

Biochemical studies on cotton leafworm *Spodoptera littoralis* (boisd.)

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V. SUMMARY

5.1. Toxicological studies: Bioassays were carried out using leaf-dipping technique for monitoring resistance spectrum toward Spinosad as naturally-derived compound, Chlorpyrifos as organophosphates and Methoxyfenozide as ecdysteroid agonist in four different strains of the cotton leafworm *Spodoptera littoralis*, (Boisecl.) The results of toxicological studies could be summarized as follows: a) Based on LC50 values, the field strain (F-) tolerated moderately the tested insecticides, recording tolerance ratios of 3.53, 2.26 and 4.52, for Spinosad (Sps), Chlorpyrifos (Cpf) and Methoxyfenozide (Mfz) respectively relative to the standard laboratory (L) strain. b) The RR genotype strains obtained from the isolated egg-masses (families) exhibited resistance ratios of 2.66, 130 and 2.93 fold relative to field strain versus 9.4, 7.45 and 13.36 fold when compared to laboratory (L) strain, for the same compounds, respectively. c) As for SS genotype strains obtained from the isolated egg-masses (families), resulted in resistance ratios of 0.40, 0.81 and 0.55 folds relative to field strain (F) versus 1.43, 3.62 and 1.24 folds when compared to laboratory (L) strain for Sps, Cpf and Mfz, respectively. d) Summarizing the forementioned results, it could be concluded that, using the discriminating doses through such approach of rearing isolated egg-masses (families) could save time and costs in producing easily resistant or/and susceptible strains from any field population. However, such procedure can help in carrying biochemical studies for the already resistance genotypes distributed in a field population and thus can be accordingly linking the enzymatic activity with the genotype specific for resistance to an insecticide early in the season before build up of resistance to such insecticide.

5.2. Biochemical Studies: The biochemical studies were performed to evaluate the relation between resistance level of *S. littoralis* to the three tested insecticides and some biochemical parameters thought to be involved in resistance mechanism. The results could be summarized as follows:

5.2.1. The main components:

5.2.1.1. Total protein: The Sps-R, F- and S-strains were significantly lower than that of L-strain, the lowest value was recorded in Sp's-R strain (40.84% of L-strain). A negative correlation was obtained (-0.9476) between resistance level and total protein. The same trend was observed in the case of Cpf and Mfz-resistant strains, where the percentages values were 51.03% and 48.24% of L-strains while the correlation coefficients were -0.9135 and -0.8476 respectively.

5.2.1.2. Total lipids: The total lipids in the Sps-R, F- and S-strains were higher than that of L-strain, the highest value was recorded in Sps-R strain (217.56% of L-strain). A positive correlation was obtained (0.8263) between resistance level to Sps and lipid content. The same trend was observed in the case of Cpf and Mfz-resistant strains, where the highest values were recorded in Cpf-R and Mfz-R strains with percentages 224.89% and 302.92% of L-strains, while the correlation coefficients were 0.7740 and 0.8830 respectively.

5.2.1.3. Total Carbohydrates: The total carbohydrates in the Sps-R, F- and S-strains were higher than that of L-strain, the highest value was recorded in Sps-R strain (157.88% of L-strain). A positive correlation was obtained (0.9002) between resistance levels and carbohydrates content. The same trend was observed in the case of Cpf- and Mfz-resistant strains, where the highest values were recorded in Cpf-R and Mfz-R strains with percentages 160.67% and 176.62% of L-strains, while the correlation coefficients were 0.8887 and 0.8972 respectively.

5.2.2. Carbohydrate hydrolyzing enzymes:

5.2.2.1. Invertase enzyme activity: Invertase activities in the Sps-R, F- and S-strains were lower than that of L-strain, the lowest activity was recorded in Sps-R strain (65.47% of L-strain). A negative correlation

coefficient was obtained between resistance level to Sps and invertase activities, (- 0.8654). The same trend was observed in the case of Mfz-resistant strains, where the lowest activity was recorded in Mfz-R strains with percentage. 56.15 % of L-strains, while the correlation coefficient was - 0.6437. On the other hand a different pattern was observed in the case of chlorpyrifos, where a low positive correlation coefficient was recorded (0.5194) and the invertase activity in Cpf-R was significantly higher than L-strain (119.44 5%)

5.2.2.2. Trehalase enzyme activity: The obtained results revealed high significant levels of trehalase in resistant strains when compared with L- strain as reference the activities percentages were 141.76, 212.91 and 146.03 for (Sps- R, Cpf-R and Mfz R, respectively). The correlation coefficient between resistance level and trehalase activities, showed a positive correlation for the three compounds (0.7064, 0.8928 and 0.8060) for Sps, Cpf and Mfz respectively it could be easily observed that the highest correlation coefficient was recorded in the case of Cpf suggesting a high relationship between resistance level to organophosphates and trehalase activity.

5.2.2.3. Amylase enzyme activity: Amylase activities in Sps- R, F-.and S-strains were lower than that of L-strain. The lowest activity was recorded in Sps-R strain (53.49 % of L-strain). The same trend was observed in the case of Cpf and Mfz-resistant strains, where the lowest values were recorded in Cpf-R and Mfz-R strains with percentages 40.57 % and 26.84 % of L-strains, A negative correlation coefficient was obtained between resistance level and amylase activities, (- 0.8419, - 0.8863 and -0.9352) for Sps, Cpf and Mfz respectively. The lowest amylase activity and the highest negative correlation coefficient recorded in the Mfz-R, suggesting a high significant relationship between resistance level to Mfz and amylase activity.

5.2.3. Transaminases activities:

5.2.3.1. Aspartic aminotransferase (ASAT) : ASAT activities in Sps, the Sps- R, F-.and S-strains were lower than that of L-strain, the lowest activity was recorded in Sps-R strain (30.37 % of L-strain). The same trend was observed in the case of Cpf and Mfz-resistant strains, with percentages 6.60% and 12.51 % of L-strains, respectively. A negative correlation coefficient was obtained between resistance level to the three insecticides and ASAT activities, (- 0.8457, -0.8860 and - 0.7222) for Sps, Cpf and Mfz respectively.

5.2.3.2. Alanine aminotransferase (ALAT) : ALAT activities in Sps, the Sps- R, F-.and S-strains were higher than that of L-strain, The highest activity was recorded in Sps-R strain (252.73 % of L-strain). The same trend was observed in the case of Cpf and Mfz-resistant strains, where the highest activities were recorded in Cpf-R and Mfz-R strains with percentages 283.64 % and 254.55 % of L-strains, A positive correlation coefficient was obtained between resistance level to the three insecticides and ALAT activities. (0.7502, 0.8235 and 0.6003) for Sps, Cpf and Mfz respectively.

5.2.4. Activities of Non-specific esterases:

5.2.4.1. a-Esterase: a-Esterase activities in Sps- R, F-.and S-strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (501.63 % of L-strain). The same trend was observed in the case of Cpf and Mfz-resistant strains, where the highest activities were recorded in Cpf-R and Mfz-R strains with percentages 644.38 % and 490.44 % of L-strains, A positive correlation coefficient was obtained between resistance level to the three insecticides and a-Esterase activities. (0.8771, 0.9285 and 0.7686) for Sps, Cpf and Mfz respectively.

5.2.4.2. 13-Esterase: (3-Esterase activities in Sps- R, F-.and S-strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (416.34 % of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest activities were recorded with percentages 516.89 % and 355.14 % of L-strains, respectively. A positive correlation coefficient was obtained between resistance level to the three insecticides and 13 -Esterase activities, (0.9172, 0.9399 and 0.7326) for Sps, Cpf and Mfz respectively. From the results of both a and 13-Esterase, it is clearly obvious that the organophosphorus resistant strain had the highest activity and in the same time the highest correlation coefficient.

5.2.5. Phosphatases activities:

5.2.5.1. Alkaline phosphatase: Alkaline phosphatase activities in the Sps- R, F-.and S-strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (444.09 % of L-strain). The same trend was observed in the case of Cpf and Mfz-resistant strains, where the highest activities were recorded in Cpf-R and Mfz-R strains with percentages 522.84 % and 451.30 % of L-strains. A positive correlation coefficient was obtained between resistance level to the three insecticides and alkaline phosphatase activities. (0.7713, 0.8987 and 0.8228) for Sps, Cpf and Mfz respectively. The highly significant alkaline phosphatase activity as compared to L- and S- and F- in addition to the positive correlation

coefficient observed in the three insecticides, could lead to the suggestion that alkaline phosphatase play an important role in the resistance mechanism to the three tested compounds. 5.2.5.2. Acid phosphatase: In the three insecticides used the acid phosphatase activity was higher in the resistant strain than the other three strains and the activity can be arranged descendingly as follows: R->F->S->L-strain. a) The highest acid phosphatase activity was recorded in Cpf R-strain (800% of L-strain). b) There was a positive correlation coefficient between resistance level to the three insecticides and acid phosphatase activities. (0.7784, 0.9780 and 0.9111) for Sps, Cpf and Mfz respectively. Summary 134 c) It was concluded that acid phosphatase may play an important role in the resistance mechanism to the three tested compounds and this role is obvious in organophosphate compound.