 16:8 (light: dark) photoperiod as described by Noppun *et al.* (1983). Cabbage seedlings were provided for an adult oviposition substrate. For susceptibility bioassay collection of cabbage plant infested with larvae of *P. xylostella* was carried out from Menufia and Kalubia just before the bioassay of the candidate insecticides.

**Bioassay Method:**

The leaf residue technique was carried out to perform the Susceptibility of field populations according to (Magaro and Edelson, 1990 and Fahmi, *et al.* (1991). Cabbage *Brassica oleracea* leaves was used for exposing larvae to test insecticides. Discs (5 cm diameter) of cabbage leaves were dipped in 7concentrations of each insecticide formulations for 5 seconds and dried for 1 hour at room temperature. For the untreated test discs were dipped in water only as a control. After drying, the discs were placed in glass containers. Around thirty 3rd instars larvae were released per two discs for each concentration and the mouth of the container was secured with muslin cloth tied with rubber band. The test larvae were allowed to feed for 48 hours on treated discs. Larvae that did not show coordinated movement or did not move when touched with brush were considered dead. Thereafter, larval mortality was recorded, Data were corrected for mortality using Abbott's formula (Abbott 1925), and analyzed by the probit method (Finney 1971), using the computer programm POLO (Russel *et al.* 1977) to estimate $LC_{50}$'s and their 95% Fiducial limits, slopes and chi-squares. Strains were considered significantly different if their 95% Confidence Limits of the $LC_{50}$ did not overlap.

**Insecticides:**

Imidacloprid (confidor 20% Soluble Liquid), prothiophos (Tokuthion 50%EC), was obtained from Bayer CropScience, fenpropathrin (meothrin 20% Emulsifiable Concentrate), from Sumitomo chemical co.Ltd., spinosad (spintor 24% SL), methomyl (Lannate 90% SP) from DuPont Agricultural Products and abamectin (Vertimec 1.8 %EC), lambdacyhalothrin (Karate 2.5 %EC), were obtained from Novartis agrochemicals. The remain insecticides such as acetamiprid (mosbilan 20% EC),thiocyclam (Evisect 50%Wettable Powder), pymetrozine (Chess 25%WP), pyriproxyfen (Admiral 10% EC), fenoxycarb (Insegar 25% WP), hexaflumeron (consult 10% EC) , dinotifuran (MTI 446 20%WP), pirimicarb (Aphox 50 % DG), carbosulfan (marshal 25%WP),malathion (Malatox 57%EC), was obtained from the professors of the Central agriculture pesticide lab, Egyptian Ministry of Agriculture.

## RESULTS AND DISCUSSION

For the assessment of the potential role of insecticide resistance management of *Plutella xylostella* a survey of the susceptibility of *Plutella xylostella* to commonly used insecticides during the Cabbage season were achieved. The data of the susceptibility test to the lab reared *P. xylostella* strain were in table (1). Tables 2 and 3 showed the susceptibility data for the tested insecticides on the 3$^{rd}$ larvae of *P. xylostella* susceptible, Kalubia and Menufia population.

There is a great variation in response between the two field strains to dinotifuran, pirimicarb, spinosyn, prothiophos, imidacloprid and acetamiprid and less variation in response to hexaflumeron, malathion and pyriproxyfen. The highest $LC_{50}$ in Kalubia was only 704.8 ppm for dinotifuran, subsequently 48.9, 34.64, 30.27 ppm for Malathion, thiocyclam and pirimicarb respectively. Mostly Kalubia was considered more susceptible than Menufia population in the all tested insecticides. In general the highest $LC_{50}$ 1235, 114.37, 121.57, 86.15, 58.62 and 45.44 ppm, occurred with dinotifuran, pirimicarb, spinosad, prothiophos, imidacloprid and malathion in Menufia respectively.

Three general categories of susceptibility represented in Menufia and kalubia were established. They probably reflect the baseline susceptibility of *P. xylostella* to the candidate insecticides. The first: where Menufia population would be expected to have very little survivorship at treatment of acetamiprid, methomyl, fenpropathrin, lambdacyhalothrin, abamectin, hexaflumeron, pymetrozine, pyriproxyfen when compared with the susceptible strain. The second group represented by Kalubia population that had high survivorship at $LC_{50}$ concentrations of thiocyclam, pirimicarb, malathion, spynosad and pymetrozine. The third category of responses relatively not significantly different from the lab strain those are abamectin in Kalubia and fenpropathrin and lambdacyhalothrin in Menufia. Kalubia population exhibit 42.24 fold for thiocyclam and 20.96 for methomyl when compared with the susceptible strain and the rest of insecticides were considered very susceptible whereas very little resistance ratio value. But when we used the kalubia as a reference strain (instead of the susceptible strain) the other population (Menufia) had resistance ratios (RR2) of 15.05, 8.2, 4.71 and 4.48 fold for spinosad, abamectin, prothiophos and carbosulfan, respectively. $LC_{90}$ of this population were 684.74, 29.75, 570.25, and 160.01 for spinosad, abamectin, prothiophos and carbosulfan, respectively, this also may be considered the discriminating concentration (DC) of the test.

Resistance ratio > 10 can probably lead to control problem in the field, then there is a DC hypotheticale to be exist from resistance ratio 1-10 and this DC may be used to classify populations for susceptibility and to predict the potential usefulness of the tested insecticides. In conclusion some insecticides exhibited a tendency to build a resistance status in Menufia such as imidacloprid, prothiophos, abamectin, and carbosulfan (11.43, 17.69, 26.18 and 108.54 folds) respectively. While in Kalubia methomyl and thiocyclam exhibited 20.96 and 42.24 folds.

This results were similar to 44 populations were found to be resistant to permethrin, methomyl and methamidophos including new generation insecticides such as neo-nicotinoids, avermectins, macrocyclic lactones, synergists and (IGRs) (Shelton and Wyman, 1992). These levels of resistance might be required a long time of insecticide free application to reach the susceptibility acceptable levels. Hama 1988a reported that the stability of OP's resistance tends to increase with resistance levels whereas decreases in high levels of resistance needed 10-20 generations or more.

**Table (1): Toxicity of insecticides to the laboratory population of *P. xylostella* 3rd instar larvae.**

| Treatment | Slope±SE | $LC_{50}$ (Fiducial limits) | $LC_{90}$ (Fiducial limits) | H |
|---|---|---|---|---|
| Acetamiprid | 1.897±0.246 | 3.49 (2.7 - 4.57) | 16.51 (11.1 - 30.5) | 0.48 |
| Imidacloprid | 1.563±0.224 | 5.13 (3.65 - 6.95) | 33.9 (21.4 - 71.6) | 0.26 |
| Methomyl | 1.997±0.260 | 1.36 (1.03 - 1.75) | 5.95 (4.17 - 10.24) | 0.38 |
| Prothiophos | 1.795±0.237 | 4.87 (3.7 - 6.48) | 25.23 (16.46 - 49.4) | 0.50 |
| Thiocyclam | 1.537±0.222 | 0.82 (0.581 - 1.1) | 5.58 (3.48 - 12.057) | 0.61 |
| Fenpropathrin | 2.152±0.265 | 0.58 (0.45 - 0.74) | 2.28 (1.6 - 3.8) | 0.45 |
| Lambdacyhalothrin | 1.502±0.224 | 1.22 (.857 - 1.67) | 8.72 (5.38 - 19.62) | 0.67 |
| Abamectin | 1.642±0.229 | 0.17 (0.127 - 0.23) | 1.03 (0.64 - 2.24) | 0.40 |
| Carbosulfan | 1.917±0.247 | 2.04 (1.559 - 2.66) | 9.51 (6.47 - 17.16) | 0.36 |
| Pirimicarb | 1.629±0.228 | 14.4 (10.5 - 19.4) | 88.23 (56.5 - 180.8) | 0.46 |
| Fenoxycarb | 1.674±0.235 | 3.68 (2.713 - 4.91) | 21.44 (13.8 - 43.75) | 0.31 |
| Spinosad | 2.098±0.259 | 1.12 (.869 - 1.43) | 4.55 (3.193 - 7.751) | 0.22 |
| Malathion | 1.469±0.218 | 11.04 (7.961 - 15.44) | 82.3 (47.9 - 206.43) | 0.23 |
| Hexaflumeron | 1.838±0.241 | 0.38 (0.286 - 0.498) | 1.89 (1.27 - 3.5) | 0.04 |
| Pymetrizine | 1.679±0.232 | 1.93 (1.43 - 2.58) | 11.16 (7.17 - 22.70) | 0.70 |
| Pyriproxyfen | 1.917±0.254 | 0.59 (0.44 - 0.76) | 2.74 (1.9 - 4.83) | 0.55 |
| Dinotifuran | 1.767±.236 | 354.8 (267.1 - 471.1) | 1884.7 (1232.3 - 3683.8) | 0.18 |

**Table (2): Insecticide susceptibility in 3rd instar larvae of Kalubia *P. xylostella*.**

| Treatment | Slope±SE | $LC_{50}$(Fiducial limits) | $LC_{90}$(Fiducial limits) | H | RR |
|---|---|---|---|---|---|
| Acetamiprid | 1.614±0.229 | 15.7 (11.620 - 21.43) | 97.71(59.95 - 218.71) | 0.95 | 4.50 |
| Imidacloprid | 1.757±0.237 | 24.2 (18.040 - 31.98) | 129.55 (85.34 - 250.29) | 0.81 | 4.72 |
| Methomyl | 1.751±0.236 | 28.5 (21.349 - 37.84) | 153.83 (100.7 - 300.82) | 0.81 | 20.96 |
| Prothiophos | 1.623±0.226 | 18.3 (13.494 - 24.78) | 112.65 (70.346 - 241.78) | 0.28 | 3.76 |
| Thiocyclam | 2.387±0.289 | 34.64 (27.588 - 43.65) | 119.28 (86.88 - 190.51) | 0.69 | 42.24 |
| Fenpropathrin | 1.945±0.249 | 4.32 (3.314 - 5.616) | 19.69 (13.46 - 35.21) | 0.03 | 7.45 |
| Lambdacyhalothrin | 1.470±0.219 | 6.33 (4.510 - 8.76) | 47.13 (27.998 - 113.96) | 0.54 | 5.19 |
| Abamectin | 1.609±0.228 | 0.543 (.396 - 0.73) | 3.4 (2.144 - 7.19) | 0.46 | 3.19 |
| Carbosulfan | 2.155±0.270 | 6.63 (5.135 - 8.44) | 26.054 (18.70 - 42.72) | 0.11 | 3.25 |
| Pirimicarb | 1.641±.230 | 30.27 (22.189 - 40.66) | 182.75(116.53 - 378.47) | 0.98 | 2.10 |
| Fenoxycarb | 2.222±0.272 | 19.9 (15.683 - 25.38) | 75.1 (53.484 - 124.64) | 0.24 | 5.41 |
| Spinosad | 1.698±0.231 | 8.08 (6.011 - 10.80) | 45.9 (29.59 - 92.48) | 0.12 | 7.21 |
| Malathion | 1.621±0.228 | 48.9 (36.284 - 66.95) | 301.73 (184.7 - 675.48) | 0.28 | 4.43 |
| Hexaflumeron | 1.552±0.226 | 1.18 (.830 - 1.603) | 7.9 (5.006 - 16.686) | 0.81 | 3.11 |
| Pymetrizine | 1.609±0.227 | 11.06 (8.194 - 15.2) | 69.2 (42.08 - 157.13) | 0.23 | 5.73 |
| Pyriproxyfen | 1.842±0.244 | 1.56 (1.176 - 2.04) | 7.7 (5.18 - 14.4) | 0.61 | 2.64 |
| Dinotifuran | 1.626±0.228 | 704.8 (520.56 - 954.97) | 4328.5 (2701.69 - 9332.47) | 0.41 | 1.99 |

**H: heterogeneity factor is equal to x2 divided by d.f.**

**Resistance ratio for each insecticide is equal to $LC_{50}$ field population / $LC_{50}$ of the most susceptible population.**

**RR= $LC_{50}$ of Kalubia population / $LC_{50}$ of the susceptible strain**

**Table (3): Insecticide susceptibility in 3rd instar larvae of Menufia *P. xylostella*.**

| Treatment | Slope±SE | $LC_{50}$(Fiducial limits) | $LC_{90}$(Fiducial limits) | H | RR1 | RR2 |
|---|---|---|---|---|---|---|
| Acetamiprid | 1.591±0.228 | 2.98 ( 2.17 to 4.03) | 19.02 (11.88 to 41.19) | 0.88 | 0.85 | 0.19 |
| Imidacloprid | 2.092±0.262 | 58.62 (39.96 to 86.95) | 240.19 (145.6 to 620.3) | 1.11 | 11.43 | 2.42 |
| Methomyl | 1.840±0.25 | 4.94 (3.66 to 6.48) | 24.59 (16.772 to 44.63) | 0.61 | 3.63 | 0.17 |
| Prothiophos | 1.561±0.224 | 86.15 (63.3 7 to 119.27) | 570.25 (341.035 to 1341.91) | 0.27 | 17.69 | 4.71 |
| Thiocyclam | 1.837±0.243 | 7.66 (5.068 to 11.43) | 38.17 (22.3 4 to 106.58) | 1.00 | 9.34 | 0.22 |
| Fenpropathrin | 1.851±0..240 | 0.614 (0.47 to 0.81) | 3.025 (2.009 to 5.7) | 0.29 | 1.06 | 0.14 |
| Lambdacyhalothrin | 1.927±0.249 | 1.79 (1.375 to 2.34) | 8.28 (5.598 to 15.17) | 0.75 | 1.47 | 0.28 |
| Abamectin | 1.553±0.223 | 4.45 (3.216 to 6.06) | 29.75 (18.366 to 65.79) | 0.04 | 26.18 | 8.20 |
| Carbosulfan | 1.753±0.235 | 29.72 (22.12 to 39.33) | 160.01 (105.9 4 to 305.253) | 0.21 | 14.57 | 4.48 |
| Pirimicarb | 1.811±0.239 | 114.37 (86. 8 to 151.54) | 583.44 (383.38 to 1126.98) | 0.18 | 7.94 | 3.78 |
| Fenoxycarb | 2.024±0.256 | 35.85 (27. 9 to 46.4) | 154.05 (106.3 5 to 270.78) | 0.49 | 9.74 | 1.80 |
| Spinosad | 1.707±0.232 | 121.57 (90.44 to 162.2) | 684.74 ( 443.26 to 1367.093) | 0.10 | 108.54 | 15.05 |
| Malathion | 1.389±0.214 | 45.44 (32.30 to 64.88) | 380.017(210.898 to 1066.24) | 0.17 | 4.12 | 0.93 |
| Hexaflumeron | 1.601±0.226 | 0.66 (0.475 to .89) | 4.17 (2.663 to 8.627) | 0.15 | 1.74 | 0.56 |
| Pymetrizine | 1.883±0.295 | 1.83 (1.38 to 2.47) | 8.77 (5.53 to 19.7) | 0.49 | 0.95 | 0.17 |
| Pyriproxyfen | 1.948±0.249 | 0.95 (0.73 to 1.24) | 4.3 (2. 94 to 7.836) | 0.06 | 1.61 | 0.61 |
| Dinotifuran | 1.553±0.223 | 1235.06( 893.3to1683.19) | 8262.9 (5101.75 to 18275.97) | 0.04 | 3.48 | 1.75 |

**RR1= $LC_{50}$ of Menufia population / $LC_{50}$ of the susceptible strain**
**RR2= $LC_{50}$ Menufia population / $LC_{50}$ Kalubia population**

The variation observed of $LC_{50}$'s obtained could reflect the history of insecticide application and/or cross-resistance due to other pyrethroids in cruciferous crop season. The folds of *P. xylostella insecticide* resistance have been reported in many areas. In southern Australia, 19 of 28 *P. xylostella* populations from non-vegetable crucifers were significantly tolerant (2.1–6.9-fold) to permethrin while all the five populations from vegetable crucifers showed 3.6–13.0-fold resistance to this pyrethroid (Endersby *et al.,* 2004). A field strain from Japan was 9.5-fold resistant to acetamiprid, exhibited up to 110-fold resistance to this nicotinoid insecticide after laboratory selection for five generations (Ninsin, 2004). The field-collected populations from Adelaide (Australia) were eight to 400-fold resistant to pyrethroids (esfenvalerate and permethrin), five to 200-fold to OPs (chlorpyrifos, methamidophos and mevinphos) and six-fold to carbamate (methomyl) (Baker and Kovaliski, 1999). Mohan and Gujar (2003) reported complete failure of fenvalerate and flufenoxuron for *P. xylostella* control in India and Resistance to deltamethrin, bifenthrin and lambdcyhalothrin was high throughout the seasons. There is a solve for controlling this pest with escaping from the high insecticide resisatnce levels that experimented by Ninsin *et al.,* 2000 where reported that Insecticides is used two times a year at transplanting, and once per growing season, this protect cabbage plants against *P. xylostella* damage through maturity whereas the granular formulation of acetamiprid which allow toxicity to be present in cabbage plants for an extended time.

The fast development of resistance probably caused by: (1) a pyrethroid resistance gene(s) might have already been present frequently in the population because heavy pyrethroid applications on closed populations. Chen and Sun (1986) found that a mostly Pyrethroid resistance in *P. xylostella* is more stable than OP's resistance in addition to Hama 1988b

observed that although a high resistance level in a few populations' decreases within 10 generations after collection; in most cases it remains for more than 15 generations. (2) Such control failure may have been influenced by environmental factors temperatures and below-normal rainfall (Harcourt 1986). (3) Migration of susceptible individuals was very limited in the areas where *P. xylostella* is present throughout most of the year. Migration of resistant individuals from surrounding areas, where cruciferous crops are being cultivated on a large scale, could be another possible reason for this fluctuation. *P. xylostella* is highly migratory and its seasonal movements have been well documented (Chapman *et al.,* 2002). (4) The differences in the intrinsic rate of growth between resistant and susceptible populations (fitness costs) (Hama 1989a). Sayyed *et al.,* (2005) study, of *P. xylostella* population collected from Pakistan showed incomplete dominance of resistance to deltamethrin (pyrethroid),it was dose dependant and unstable in the absence of selection pressure, which probably means high fitness costs. (5) The cross resistance relationships between these classes of insecticides for *Plutella xylostella may* be the cause of the potential role in development of resistance because each class has similar resistance mechanisms (Sun1992).

The OP resistance may be different between each other according to results of selection experiments (Sasaki 1982); cross-resistance to OP's insecticides is related to chemical structure of insecticides. When thiono-type insecticides were used as selecting agents, resistance level was higher than that to phosphate or dithio-types, although susceptibility to phosphate or dithio-types decreased. In another survey of OP's insecticide resistance in field populations collected throughout Japan, the resistance level to thiono-types such as cyanofenphos, prothiophos, cyanophos and isoxathion tended to be higher than that to phosphate-type dimethylvinphos or dithio-type methidathion and phenthoate (Hama 1986b).

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# درجـات المقاومـه للفراشـه ذات الظهـر الماسـي مـن سـلالات القليوبيـه والمنوفيـه لبعض المبيدات

**حنان صلاح الدين طه دياب**
**المعمل المركزي للمبيدات , دقي, جيزه, مصر.**

الفراشه ذات الظهر الماسي *Plutella xylostella* (Lep. Plutellidae) من اكثر الافات انتشارا علي محاصيل العائله الصليبيه و التي منها الكرنب والقرنبيط مسببه خساره كبيره للمجموع الخضري. لحمايه هذه النباتات من هجوم الافه نحتاج الي الرش بالمبيدات قد يصل الي مرتين في الاسبوع. مما ادي الي مقاومه هذه الافه للمبيدات . فكان لابد من عمل حصر للمبيدات التي وصلت الي مستويات مختلفه من المقاومه . ولعمل ذلك الحصر تم جمع كميات من هذه الافه من محافظتي القليوبيه و المنوفيه من محصول الكرنب لعمل اختبار حيوي بطريقه غمر اوراق النباتات في التركيزات المحدده من المبيدات المراد اختبارها للحصول علي نتائج موضحه لذلك. تمت تربيه الحشره في المعمل حتي الحصول علي سلاله معمليه غير معرضه للمبيدات يمكن بها المقارنه بالنتائج المتحصل عليها من الجرعات النصفيه لكل مبيد مختبر علي السلالتين القليوبيه و المنوفيه. واختيرت المبيدات التي تم اختبارها من مجموعات كيماويه مختلفه

**و كانت النتائج كالتالي:**

1. المنوفيه والقليوبيه كانتا حساستين للمبيدات البيروثرويد ومنظمات النمو.
2. مبيد سبينوساد في المنوفيه سجل اعلي نسبه مقاومه يليه البروثيوفوس و الابامكتين ثم الايميداكلوبريد.
3. و في القليوبيه كان اعلي مبيد في نسبه المقاومه الثيوسيكلام يليه الميثوميل.

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